CLINICAL PHARMACOLOGY OF LIDOCAINE-PRILOCaine CREAM IN INFANTS

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy, Graduate Department of Pharmacy, University of Toronto

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Clinical Pharmacology of Lidocaine-Prilocaine Cream in Infants

Doctor of Philosophy 1997

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Abstract

Introduction: Despite evidence that infants experience pain, analgesics are not administered frequently during painful procedures. The objective of this research was to determine whether procedural pain in infants could be diminished with topical lidocaine-prilocaine 5% cream (EMLA). The following specific hypotheses were tested: EMLA is efficacious for treatment of pain in infants undergoing vaccination and circumcision; EMLA is safe for treatment of pain in infants undergoing circumcision and heel lancing; and untreated pain from circumcision has long-term effects on infant pain behaviour that can be observed during routine vaccination.

Methods: Five studies were performed to test the hypotheses. The first study was a double-blind randomized controlled trial of the efficacy of EMLA for treatment of pain during routine 4 or 6 month vaccination. Pain was assessed using behaviour, crying and visual analogue scale scores. The second study was a retrospective analysis of vaccination pain scores of circumcised and uncircumcised male infants from the first study. The third study was a double-blind randomized controlled trial of the efficacy and safety of EMLA for treatment of pain during neonatal circumcision. Pain was assessed using facial activity, crying, heart rate, and blood pressure. Safety was assessed by measuring methemoglobin, lidocaine, and prilocaine concentrations. The fourth study was an observational study investigating vaccination pain responses among uncircumcised male infants and circumcised male infants who participated in the third study. Pain
was measured using infant facial activity, crying, and visual analogue scale scores. The fifth study was an open trial of the safety of EMLA in preterm neonates aged 30 to 37 weeks gestation. Safety was assessed in a similar way as in the third study.

Results: EMLA was efficacious for pain management during vaccination and circumcision. Lidocaine and prilocaine were measurable in low concentrations and methemoglobin concentrations were not elevated after administration of EMLA. Circumcised infants demonstrated an increase in pain response during routine vaccination compared with uncircumcised infants. Infants pre-treated with EMLA for pain from circumcision scored between the uncircumcised infants and the infants circumcised with a placebo.

Conclusions: EMLA decreases procedural pain in infants.
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General Introduction

Infants commonly undergo surgical procedures such as circumcision and invasive medical procedures such as vaccination, venepuncture, and heelstick without the benefits of analgesia (Toffler et al., 1990; Wellington et al., 1993; Howard et al., 1996; Tohill et al., 1990; Rutter et al., 1992; Bauchner et al., 1992). The practice of not providing analgesia is a direct result of the myths and beliefs that have been propagated about pain in infants (Walco et al., 1994; Cunningham, 1993; Schechter, 1989).

Myths and Beliefs About Pain in Infants

One of the myths that has been propagated about pain in infants is that infants are neurologically immature and cannot experience pain (Walco et al., 1994). This myth appears to persist, despite studies revealing that the nociceptive system is functional by 30 weeks gestation. Clinical evidence also supports the notion that infants have pain. Infants react to noxious stimuli with many of the same physiological, biochemical, and behavioural changes that are observed in adults in pain.

Another myth is that the benefits of pharmacologic pain management are outweighed by the risks of toxicity from the medications (Walco et al., 1994). Although it has been demonstrated that the pharmacokinetics of analgesic drugs differs in young infants when compared with adults, this difference in drug disposition does not prevent the use of analgesics in this population. The therapeutic benefits of analgesia can be achieved with a minimal risk of toxicity if changes to drug dosing and clinical monitoring regimens are made when these drugs are administered in infants.
Finally, there is the myth that young infants do not remember their pain (Schechter, 1989). That is, pain has no long-lasting consequence on the infant, and any unintentional harm resulting from painful procedures is simply forgotten. However, even prior to the research presented here, there were preliminary clinical data to suggest that early pain experiences resulted in permanent alterations to the nociceptive neural pathways, with the potential to influence future pain experiences.

In addition to myths about infant pain, there are also some beliefs that predispose clinicians to overlook pain management issues in this population. One belief is that, because infants cannot articulate their pain using words, the severity of their pain cannot be measured accurately. However, previous observational studies have demonstrated that infants communicate their pain in many ways that can be objectively measured (Grunau et al., 1987; Craig et al., 1984; Craig et al., 1993; Johnston et al., 1988). These responses have been characterized and used to develop pain assessment tools which measure the intensity of infant pain.

Finally, another prevalent belief undermining infant pain management is that infants experience less pain than older patients in response to a given noxious insult (Quinn et al., 1993; Schechter, 1989). Again, the evidence to refute this belief is available, and there are no data that would appear to support it.

**Procedural Pain**

The skin is the most common site of noxious input. All infants will undergo routine medical procedures which are associated with skin (cutaneous) pain, such as heel lancing or vaccination. In addition, a substantial number of newborn Canadian male infants (approximately 1/3) will experience pain from circumcision. Pharmacologic agents commonly used to manage
major surgical pain are not considered appropriate for use in these procedures. As a result, pharmacologic agents are not routinely utilized for the management of procedural pain in clinical practice. Opioid analgesics, for instance, are used in ventilated infants. They are not routinely used in non-ventilated infants due to fears of respiratory depression and muscle rigidity. Local anesthetics traditionally have required parenteral administration, which is perceived to be more painful than the procedure itself. In addition, administration of local anesthetics often require technical skills (e.g., infiltration, regional techniques) that are not routinely acquired by most physicians.

Recently, a new topical local anesthetic cream (lidocaine 2.5% - prilocaine 2.5% cream; EMLA 5% cream, Astra) has become commercially available that offers an option for a non-invasive method of treatment of procedural pain in infants. EMLA cream penetrates intact skin and has been shown to decrease pain from numerous cutaneous procedures in children and adults (Buckley et al., 1993). To date, few studies have investigated the safety and efficacy of EMLA in young infants. There has been apprehension among clinicians and researchers about using EMLA in this population due to concerns about the risk of methemoglobinemia, a toxic effect of prilocaine that can occur after its administration in young infants. At the time this research was undertaken, the efficacy and safety of EMLA for procedural pain management in young infants had not been well studied.

**Overall Hypothesis and Scope of Research**

The overall working hypothesis of this research was that procedural pain in infants could be successfully decreased with pharmacologic intervention. The pharmacologic agent investigated was EMLA cream. In 5 studies of this thesis, the following research questions were addressed: is
EMLA safe and efficacious, and are there long-term effects of untreated procedural pain? The results dispel and refute some of the commonly held myths and beliefs regarding the clinical management of pain in infants.

**Working Hypotheses**

The following hypotheses were tested:

1) EMLA decreases pain from routine vaccination in 4 to 6 month-old infants
2) EMLA decreases pain from newborn male circumcision
3) EMLA is safe in full-term neonates for treatment of circumcision pain
4) EMLA is safe in preterm infants for treatment of pain from heel lancing
5) Untreated pain from newborn male circumcision affects pain behaviour 4 to 6 months later at routine vaccination.

The investigations which are presented in this thesis are based on a thorough review of pain theory, pain measurement and the pharmacology of local anaesthetics.

**Theories of Pain**

Many theories have been proposed over the last century to explain the phenomenon of pain. These theories have been critically reviewed (Melzack et al., 1988), and are briefly summarized here. The traditional theory of pain, *specificity theory*, was originally described by Descartes in 1664. Descartes proposed that a specific pain system carried all nociceptive information from pain receptors in the skin to a pain centre in the brain. The main limitation of specificity theory was that it could not explain different qualities of pain sensation. The theory was subsequently modified by Muller (1842), who proposed that the quality of sensation was
given by the location of the nerve terminals in the brain, i.e., the brain was responsible for the sensation.

Specificity theory was further modified by Von Frey in 1895. He proposed that four major cutaneous modalities, or receptors, (i.e., touch, warmth, cold, pain) were responsible for the quality of sensations, and that each modality had its own pathway to a specific location in the brain. Von Frey's theory had three main assumptions. The first assumption was that receptors were specialized and each of the four modality receptors had a specific form of stimulus energy to which it was sensitive. This assumption of an "adequate stimulus" was supported by experimentation. The second assumption was that there were single specific receptors on the skin for each of the four modalities. The third assumption was that there was a fixed, one-to-one relation between the skin and the brain, so that each modality had its own distinct neural pathway to different parts of the brain. Both the second and third assumptions were not supported by physiological, psychological and clinical experimentation, leading to the development of the pattern theory of pain.

Pattern theory, as described by Goldscheider in 1894, proposed that pain was a result of stimulus intensity as well as central summation (i.e., patterning of the input). Several additional theories emerged from this original concept, including the peripheral pattern theory, central summation theory and sensory interaction theory. All of these theories recognized patterning as important for pain. However, they did not apply consistently concepts such as physiological specialization into their mechanisms.

An alternate theorist proposed that pain was a sensory modality. This theory is known as the affect theory of pain, and was described by Marshall in 1894. The theory assumed that pain was a primary sensation and that motivational and cognitive processes were reactions to pain.
However, it is currently believed that motivational-affective processes are part of the experience of pain (Melzack et al., 1988).

All of the theories described above have made important contributions to our understanding of pain. Each has provided a mechanism for explaining the complex experience of "pain." However, no single theory adequately describes how all pain messages are transmitted and/or modulated by psychological factors. The gate control theory was introduced by Melzack and Wall to compensate for the limitations of the previous theories and to provide a more wholistic approach to the phenomenon of pain (Melzack et al., 1965).

**Gate Control Theory**

The gate control theory proposes that pain perception involves modulation of nociceptive input by the brain. The sequence of events begins with transmission of nociceptive information from the periphery to the spinal cord by myelinated A-δ (delta) and unmyelinated C peripheral afferent nerve fibres. These neurons transmit information to second-order neurons in the spinal cord. At the level of the spinal cord, this information can either be inhibited or facilitated (i.e., gated) by interneurons which regulate the transmission of nerve impulses. The brain can also inhibit or facilitate the transmission of messages from the spinal cord. Thus, psychological and evaluative factors can also modulate the perception of pain, making the pain experience the result of a complex interaction between afferent fibre input and modulating systems. Additional mechanisms have been added to the original model to help explain long-term changes in pain sensitivity. One of these is referred to as the "impulse-triggered prolonged pain mechanisms" which proposes that changes in pain sensitivity can result from nociceptive inputs. The second is
referred to as “transport-controlled prolonged pain mechanisms” which proposes that chemicals transported within the axons of sensory fibres can induce long-term changes.

Melzack and Casey (1968) propose that there are three dimensions of activity that interact with one another to determine the experience of pain. These dimensions are: sensory-discriminative, evaluative-cognitive, and affective-motivational. The sensory-discriminative (i.e., perceive/feel) dimension refers to the ability to identify and characterize the noxious stimulus. The evaluative-cognitive (i.e., thinking) dimension refers to the ability to interpret the noxious stimulus. The motivational-affective (i.e., acting) dimension refers to escape and avoidance behaviours that occur as a consequence of the stimulus.

**Defining Pain in Infants**

The first dimension of pain, sensory-discrimination, is known to be present in infants (Wachter-Shikora, 1981). Previous research has shown that the infant’s nervous system is sufficiently mature at the time of birth to respond to noxious stimuli. There is limited evidence to support the presence of evaluative-cognitive and motivational-affective aspects of pain in infants. Infant factors such as sleep/wake state have been shown to modulate infant pain response (Grunau et al., 1987). Craig et al. (1988b) suggest that variability in infant pain responses would not occur if pain response were merely reflexive. It has been suggested that crying is also a higher nervous system function because it involves more than a reflex at the spinal cord level (Wachter-Shikora, 1981).

In accordance with the gate control theory, pain has been defined by the International Association for the Study of Pain (IASP) as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain is
always subjective; each individual learns the application of the word through experiences related
to injury in early life" (Merksey et al., 1994). This definition of pain has limited applicability to
young (preverbal) infants because it states that each individual learns the meaning of pain through
life experiences and it imposes the requirement of self-report of pain.

A modification of the IASP definition of pain has been proposed that can be applied to
young (preverbal) infants (Anand et al., 1996). Anand proposed that the perception of pain is an
inherent quality of life present at the time of birth. In addition, self-report is not a necessary
component of pain assessment. Pain in young (preverbal) infants can be inferred from biological
and behavioural responses, as they constitute the infants' self report at this developmental stage.

This modified definition of pain (Anand et al., 1996) has been criticized because it does
not account for consciousness, (i.e., evaluative, emotional and cognitive components in the
appreciation of pain) but suggests that pain can be inferred from the observed response
(Derbyshire, 1996). Rather, the neurophysiological system of young infants may react to noxious
stimuli without the presence of consciousness. The term ‘infant pain response’ is used throughout
this manuscript but it is recognized that infant pain experience is assumed to underlie the infant
pain response.

**Nociception**

An overview of the sensory-discriminative aspect of pain, that is, nociception, is now
provided. This background is necessary before current theories of pain plasticity and the
pharmacology of local anesthetics are discussed. Nociception is defined as: the detection of a
noxious stimulus and the transduction/transmission of information about the presence and quality
of that stimulus from the site of stimulation to the brain (McGrath, 1990). Nociception does not
include either the evaluative or affective components of pain that are included in the current IASP definition of pain.

**The Action Potential**

Messages are transmitted from peripheral receptors in the skin, muscle and bone by nerve cells by way of action potentials generated from electrical, mechanical, thermal or chemical stimuli. Nerve cells are made up of cell bodies and elongated processes called axons. The axonal membrane is made up of a phospholipid bilayer and membrane proteins with interspersed ion channels. These ion channels allow trans-membrane flux of selective ions (sodium or potassium) and are responsible for the generation of action potentials. Schwann cells surround all nerves. These cells are not believed to be involved in impulse conduction. They do, however, manufacture myelin, which is a lipid material that encircles many neurons. Gaps, called nodes of Ranvier, interrupt the insulating myelin layer at regular intervals along the axonal membrane between adjacent Schwann cells and their associated myelin (DeJong, 1994).

Under normal conditions, there is a negative resting membrane potential (interior of cells is negatively charged with respect to the exterior environment). The resting membrane potential results from the combined effects of the sodium-potassium pump (which actively pumps sodium out of the cell and potassium in) and a high resting membrane leakage of potassium. Following an increase in the resting membrane potential, sodium channels open, allowing sodium influx into the cell. This influx of sodium causes depolarization of the membrane. Shortly thereafter, potassium channels open, allowing potassium to efflux from the cell down its concentration gradient. The sodium channels inactivate quickly, and outflowing potassium ions restore the membrane potential (DeJong, 1994).
The impulse self-propagates along the axonal membrane from activated to resting membrane segments. The spread of the impulse does not depend on continued external stimulation of the nerve. In nonmyelinated axons, the impulse is propagated by sequential depolarization of membrane segments. In myelinated axons, however, impulses are conducted from node to node. This type of conduction is referred to as saltatory (jump-wise) conduction, and allows more rapid and efficient impulse conduction. Retrograde conduction is prevented by the nerve's inability to respond to a new stimulus (refractory state) immediately following depolarization (DeJong, 1994).

**Afferent Neurons Involved in Nociception**

Nerve conduction through the nociceptive system has been extensively reviewed (Woolf, 1987; Wilcox, 1991; Jones, 1992; Markenson, 1996). Nociceptive information is transmitted to the brain via peripheral and central neurons where pain is perceived. Under normal conditions, the peripheral afferent nerve fibres which transmit noxious information are myelinated A-δ (group III) and unmyelinated C (group IV) fibres. The A-δ and C afferent fibres conduct information in cutaneous and muscle nerves. In both types of nerve, the non-myelinated afferents outnumber the myelinated ones (Wall et al., 1984). The A-δ afferents respond to mechanical and thermal input. They transmit information quickly (5-25 meters per second) and mediate "first" or sharp pain. C-fibre afferents respond to mechanical, thermal, and chemical input. They conduct information at a slower rate (< 2 meters per second), and are responsible for "second" or dull pain. The A-δ and C-fibre afferents terminate in the dorsal horn (DH) of the spinal cord. A-δ fibres terminate mainly in Rexed's laminae I, V, and X. Cutaneous C-fibres terminate in lamina II (substantia gelatinosa) and muscle C-fibres terminate in lamina I and V (Wall et al., 1984).
**Processing of Nociceptive Information at the Spinal Level**

Nociceptive input is somatotopically organized at the level of the DH (Jones, 1992). Thus, a structural framework exists for the transfer of information from cutaneous afferent neurons to DH neurons. In the DH, information is mediated by two classes of neurons; nociceptive specific (NS, class 3) neurons, and wide dynamic range (WDR, class 2) neurons. NS neurons respond to A-δ and C-fibre input only. WDR neurons respond to nociceptive (C-fibre) and non-nociceptive (A-β, low threshold, tactile) input. The response properties of the DH neurons are complex. Dermatomal regions responding to A-β input overlap with regions that respond to C-fibre input. The size of the receptive field may be increased or decreased by different NT. Repetitive activation of C-fibre input increases the size of the receptive field, leading to nociceptive input from a previous low threshold region.

Conduction of nociceptive information is transmitted rostrally mainly via axons that ascend in the contralateral spinal cord. Information is transmitted to the ventroposterior and medial thalamic nuclei and to somatosensory areas of the cerebral cortex where the message is interpreted. Neurons also project into the limbic system, where arousal, affective, neuroendocrine and autonomic responses ensue.

**NMBA and non-NMDA Receptors**

Nociceptive afferent neurons release neurotransmitters (NT) from vesicles which cause excitation of second order neurons in the DH. Two classes of NT dominate transmission of nociceptive afferent input to DH neurons; excitatory amino acids (EAA), and neuropeptides. EAA mediate excitatory postsynaptic potentials between dorsal root ganglia and spinal neurons (Dickenson et al., 1990). EAA (e.g., glutamate, aspartate) act on at least two post-synaptic
ligand-operated ion channel receptor systems; NMDA (N-methyl-D-aspartate) and non-NMDA receptors (e.g., AMPA; \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, kainate, and quisqualate). Ligand binding to the non-NMDA receptor leads to the opening of the sodium-potassium channel. Influx of sodium into the cell causes depolarization of the membrane. The presence of ligand binding (e.g., EAA such as glutamate, aspartate) alone, however, does not lead to opening of the NMDA receptor channel. There is a voltage-dependent block at physiologic membrane potential due to the presence of magnesium ions (Mg\(^{++}\)) in the NMDA channel (Mayer et al., 1984; Nowak et al., 1984). In order to overcome the magnesium ion block, prior depolarization is necessary. Moreover, glycine must be present for the channel to open and there appears to be a separate binding site for glycine, distinct from the EAA site. Thus, three conditions must be met before the NMDA channel can operate: 1) prior depolarization to remove the magnesium block; 2) glutamate binding; 3) glycine binding. Co-release of neuropeptides and EAA leads to activation of the NMDA receptor through depolarizations of other receptor systems which remove the magnesium block. The NMDA receptor is believed to play a role in altering cell responsivity in the presence of repetitive noxious stimulation. Changes in cell responsivity may lead to long-term alterations in the processing of nociception and possibly underly some clinical pain states (discussed in further detail later).

Glutamate binding sites and NMDA receptors are abundant in the substantia gelatinosa of the DH, where nociceptive afferents terminate (Dickenson et al., 1990). Unlike the non-NMDA receptor, the NMDA receptor ion channel system lets in sodium, potassium, as well as divalent cations such as calcium and magnesium. Influx of calcium is believed to initiate a cascade of biochemical events in the cell. These include induction of oncogenes such as c-fos, long-term potentiation, synaptic remodelling and neurotoxicity. Due to the complexity of NMDA receptor
channel activation, it is believed to play a minor role in normal synaptic transmission. Instead, the NMDA receptor is believed to play a role in altering cell responsivity in the presence of repetitive noxious stimulation. Only stimuli that are sufficient, with respect to intensity or due to temporal summation, can induce NMDA receptor activity. NMDA receptor activity, in turn, amplifies, enhances and prolongs activity in spinal cord DH neurons through a phenomenon called “wind up” and contributes to a state of central hypersensitivity or sensitization (Dickenson, 1994).

The neuropeptides that dominate transmission of information from primary afferents to second order neurons include substance P (SP), neurokinin A, and neurokinin B (Wilcox, 1991). These neuropeptides act on two classes of post-synaptic receptor systems: neurokinin-1 (NK-1) and neurokinin-2 (NK-2). SP acts mainly at NK-1. Its mechanism of action includes activation of phospholipase C which leads to an increase in the production of inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 promotes intracellular calcium release. Moreover, there is an increase in calcium influx and a decrease in potassium efflux, causing hyperresponsivity of the cell to other neurotransmitters. Other NT that are involved in pain processing at this level are calcitonin gene-related peptide (CGRP), somatostatin, bombesin, neotensin, neuropeptide Y, GABA, acetylcholine, and adenosine.

C-fibres containing SP have been shown to contain glutamate as well. Thus, it is likely that noxious stimulation leads to release of both neuropeptides and EAA from the central terminals of primary afferent neurons. There may, however, be a differential release pattern of neuropeptides and EAAs. For instance, EAA are released after acute and prolonged noxious stimulation, while neuropeptides may only be released when stimuli are of sufficiently long duration. Synaptic transmission occurs over wide range of time epochs from tens of milliseconds for fast transmitters such as EAA acting on AMPA receptors, hundreds of milliseconds for EAA
acting on NMDA receptors, to tens of seconds for tachykinins acting on neurokinin receptors (Wilcox, 1991). Under normal circumstances, it is believed that most synapses operate to produce subthreshold responses of varying amplitudes and action potentials in postsynaptic cells are generated by multiple inputs (Woolf, 1991).

**Modulation of Nociceptive Input**

The excitatory effects of primary afferent discharge are subject to pharmacologic modulation at all levels of the nervous system. This modulation either increases or decreases the effects of primary afferent input. In the spinal cord, the dominant endogenous antinociceptive NT are the opioid peptides, serotonin, and adrenergic agonists. The opioid peptide ligands are β-endorphin, met-enkephalin and dynorphin. The three main types of receptors they act upon are μ (mu), κ (kappa), and δ (delta). There is a high concentration of μ-receptors in the substantia gelatinosa. It is important to note that no endogenous ligand is specific for any one receptor.

Antinociceptive medications are also available to diminish pain. The mostly commonly used medications include the opioid analgesics, local anesthetics, nonsteroidal anti-inflammatory drugs (NSAIDS) and acetaminophen. Opioids have activity both pre- and post-synaptically. Pre-synaptically, they inhibit release of NT. Post-synaptically, they hyperpolarize cells. In both cases, the mechanism of action for mu and delta receptors involves activation of a G-protein, which leads to an increase in potassium efflux from the cell. Kappa receptors act by closing calcium channels (Dickenson, 1991). Local anesthetics inhibit the generation and conduction of nerve impulses by blocking sodium flux into the neuron. The pharmacology of local anesthetics is discussed in more detail later. NSAIDS and acetaminophen do not directly block nociceptors. Instead, they inhibit synthesis and release of eicosanoids (prostaglandins, leukotrienes) which are
inflammatory chemicals that are released during injury. Inflammatory mediators may stimulate nociceptors directly, or enhance the sensitizing effects of other stimuli (Rang et al., 1991).

**Fetal Development of the Nociceptive System**

Studies of the development of pain pathways suggest that the functional capability for the perception of nociception is present in the newborn neonate. A brief summary of these data, obtained from more extensive reviews (Fitzgerald, 1993; Anand et al., 1989) is provided below.

The development of the nociceptive system occurs throughout gestation and involves a complex series of changes in molecular, cellular and organizational units. Cutaneous sensory perception, present in the seventh week gestation, increases gradually in responsiveness from the perioral area to all cutaneous surfaces by 20 weeks gestation. The formation of central synapses between the first primary afferent terminals and neurons in the spinal cord precedes sensory perception, beginning at six weeks gestation. A-fibre terminals are believed to enter the superficial DH before C-fibre terminals. C-fibre growth into the spinal cord occurs between 8 and 10 weeks gestation. Morphologic differentiation of the DH neurons begins at around 13 weeks. Rexed’s laminae, synaptic connections and most specific NT vesicles approximate the adult spinal cord by 30 weeks (Fitzgerald, 1993; Anand et al., 1989).

Myelination in the spinal cord and brain stem is also completed by 30 weeks gestation. In the thalamocortical tracts, myelination is completed by 37 weeks (Gilles et al., 1983). It has been suggested that the incomplete myelination of nerves at this stage in fetal development is indicative of a lack of nociceptive transmission (Anand et al., 1989). However, nociceptive impulses are also transmitted by unmyelinated neurons in adults, and incomplete myelination results in a slower conduction velocity in the fetus, not a decrease in conduction.
The production of NT involved in the transmission of nociceptive information in the neonate has also been investigated. NT are present in the spinal cord in small amounts at 8-10 weeks gestation (Anand et al., 1989). The concentrations, however, are low and increase through fetal life. There is a marked increase in NT levels during the perinatal period thus suggesting a degree of maturity of the system at birth (Fitzgerald, 1993).

The fetal neocortex begins to develop at 8 weeks gestation, and by 20 weeks gestation there is a full complement of neurons (Anand et al., 1989). The development of thalamocortical synaptic connections are established between 20 and 24 weeks gestation (Kostovic et al., 1983; Kostovic et al., 1984). The functional maturity of the cerebral cortex has been measured using various techniques including studies of EEG patterns, cerebral glucose utilization, and sleep-wake periods. Somatosensory evoked potentials recorded in premature infants suggest that afferent thalamic inputs reach the somatosensory cortex at 29 weeks gestation (Klimach et al., 1988). They are slow in premature neonates but by 40 weeks gestation, the latency is decreased and the pattern quite complex (Hrbek et al., 1973). Anatomical studies substantiate these findings by revealing that thalamocortical fibres penetrate the cortical plate between 26 and 34 weeks gestation (Mrzljak et al., 1988). Cerebral glucose utilization studies show that maximal metabolic activity appears in sensory areas of the brain by 32 weeks gestation. Well-defined states of sleep and wakefulness begin at 28 weeks gestation (Anand et al., 1989).

Together, these data suggest that the functional capability for the perception of nociception is present in the newborn neonate. Even in the presence of such evidence, however, infants continue to be denied adequate pain management (Toffler et al., 1990; Wellington et al., 1993; Howard et al., 1996; Tohill et al., 1990; Rutter et al., 1992; Bauchner et al., 1992).
Central Neural Plasticity

One of the myths regarding infant pain that may be contributing to lack of pain treatment is the belief that untreated infant pain has no long-term sequelae. However, this myth is questionable based on a growing body of evidence that injury to the peripheral and/or central nervous system can cause persistent changes in how the nervous system responds to subsequent somatosensory inputs (Coderre et al., 1993; Woolf, 1991).

Activation of nociceptive afferent fibres may induce changes in the responsiveness of the DH neurons to subsequent input (i.e., neural plasticity). A distinction has been made between stimuli that do not alter the responsiveness of the nociceptive system and those that do. The former are believed to contribute to physiological pain, and the latter, pathological pain (Woolf, 1989). Physiological pain results from activation of high threshold afferent nociceptors which conduct information to the spinal cord and brain. Pathological pain is the result of changes to the somatosensory system from high threshold afferent nociceptive input that may persist over time. Four categories of change are believed to contribute to development of pathological pain: 1) peripheral sensitization of primary afferents, 2) central sensitization of DH neurons, 3) abnormal properties of central nervous system pathways, and 4) permanent changes in the central nervous system (Woolf, 1989). Pathological pain is manifested by changes in the response properties of DH neurons such as an exaggeration in amplitude and/or duration of the response to suprathreshold stimuli (hyperalgesia), diminished threshold for eliciting pain (allodynia), spreading of the sensation of pain to an un-injured site (referred pain and secondary hyperalgesia), and a prolonged response to a transient stimulus (persistent pain) (Woolf, 1989). In pathological pain, a new state of excitability leads to excessive responses to subsequent inputs that would not be painful if the spinal cord were not primed (i.e., allodynia), and can be sustained by mechanisms
within the spinal cord (Wall, 1988). A review of some clinical pain states and the proposed mechanisms for them, are briefly reviewed.

Hyperalgesia is a condition in which there is a decrease in pain threshold and an increase in pain to suprathreshold stimuli, or spontaneous pain, that occurs after peripheral injury (Merksey et al., 1994). There are two types of hyperalgesia, depending on the site of injury. Primary hyperalgesia refers to an increase in sensitivity to noxious stimuli at the site of the injury. Secondary hyperalgesia refers to increased sensitivity in an area extending beyond the site of the injury. Several peripheral mechanisms have been postulated to explain primary hyperalgesia (Treede et al., 1992). The first mechanism postulates that an increase in responsiveness of primary afferent nociceptors, called “peripheral sensitization,” may occur. Peripheral sensitization of A- and C-fibres increases central input without an enhancement of corresponding central neuron responses. A second mechanism postulates that hyperalgesia is caused by increases in receptive field sizes of primary afferents. This increase in receptive field size leads to spatial summation and increases the afferent input to the central neurons per given stimulus. The result is an increase in responsiveness to nociceptive input or increased responsiveness to non-noxious stimuli (i.e., allodynia). Thus, a stimulus that is applied within the site of injury activates more nociceptors than one that is in an adjacent non-injury site, increasing nociception. Alternatively, a third mechanism suggests that there may be a decrease in inhibitory input to WDR neurons from Aβ-fibres that have been injured. The consequence is therefore, an increase in synaptic efficacy of nociceptive input and hyperalgesia (Treede et al., 1992).

Peripheral sensitization is believed to be mediated by inflammatory chemicals that are released during injury. These chemicals include histamine, serotonin, bradykinin, SP, metabolites of arachidonic acid such as prostaglandins, leukotrienes, platelet activating factor, and free
radicals. Inflammatory mediators may stimulate nociceptors directly, or enhance the sensitizing effects of other stimuli (Rang et al., 1991).

Secondary hyperalgesia is a condition characterized by enhanced pain to mechanical stimuli in an area that immediately surrounds an injury site, but does not include the injury site (Treede et al., 1992). Both peripheral and central mechanisms have been postulated. The peripheral mechanism suggests that activated nerve terminals release chemicals that sensitize other local afferent fibres through a process called axon reflex. Stronger evidence, however, points to a central mechanism, whereby action potentials generated by nociceptive afferents in injured sites cause sensitization in the spinal cord and hyperalgesia.

**Evidence of Central Neural Plasticity in Adults**

Central mechanisms are believed to contribute to clinical pain states such as “phantom limb” pain (Coderre et al., 1993; Katz, 1993; Katz et al., 1990). Phantom limb pain refers to the persistence of pain from deafferented (amputated) limbs. Phantom limb pain cannot be explained in terms of peripheral or psychological mechanisms alone as blockade of input from either the periphery or somatosensory areas of the cortex fail to relieve it. It has been hypothesized that one type of phantom limb pain is due to the development of pain memories in the central nervous system during amputation. These pain memories are induced by the action of peripheral nociceptive afferents on central neurons due to pre-amputation pain or due to noxious inputs at the time of amputation and continue to exist after amputation. The involvement of a central mechanism in phantom limb pain is supported by clinical studies demonstrating a decrease in the incidence of phantom limb pain in patients who were kept pain-free prior to amputation (Bach et al., 1988; Jahangiri et al., 1994).
Central sensitization also contributes to postoperative pain in surgical patients (Coderre et al., 1993; Niv et al., 1993; Woolf et al., 1993; Dahl, 1994). "Pre-emptive analgesia," which involves the administration of analgesics prior to surgery in order to interrupt (prevent) the afferent nociceptive input that is induced by surgery and the resulting central sensitization of DH neurons, may decrease postoperative pain. Studies have compared the effects of pre- versus post-injury administration of opioids, local anaesthetics, and NSAIDS on postoperative pain and analgesic requirements. At present, the evidence in support of pre-emptive analgesia is accumulating. Studies that have shown significant effects have reported pain scores and analgesic requirements to be lower in the groups receiving the intervention (Katz et al., 1994; Katz et al., 1992; Richmond et al., 1993). These differences, although relatively small in magnitude and short-lived (lasting up to 72 hours), suggest that changes in central neural function induced by injury can be prevented if administration of pharmacologic agents that block nociceptive input occurs prior to the injury.

To summarize, then, activation of afferent fibres can lead to changes in the excitability of the nervous system to subsequent inputs, a phenomenon termed central sensitization. Central sensitization is believed to contribute to different clinical pain states. Adequate treatment of pain may prevent the central sensitization and the consequences of this altered state on future experiences of pain (Wall, 1988). The molecular mechanisms of central sensitization are briefly reviewed in the next section.

Central Sensitization

It has been shown that with continued input sufficient to stimulate C-fibres, there occurs in WDR DH neurons a progressive increase in the number of action potentials evoked per stimulus
(Mendell, 1966). This phenomenon is known as "wind up," in which a state of hyperexcitability is induced in WDR DH neurons. Wind up is dependent on the frequency of stimulation of DH neurons. It can occur following tissue injury, inflammation, or repeated stimulation of C-fibre afferents (Coderre et al., 1993). The spinal hyperexcitability has been shown to outlast afferent input, suggesting that once initiated, it can persist independently of afferent input (Woolf, 1983).

Central sensitization is induced by C-fibre inputs, and manifests as an expansion in size and increase in responsiveness of the receptive fields of DH neurons (Woolf, 1991; Woolf, 1987). C-fibres innervating different target tissues (e.g., skin versus muscle) have different efficacies in producing hypersensitivity (Woolf et al., 1986).

The NMDA receptor system is believed to mediate wind up and central sensitization. It is hypothesized that repetitive stimulation of C-fibres temporarily alleviates the magnesium block of the NMDA receptor channel. Removal of the magnesium block allows the NMDA receptor to participate in synaptic transmission. The cumulative depolarizations (i.e., wind up) then lead to the induction of a state of central sensitization. Central sensitization could be the result of: 1) uncovering a new polysynaptic pathway involving NMDA receptors, 2) tonic release of NT and tonic activation of NMDA receptors, 3) prolonged effects mediated by second messengers, such as c-fos gene expression (Woolf et al., 1991). The importance of the NMDA receptor in the development wind up is shown by electrophysiological studies revealing a reduction in wind up by NMDA receptor antagonists without an effect on A- and C-fibre evoked inputs and fast excitatory inputs (Dickenson et al., 1987). NMDA antagonists diminish the flexor reflex after C-fibre strength electrical or chemical stimulation and prevent wind up in motorneurons.

EAA and C-fibre neuropeptides are believed to play a significant role in central sensitization. Both types of NT are released after excitation of peripheral nociceptive afferents.
EAA are fast excitatory transmitters that act on AMPA (non-NMDA) receptors by causing fast depolarizations. Neurokinin peptides such as SP act by producing prolonged depolarizations. Together, the fast and slow postsynaptic potentials can summate to remove the magnesium block in the NMDA receptor channel. Slow potentials are particularly important because of the possibility for temporal summation. Summation of potentials produces NMDA receptor activation and depolarization (Woolf, 1989).

NMDA-mediated synaptic transmission may induce changes in gene expression in certain postsynaptic neurons. Induction of c-fos protein in superficial layers of the DH has been detected following stimulation of primary nociceptive afferent neurons (Hunt et al., 1987). Production of proto-oncogenes like c-fos is hypothesized to be involved in prolonged alterations in central transmission of nociceptive information and chronic neuropathic pain.

Since neonates are functionally capable of perceiving pain, and untreated pain can lead to central sensitization, prevention of pain in infants appears to be clearly warranted. Pain in infants, however, continues to be undertreated in clinical practice. One of the reasons used to justify current practice is that pain cannot be accurately measured in this population.

**Measurement of Pain in Infants**

While it is accepted that the pain experience of an infant cannot be directly measured due to its subjective nature and reliance on others for quantification, there are many observable signs that can be used to make objective assessments of pain in infants. In order to make pain assessment more reliable among different observers, there has been a considerable amount of research performed on the characterization of neonatal responses to noxious stimuli and the development of pain measures for this age group. Infant pain responses encompass a variety of
dimensions, including physiologic (e.g., heart rate, blood pressure), biochemical (e.g., cortisol, adrenaline), and behavioural (e.g., facial activity, body movements, crying). These responses are described in more detail below.

**Infant Physiologic and Biochemical Responses to Painful Stimuli**

Common medical procedures such as heel lancing, vaccination and circumcision have been shown to elicit characteristic physiologic changes in infants (Owens et al., 1984; Dale, 1986; Harpin et al., 1983b; Stevens et al., 1994a; Maxwell et al., 1987; Stang et al., 1988; Williamson et al., 1983; Porter et al., 1988). These responses consist of cardiovascular, respiratory, metabolic and hormonal changes. Increases in heart rate, blood pressure, respiratory rate, intracranial pressure and palmar sweating have been observed. Decreases in oxygen saturation and vagal tone have been also observed. Biochemical changes including increases in growth hormone, catecholamines, glucagon and corticosteroids (e.g., cortisol) have also been reported.

These responses are consistent with a stress response. Surgical stress activates the brain stem and limbic neural pathways, which stimulate the hypothalamus to secrete hormones. Systems that control the surgical stress response are integrated with nociceptive systems. Thus, analgesics such as opioids, which act by inhibiting nociceptive transmission, also blunt the stress response during major surgery when administered in high dose, thus decreasing adrenocortical, adrenomedullary, and centrally mediated stress hormone secretion.

In adults, the stress response can lead to various clinical complications, including persistent metabolic acidosis, thromboembolic complications, pulmonary insufficiency, impaired immune responses, cardiac insufficiency, dysrhythmias, and mortality (Kehlet, 1988). In infants, the presence of physiologic and metabolic immaturity makes maintaining metabolic homeostasis
Thus there may be a greater magnitude of change compared with adults and a higher incidence of postoperative complications. Levels of adrenaline, beta-endorphin, insulin, and glucagon have been used as indices of the surgical stress response (Anand et al., 1987b; Anand et al., 1988; Anand et al., 1992). The magnitude of the metabolic stress response correlates with the severity of the surgical stress (Anand, 1990). Administration of anaesthesia in infants undergoing major surgery diminishes the stress response, and decreases postoperative morbidity and mortality (Anand et al., 1987b; Anand et al., 1988; Anand et al., 1992). For minor surgery such as circumcision, infiltration of local anaesthesia has been shown to minimize physiologic instability and hormonal changes (Masciello, 1990; Stang et al., 1988; Maxwell et al., 1987; Holve et al., 1983; Williamson et al., 1983).

Physiologic and biochemical indicators, although objective and well correlated with pain, are not specific to pain (Chapman et al., 1985). Changes due to other factors, such as medical illness, must be ruled out. Pain responses may not be easily distinguished from other stress responses. There are other disadvantages with the use of physiologic and biochemical indicators as measures of pain. Physiologic parameters may be difficult to interpret in situations where there are technical problems relating to the monitoring devices used. In fact, application of physiologic monitors on the very young infant may modulate the response. With respect to the measurement of hormones and other chemicals in infant plasma, practicality is an issue. In situations where minor surgical or other medical procedures are being performed, the additional pain from blood sampling and the quantity of blood needed to perform the tests may not be ethically justified.
Infant Behavioural Responses to Painful Stimuli

Facial Expressions

Detailed descriptions of infant facial expressions, gross motor activity and cry have been undertaken to determine whether tissue damage or injury results in a unique pattern of behavioural response. Facial expressions have emerged as the most promising univariate indicator of pain in infants (Craig et al., 1994). Facial expressions in response to injury show consistent and temporally integrated patterns for different types of stimulation and tissue injury. They also show a strong correspondence to the facial patterns expressed by adults in pain. Facial expressions are the most widely utilized behavioural approach of infant pain assessment.

Izard (1982; 1987) developed a coding system to classify infant facial reactions according to emotional states, including pain (Maximally Discriminative Facial Movement Coding System, MAX). The pain state is characterized by facial movements in three areas: brow (lowered and drawn together), eyes (closed tightly) and mouth (angular, squarish with nasal root widening and bulging). Pain assessments are made by trained personnel that view infants' responses from videotapes.

Grunau and Craig (1987) adapted an existing facial coding system for use in neonates (Neonatal Facial Coding System; NFCS). Like the MAX, the NFCS uses discrete facial actions to infer infant pain from noxious stimulation. Unlike the MAX, however, there was no attempt to describe emotional states. Infants are scored for the presence or absence of the following facial actions: brow bulge, eye squeeze, naso-labial furrow, open lips, vertical stretch mouth, horizontal stretch mouth, lip purse, taut tongue, chin quiver, and tongue protrusion. The NFCS has demonstrated sensitivity and specificity. Facial activity scores correlate with scores obtained from
other pain measures and with parental pain ratings (Craig et al., 1988a; Craig et al., 1994). The amount of infant facial action following noxious stimulation varies with the nature of the medical procedure (i.e., invasive or noninvasive) (Craig et al., 1993; Grunau et al., 1990) and the gestational age of the infant (Craig et al., 1993). Like the MAX, the NFCS is scored from videotapes by trained coders. Both measures are labour-intensive and not practical in a clinical setting.

**Crying**

Infant vocalizations have been used to assess pain (Johnston et al., 1988; Grunau et al., 1987; Rushforth et al., 1994; Grunau et al., 1990; Owens et al., 1984; Dale, 1986; Fuller et al., 1988; Porter et al., 1986; Maikler, 1991). Cry characteristics included in pain assessments are: duration, fundamental frequency, pitch pattern and harmonic structure. Spectrographic analyses have demonstrated differences between pain cries and other cries, although the cry sounds from painful situations are not uniquely different from those of other types of cries (Zeskind et al., 1985; Fuller, 1991; Johnston et al., 1988; Porter et al., 1986). Cry is considered a valid measure of pain in healthy infant populations. It is problematical in premature infants because they do not consistently exhibit this behaviour following noxious stimulation (Stevens et al., 1994b).

**Body Movements**

Observations of infant body movements have also been used by investigators to assess pain. Infants react to acute noxious stimuli by thrashing, jerking, wiggling, withdrawing, kicking or exhibiting torso rigidity (Johnston et al., 1986; Craig et al., 1984; Mills, 1989; Dale, 1986; Maikler, 1991; Bozzette, 1993). Craig (1993) developed the Infant Body Coding System (IBCS) to describe neonatal body activity. The IBCS is used in a similar fashion as the NFCS; trained
coders score the presence or absence of hand, foot, arm, leg, head and torso movements. As with the NFCS, infant body activity varies with the type of procedure and gestational age (Craig et al., 1993).

The flexor reflex has also been used as a measure of pain in neonates. The flexor reflex is a nociceptive reflex that involves withdrawal of a limb from a painful stimulus. The pattern of the flexor reflex response correlates with noxious sensory input and with pain perception in the adult (Woolf, 1987). In infants, the flexor response involves a diffuse pattern which may include the entire body. Responses are greater in magnitude and duration than in adults. The threshold for reaction is also lower, so that elicitation of a response can occur with non-noxious stimulation (Fitzgerald et al., 1988). The flexor reflex response becomes more restricted (i.e., specific), as the neonate matures.

**Multidimensional Pain Assessment**

Multidimensional pain assessment scales have also been developed to assess infant pain. They include variables from more than one dimension (e.g., facial action, cry, heart rate) in order to facilitate a more complete description of the pain response. The multidimensional approach is believed to improve the validity of the pain assessment. In general, these scales have concentrated on behavioural responses; namely, facial, cry and body behaviours due to the frequency of occurrence of these reactions and the ease with which they are observed. A pain score is assigned to each item on the measure, and the overall pain score is computed by summing the scores from the individual items.

Several different measures have been developed to reliably measure pain in infants of different ages (Stevens et al., 1996a; Pokela, 1994; Lawrence et al., 1993; Robieux et al., 1991;
Barrier et al., 1989). Some neonatal scales include infant behavioural state (i.e., sleeping vs awake) in the assessment because behavioural state can modify infant pain response (Grunau et al., 1987; Stevens et al., 1994a).

In summary, infants' responses to noxious stimuli include physiologic, hormonal, and behavioural changes. Since these responses are both objectively and reliably measured, they can be used as legitimate measures of infant pain. Although it is believed that the validity of infant pain assessment may be improved if a multidimensional approach is used, univariate measures can also be used. Together, these data do not support the claim that infant pain cannot be accurately assessed.

Clinical Evidence of Persistent Effects of Pain in Infancy

Untreated pain can have serious consequences in adults including the development of chronic clinical pain states. Several studies recently examined whether there are long-term effects of pain and stress in infants. The results from these studies suggest that untreated pain may have long-term effects on infants as well. However, these studies suffer from many methodologic flaws which prevent definite conclusions from being made, and they are reviewed below.

Short-term Effects on Infant Behaviour from Untreated Circumcision Pain

Several studies have investigated the effects of untreated circumcision pain on infant behaviour. In the first study, the sleeping patterns of circumcised and uncircumcised healthy full-term male infants was compared (Emde et al., 1971). Twenty infants were randomized to one of two study groups; circumcision during or circumcision after the study period. All infants were observed on 2 successive nights, beginning at 24 hours of age for 10-hours (from 2200h-0800h). Study conditions were identical in both groups, except that infants in the experimental group had
undergone application of the Plastibel. The Plastibel consists of a ligature used to promote ischemic necrosis of the foreskin over several days. In this study, the same infants were compared before and after circumcision and circumcised infants were compared with uncircumcised infants. The circumcised infants demonstrated a significant increase \((p<0.01)\) in non-rapid-eye-movement (REM) sleep, indicative of diminished arousal, on the second observation day compared to the first day. This increase in non-REM sleep was balanced by an decrease in REM sleep. There was also an decrease in the latency to the first non-REM sleep period and an increase in the number of non-REM sleep periods. No differences were observed from day one to two in the uncircumcised infants. It was postulated that the differential sleep pattern observed between the study days for the circumcised and uncircumcised groups were due to the effects of stressful stimulation. The results suggested the theory of conservation-withdrawal in which the neonate is considered to have a limited capacity to avoid stressful stimulation, and responds by increasing sensory thresholds, withdrawing attention, decreasing motoric activity and sleeping.

In another study, the effect of circumcision on sleep-wake states was investigated. Eleven neonates were observed by investigators for 1-hour periods at three different times: immediately following a feeding and preceding circumcision, immediately following the circumcision, and immediately following the first feeding after circumcision (Anders et al., 1974). Compared to activity prior to circumcision, infants demonstrated a significant \((p<0.05)\) increase in wakefulness, particularly fussy-crying behaviour, immediately following circumcision. A decrease in sleep onset latency and increase in quiet sleep was observed in the third observation period which investigators speculated may have been caused by the prolonged wakefulness after circumcision. No differences in the relationship between REM and non-REM sleep were noted.
Brackbill (1975) studied the behavioural and physiological effects of different levels of sound intensity on three groups of full-term neonates: uncircumcised males, circumcised males (who had been circumcised on average, 37 hours prior to testing), and females. The experimental design involved exposure of 10 neonates from each group to one of three levels of sound intensities (60, 70, or 80 db). Infants were exposed to a 30-minute control and 30-minute experimental period during which the ambient noise level was either 55 db or the experimental level. The results showed that as sound intensity increased, heart rate decreased and the percentage of time infants spent in quiet sleep (non-REM) significantly increased. Circumcised males, however, differed (p<0.05) in their response patterns compared with the other two groups: they demonstrated more time awake and greater decreases in heart rate.

In another study, a double-blind design was employed to investigate the effects of circumcision on infant reactions to different stimuli (Marshall et al., 1980). In that study, 26 male newborns whose parents chose for them to be circumcised were randomized to early circumcision (performed on the second day of life) or delayed circumcision (performed at 3 weeks of age). Blinded observers recorded infant behaviour on three separate occasions from 47 to 71 hours after birth. Changes in behaviour were measured using the Brazelton Neonatal Assessment Scale (BNAS). Changes between the preoperative and first post-operative observation periods occurred significantly more often in the early circumcision group compared to the delayed circumcision group (87% vs 16%, p<0.0001). Infant state at the beginning of the circumcision predicted how the behaviour changed. Changes in behaviour persisted to the third observation period (i.e., 22 hours after the circumcision) in approximately one-third of the sample. The investigators speculated that newborn circumcision might also affect the mother-infant interaction.
In a separate blinded randomized controlled study, the effect of circumcision on mother-infant interaction was investigated (Marshall et al., 1982). Fifty-nine mother-infant dyads were observed for 15 minutes during 4 feeding sessions over a 24-hour period. During each observation period, maternal and infant behaviours such as facial expressions, vocal expressions, feeding and touch were recorded onto a scoring sheet by study observers. Infants in the experimental group (n=27) were circumcised between the second and third observation periods, and those in the control group (n=32) were circumcised after the fourth observation period. The experimental group exhibited fewer intervals of uninterrupted feeding during the third observation period than the control group. However, the experimental group appeared to focus their attention on feeding more intensely, with less ability to socialize with the mother. The differences between groups did not persist beyond the third observation period.

In the most recent study, the effects of circumcision on infant behaviour were investigated (Dixon et al., 1984). Thirty-one male newborns were randomized to one of three study groups: circumcision using dorsal penile nerve block (DPNB) with lidocaine, circumcision using DPNB with saline, and circumcision without any injection. Behaviour assessments using the BNAS were made on 16 of the infants by observers that were unaware of the treatment group. These assessments were made on three occasions between feeding periods; the first was prior to circumcision, the second immediately following circumcision, and the third on the day following circumcision. Immediately after circumcision, infants who received lidocaine were more attentive and alert, and showed better motor coordination. In addition, they were better able to quiet themselves after distress (p<0.05). Thus, lidocaine prevented the changes in behaviour observed in unanesthetized infants.
In summary, a number of studies have revealed that circumcision without analgesia is associated with a disruption in infant behaviour that may make infants less available for social interaction. Administration of dorsal penile nerve block may prevent these changes. These changes persist for up to one or two days post-operatively and may be due to ongoing pain in the infants.

**Long-term Pain Behaviour from Repeated Noxious Stimulation**

Studies have investigated the development and responsiveness of the neonatal flexion reflex in neonates. In one study, 103 neonates aged 27.5 to 39.5 weeks post-conceptional age were observed following application of graded von Frey hairs to the lateral plantar surface of the foot (Fitzgerald et al., 1988). Younger post-conceptional age infants had lower thresholds than older infants and demonstrated an increase in the reflex response (i.e., hypersensitivity) with repeated stimulation. This behaviour was visible until 32 weeks post-conceptional age, when a pattern of habituation began to appear (i.e., no response) with the same stimulus (Fitzgerald et al., 1988). In another study (Andrews et al., 1994) of the flexion reflex responses of 50 infants (23-43 weeks post-conceptional age), similar results were observed. There was an increase in threshold with increasing post-conceptional age. The largest increase in threshold occurred after 35 weeks. Repeated stimulation resulted in decreasing thresholds for infants up to 35 weeks post-conceptional age (Andrews et al., 1994).

Hypersensitivity in the reflex response has been observed following tissue injury as well. In a study where premature neonates were subjected to repeated heel lances (for routine blood samples) on one foot only, the threshold for the flexor reflex decreased in the lanced foot
(Fitzgerald et al., 1989). In a double-blind study, chronic application of local anaesthesia (EMLA cream) prior to heel lancing prevented hypersensitivity of the injured foot (Fitzgerald et al., 1989).

The changes observed in the flexor reflex response have been attributed to developmental changes in the neonate in both peripheral and central nociceptive pathways. It has been postulated that the plasticity in flexion reflex threshold results from cutaneous hyperinnervation of the injured tissue. An animal study of the developmental and response patterns of cutaneous afferent fibres to skin injury revealed that neonatal animals have a more pronounced hyperinnervation of wounded tissue than adults, and that this hyperinnervation persists for long after the wound has healed. The innervation has been shown to be comprised of A- and C- fibres, and is associated with a decrease in mechanical threshold in the wounded area (Reynolds et al., 1995).

Differences in central nociceptive pathways also may explain the observed results. The interneurons in the DH of the spinal cord and descending inhibitory pathways are thought to develop in late gestation in the human fetus. These interneurons inhibit inputs to DH neurons, thus reducing the size of receptive fields (Fitzgerald, 1993). Descending inhibitory pathways, which link with interneurons, are also immature in the newborn rat. Moreover, serotonin and norepinephrine, both of which are known NT in descending pathways, appear postnatally.

In summary, the exaggerated neonatal flexion responses to noxious stimulation observed by Fitzgerald and colleagues (1989) may be at least partially due to the combined effects of hyperinnervation of the wound site and a lack of central inhibition. However, it is also possible that differences in thresholds between younger and older infants are due to younger infants receiving intensive care. Noxious stimuli associated with treatments in intensive care units may induce a change in central excitability and hyperalgesia in younger infants. Johnston and Stevens
(1996) recently tested the hypothesis that prolonged hospitalization, which is associated with repeated painful interventions, could affect infant pain behaviour.

Using a cross-sectional study design, the pain responses of newly born infants of 32 weeks gestation undergoing heel lancing were compared with the pain responses of infants of the same post-conceptional age delivered 4 weeks earlier (i.e., born at 28 weeks gestation). The two groups of infants differed significantly in their responses to heel lancing. The earlier-born infants showed less behavioural (i.e., facial) activity, but greater physiologic changes (lower oxygen saturation values, higher heart rate values) during the procedure than later-born infants. The factors that significantly affected behavioural response were the number of painful procedures the infant had, and Apgar score at delivery. For physiological responses, birth factors such as gestational age at delivery and birthweight were significant predictors. The investigators concluded that earlier-born infants had greater behavioural and physiological ‘immaturity’, due to past painful experiences and birth factors, respectively (Johnston et al., 1996).

The Johnston and Stevens study (1996) suggested that previous pain experience may affect infant pain behaviour later on. However, no definite conclusions could be made due to the observational nature of the study, and the lack of appropriate control groups. It would have been interesting to investigate the pain responses of newly born 28 week gestational age infants and infants of postconceptual age 36 weeks who were born 4 weeks earlier and compared these responses to those of the other two groups.

Clinical and Neurobehavioural Outcomes from Prolonged Hospitalization

In addition to the changes in pain response noted above, investigators have hypothesized that pain and stress may influence other aspects of infant development. Several studies have been
undertaken to determine the effects of cumulative stress and pain associated with prolonged hospitalization on infant outcomes. The results of these studies have demonstrated that infants who received care aimed at minimizing pain and stress had significantly better clinical and neurobehavioural outcomes than infants who received standard care. The improvement in infant outcome by minimizing noxious stimulation has implications for the prevention of pain during all noxious medical procedures.

Several studies have assessed the effects of “Developmental Care,” an individualized approach to caring for hospitalized preterm infants (Als et al., 1986; Als et al., 1994; Becker et al., 1991; Becker et al., 1993; Fleisher et al., 1995; Buehler et al., 1995). The individualized approach (care plan) is based on formal observation and evaluation of infant behaviours before, during, and after various care-giving procedures. A plan is designed and implemented which aims to alter the physical environment and care giving procedures in order to avoid stress and better coincide with infant maturity and natural sleep-wake states. The first study of developmental care used a phase-lag (i.e., before and after) study design and involved 16 preterm infants weighing <1250 g (Als et al., 1986). It showed that infants who received individualized care plans with minimization of inappropriate input had a reduction (p<0.05) in the number of days of supplemental oxygen and ventilation, and the number of days before bottle-feeding. Neurodevelopmental outcome, as measured by blinded testers at up to 9-months post-conceptional age using the Bayley Scales of infant development, was significantly better in the experimental group (Als et al., 1986). Two other small studies of similar design (i.e., phase-lag) in infants weighing <1501 g also reported clinical benefits with this approach (Becker et al., 1991; Becker et al., 1993).
The effectiveness of developmental care has been evaluated in randomized controlled trials as well. In one study, 38 infants weighing <1250 g were randomized to receive developmental care by specially trained nurses or to receive standard care. The control group consisted of staffing by non-trained nurses who were unaware of study group status. Compared to the control group, the individualized care group had a significantly shorter duration of ventilation and supplemental oxygen support, and a decrease in the incidence of medical complications such as severe bronchopulmonary dysplasia and intraventricular hemorrhage. The number of days in hospital was reduced and neurodevelopmental outcome at 9 months was significantly improved (Als et al., 1994). In another randomized controlled study of 40 infants ≤ 1250 g, however, no significant differences were observed in medical outcomes between groups (Fleisher et al., 1995). Developmental outcome, measured at 42 weeks post-conceptional age, was significantly better in the experimental group (p<0.05) (Fleisher et al., 1995).

Only one study investigated the effectiveness of developmental care in low-risk preterm infants (i.e., <2500 g) (Buehler et al., 1995). Infants were randomly assigned to experimental and control groups. There were no differences observed between the groups in medical outcomes, however, preterm infants who received developmental care had significantly improved behavioural scores (i.e., autonomic, motor, attentional functioning) at the two-week post-term follow-up when compared to preterm infants randomized to standard care. Electrophysiologic evaluations were consistent with the behavioural findings (Buehler et al., 1995).

Although the concept of developmental care has increased awareness of infant pain and stress, the magnitude of the benefit(s) of this approach are uncertain due to limitations in the study designs (Ohlsson, 1995; Lacy, 1995; Garland, 1995; Saigal, 1995). For instance, phase-lag study designs do not allow one to discern differences due to the intervention and changes that occur
over time. Lack of blinding may also contribute to differences between groups due to caregiver bias. Studies of developmental care have typically utilized narrow inclusion/exclusion criteria, which prevent extrapolation of results to all preterm neonates. In addition, most studies pre-date widespread use of pharmacologic interventions such as antenatal steroids and postnatal surfactant, which may significantly impact on infant ventilatory requirements and well-being. All of the studies utilized fairly small sample sizes, and although there were no statistically significant differences reported at study entry in demographic characteristics between treatment groups, entry characteristics consistently favoured the intervention groups. Moreover, differences in outcomes between treatment groups cannot be attributed specifically to pain.

Another avenue of research has focused on investigating the effect of prolonged hospitalization in a neonatal intensive care unit on childhood behaviours. One study prospectively examined the differences in pain-related behaviour between children at 4.5 years of age who had a normal infancy and those who experienced prolonged neonatal intensive care (Grunau et al., 1994b). The former group included extremely low birth weight (ELBW; <1000g) infants who were being monitored by the hospital neonatal follow-up programme. The full-term infants were recruited from community health centres. Seventy-two children participated (36 per group). The ELBW group had higher somatic complaints of unknown origin than full-term children. Somatization was explained in terms of prior painful experiences, however, familial factors were also associated with somatization. In addition, somatization data were obtained from the mother, and may have been biased (Grunau et al., 1994b).

In another prospective study, parental ratings of pain sensitivity in their 18 month-old infants were studied (Grunau et al., 1994a). Infants were divided into 4 groups according to birth weight: 480-800 g (n=49), 801-1000 g (n=75), 1500-2499 g (n=42), > 2500 g (n=29). Both
groups of ELBW infants were rated by parents to have significantly lower pain sensitivity thresholds than infants in the other groups. Temperament was also significantly associated with pain sensitivity ratings, except in the lowest birthweight category. Parenting style, on the other hand, was not related to pain sensitivity. The investigators concluded that pain behaviour was related to prior painful experiences. These conclusions are limited by the observational nature of the study, in that the observed differences may be due to factor(s) that were not measured in the study. Also, pain behaviour was not directly measured in this study. Parents rated their infants' pain sensitivity (Grunau et al., 1994a).

In a recent study, the association between prior nociceptive experiences and 8 1/2 year-old childrens' interpretations of pain-producing situations was evaluated (Grunau et al., 1996). Two groups of children were asked to judge the amount of pain depicted in pictures of medical, recreational, daily living, and psychosocial situations using two pain measures: faces scale and pain thermometer. One group had been prematurely born (n=47, birthweight 808 g), and the other group was full-term at birth (n=37, birthweight 3487 g). There were no differences between groups in "overall" perception of pain. However, the prematurely-born infants rated medical pain significantly higher than psychosocial pain using the pain thermometer (p=0.004). The duration of hospitalization was significantly correlated with pain ratings for recreational and daily living settings using the faces scale (p<0.05).

Together, these data suggest that there may be differences in childhood behaviours associated with prolonged hospitalization. It has not been determined, however, if these changes are specific to pain. Differences due to gestational age at the time of delivery, medical complications associated with hospitalization, parental and environmental situations may all be important factors explaining the observed results.
Summary: Clinical and Behavioural Importance of Infant Pain

Studies show that newborns can competently respond to painful and/or stressful experiences. In fact, they may be more sensitive to the effects of pain due to differences in cutaneous wound healing patterns and central nociceptive pathways (Reynolds et al., 1995; Fitzgerald, 1993). Painful experiences may lead to long-term changes in behaviour and neurological outcomes, suggesting the capacity for memory of pain.

However, no definite conclusions about the possible long-term effects of pain can be made from these studies due to several design limitations. Studies investigating the short-term effects of circumcision on infant behaviour, for instance, have not ruled out continued pain as the cause of altered infant behaviours. Studies investigating the pain responses of hospitalized preterm infants can not extrapolate their results beyond the sample studied due to the nature of the study as well as the extreme developmental immaturity of the infants included. Studies investigating the effects of prolonged hospitalization have been unable to tease out the effects of pain from the effects of illness and prematurity.

In summary, the available evidence suggests that untreated infant pain can lead to long-term alterations in infant behaviour. This evidence runs counter to the myth that untreated pain in infancy has no long-lasting consequences on the infant. Stronger evidence, however, is necessary before any definite conclusions can be made. Further study of the possible effects of untreated pain in infants is warranted. Studies that investigate the effects of an isolated painful experience (e.g., circumcision) on future infant pain behaviour and whether pre-emptive analgesia can prevent any long-term changes in infant behaviour are needed.
Lidocaine-Prilocaine Cream (EMLA)

The opportunity to investigate procedural pain in infants was provided by the introduction of a pharmacologic agent expected to minimize the infant's pain response. This agent was lidocaine 2.5% - prilocaine 2.5% cream (EMLA 5% cream, Astra), a new topical local anesthetic preparation with established clinical efficacy in older infants and adults (Buckley et al., 1993).

EMLA is an oil:water emulsion comprised of equal parts of lidocaine and prilocaine. The name EMLA is derived from “eutectic mixture of local anesthetics,” because a liquid mixture is produced at room temperature when the crystalline bases of lidocaine and prilocaine are mixed together in a 1:1 ratio. EMLA has unique characteristics which favour its absorption through intact skin, including high water content (increases penetration through stratum corneum), high concentration of drug in each oil droplet (80%) in contact with the skin, small microdroplet size (1μm), and a favourable release rate (since drugs are in the liquid phase). The low concentration in the whole preparation (5%) reduces the risk of toxicity. The chemical formulation of EMLA is displayed in Table 1.

Prior to the introduction of EMLA, previously investigated topical anaesthetic formulations were unsuccessful for two main reasons: insufficient amounts of water, and low concentration of local anaesthetic in basic form. To remedy these limitations, the use of preparations containing skin penetrants (e.g., dimethylsulphoxide, DMSO) or high concentrations of local anaesthetics have been employed. These attempts have been limited by concerns about severe skin reactions, systemic toxicity (especially if applied to large surface areas) and inadequate analgesia. At the time this research was undertaken, topical formulations of local anaesthetics other than EMLA, had not been shown to provide both reliable cutaneous anaesthesia and a low risk of adverse effects. In addition, injectable local anaesthetic solutions, which are widely used to
provide anaesthesia for minor operative and medical procedures, are associated with discomfort and pain during their administration and are not feasible for superficial procedures.

Table 1. Chemical Formulation of EMLA

<table>
<thead>
<tr>
<th>Active Ingredients</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine</td>
<td>25 mg</td>
</tr>
<tr>
<td>Prilocaine</td>
<td>25 mg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vehicle Ingredients</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Arlatone</td>
<td>19 mg</td>
</tr>
<tr>
<td>Carbopol</td>
<td>10 mg</td>
</tr>
<tr>
<td>Sodium hydroxide to pH</td>
<td>9.6</td>
</tr>
<tr>
<td>Purified water sufficient to produce</td>
<td>1 g</td>
</tr>
</tbody>
</table>

The active ingredients of EMLA, lidocaine and prilocaine, are both aminoacyl amide local anesthetics. Prilocaine is a secondary amine and lidocaine is a tertiary amine. Their molecular structures are shown in Figures 1 and 2. The molecular weight is 234 for lidocaine and 220 for prilocaine. Prilocaine has a lower lipid solubility than lidocaine. Both drugs are weak bases with pKa values of 7.9 (Mather et al., 1979). They separate in the body predominantly in the ionized form.

Figure 1. Lidocaine

![Lidocaine molecule diagram]
Mechanism of Action

Percutaneous Absorption

Lidocaine and prilocaine must diffuse through the skin’s surface (epidermis) in order to be effective. The stratum corneum comprises the outermost membrane in the unvascularized epidermis and provides the major barrier to percutaneous absorption. The stratum corneum is comprised of tightly packed dead keratinocytes arranged in flat plates whose plasma membrane is thickened and cytoplasm is filled with proteins. The thickness of this membrane is 0.01-0.05 mm. The total epidermal thickness is 0.05-0.1 mm. Underneath the epidermis is the highly vascularized dermis. The dermis contains blood vessels, nerves and muscle. The thickness of the dermis is 2-4 mm (Shaw et al., 1991; West et al., 1981).

The main mode of percutaneous drug absorption is believed to be by intracellular passive diffusion through the lipid cell membranes and aqueous cell cytoplasms (Rutter, 1987; Rasmussen, 1979). Other minor routes of absorption are intercellular (i.e., between cells) and transappendageal (i.e., through sweat ducts, hair follicles) (Rutter, 1987).

The physico-chemical factors involved in the penetration of drugs through the skin are: hydration of the stratum corneum, temperature, concentration of penetrant in vehicle and rate of release from vehicle, characteristics of the penetrant (e.g., lipid/water partition coefficient,
molecular weight), and vehicle characteristics. The physiological factors involved in the penetration of drugs through the skin are: skin condition, skin age, regional skin site, and blood flow (Idson, 1971).

**Infant Skin**

At the time of birth, the stratum corneum of infants is fully developed and virtually indistinguishable from that of adult skin. Preterm infants, however, have skin that may be more permeable to drugs (West et al., 1981). Several studies which investigated the effect of gestational age and postnatal age on the histological development of the epidermis have demonstrated that infants of gestational ages less than 32 weeks have a thinner epidermis and stratum corneum (Evans et al., 1986; Holbrook, 1982). Evans & Rutter (1986) found that considerable maturation occurs in preterm infants until approximately 34 weeks gestation, when the epidermis resembles that of a term infant. Development is expedited in fetuses that have been prematurely delivered, so that the epidermis of even the most premature infants resembles that of a term infant after about 2 weeks of age (Evans et al., 1986).

The structural maturation of the epidermis has been shown to closely parallel the development of the barrier properties in newborn skin. Nachman and Esterly (1971) and Harpin and Rutter (1983a) performed studies investigating the permeability of neonatal skin to the vasoconstrictive agent phenylephrine. In both studies, the incidence of blanching (i.e., vasoconstriction) due to topically applied phenylephrine was greater for preterm neonates than full-term neonates. Infants of lower gestational age responded more dramatically and more quickly than older infants. The response to phenylephrine was related to postnatal age as well; the incidence of blanching decreased over time, so that by between 2 and 3 weeks of postnatal life,
the preterm infant's response was similar to that of the mature neonate (Nachman et al., 1971; Harpin et al., 1983a).

Similar results have been obtained when investigators used transepidermal water loss as an indicator of skin barrier function. Preterm infants were found to have significantly greater water losses than older gestational age preterm infants and full-term infants. In addition, older preterm infants had lower values than newly-born preterm infants (Rutter et al., 1979; Harpin et al., 1983a; Wilson et al., 1982).

**Pharmacologic Activity**

After penetrating the epidermis, lidocaine and prilocaine diffuse through the dermis to act upon the sensory nerve endings. They inhibit the generation and conduction of nerve impulses in a reversible, concentration-dependent manner (DeJong, 1994). The non-ionized form of the local anesthetic diffuses from the site of administration across cell membranes to the axoplasm, and once inside the neuron, re-equilibrates between ionized and non-ionized form according to the local pH.

Unlike lidocaine, prilocaine is an optically active compound, and administered clinically as the racemate mixture. The enantiomers of prilocaine do not differ significantly in either neuronal blocking activity or toxicity (Akerman et al., 1970). In vitro studies on isolated nerve have shown that the anesthetic potency of prilocaine is approximately 0.6 compared to lidocaine (Astrom et al., 1961). In vivo studies, however, have revealed that both anesthetics share similar clinical efficacy, but that prilocaine is less toxic (Astrom et al., 1961; Englesson et al., 1965). The observed differences in pharmacological action between *in vitro* and *in vivo* techniques may be
explained on the basis of differences in regional blood flow and physiologic drug distribution (Akerman et al., 1966).

Local anesthetics have a low therapeutic-to-toxic index. Their pharmacological effects are dose-dependent: plasma/blood concentrations closely predict tissue (i.e., brain and myocardium) concentrations during toxicity. It is important to note, however, that total drug concentration does not entirely predict local anesthetic toxicity because it does not account for the concentration of the different dissociation forms of the local anesthetics within the tissues. Acidosis, for example, may lead to higher intracellular concentrations of the local anesthetic due to ‘ion trapping’ (Widman, 1975) and a greater tendency for adverse effects (Astrom, 1971).

**Site of Action**

Local anesthetics arrive at the receptor site (sodium channel) in one of two ways; diffusion of the unionized form through the membrane via a hydrophobic pathway in the membrane to the channel, or transfer of the ionized form through a hydrophilic pathway in the channel pore. Binding of local anesthetics to the receptor site in the sodium channel causes a block of inward sodium ion flux through the channel. The majority of pharmacological activity is due to the ionized form. The unionized form may contribute to neural blockade by incorporating itself into the lipid cell membrane and restricting conformational changes in sodium channel proteins, thus preventing their opening. The duration of the block is determined by disposition characteristics of the local anesthetic (DeJong, 1994).

Local anesthetics inhibit sodium current in two ways: tonic and phasic. Tonic inhibition refers to inhibition during infrequent depolarization. Phasic inhibition refers to inhibition when the frequency of depolarization is increased. The distinction between tonic and phasic inhibition may
arise from differences in local anaesthetic receptor binding characteristics. Three general mechanisms of channel inhibition have been proposed. The first proposes that the local anaesthetic inactivates the channel when it first opens. The second and third, however, propose that local anaesthetics inhibit the receptor activation process. Tonic and phasic modes of inhibition are implicit in all three mechanisms. During tonic inhibition, local anaesthetics may bind to channels that have spontaneously activated at rest, or to nonactivated, closed channels. During phasic inhibition, local anaesthetics may bind to either activated (but not conducting), open (conducting), or inactivated (nonconducting) states of the channel. Selective binding of local anesthetics to a particular channel conformation may occur due to enhanced binding affinity during a particular conformational state (Butterworth et al., 1990).

Threshold Concentration

The quality of the block is determined by the concentration of the local anesthetic in the fibre and the length of exposure along the fibre. There is a threshold concentration of the local anesthetic that is necessary to block impulse conduction. When the concentration is below this threshold value, function returns to the nerve. C-fibres and A-δ fibres are blocked with similar concentrations of local anesthetics.

Local anesthetics must be present across a minimum distance along the axon to be effective. This is because ion fluxes from the initiating site can induce depolarizations in adjacent membrane as well. To achieve a complete nerve block in nonmyelinated fibres, between 3 and 5 mm of the membrane should be occupied by the local anesthetic. For myelinated nerves, local anesthetics must span at least two or three successive nodes of Ranvier (range, 2-8mm) because impulses can skip over one or two blocked nodes and still continue to be propagated. Larger
myelinated fibres require a greater blocking length because the inter-nodal distance increases as axonal diameter increases. The minimal length to completely block impulse conduction is not static, and changes according to the concentration of local anesthetic present (length-dependent block). When the fibre is exposed to sub-threshold concentrations of local anesthetic, a larger distance is required to achieve complete impulse blockade. Inversely, higher concentrations of local anesthetic require shorter exposure lengths for impulse blockade (DeJong, 1994).

Local anesthetics do not alter resting membrane potential and therefore produce a nondepolarizing block. In addition, local anesthetics do not permanently alter nerve function or anatomy unless their administration is associated with significant tissue trauma (Lofstrom, 1970). Intracellular neuronal processes are affected at concentrations 5 to 20 times the therapeutic amount.

Clinical Pharmacology

The analgesic efficacy of EMLA has been demonstrated in adult and pediatric patients using double-blind randomized controlled trials; see reviews by (Gajraj et al., 1994; Buckley et al., 1993). It has proven clinical efficacy for a variety of cutaneous procedures. These procedures can be divided into two categories; those involving needle pain (Table 2) and those involving minor dermatologic procedures (Table 3) (Gajraj et al., 1994; Buckley et al., 1993; Freeman et al., 1993; Juhlin et al., 1990).
Table 2. Procedures Involving Needle Pain

- Venipuncture
- Venous Cannulation
- Arterial puncture
- Lumbar puncture
- Subcutaneous drug reservoir puncture
- Subcutaneous injection
- Intramuscular injection
- Intradermal injection

Table 3. Superficial Surgical Procedures

- Curettage of minor skin lesions (e.g., molluscum contagiosum)
- Harvesting of split-thickness skin grafts
- Port wine stain removal
- Genital wart removal
- Debridement of leg ulcers

EMLA is commercially available as a 5% cream. The usual dose is 1-2 g of cream applied per 10 square centimetres of skin (Astra USA Inc. 1993). EMLA is kept on the skin with an occlusive dressing (Tegaderm or Saran wrap). The usual application time is 1 hour for procedures involving needle pain and 2 hours for dermatologic procedures (Astra USA Inc. 1993). After the appropriate application time, the cream and dressing are removed and the site is prepared for the procedure.
The purpose of applying the occlusive dressing is to prevent the cream from being wiped off and to hydrate the skin. Hydration swells the horny layer, making it more permeable to lipid soluble molecules such as local anesthetics, which already have some capacity for diffusion. In addition, water temporarily becomes part of the membrane and increases its diffusivity. Occlusion also raises the surface temperature (from 32 to 37 degrees), which increases diffusion slightly. Temperature elevations, however, can also increase blood flow and absorption of chemicals (Kligman, 1983).

The efficacy of EMLA depends on the dose administered, the site of application and the duration of application. In a study of pain from venepuncture, a thicker layer (i.e., larger dose) of cream was demonstrated to be more effective than a thinner layer (Sims, 1991). Regional variation in absorption of EMLA occurs according to the cutaneous application site. The site of application influences the onset of action, efficacy and duration of analgesia of EMLA. This is because of regional differences in skin blood flow, epidermal and dermal thickness and skin pathology. Arendt-Nielsen et al. (1990) found that efficacy and duration of action were decreased in areas of high vascularity (e.g., face, forehead) compared to those with relatively low vascularity (e.g., hand, cubital fossa, back) due to increased systemic absorption. The thickness of the epidermis is approximately 0.05-0.1 mm (Shaw et al., 1991) but varies dramatically with body site. Areas with a thicker epidermis (e.g., hand) have a prolonged onset and duration of action of EMLA compared with areas with a thinner epidermis (e.g., face) due to increased diffusion distance (Arendt-Nielsen et al., 1990). Conversely, areas with a thinner epidermis such as the foreskin, allow faster drug penetration. In a trial of anaesthesia for thermocautery of condylomata of penile and scrotal skin, a mean application time of 35 minutes (range, 20-70 minutes) was adequate (Hallen et al., 1987).
The stratum corneum is not a homogeneous structure; both qualitative and quantitative differences exist in different anatomic sites. The palms and soles, for instance have a thicker stratum corneum (approximately 40 times) than other body regions, however, qualitatively, the cells are less densely packed and spheroidal in shape (Kligman, 1983). The condition of the skin has also been shown to affect analgesia (Juhlin et al., 1989).

The efficacy of EMLA varies according to the duration of application. Bjerring and Arendt-Nielsen (1990) found that the depth of analgesia to needle insertion on the forearm increased with increasing application time for 30, 60, 90 and 120 minutes. The maximum depth of analgesia (up to 5 mm) was achieved 30 minutes after a 90 minute application time, or 60 minutes after a 120 minute application time (Bjerring et al., 1990). Of note, longer application times of EMLA can lead to a decrease in its effectiveness (Ohlsen et al., 1985). This is believed to be due to depletion of the drug in the cream layer while it is in direct contact with the skin. It has been recommended that the dressing (and cream) be massaged at hourly intervals in order to maintain adequate drug concentrations at the cream-skin interface (Ohlsen et al., 1985).

The duration of analgesia is dependent on the concentration of local anesthetic around the nerve endings. The finding of increasing analgesia with time following removal of the cream from the skin suggests continuing penetration of EMLA from the superficial layers of the skin. Because of its relative impermeability, the skin can function as a depot which slowly releases drug into the circulation. Thus in areas of relatively low blood flow, analgesia may continue for some time after the drug is removed from the skin surface because the skin acts as a drug reservoir. In areas of higher vascularity, the rate of uptake may be equal to rate of influx through the skin and no reservoir is formed. In these areas, the effect begins to decline as soon as the drug is wiped off the skin.
Pharmacokinetics

The local anesthetic concentrations achieved after application of EMLA are closely associated with the applied dose (and the total surface area of the applied dose), and the duration of application (Ohlsen et al., 1985). When used according to the manufacturer’s guidelines, plasma concentrations of lidocaine and prilocaine achieved in adult patients are generally low (Evers et al., 1985; Malmros et al., 1990; Juhlin et al., 1989), with peak blood concentrations approximately 1/20th-1/40th the toxic values (Astra USA Inc. 1993). Limited data in pediatric patients have also revealed low systemic concentrations following the use of EMLA (Table 4). Of note, the anaesthetic effect has not been shown to correlate with blood local anaesthetic concentrations (Ohlsen et al., 1985).
Table 4. Plasma Concentrations of Lidocaine and Prilocaine After Application of EMLA in Infants

<table>
<thead>
<tr>
<th>Reference</th>
<th>Dosage Regimen*</th>
<th>Age of Subjects</th>
<th>Timing of Samples (hours)</th>
<th>Lidocaine (mcg/mL)</th>
<th>Prilocaine (mcg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manner 1987</td>
<td>2.5g for 1.63 hours</td>
<td>4-10 years</td>
<td>0.0, 0.25, 0.42, 0.67, 1.5</td>
<td>0.02-0.05</td>
<td>-</td>
</tr>
<tr>
<td>Haugstvedt 1990</td>
<td>10g/100cm² for 2 hours</td>
<td>2-3 years</td>
<td>0.2, 3, 4, 5 (0.092-0.315)</td>
<td>(0.045-0.215)</td>
<td></td>
</tr>
<tr>
<td>Haugstvedt 1990</td>
<td>10g/100cm² for 2 hours</td>
<td>6-8 years</td>
<td>0.2, 3, 4, 5 (0.07-0.299)</td>
<td>(0.024-0.110)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16g/160cm² for 2 hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Engberg 1987</td>
<td>2g/16cm² for 4 hours</td>
<td>6-12 months</td>
<td>0.2, 4, 8 (0.155 (maximum))</td>
<td>0.079 (maximum)</td>
<td></td>
</tr>
<tr>
<td>Engberg 1987</td>
<td>2g/16cm² for 4 hours</td>
<td>3-6 months</td>
<td>0.2, 4, 8 (0.127 (maximum))</td>
<td>0.131 (maximum)</td>
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</tr>
<tr>
<td>Nilsson 1990</td>
<td>2g/16cm² for 4 hours</td>
<td>1.5-3 months</td>
<td>0.4, 8, 12 (0.05-0.412) (0.017-0.078)</td>
<td>0.045 (0.017-0.078)</td>
<td></td>
</tr>
<tr>
<td>Ramaioli 1991</td>
<td>1g for 0.5 hours</td>
<td>full-term infants</td>
<td>0.5 &lt;0.04</td>
<td>&lt;0.04</td>
<td></td>
</tr>
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Values are mean/median and (range), unless otherwise specified.

* surface area of drug application not specified in all studies.
Intravenous Use of Lidocaine and Prilocaine

When lidocaine and prilocaine are administered intravenously, both anesthetics exhibit multicompartment distribution characteristics (Boyes et al., 1971; Thomson et al., 1987; Arthur et al., 1979). The pharmacokinetic parameters for lidocaine and prilocaine in adults are shown in Table 5. Prilocaine has a higher volume of distribution than lidocaine. When equal amounts of lidocaine and prilocaine are administered, the larger volume of distribution of prilocaine results in a lower plasma concentration for prilocaine than for lidocaine. Animal studies have revealed similar distribution patterns in the spleen, liver, kidney, heart and brain. However, distribution in the lung is significantly higher for prilocaine (Akerman et al., 1966). The plasma/erythrocyte concentration ratio for lidocaine is 1.3 (Englesson et al., 1962). For prilocaine, the plasma/erythrocyte concentration ratio is 0.9 (Englesson et al., 1962). Both lidocaine and prilocaine are mainly bound to alpha-1 acid glycoprotein (Tucker, 1986) and protein binding is relatively unchanged at therapeutic concentrations (Tucker et al., 1970b). The half-life of prilocaine is slightly shorter than that of lidocaine.
Table 5. Pharmacokinetics of Lidocaine and Prilocaine in Adult Subjects

<table>
<thead>
<tr>
<th></th>
<th>Lidocaine</th>
<th>Prilocaine</th>
</tr>
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<tbody>
<tr>
<td>Steady state volume of distribution (L)</td>
<td>87-93</td>
<td>261</td>
</tr>
<tr>
<td>Protein binding (%)</td>
<td>64</td>
<td>55</td>
</tr>
<tr>
<td>Plasma clearance (L/min)</td>
<td>0.7</td>
<td>2.84</td>
</tr>
<tr>
<td>Terminal half-life (hours)</td>
<td>1.7-1.9</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Data obtained from: (Arthur et al., 1987; Thomson et al., 1987; Tucker et al., 1979; Mather et al., 1979; Arthur et al., 1979; Thomson et al., 1973; Adjepon-Yamoah et al., 1973; Rowland et al., 1971).

In general, the lidocaine and prilocaine blood concentrations achieved following parenteral administration vary according to the interaction between the rate of absorption from the site of administration into the systemic circulation, distribution in the tissues, and elimination from the body. Scott (1972) found that the peak lidocaine and prilocaine plasma concentrations are linearly correlated with the administered dose. Rapid injections are associated with higher peak concentrations than slower injections. The plasma concentration is related to the vascularity of the injection site as well; the more vascular the site, the higher the absorption rate. Since absorption is also influenced by regional blood flow, addition of a vasoconstrictive agent such as epinephrine can decrease the absorption of local anesthetics, particularly those with vasodilatory action such as lidocaine.

As distribution throughout the body tissues occurs, local anesthetic concentrations fall quickly at first, and then more slowly as elimination takes place. Hepatic and cardiovascular disease may be associated with clinically significant reductions in lidocaine clearance (Thomson et al., 1973). Concomitant therapy with drugs that inhibit enzyme activity or decrease blood flow
(e.g., cimetidine, propranolol, halothane) may also decrease clearance, whereas enzyme-inducing agents (e.g., phenytoin) may increase it. Other variables such as gender, race, and renal disease have not been shown to have a significant influence the clearance of lidocaine (Tucker, 1986).

**Metabolism of Lidocaine and Prilocaine**

The skin possess some drug-metabolizing activity (Pannatier et al., 1978; Bickers, 1991), however, it is not known whether lidocaine or prilocaine are appreciably metabolized by the skin (Astra USA Inc. 1993). The liver accounts for the majority of lidocaine metabolism (Stenson et al., 1971). The estimated hepatic extraction ratio is 0.65 (Tucker et al., 1979), which is indicative of perfusion-limited clearance. Less than 10% of the dose is excreted as the parent compound in the urine, and only a trace amount is found in the feces. Acidification of the urine increases the proportion of protonated cation species (due to pKa of 7.9) and results in an increase in the fraction of unchanged lidocaine eliminated. Approximately 80% of the dose is renally excreted as metabolites (Keenaghan et al., 1972). The major pathway involves oxidative deethylation to mono-ethylglycine xylidide (MEGX) and acetaldehyde (DeJong, 1994), which is believed to be mediated by P450IIIA4 (Bargetzi et al., 1989). MEGX is an intermediary lidocaine metabolite that has both local anesthetic and antidysrhythmic activity (Burney et al., 1974).

MEGX has a similar half-life to lidocaine (Bennett et al., 1982; Thomson et al., 1987) and undergoes further hydrolysis by amidases to 2,6-xylidine and N-ethyl glycine, or deethylation to glycine xylidide (GX) (DeJong, 1994). GX also possesses some antidysrhythmic activity (Burney et al., 1974), and has a longer half-life (10 hours). GX is further degraded to 2,6-xylidine, which then undergoes parahydroxylation to 4-hydroxy-2,6-xylidine (DeJong, 1994) and conjugation.
The 4-hydroxy-2,6-xylidine metabolite accounts for over 70% of the administered lidocaine dose that is recovered in urine (Keenaghan et al., 1972).

Prilocaine is metabolized in the liver, kidney, and lung by amidases to N-propylalanine and o-toluidine (DeJong, 1994). Animal studies have revealed that the metabolism of prilocaine is stereospecific; the D-form is metabolized more quickly than the L-form (Akerman et al., 1970). The major elimination products are metabolites which are renally excreted (Akerman et al., 1966). Prilocaine undergoes passive and active renal elimination (DeJong, 1994). As with lidocaine, alkalization of the urine decreases the elimination of unchanged prilocaine (Eriksson, 1965).

O-toluidine is further hydroxylated at the 3- and 5-positions to o- and p-aminophenol and then conjugated by sulphate or glucuronide (DeJong, 1994). In adult plasma, the p-hydroxytoluidine metabolite is present in higher concentrations than other metabolites (Hjelm et al., 1972). In an animal study of the elimination products of o-toluidine, 37% of the administered dose was recovered from urine as unchanged drug and 52% as p-aminophenol conjugates (Cheever et al., 1980). Of note, the production of hydroxytoluidine metabolites can be decreased by general anesthetics due to a decrease in hepatic blood flow (DeJong, 1994).

In general, drugs display altered pharmacokinetics in neonates. Neonates, for instance, exhibit lower plasma binding of drugs, which results in higher free drug concentrations and possibly enhanced pharmacologic effects. Metabolic systems such as the cytochrome P450 system and glucuronidation system are also deficient, which results in a decrease in clearance and prolongation in half-life. Renal function (glomerular filtration and tubular secretion) is also immature, so that drugs which require renal excretion for their elimination will also have decreases in clearance and increases in half-life. If these differences are not taken into consideration in dosing regimens, then the infant may be at risk of toxicity (Morselli, 1976).
review of the pharmacokinetics of lidocaine and prilocaine in young infants is therefore warranted because any differences in drug handling should be known prior to the use of EMLA in this population.

**Pharmacokinetics in Young Infants**

Although lidocaine is routinely used in young infants for regional anaesthesia, surprisingly few investigators have studied its pharmacokinetics in this population. The most comprehensive study of the pharmacokinetics of lidocaine was performed by Mihaly and colleagues (1978). In that study, the pharmacokinetics of lidocaine were investigated in neonates (n=4; 26-38 weeks gestational age at delivery, 5-42 days postnatal age) and adults (n=10). The plasma clearance was 0.61 l/h/kg for infants compared to 0.55 l/h/kg in adults (adult value obtained from literature values), which was not different (p>0.05). The terminal half-life, however, was longer in the infants (3.16 hours vs 1.80 hours; p=0.025), perhaps due to a larger volume of distribution.

Relative to the total administered dose, neonates excreted a significantly (p=0.025) greater fraction of unchanged lidocaine than the adults (19.7% vs 4.3%), MEGX (19.7% vs. 5.0%), and a lower fraction of conjugated 4-OH xylidine (8.9% vs. 63.8%) over a 48 hour period compared to adults, p<0.05. The fraction of xylidine was not significantly different (2.7% vs 2.5%).

Tucker and colleagues (1970a) observed lower lidocaine protein binding in umbilical blood compared to maternal blood in samples obtained from mothers who received lidocaine anaesthesia at the time of delivery. In another study by Brown et al. (1975), the half-life of lidocaine was 3 hours for newborns who had been exposed in-utero at the time of delivery.

The ability of the neonate to metabolize lidocaine was studied in 36 neonates born to mothers who received epidural or pudendal lidocaine anaesthesia during delivery (Kuhnert et al.,
Neonates and mothers provided three consecutive 24-hour urine samples for lidocaine, MEGX, and GX analysis. The results revealed that lidocaine, MEGX and GX were excreted by the neonate. By the second day, very little unchanged lidocaine was excreted, and by the third day, levels of all drugs were low. The pattern of excretion in the neonate resembled the pattern observed in the mother (Kuhnert et al., 1979). A similar study was performed by Blankenhaker and colleagues (1975), who measured the urinary excretion of lidocaine, MEGX, and hydroxyxylidine in newborns whose mothers received epidural lidocaine anaesthesia. They found that the fraction of the lidocaine dose recovered as metabolites increased over the first 12 hours of life from 50% to 77% for the second 12-hour (12-24 hours) collection period. Together, the data from these studies are supportive of lidocaine metabolism in the neonate.

No studies that examined the pharmacokinetics of prilocaine in infants were located, likely due to the recommendation that prilocaine not be used in young infants (reviewed below).

**Adverse Effects**

The adverse effects that may occur with the use of EMLA are usually limited to transient local skin reactions. Methemoglobinemia is the main systemic adverse effect that may be associated with its use in young infants, due to known deficiencies in specific oxidation/reduction systems in this population. Other systemic effects which occur following parenteral administration of lidocaine and prilocaine, such as central nervous system and cardiovascular effects, have not been observed with EMLA. Nevertheless, they are briefly discussed to provide a more complete review of the adverse effect profile of local anesthetics.
Methemoglobin

Methemoglobin is the name given to hemoglobin when the heme iron is present in the oxidized, or ferric, state instead of the ferrous state. This form of hemoglobin is unable to carry oxygen. It is important to note that hemoglobin is continuously being oxidized to methemoglobin by endogenous oxidizing agents, and that there are complementary endogenous reducing systems that continuously reduce the methemoglobin back to hemoglobin. The most important of these is the cytosolic enzyme, NADH cytochrome b5 reductase, which is responsible for up to 70% of the required reducing activity. Under normal conditions, the methemoglobin concentration represents a balance between the oxidizing and reducing systems, and is less than 2% of total hemoglobin. Such low concentrations of methemoglobin do not cause any adverse systemic effects.

Methemoglobin concentrations are slightly higher in infants than in adults (Kunzer et al., 1951). In addition, premature infants have higher methemoglobin concentrations compared to full-term infants (2.3% vs. 1.5%, respectively) (Kravitz et al., 1956). This variability in methemoglobin concentration may reflect maturational differences among preterm infants, full-term infants and adults.

The concentration of methemoglobin may increase in the presence of additional oxidizing agents and in certain inherited diseases. When the concentration of methemoglobin rises above 5% of total hemoglobin, then methemoglobinemia is said to be present. Very high concentrations of methemoglobin (> 40%) can impair tissue oxygenation because a significant amount of hemoglobin cannot carry oxygen. This can result in hypoxemia and death.

Fortunately, methemoglobinemia is rarely associated with significant morbidity. The condition is associated with characteristic changes in skin colour (marked cyanosis of the extremities, especially the lips and nailbeds) that facilitate its diagnosis. When present,
methemoglobinemia can be reversed by administering the antidote, methylene blue (Crawford, 1965; Scott, 1965). Methylene blue utilizes an alternate biochemical pathway to reduce the excess methemoglobin back to hemoglobin.

Methemoglobinemia occurs with administration of D- and L- enantiomers of prilocaine (Akerman et al., 1970). In adults, there is a positive correlation between prilocaine dose and methemoglobin concentration (Hjelm et al., 1965; Nishimura, 1971; Crawford, 1965; Lund, 1965). Methemoglobinemia has been observed with peak prilocaine blood concentrations of greater than 3 ug/ml (Crawford, 1965; Debruyne et al., 1985). Methemoglobin increases for up to 6 hours after injection (Sadove et al., 1965; Hjelm et al., 1965; Crawford, 1965; Onji et al., 1965) and declines towards baseline values by 24 hours (Onji et al., 1965; Hjelm et al., 1965). Clinically significant increases in methemoglobin can be prevented by limiting the total dose of prilocaine in adults to 600mg (Hjelm et al., 1965; Lund, 1965; Crawford, 1965) or by administering methylene blue (1mg/kg) prophylactically (Hjelm et al., 1965).

Since methemoglobin only rises after prilocaine concentrations begin to decline (Nishimura, 1971; Crawford, 1965), the metabolite(s) of prilocaine have been suspected to be involved. In vitro studies which have have been performed have confirmed this hypothesis (Onji et al., 1965). In vivo studies have demonstrated that o-toluidine induces methemoglobinemia over a similar time frame as prilocaine (Onji et al., 1965; Nishimura, 1971) and that the o- and p-hydroxylated (i.e., aminophenol) metabolites of o-toluidine also cause methemoglobinemia in man and animals (Hjelm et al., 1972). In a study of human volunteers, a positive correlation was demonstrated between plasma concentration of p-hydroxytoluidine and methemoglobinemia after a subcutaneous injection of 20 mg/kg of prilocaine (Hjelm et al., 1972). The peak methemoglobin concentration (15-20 percent of total hemoglobin), occurred after approximately one hour, when
peak p-hydroxytoluidine plasma concentrations (4-5 ug/ml) were achieved (Hjelm et al., 1972). The mechanism by which prilocaine metabolites induce methemoglobinemia is unknown but may involve production of quinonimines or nitrosotoluene (Kiese, 1965) and then oxidation of hemoglobin. In vitro, a positive correlation was found between the amount of nitrosotoluene and methemoglobinemia. In addition, nitrosotoluene is a more potent inducer of methemoglobinemia than o-toluidine and p-aminophenol (Hjelm, 1965).

Therapeutic doses of lidocaine do not cause clinically significant increases in methemoglobin concentrations (Hjelm et al., 1965). Toxic concentrations (> 5 ug/ml) may be associated with a risk of methemoglobinemia (Weiss et al., 1987). Methemoglobinemia has been described in several adults following epidural administration (Burne et al., 1964), local injection for dental extractions (Deas, 1956), and topical administration to the mucous membranes (O'Donohue et al., 1980).

**Risk Factors for Methemoglobinemia**

The risk of developing methemoglobinemia from local anesthetics is not only dependent on total local anesthetic dose but also on individual factors. These factors include the equilibrium state of red cell methemoglobin oxidation and reduction, the functional status of cellular methemoglobin reducing mechanisms (e.g., NADH cytochrome b5 reductase), and the variation in elimination pathways for prilocaine metabolism (Sadove et al., 1965).

Local anesthetics, including EMLA, are contraindicated in patients with hemoglobin abnormalities (e.g., hemoglobin M), enzyme deficiencies (e.g., NADH cytochrome b5 reductase), or enzyme co-factor deficiencies (e.g., cytochrome b5) that predispose them to methemoglobinemia. Fortunately, these conditions are rare.
In addition, prilocaine has not been recommended for clinical use in young infants. The erythrocytes of infants are more vulnerable to methemoglobin formation from oxidizing substances than those of adults. Infants have reduced activity of the enzyme NADH cytochrome b₅ reductase, which is responsible for the conversion of methemoglobin to hemoglobin (see Figure 3). Activity of this enzyme is reduced for the first 3-12 months of life. In newborns, it retains from 40 to 80% of adult activity (Ross, 1963; Hibbard et al., 1978; Eng et al., 1972; Agar et al., 1972; Bartos et al., 1966; Nilsson et al., 1990; Board, 1981). Low birth weight newborn infants have lower enzyme activity than heavier newborns (Eng et al., 1972; Ross, 1963; Bartos et al., 1966). Cytochrome b₅ is present in lower amounts in newborns compared to adults (Takeshita et al., 1980). Fetal hemoglobin, which makes up most of the hemoglobin concentration in the first few months of life, is more susceptible to oxidation than adult hemoglobin (Martin et al., 1963). Infants may also have other risk factors that predispose them to methemoglobinemia such as infection, acidosis, formula feeding, and failure to thrive (Hanukoglu et al., 1996).

Figure 3. Reduction of Methemoglobin

\[
\text{NADH} \quad \text{cytochrome } b_5 \text{ (oxidized)} \quad \text{hemoglobin}
\]
\[
\text{NAD} \quad \text{cytochrome } b_5 \text{ (reduced)} \quad \text{methemoglobin}
\]

NADH cytochrome b₅ reductase Nonenzymatic reaction

Methemoglobinemia has been reported in newborn infants following maternal use of prilocaine for analgesia during labour (Climie et al., 1967; Arens et al., 1970). In addition, methemoglobinemia has been observed in young infants (Mandel, 1989; Lloyd, 1992; Duncan et al., 1983; Ozbek et al., 1993) following administration of 6-7 mg/kg for local anaesthesia. Since EMLA contains prilocaine, there is a risk that it may cause methemoglobinemia as well.
Research with EMLA in young infants has been limited by the concerns regarding methemoglobinemia in this population. Besides immaturity of the NADH cytochrome b5 reductase enzyme system, which leads to a diminished capacity to reduce methemoglobin, infants may be at risk of methemoglobinemia from EMLA due to developmental factors such as impaired drug clearance. Infants also have a larger surface area relative to body weight, which results in greater doses of local anesthetic administered per kg of body weight. Full-term neonates have similar skin barrier characteristics as adults, so that the absorption of drug per square centimetre is similar to adults. In preterm neonates, on the other hand, the epidermis is neither structurally nor functionally mature (Holbrook, 1982; Evans et al., 1986; Harpin et al., 1983a). Absorption of EMLA may be enhanced in preterm infants, resulting in greater doses (mg/kg) of drug than in the adult.

**Methemoglobin Concentrations Following Use of EMLA in Infants**

At the time this research was undertaken, five studies had investigated methemoglobin concentrations after administration of EMLA in infants and young children. In all studies, clinically important increases in methemoglobin concentrations were not observed following single dose administration. The results of these studies are presented below.

Frayling and colleagues (1990) investigated methemoglobin concentrations in 48 children between 1 and 6 years of age. Infants were treated with 5 g of EMLA on the arm for 2 hours. Blood methemoglobin concentrations were measured from a control group that did not receive EMLA and a subsample of the infants who were treated with EMLA at 2, 4, 6, 8, 10, 20 and 24 hours after drug administration. Methemoglobin concentration was higher in the EMLA-treated infants than in the control group: the maximum mean methemoglobin concentration was 0.85% in
the EMLA-treated infants compared to 0.46% in the control group (p<0.001) and occurred 10 hours after application of EMLA. The methemoglobin concentration decreased to 0.58% by 24 hours, but remained higher in the EMLA group than in the control group (p<0.05).

Engberg and colleagues (1987) used an open study design to investigate methemoglobin concentrations in 22 infants aged 3 to 12 months. The infants were stratified into 2 groups: those < 6 months of age and those ≥ 6 months of age. All infants received 2 g of EMLA on 4 separate cutaneous sites (most commonly the foot and cubital fossa) over a surface area of 4 x 4 cm. The duration of cream application was 4 hours. Methemoglobin concentrations were measured at baseline, then at 2, 4, and 8 hours after drug administration. Methemoglobin concentrations were not increased at any time after EMLA for infants ≥ 6 months of age (p>0.05). For infants < 6 months of age, however, the 8-hour methemoglobin concentration was higher than the baseline concentration (1.7% vs 0.8%, p<0.05). The highest individual value was 2%.

Nilsson et al. (1990) measured methemoglobin after application of EMLA in 10 infants < 3 months of age. A total of 2 g of EMLA was applied to 4 different cutaneous sites for 4 hours (total surface area 16 cm²). Methemoglobin concentration was measured before the application of EMLA and then at 4, 8 and 12 hours afterward. The maximum observed methemoglobin concentration after EMLA was higher than the baseline concentration (1.32% vs 2.24%, p<0.001). The maximum observed methemoglobin concentration was 3.37%, and occurred 8 hours after administration. Of note, maximum methemoglobin concentration were observed to be negatively correlated with methemoglobin reductase (NADH cytochrome b₅ reductase) activity (r = -0.72, p-value not reported).

Ramaioli and colleagues (1991) investigated methemoglobin concentrations in full-term neonates who received EMLA prior to heel lancing. Altogether, 30 neonates participated. Half
of the infants were randomly assigned to treatment with 1g of EMLA for 30 minutes, and the other half received a placebo. Methemoglobin concentrations were measured 30 minutes after application of the study medication. The results revealed that the mean methemoglobin concentration was 0.85% (0.2) in the EMLA group and 1.18% (0.27) in the placebo group (p>0.05).

The same research team investigated the safety of EMLA in preterm infants aged 29-36 weeks gestation (Ramaioli et al., 1993). Methemoglobin concentrations were measured 10 infants who were randomized to treatment with 0.5 g of EMLA for 30 minutes and in 10 infants who were randomized to glycerine placebo. Mean methemoglobin concentrations, measured 30 minutes after application, were 0.6% (0.2) in the EMLA group and 0.6% (0.8) in the placebo group, p>0.05.

To date, three cases of clinically significant methemoglobinemia have been reported following the use of EMLA in young infants (Jakobson et al., 1985; Nioloux et al., 1995, Kumar et al., 1997). In all cases, the infants were given suprathertapeutic doses of EMLA. In the first report, a 12-week-old prematurely born infant was treated with 5 g of EMLA for 5 hours. In addition, the infant received concomitant therapy with sulfamethoxazole, another oxidizing agent (Jakobson et al., 1985). In the second report, a 34-week gestation 5-day old infant received two simultaneous applications of EMLA at two different sites for a total of 3 hours (dose not reported) (Nioloux et al., 1995). Methemoglobinemia was reversed in both infants with methylene blue and no adverse sequelae were reported. In the third report, a full-term, 2-day old infant received 3.5 g of EMLA for 60 minutes on the outside of the prepuce prior to circumcision (Kumar et al., 1997). The infant was treated with 100% oxygen with no adverse sequelae.
In summary, studies have shown that methemoglobin concentrations may increase over the first 8 to 10 hours after administration of EMLA, and return to baseline by about 24 hours. However, in all cases where increases in methemoglobin concentrations were observed, supratherapeutic doses of EMLA were used. In two small studies of full-term and preterm neonates that utilized therapeutic doses of EMLA, methemoglobin concentrations were measured before the time when methemoglobin is expected to peak (i.e., 8 hours) and it is unknown whether methemoglobin concentration was increased. Together, these data suggest that there are inadequate data for judging the safety of single therapeutic doses of EMLA in preterm and full-term neonates.

**Local Skin Reactions**

Local skin reactions have been reported to occur in approximately half of the patients treated with EMLA (Buckley et al., 1993). The most common local skin reactions are blanching (pallor) and redness. Application times of up to 2.5 hour are usually associated with blanching, while longer application times are associated with erythema (Ohlsen et al., 1985). The erythema response may succeed the blanching response and may be due to a reactive or direct vasodilatory response (Bjerring et al., 1989; Ohlsen et al., 1985; Freeman et al., 1993). Alterations in temperature sensation, edema, itching and rash have also been reported (Buckley et al., 1993). These effects are generally mild and transient, lasting only one or two hours (Buckley et al., 1993). Repeat applications have not been associated with irritation or hypersensitivity (Freeman et al., 1993). Of note, the anaesthetic effect has not been observed to correlate with the appearance of the skin (Ohlsen et al., 1985).
Similar reactions are observed in young infants. In previous studies, the incidence of redness ranged from 13%-20% (Engberg et al., 1987; Nilsson et al., 1990). The incidence of pallor and edema were reported to be 3% and 1%, respectively (Engberg et al., 1987).

EMLA has antibacterial properties in vitro (Powell et al., 1991). The effect of EMLA on wound healing, however, has revealed inconsistent results. In one animal study, application of EMLA in contaminated wounds was associated with an increase in the incidence of infection (p<0.05) when compared to application of 0.9% saline (Powell et al., 1991). In another animal study, application of EMLA to the wound did not lead to a higher incidence of infection, and its effect was comparable to that of 1% lidocaine infiltration (Nykanen et al., 1991).

**Central Nervous System Adverse Effects**

The most frequent adverse effects observed in adults following parenteral administration of local anesthetics involve the central nervous system (Benowitz et al., 1978). Therapeutic doses can cause mild adverse effects such as drowsiness, dizziness, paresthesia and euphoria. Higher doses can cause confusion, agitation, dysarthria, vertigo, visual disturbances, tinnitus, nausea, sweating, muscle tremor, psychosis, seizures, respiratory depression, and coma. Mild central nervous system adverse effects are observed at blood concentrations of 3-6 µg/ml, which is within the therapeutic range for treatment of ventricular dysrhythmias (1.2-6.5 µg/ml) (Lie et al., 1974; Harrison et al., 1971). Severe toxicity is usually observed at higher blood concentrations (6-10 µg/ml) (Benowitz et al., 1978). Of note, MEGX has also been shown to have convulsant potential (Blumer et al., 1973). Moreover, GX can potentiate the convulsant activity of lidocaine and MEGX when given concomitantly (Blumer et al., 1973). It should also be noted that patients with primary liver disease, decreased hepatic blood flow, or kidney disease may be at a higher risk
for toxicity than other patients due to accumulation of lidocaine and/or its more polar MEGX and GX metabolites (Thomson et al., 1973).

Prilocaine produces fewer symptoms of toxicity than lidocaine when the same dose is administered intravenously (Englesson et al., 1962). Animal studies revealed that twice as much prilocaine is required in order to produce comparable degrees of convulsions as with lidocaine (Akerman et al., 1966). The reported toxic blood concentration for prilocaine is 7-9 ug/ml (Mather et al., 1979). The reduced toxicity potential of prilocaine may be due to higher volume of distribution and enhanced metabolic rate.

**Cardiovascular Adverse Effects**

At toxic doses, local anesthetics can affect the cardiovascular system. They cause depression of myocardial contractility, peripheral vasodilation, and hypotension. The lidocaine metabolite, MEGX, has been shown to share lidocaine’s cardiovascular activity in animal studies, however, the N-ethyl-glycine and xylidine metabolites have not (Astrom, 1971). Clinically significant effects are rare, and appear limited to specific populations such as those with acute myocardial infarction or other cardiac disturbances (Benowitz et al., 1978).

**Allergies**

Allergic reactions to local anesthetics are rare, comprising less than 1% of all adverse reactions. Most of the complications arising from their use are due to direct pharmacologic activity of the local anesthetic, the presence of a vasoconstrictor and preservatives co-administered with the local anesthetic, concomitant drug therapy, and/or patient anxiety (Giovannitti et al., 1979).
Summary and Research Strategy

Analgesics are rarely administered to young infants undergoing relatively minor, yet painful, procedures. There are many myths and beliefs surrounding infant pain that may contribute to this practice. Some of the more commonly cited myths include: infants are neurologically immature, medications are too toxic, pain cannot be measured accurately, and pain is not remembered and therefore has no consequence. These myths persist despite the growing body of evidence that would disprove them.

EMLA is a new topical local anesthetic that can be used to decrease procedural pain. EMLA does not require injection, thus its use is not associated with the pain that is typical of previous local anesthetic preparations. The efficacy of EMLA is established in older children and adults. At the time this research was undertaken, data were lacking for young infants. Since infants undergo painful procedures such as vaccination, heelstick, and circumcision, without the benefits of analgesia, EMLA offered the potential to be very useful for pain management in these common procedures.

Before EMLA could be routinely used to manage pain in young infants, studies establishing both its safety and efficacy in this population were required. Demonstrating the safety and efficacy of EMLA would provide an opportunity to disprove the myth that the risks of toxicity from analgesic agents outweigh the potential benefits. EMLA also provided a probe for investigating whether painful experiences in infancy are associated with long-lasting alterations in infant pain behaviour. If untreated pain was shown to have long-lasting effects on infant pain behaviour, then it would be evidence against the myth that infants do not have pain memories.
Chapter 1. EMLA in Infant Vaccination

At the time this research was undertaken, no studies had investigated the efficacy of EMLA in young infants. The objective of this study was to determine the efficacy of EMLA in decreasing vaccination pain in infants aged 4 to 6 months. Infant vaccination was chosen for study as it is the most common medical procedure performed in young healthy infants and causes them pain (Maikler, 1991; Dale, 1986; Dale, 1989; Craig et al., 1984).

The study included infants aged between 4 and 6 months as previous studies demonstrated that EMLA was safe in young infants (Nilsson et al., 1990; Engberg et al., 1987; Ramaioli et al., 1993; Ramaioli et al., 1991) and that behavioural responses to vaccination were similar within this age range (Lewis et al., 1995; Maikler, 1991). Infants do not demonstrate anticipatory fear until they are older than six months of age (Craig et al., 1988b; Izard et al., 1987; Levy, 1960), thus infant fear would not require assessment and/or consideration in the study.

Prior to this study, we investigated the efficacy of EMLA in decreasing injection pain in adults (Appendices 1 and 2). In two separate double-blind randomized placebo-controlled studies of subcutaneous and intramuscular injection pain, EMLA was demonstrated to significantly reduce pain resulting from penetration of the needle through the skin (Taddio et al., 1992b; Taddio et al., 1992a). EMLA decreased the pain from the intramuscular injection of Influenza virus vaccine as well (Taddio et al., 1992a). In a recent study, EMLA was demonstrated to decrease pain from needle puncture and infiltration of saline into the deltoid muscle (Himelstein et al., 1996). Taken together, these data demonstrated that EMLA was beneficial for reducing adult vaccination pain. It also was postulated that EMLA would be beneficial for infant vaccination pain.
The dose of EMLA used in this study was the same as the dose used in previous studies of pain from injections (Taddio et al., 1992b; Taddio et al., 1992a) and similar to doses used for pain from venepuncture in children (Sims, 1991).

As part of this research, a behavioural pain scale was adapted from the Children’s Hospital of Eastern Ontario Pain Scale (McGrath et al., 1985) to assess pain in infants during vaccination (Taddio et al., 1995b). The scale encompassed three categories of infant pain behaviours: facial activity, crying and motor responses. The details regarding development and psychometric testing of this scale have been described in detail (Appendix 3). In addition, two other methods of infant pain assessment were used: infant crying, and visual analogue scale scores.

**Hypothesis 1**

The null hypothesis in this double-blind randomized controlled study was that there would be no difference in infant pain response during routine 4 or 6 month vaccination between infants treated with EMLA and infants treated with a placebo.

Please note that there are additional notes to this chapter in Appendix 4.
Use of Lidocaine-Prilocaine Cream for Vaccination Pain in Infants


Abstract

Purpose: To assess whether use of lidocaine-prilocaine 5% cream (EMLA) decreases pain associated with diphtheria-pertussis-tetanus (DPT) vaccination in infants.

Methods: Randomized, double-blind controlled trial in outpatient pediatric practice, Toronto, Ontario, Canada. Before vaccination, parents applied 2.5 gm of EMLA or placebo to the infant's leg and covered it with an occlusive dressing for at least 60 minutes. The infant received a 0.5 mL intramuscular injection of DPT at 2° to 8° C with a 1.6 cm 25-gauge needle; the infant was videotaped. The Modified Behavioral Pain Scale (MBPS) was used to assess baseline and postvaccination pain scores. Latency and duration of infant cry were measured.

Results: A total of 49 evaluable infants received EMLA, and 47 infants received placebo. There were no significant differences in demographic data; mean age was 5 months; and 50% of subjects were male. The median difference in prevaccination and postvaccination MBPS scores was lower for EMLA than placebo (p=0.001). The latency to the first cry was longer for subjects who were treated with EMLA (p=0.0004), but the total crying time was shorter (10.3 seconds vs 25.2 seconds; p=0.027). Of the study group, 90% (45/50) of subjects treated with EMLA and 12% (6/49) of subjects treated with placebo subjects had local skin reactions (p<0.0001), namely skin blanching.
Conclusions: Pretreatment with EMLA decreases infant pain from DPT vaccinations. Application of these data is limited to healthy infants receiving DPT vaccinations.

Introduction

Although routine intramuscular vaccinations are the most common source of iatrogenic pain in infants and children, methods to reduce this pain have not yet been adequately tested. Pretreatment with lidocaine-prilocaine cream, 5% strength (EMLA, Astra Pharma Inc.), has become a useful tool in this regard. Composed of equal parts of lidocaine and prilocaine, EMLA has been shown in pediatric clinical trials to decrease the pain of venepuncture (Robieux et al., 1991; Maunuksela et al., 1986; Hopkins et al., 1988; Halperin et al., 1989), lumbar puncture (Halperin et al., 1989), and removal of molluscum contagiosum lesions (Rosdahl et al., 1988; Juhlin et al., 1980). A comprehensive review of pretreatment with EMLA for the management of procedure-related pain in children was recently published (Steward, 1993). Before evaluating EMLA's efficacy in children, we tested its effect in adults in two double-blind randomized, clinical trials (Taddio et al., 1992b; Taddio et al., 1992a). In both instances, pretreatment with EMLA decreased the pain from skin penetration by the needle.

The purpose of this study was to determine whether use of EMLA cream could decrease the pain associated with intramuscular diphtheria-pertussis-tetanus (DPT) vaccination in infants.

Methods

Subjects participated in a double-blind, randomized (in blocks of two) clinical trial between April 30, 1992 and February 11, 1993. Two team members recruited subjects from their shared pediatric outpatient clinic in Toronto, Ontario. Study subjects included healthy infants receiving their 4-month or 6-month regular DPT vaccine. Exclusion criteria included a history of
sensitivity to amide local anaesthetics, use of analgesics within 4 hours of the vaccination procedure, fever, or illness that prevented administration of the vaccine.

Each subject received either EMLA or placebo cream before the administration of the vaccine. The parent(s) were given one 5-gram tube of EMLA or a placebo cream that was visually and cosmetically identical to EMLA, except that the active ingredients were replaced by coconut oil (Miglyol 812 Oil; Nobel Industries AB, Stockholm, Sweden).

The protocol was approved by our hospital's research ethics committee. Parents were informed of the study objectives and design through an information summary sheet. Parental consent to infant participation in the trial was obtained during the prior clinic appointment (i.e., before the infant's 4- or 6-month appointment). A study nurse explained and showed the parents how to apply the cream, which was then covered by an occlusive transparent dressing (Tegaderm; 3M Health Care, St. Paul, Minn.). The parents then practised applying the cream and dressing. Parents were given an instruction sheet to keep for reference. Parents were also contacted by one of the investigators within 1 or 2 days of the study day to review the protocol.

On the day of vaccination, the parent applied approximately 2.5 gm to the upper part of the infant's thigh approximately 60 minutes before the scheduled appointment. The parent noted the time that the cream was applied or wrote the time directly on the dressing. The dressing and cream were both removed in the clinic by one of the investigators after 60-120 minutes. The cream was wiped from the skin with a paper tissue; a water-soluble marker was used to mark four dots on the skin where the cream had been applied.

Within several minutes of removal of the cream, the infant was placed in a supine position on the examining table. If the infant was unsettled, he or she sat on the table or was held by a parent. Immediately before the injection, the site was wiped with an alcohol swab by a
pediatrician who then administered one 0.5 mL intramuscular injection of DPT vaccine at 2° to 8°C with a 1.6 cm 25-gauge needle. Two pediatricians unaware of the treatments performed all vaccinations.

The vaccination procedures were videotaped with a color camera. A mirror was mounted on the wall behind the examining table so that the videographer could film the infant's reaction both face on and from the mirror image. The videographer stood approximately 3 feet from the infant and did not interfere with the procedure. The entire vaccination procedure was taped until the baby settled down. Parents were told that "[they could do] whatever they normally would do after the vaccine was given, and that [they] did not have to do anything different because of the videocamera."

Parents also completed two questionnaires. The revised Carey Infant Temperament Questionnaire (Carey et al., 1978) for infants 4 to 8 months of age was completed within 2 weeks of the scheduled vaccination appointment. According to the scoring method, infants were assigned to one of five possible categories: easy, intermediate low, slow to warm up, intermediate high, and difficult. These five categories were then ranked in increasing order from one to five for statistical analyses. A questionnaire regarding parental opinion of the cream was completed on the study day.

The pain from the vaccination was assessed according to two methods: the Modified Behavioral Pain Scale and a 100 mm unmarked Visual Analogue Scale (VAS). On the VAS, a score of 0 denotes no pain, and 100 mm denotes maximal possible pain. The VAS was scored by an uninformed investigator at the time of the procedure, within 15 seconds of the injection. After the videotapes were reviewed, the infant pain scores were assessed by the same investigator.
according to the MBPS (Table 6), which was modified from the Children's Hospital of Eastern Ontario Pain Scale (McGrath et al., 1985) to score pain in infants.

The MBPS was used to score baseline pain and postvaccination pain for each vaccination procedure. The main outcome measure was the difference between the pre- and post- MBPS pain scores (i.e., the net increase in pain). In all instances, the prevaccination pain scores were assessed within 5 seconds of the vaccination and the postvaccination pain scores were assessed within 15 seconds (i.e., maximal pain response) by one investigator. The same investigator also scored whether the infant had a general "startle" reaction after receiving the vaccination. Finally, infants' cry patterns were also analyzed by viewing the videotapes. With a hand-held stopwatch, the investigator recorded the latency to the first cry after the injection, the length of the first cry, and the total crying time.
<table>
<thead>
<tr>
<th>Behavior Observed</th>
<th>Score (0-10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facial Expression</td>
<td></td>
</tr>
<tr>
<td>Definite positive expression: (i.e., smiling neutral expression)</td>
<td>0</td>
</tr>
<tr>
<td>Slightly negative expression: (i.e., grimace)</td>
<td>1</td>
</tr>
<tr>
<td>Definite negative expression: (i.e., furrowed brows, eyes closed tightly)</td>
<td>3</td>
</tr>
<tr>
<td>Cry</td>
<td></td>
</tr>
<tr>
<td>Laughing or giggling</td>
<td>0</td>
</tr>
<tr>
<td>Not crying</td>
<td>1</td>
</tr>
<tr>
<td>Moaning, quiet vocalizing, or gentle or whimpering cry</td>
<td>2</td>
</tr>
<tr>
<td>Full lunged cry or sobbing</td>
<td>3</td>
</tr>
<tr>
<td>Full lunged cry, clearly more than baseline full lunged cry*</td>
<td>4</td>
</tr>
<tr>
<td>Movements</td>
<td></td>
</tr>
<tr>
<td>Usual movements and activity</td>
<td>0</td>
</tr>
<tr>
<td>Resting and relaxed</td>
<td>0</td>
</tr>
<tr>
<td>Partial movement or attempt to avoid pain by withdrawing the limb where the puncture is done</td>
<td>2</td>
</tr>
<tr>
<td>Agitation with complex movements involving the head, torso or the other limbs, or rigidity</td>
<td>3</td>
</tr>
</tbody>
</table>

* Used only for post-procedural pain.
The infant's reaction to removal of the Tegaderm dressing was assessed according to three categories: no reaction, mild (i.e., movement), or severe (i.e., crying). Within one to five minutes after the removal of the cream, local skin reactions were also assessed using a four-point rating scale (none, mild, moderate, severe).

From a previous study (Robieux et al., 1991), we calculated a sample size of 48 subjects in each group to show a 50% difference in pain scores with a standard deviation of twofold this difference. Differences in infants' characteristics between subjects who were treated with EMLA and subjects who received placebo were analyzed by means of the Chi square test, Mann-Whitney U test, or student's t-test, where appropriate. Differences in pain scores, cry characteristics, and adverse effects were similarly analyzed. Correlations between pain scores and infant cry patterns were assessed according to the Spearman method. Backward elimination multiple linear regression was used to assess the contribution of potential confounding factors on pain scores and duration of infant cry. The significance level (probability) was 0.05.

Results

There were 112 participants randomly assigned to the study groups, but 12 withdrew from the study or did not come on a scheduled study day for vaccination. Of the 100 study subjects, 51 were randomly assigned to the EMLA group and 49 to the placebo group. For two subjects in each group, there was deviation from the study protocol (i.e., the parent did not apply enough cream, cream was applied for <60 minutes, or occlusive dressing did not cover the skin adequately). Thus, 96 subjects were available for study.

The mean age of infants was 5 months. Of the infants, 50% were male, and 96% were white. There were no significant differences between the groups in any of the demographic
characteristics, temperament scores or other factors measured (data available on request). In the EMLA group, the cream was applied on the skin for an average of 83 minutes (SD=14 minutes).

The pain scores for EMLA and placebo groups are shown in Table 7. The prevaccination MBPS scores did not differ between the two groups (p=0.975); however, both the postvaccination MBPS scores and the difference between pre and post-vaccination MBPS scores were lower for the EMLA group (p=0.001). Similarly, the VAS scores were lower in the subjects treated with EMLA (p=0.002). Correlation of the differences in prevaccination and postvaccination MBPS scores with VAS scores yielded a Spearman correlation coefficient of 0.608 (p=0.001). The incidence of startle reaction in the infants who received EMLA was 22 (44.9%) compared with 25 (53.2%) in the placebo group (p=0.416).

Table 7. MBPS and VAS Pain Scores for EMLA and Placebo

<table>
<thead>
<tr>
<th>Pain Scores (SD)</th>
<th>EMLA group (n=49)</th>
<th>Placebo group (n=47)</th>
<th>p *</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBPS score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before vaccination</td>
<td>2 (0-4)</td>
<td>2 (0-5)</td>
<td>0.975</td>
</tr>
<tr>
<td>After vaccination</td>
<td>7 (3-9)</td>
<td>8 (3-9)</td>
<td>0.001</td>
</tr>
<tr>
<td>Difference between before and</td>
<td>5 (1-8)</td>
<td>6 (1-9)</td>
<td>0.001</td>
</tr>
<tr>
<td>after scores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS score in (mm)</td>
<td>26.0 (0-94.0)</td>
<td>48.0 (0-97.0)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Values (except p values) are expressed as median (range); * Mann-Whitney U test

A backward elimination multiple regression analysis was performed to assess if magnitude of pain (defined by the difference in the pre- and post-MBPS scores) could be attributed to infant
sex, age or temperament; the physician who administered the vaccine; or preadministration with EMLA or placebo cream. Administration of EMLA cream and female sex were the only two factors associated with lower pain scores, \(F=8.31; \text{df}=2, 93; p=0.001\). The multiple correlation coefficient \((R)\) was 0.390, \((R^2=0.152)\). There was no interaction between infant sex and randomization code in regard to the difference in the pain scores \((F=0.34; \text{df}=1; p=0.563)\).

The subjects who were treated with EMLA had a longer latency period between needle puncture and the start of crying \((p=0.0004)\). The total time that infants cried after the vaccination procedure was less for the EMLA group than for the placebo group \((p=0.027)\). The difference in prevaccination and postvaccination MBPS scores was correlated with the latency to the first cry \((\text{Spearman's rho}=-0.22; p=0.031)\) and total duration of infant crying \((\text{Spearman's rho}=0.509; p<0.001)\) (Table 8).
Table 8. Cry Characteristics for Subjects receiving EMLA and Placebo

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Duration (seconds)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EMLA group (n=49)</td>
<td>Placebo group (n=47)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>3.4 (1.2)</td>
<td>2.5 (1.0)</td>
</tr>
<tr>
<td>latency of first cry b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median c</td>
<td>3.3 (1-6.4)</td>
<td>2.4 (0.5-5.5)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>4.7 (2.8)</td>
<td>6.0 (3.4)</td>
</tr>
<tr>
<td>duration of first cry d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median c</td>
<td>4.7 (0.6-10.3)</td>
<td>5.9 (0.7-14.2)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>31.2 (37.4)</td>
<td>35.4 (29.5)</td>
</tr>
<tr>
<td>total duration of cry c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median c</td>
<td>10.3 (0-145.1)</td>
<td>25.2 (0-117.4)</td>
</tr>
</tbody>
</table>

* Mann-Whitney U test
b one subject in the EMLA group and two subjects in the placebo group did not cry and are not included in the analysis.
c values in parentheses are ranges
d data also not available for one subject in the EMLA group.
e data not available for one subject in the EMLA group.

Multiple regression was again used to identify factors associated with duration of crying.
The log of the total duration of infant crying was analyzed with the same factors tested in the previous analysis. Only pretreatment with EMLA was associated with decreased duration of infant crying (F=6.64; df=1, 90; p=0.012, R²=0.069).
The parent who accompanied the infant to the clinic appointment was usually the mother; a father comforted the infant after the vaccination instead of the mother in only two instances. Most infants were touched by the parent during the vaccination procedure. The parent usually held the infant's hand(s), but some touched other parts of the infant. Only four subjects in the placebo group and one in the EMLA group were not touched by a parent (p=0.199). After the injection, 26 parents (55.3%) in the placebo group picked up their infants compared with 23 (46.9%) in the EMLA group (p=0.411). Picking up the child was correlated with the duration of the infant crying (Spearman correlation coefficient=0.340; p=0.001); infants whose mothers picked them up cried longer than those whose mothers did not.

Adverse effects could be evaluated in 50 subjects in the EMLA group and 49 subjects in the placebo group. Ninety-two subjects (93.0%) did not react to removal of the patch. Only three infants cried when the dressing was removed. Thirty subjects (30.3%) had minor skin redness where the dressing had been applied. Of the subjects in the EMLA group, 90% had local skin reactions compared with 12% of subjects in the placebo group (p<0.0001). The most common adverse reaction to pretreatment with EMLA was minor skin blanching (60%) followed by redness (30%).

When parents were asked whether they thought the cream was difficult to apply, 87 (90.6%) responded that it was not. When asked whether it was difficult to fit application of the cream into their schedules, 84 (87.5%) responded that it was not.

**Discussion**

Current methods of measuring infant pain include observations of infant behaviour, physiologic responses or both (Owens, 1984). We used a behavioural approach to measure the
pain of the vaccination procedure. Other investigators have used similar techniques to measure infant pain (Robieux et al., 1991; McGrath et al., 1985; Fradet et al., 1990). Our data are limited to healthy infants and DPT vaccines. Further research will be necessary to determine whether pretreatment with EMLA is effective with other vaccines and in other patient populations.

We scored the MBPS pain scores by means of videoanalysis. This provided a precise way of observing infant behavior. The MBPS scores obtained from video analysis were significantly correlated with VAS pain scores obtained from direct observation, suggesting that both scales measured similar responses.

Our study was not able to discern between the pain from the needle prick and that from the injection of the vaccine, however, the longer latency between the first cry for the EMLA subjects suggests that this agent causes superficial anesthesia. The overall lower pain scores suggest that it minimizes the pain from the entire procedure as well. There was a large variation in the VAS and MBPS pain scores obtained between subjects. In fact, scores varied from minimal pain to almost maximum pain for subjects in both groups. Pretreatment with EMLA may not have substantially reduced pain in all infants because it penetrates to about 5 mm below the skin surface (Bjerring et al., 1990) and vaccination involved needle insertion into the muscle.

In addition to the behavioral scores and VAS scores, we measured differences between the subjects in the EMLA and placebo groups in the latency to the infant's first cry, duration of first cry, and total crying time, which are regarded as important measurements in the overall assessment of infant pain (Grunau et al., 1990; Johnston et al., 1986). Skin anesthesia with EMLA cream was associated with a longer latency to the first cry, suggesting that infants who received EMLA, similar to adults, had not felt the needle penetrating the skin. Similarly, subjects in the EMLA group cried for a shorter period than subjects given placebo, indicating that use of
EMLA decreased the pain from the entire procedure not just the needle penetration. Some investigators have suggested that stressful events in infancy can affect developmental outcome (Tyson, 1984; Als et al., 1986). Pretreatment with EMLA may thus affect an infant's long-term conditioning to painful procedures. Decreasing infant pain may also lead to a decrease in parental anxiety during vaccination. The psychologic consequences of decreasing pain during procedures warrants further study.

Most parents in the study were able to apply the cream correctly before the child's vaccination. Although this implies that EMLA may be routinely used at home before the infant's arrival at the physician's office, our results are limited to the sample of parents studied and their level of understanding. The pediatric practice setting was in an upper class neighborhood with mature mothers (average age 33 years).

In this study, female sex was associated with lower pain scores. Other investigators have not consistently found this to be true (Fradet et al., 1990; Grunau et al., 1987). The effect of gender on pain behavior requires more study. Infant temperament was measured to try to explain infant pain behavior; no statistical associations were found. Perhaps the limitations of temperament scales are partially responsible, or infant temperament may not predict pain behavior.

Minor local skin reactions were observed in almost all subjects who received EMLA cream. The most common reactions were minor pallor and redness, which are recognized reactions to EMLA cream (Astra Pharma Inc. 1990). No serious adverse reaction was observed in any study subject.

EMLA is not currently licensed in the United States for use in neonates because there is a lack of safety data in this population, together with a concern about possible development of
methemoglobinemia from the prilocaine metabolite, o-toluidine. Systemic bioavailability of EMLA is believed to be small in adults but has not been studied in children. There is one report of methemoglobinemia in a 3 month old infant treated with trimethoprim-sulfamethoxazole and a large dose of EMLA cream (5 gm for 5 hours) (Jakobson et al., 1985), suggesting significant absorption of EMLA, but a variety of other agents may cause methemoglobin formation in neonates and the concurrent use of the sulfonamide may have contributed to the observed toxic effects (Davies, 1985). Para-aminophenol derivatives, such as acetaminophen, can also cause methemoglobin formation (Flower et al., 1985). In infants exposed to one or more of these agents there may be a potential for drug interactions with EMLA. Practitioners who treat infants with EMLA should be aware of these concerns.

We limited our study to infants more than 3 months of age who were not using other medications known to cause methemoglobin formation, because there are published data which show that for these patients, the risk for methemoglobinemia is small (Engberg et al., 1987). Until there is more evidence of EMLA's safety in younger infants, however, it should not be recommended for routine use in those patients.

The wholesale U.S. price of one 5 gm tube of EMLA cream, which yields two doses, is $5.25. The preparation is available only with a prescription; the added cost of a pharmacy dispensing fee can make it a relatively expensive alternative. Conversely, the cost of pain has yet to be defined, and may be much greater in the infants who experience it than the mere cost of the medication.

We conclude that skin anesthesia with EMLA was associated with lower pain scores and less crying in infants vaccinated with DPT vaccine. The EMLA cream may thus be useful for premedication of infants before such procedures. The method of administration is crucial to the
efficacy of EMLA; the fact that most parents were able to use it correctly suggests that it can be utilized in this setting.

Statement of Significance

Our research demonstrated that EMLA is efficacious for reduction of vaccination pain in infants. The results have been confirmed by another study (Uhari, 1993). In that study, 155 infants aged 3 to 28 months (mean, 9 months) were randomized to receive EMLA (n=79) or placebo (n=76) prior to vaccination. Most of the infants (98%) received diphtheria-tetanus-pertussis vaccine. The dose used was not specified, however, the mean time between application of cream and vaccination was 65 minutes. Both parents and nurses rated infant pain using a 10 cm visual analogue scale. Parents rated infant pain as lower in the EMLA group compared to the placebo group (4.8 vs 2.9 cm; p<0.001). Nurses also rated infant pain lower for infants treated with EMLA (2.5 vs 3.8 cm; p<0.003). Local skin skin pallor was more common in the EMLA group (p<0.01), although the incidence was not reported. However, it is important to note that the Uhari study (1993) did not report any differences in infant pain response due to gender.
Chapter 2. Association Between Infant Vaccination Pain and Neonatal Circumcision

The observed differences in pain responses between male and female infants at routine 4 or 6 month vaccination observed in Chapter 1 can be due to several reasons, such as: chance difference between the groups, parental upbringing, genetic neural differences, and previous painful experiences. It is possible that the observed differences are not really there, but are instead due to chance, that is, a type I error. Parental upbringing may differ between male and female infants and this may account for differences in pain behaviours at vaccination. However, previous clinical studies have not revealed differences in the mothering and fathering of male and female infants (Belsky et al., 1987; Ninio et al., 1988; Graham, 1993). Gender differences in neural structures subserving pain may be present. To date, however, clinical studies have not demonstrated consistent differences in newborn pain responses between males and females (Worobey et al., 1989; Owens et al., 1984; Grunau et al., 1987). Finally, differences in previous pain experiences between male and female infants may account for the observed differences in pain behaviour during vaccination. The nociceptive system is plastic and noxious input can alter how the brain responds to subsequent input. Studies have demonstrated that traumatic child medical experiences influence adult behaviours (Pate et al., 1996).

In this study, it was hypothesized that the pain behaviour of male infants differed from female infants due to differences in past experiences with pain, such as circumcision. Circumcision is a surgical procedure performed in a significant number of male newborns (Fetus and Newborn Committee. Canadian Paediatric Society, 1996). The procedure involves removing the prepuce (outer fold of skin and mucosa) from the penis. Since the prepuce is highly innervated (Taylor et al., 1996), circumcision is expected to be painful. Clinical studies have
demonstrated that neonatal circumcision is associated with extreme changes in behavioural (e.g., crying), physiological (e.g., heart rate, blood pressure), and biochemical (e.g., cortisol) parameters that are indicative of intense pain.

Early pain experiences such as circumcision have been postulated to lead to behavioural differences between male and female infants (Richards et al., 1976). To date, however, no studies have assessed whether circumcision is associated with alterations in infant pain behaviour later on. In the current study, the effect of circumcision on infant pain response during routine vaccination was investigated in male infants. This study was a post-hoc analysis of the randomized controlled trial presented in Chapter 1.

**Hypothesis 2**

The null hypothesis tested in this retrospective analysis of a double-blind randomized controlled trial was that the pain response from vaccination of circumcised male infants did not differ from the pain response from vaccination of uncircumcised male infants.
Effect of Neonatal Circumcision on Pain Responses During Vaccination in Boys


Abstract

Using data from one of our randomised trials, we investigated post-hoc whether male neonatal circumcision is associated with a greater pain response to routine vaccination at 4 or 6 months. Pain response during routine vaccination with diphtheria-pertussis-tetanus (DPT) alone or DPT followed by Haemophilus influenzae type b conjugate (HIB) was scored blind. 42 boys received DPT and 18 also received HIB. After DPT, median visual analogue scores by an observer were higher in the circumcised group (40 vs. 26 mm, p=0.03). After HIB, circumcised infants had higher behavioural pain scores (8 vs. 6, p=0.01) and cried longer (53 vs 19 s, p=0.02). Thus neonatal circumcision may affect pain response several months after the event.

Introduction

In our randomised controlled trial of a topical anaesthetic cream (EMLA, eutectic mixture of local anaesthetics; Astra) on infant pain in routine diphtheria-pertussis-tetanus (DPT) vaccination, boys had higher pain scores than girls (Taddio et al., 1994). If this sex difference is a real effect, it may be partly related to previous experience with acute pain, such as circumcision. Circumcised babies have short-term alterations in behaviour, sleep patterns, frequency of feeding, crying, fussiness, and heart rate (Marshall et al., 1980; Brackbill, 1975; Anders et al., 1974; Emde et al., 1971; Marshall et al., 1982; Dixon et al., 1984). Effects beyond the first few hours after the painful event have not been investigated. We have done a post-hoc analysis from our trial
(Taddio et al., 1994) to investigate whether circumcision was associated with pain scores during vaccination.

Methods

Healthy boys aged 4-6 months (mean 5) who were vaccinated with DPT alone, or DPT and Haemophilus influenzae type b conjugate (HIB), were included. DPT was administered intramuscularly on the upper thigh 60 min after treatment with EMLA or placebo. Infants who also received HIB were given an intramuscular dose several minutes after the DPT and on the opposite leg without EMLA or placebo. All injections were made by one of two paediatricians.

During the DPT injection, an observer and the paediatrician who administered the vaccine (both unaware of treatment allocations) rated the infant’s pain response on a 100 mm ungraded visual analogue scale (VAS: 0=no pain, 100=worst possible pain). We videotaped the infants during their one or two injections, until they settled. A coder who was unaware of the treatments rated the pain responses for all vaccinations on a modified behavioural pain scale (face, cry, and body movements). The total pain score was obtained by adding the scores of the components and varied between 0 and 10 (Taddio et al., 1994). Baseline pain scores, post-procedural scores, net pain (ie, difference between baseline and post-vaccination score), and duration of cry were assessed for all injections. Infants who had undergone painful procedures that were not routine (eg, lumbar puncture, surgery) were excluded from analyses. Infants who were in discomfort before the vaccine (ie, baseline behavioural pain scores greater than 2) were also excluded from analysis of pain for that vaccination. Statistical differences were calculated with Mann-Whitney, \(X^2\), or Fisher’s exact tests. Correlations were done with Spearman’s method.
Results

Of the 42 boys who received DPT, 30 (71%) had been circumcised. No significant differences were found between circumcised and uncircumcised infants in demographic characteristics. Observer’s VAS pain ratings were higher for the circumcised boys (median 40 vs 26 mm, p=0.03). Paediatricians’ VAS scores showed a similar trend but were not significant (56 vs 30 mm, p=0.07). There was also a trend towards less crying for the uncircumcised boys (7.3 vs 22.3 s, p=0.06). The median post-vaccination behavioural pain score was 8 for the circumcised group compared with 7 in the uncircumcised group (p=0.2).

We stratified the infants by whether they were premedicated with the local anaesthetic (n=24) or placebo (n=8) and repeated the analyses. In the local anaesthetic group, both observer’s and paediatricians’ VAS scores were higher for circumcised boys (32 vs 10 mm, p=0.004; and 35 vs 13 mm, p=0.01, respectively). The total duration of crying was longer (14 vs 5 s, p=0.004), and there was a trend toward higher post-vaccination pain scores (7 vs 6, p=0.1) in the circumcised group. These results suggest that the local anaesthetic was more effective on infants that were uncircumcised (ie, may not already be conditioned to pain). No significant differences were observed in the pain responses of the placebo-treated infants.

18 infants received the HIB vaccine; 13 (72%) had been circumcised. These infants were younger than the other boys who participated in the trial (126 vs 140.5 days, p=0.02). They did not differ, however, in other demographic characteristics, or in pain scores and cry duration for the first vaccine. Circumcision was positively associated with post-vaccine behavioural pain scores (r=0.61, p=0.007), net pain scores (r=0.67, p=0.002), and duration of crying (r=0.57, p=0.02). Circumcised boys had higher scores and cried for longer (Figure 4).
We also looked at cultural background (eg, being Jewish) and maternal intervention on pain response. No significant associations were found. Since 11 infants were initially excluded from the HIB analyses because of discomfort before injection, which may have introduced bias, analyses were repeated including these infants: the effects of circumcision status on pain response were even more statistically significant.

Discussion

Male circumcision is the most common neonatal surgical procedure. It causes intense pain and measurable changes in behaviour that last for up to 1 day. We found that circumcision status was associated with increased infant pain response to routine vaccination at 4-6 months. Circumcised boys had significantly longer crying bouts and higher pain scores. That both outcome measures, pain index, and cry duration, were influenced by circumcision status lends credibility to our observations. During the second (HIB) vaccination, circumcision status was more clearly associated with the observed pain response than after DPT. The DPT injection might have had a priming effect in circumcised infants which led them to exhibit even more pain after the HIB injection. The effects of memory and reinforcement on later nociceptive experience in neonates are not known (Zeltzer et al., 1992). Because memory of pain is believed to be important in subsequent pain perception, and the main structures for memory are functional in the neonatal period (Anand et al., 1987a), it is conceivable that pain from circumcision may have long-lasting effects on pain response and/or perception.

The post-hoc nature of our analyses and the small sample sizes make our conclusions speculative. Nevertheless, we suggest that analgesia should be routine for circumcision to avoid possible long-term effects in infant boys' pain responses.
Figure 4. Pain Response During HIB Injection in Circumcised and Uncircumcised Boys

Data not available for cry on 1 infant. p=0.02 for cry, p=0.01 for behavioural pain score.

Statement of Significance

This retrospective analysis of our vaccination study provides sufficient evidence to require further investigation of the long-term effects of untreated pain in infants. There are grounds to justify routine analgesia in newborn infant boys undergoing circumcision. The effects of analgesia may be to not only ameliorate the infant's pain at the time of surgery, but also to moderate the apparent behaviour differences that we observed. More evidence is needed, however, due to the limitations of the study design that was employed in this study.
Chapter 3. EMLA in Neonatal Circumcision

The results from Chapter 2 suggest that analgesia should be routinely administered to newborn infants undergoing circumcision to prevent pain and possible alterations in future pain behaviours. This recommendation is at variance with current practices regarding circumcision pain management. Surveys of physicians who perform neonatal circumcision demonstrated that analgesics are rarely administered (Toffler et al., 1990; Wellington et al., 1993; Howard et al., 1996). Even after the institution of an educational program aimed at physicians performing circumcisions, an audit performed 12 months after the program showed that local anaesthesia was administered in only 66% of cases (Ryan et al., 1994). In a recent survey, the median percentage of neonates who received analgesics was: dorsal penile nerve block 10%; EMLA or lidocaine cream 0%; oral analgesics (unspecified, but assumed to be acetaminophen) 0%; and comfort measures such as sucrose pacifier and swaddling 0% (Howard et al., 1996). Oral ethanol has also been used (Wellington et al., 1993).

The first report describing pharmacologic attenuation of neonatal pain during circumcision was published almost 20 years ago. Investigators reported their experience with the use of a superficial regional nerve block called a dorsal penile nerve block (Kirya et al., 1978). They observed that dorsal penile nerve block with lidocaine led to a decrease in infant pain during the circumcision. Since that report was published, several randomized controlled studies using this anesthetic technique have been performed; all have shown substantial and significant decreases in infant pain (Masciello, 1990; Stang et al., 1988; Maxwell et al., 1987; Holve et al., 1983; Williamson et al., 1983). However, physicians have been reluctant to implement this technique. Dorsal penile nerve block requires technical skills that are not acquired by most physicians. It is
perceived as an additional "unnecessary" procedure that may be associated with adverse effects, time delays, and infant pain. Moreover, the surgical procedure itself is perceived to be relatively minor, and unworthy of pain management.

Alternative pharmacologic agents have not been well studied. Two studies demonstrated efficacy with topical lidocaine preparations (Weatherstone et al., 1993; Mudge et al., 1989). However, the agents used in these studies are not commercially available, and the results have limited clinical utility. EMLA is a commercially available preparation that offers an alternative to dorsal penile nerve block for the management of neonatal circumcision pain.

In the current study, the efficacy and safety of EMLA for the management of neonatal circumcision pain was investigated. At the time the study was undertaken, one previous small randomized controlled trial had been published which investigated the efficacy of EMLA for this procedure. In that study, 0.5 g of EMLA applied to the penis for 45 to 65 minutes prior to circumcision significantly decreased infant pain when compared to a petrolatum placebo (Benini et al., 1993). However, the results of that study did not lead to widespread clinical use of EMLA for several reasons. These include: the relatively small sample size tested (only 27 neonates), potential bias during data collection, lack of clinical interpretation of results, and lack of safety assessments. Data collection was performed by an observer that was not blinded to treatment allocation. There is some evidence suggesting that this design created bias: the infants treated with EMLA had lower pain scores than infants treated with the petrolatum placebo during the baseline and restraining phases (i.e., before the circumcision) when no differences are expected to exist. Investigators expressed the magnitude of EMLA's effect on infant pain for the various components of the circumcision separately, not the entire procedure. Thus, the results were difficult to understand in clinical terms (i.e., clinicians would not be able to discern whether the
differences had any clinical meaning). Finally, methemoglobin concentrations were not monitored in the study, and there is a concern that EMLA may cause methemoglobinemia in this population.

Before the clinical trial was undertaken, we performed a study in neonatal pigs to determine the safety and bioavailability of EMLA when applied to the penile area (Gazarian et al., 1995) (Appendix 5). In that study, 1 g of EMLA was applied for an hour. Methemoglobin concentrations, measured prior to administration of EMLA and 1, 2, 4, 6 and 8 hours afterwards, were not found to be significantly elevated from baseline values. The bioavailability of lidocaine and prilocaine were low; 4% and 7%, respectively (Gazarian et al., 1995). The results provided some reassurance that 1 g of EMLA would not lead to a clinically observable risk of methemoglobinemia if applied on the penis of newborn infants for 1 hour prior to circumcision.

The dose of EMLA utilized in the clinical trial was 1 g. This dose was based on the surface area of the procedure site (i.e., amount of skin covered by the cream) which was estimated to be approximately 8 square centimetres, and the demonstrated safety of this dose in the pig study (Gazarian et al., 1995).

**Hypotheses 3 and 4**

The following hypotheses were tested in this double-blind randomized controlled trial:

There would be no difference in pain response during circumcision between male newborn infants treated with EMLA and those treated with a placebo, and

There would be no difference in methemoglobin concentrations between male newborn infants treated with EMLA and those treated with a placebo for circumcision.

Please note that there are additional notes to this chapter in Appendix 6.
Efficacy and Safety of Lidocaine-Prilocaine Cream for Pain During Circumcision

Originally published in "Efficacy and Safety of Lidocaine-Prilocaine Cream for Pain During Circumcision" by Anna Taddio, Bonnie Stevens, Kenneth Craig, Pratap Rastogi, Shlomit Ben-David, Andrew Shennan, Peggy Mulligan, Gideon Koren. THE NEW ENGLAND JOURNAL OF MEDICINE 336:1197-1201. Copyright 1997. Massachusetts Medical Society. All rights reserved. Reprinted with permission (000747).

Abstract

Background: Neonatal circumcision is a painful surgical procedure often performed without analgesia. We assessed the efficacy and safety of 5 percent lidocaine-prilocaine cream (EMLA) in neonates undergoing circumcision.

Methods: We carried out a double-blind, randomized, controlled trial in 68 full-term male neonates: 38 were assigned to receive lidocaine-prilocaine cream, and 30 to receive placebo. One gram of lidocaine-prilocaine or placebo cream was applied on the penis under an occlusive dressing for 60 to 80 minutes before circumcision. Behavioral (facial activity and percentage time spent crying) and physiologic (heart rate and blood pressure) responses were recorded during the procedure. Blood samples were obtained at various times after drug application for measurements of methemoglobin and plasma lidocaine, prilocaine, and o-toluidine (a metabolite of prilocaine).

Results: A total of 68 and 59 neonates were included in the safety and efficacy analyses, respectively. Demographic characteristics such as gestational age and birthweight did not differ between the lidocaine-prilocaine and placebo groups. During circumcision, the neonates in the lidocaine-prilocaine group had less facial activity (p<0.01), spent less time crying (p<0.001), and had smaller increases in heart rate (p=0.007) than the neonates in the placebo group. Facial
activity scores were 12 to 49 percent lower during various steps of the procedure in the lidocaine-prilocaine group. As compared with neonates in the placebo group, infants in the lidocaine-prilocaine group cried less than half as much and had heart rate increases of 10 beats per minute less. Blood methemoglobin concentrations (expressed as a percentage of the hemoglobin concentration) were similar (1.3 percent) in both groups. Lidocaine and prilocaine were detected in plasma in 23 (61 percent) and 21 (55 percent) of infants treated with lidocaine-prilocaine cream, respectively.

Conclusions: Lidocaine-prilocaine cream is efficacious and safe for the prevention of pain from circumcision in neonates.

Introduction

Many physicians are reluctant to administer analgesic drugs to neonates undergoing circumcision despite evidence that this procedure causes intense pain (Wellington et al., 1993; Toffler et al., 1990; Howard et al., 1996). The most frequently cited reasons are lack of familiarity with the administration techniques, and with the actions and side effects of analgesic drugs in this age group. There also appears to be a perception that circumcision is a minor procedure that is unworthy of analgesia (Wellington et al., 1993; Toffler et al., 1990).

A safe and effective analgesic that is also easy to administer is needed. One alternative is a 5 percent lidocaine-prilocaine cream (2.5 percent lidocaine and 2.5 percent prilocaine, EMLA, Astra, Mississauga), which is a eutectic mixture of local anesthetics - that is, a mixture whose melting point is lower than the melting points of either lidocaine and prilocaine. Unlike previous formulations, this formulation allows the use of high concentrations of the anesthetic bases without concern about local irritation, uneven absorption, or systemic toxicity. Lidocaine-
prilocaine cream produces reliable analgesia for various cutaneous procedures including
venipuncture (Robieux et al., 1991; Maunuksela et al., 1986), lumbar puncture (Halperin et al., 1989), and vaccination (Taddio et al., 1994; Uhari, 1993).

In the one reported trial of lidocaine-prilocaine cream for circumcision, neonates who received the anesthetic had significantly lower levels of facial activity, which is used to estimate pain in these patients; cried less during the procedure; and had smaller increases in heart rate and higher oxygen saturation values than neonates who received a petrolatum placebo (Benini et al., 1993). However, the data were collected by an observer who was aware of treatment allocation. Moreover, methemoglobin concentrations were not measured in the study, and there is a concern that lidocaine-prilocaine cream may cause methemoglobinemia in neonates. We therefore undertook this study to investigate the efficacy and safety of lidocaine-prilocaine cream for pain during circumcision in neonates.

Methods

The study subjects were 68 healthy white male neonates (≥ 37 weeks gestation; birth weight, ≥ 2500 g) undergoing circumcision. We excluded neonates with jaundice or methemoglobinemia and those receiving methemoglobin-inducing, analgesic or sedative drugs. The protocol was approved by the ethics boards of the Hospital for Sick Children and Women’s College Hospital, and the parents gave informed written consent for their infants to participate.

Study Procedures

The lidocaine-prilocaine and placebo creams were provided by Astra. The lidocaine-prilocaine cream was composed of a 1:1 mixture of lidocaine and prilocaine that was emulsified in
water and thickened to produce a suitable consistency. The placebo cream was identical, except that the active ingredients were replaced with coconut oil.

The neonates were randomly assigned to receive lidocaine-prilocaine or placebo cream. One mL (1 g) of study cream was drawn into a 3-mL syringe. One third of the dose was applied on the lower abdomen. The remainder of the dose was applied to a Tegaderm dressing that was placed over the penis and taped to the abdomen, so that the cream surrounded the penis. After 60 to 80 minutes, the dressing was removed and the cream was wiped away with a tissue. Local skin reactions were recorded.

Circumcisions were performed by one of three study-team pediatricians in the nursery treatment room. The procedure was standardized and divided into 13 periods for purposes of observation: base line, restraint of the infant (Circumstraint, Olympic Surgical, Seattle), application of antiseptic, application of forceps, lysis of adhesions, dorsal incision, application of the clamp (Gomco, St Louis), pulling of skin through the clamp, tightening of the clamp, cutting of foreskin, removal of the clamp (five minutes after the foreskin was cut), application of petrolatum dressing, and removal of restraints.

The infant's facial expressions were continuously recorded during the procedure with a videocamera (model PV-S770A-K, Panasonic, Mississauga, ON) positioned approximately 60 cm from his face. A model AS3 monitor (Datex-Engstrom, Helsinki, Finland) was used to monitor heart rate. Blood pressure was monitored noninvasively with a Critikon Dinamap monitor (model 1846SX, Johnson and Johnson, Peterborough, ON) interconnected with the cardiorespiratory monitor.

A heparin-treated blood sample (1 to 1.5 mL) was collected from each neonate 1.25, 2, 4, 6, 10, or 18 hours (±15 minutes) after the study drug was applied, according to a preassigned
randomization code, for measurements of methemoglobin, lidocaine, prilocaine, and o-toluidine (a metabolite of prilocaine that causes methemoglobinemia).

The neonates were observed for adverse effects every 8 hours for up to 24 hours after the circumcision. The parents were contacted by telephone two weeks after the circumcision and interviewed about adverse effects with a standardized telephone questionnaire.

Assessment of Pain

The facial activity of the neonates, which reflects the amount of pain experienced, was assessed by a research assistant unaware of the treatment assignments who was trained to use the Neonatal Facial Coding System reliably (kappa, 0.93, P<0.001) (Grunau et al., 1990; Craig et al., 1988a; Grunau et al., 1987). The presence or absence of 10 discrete facial actions—bulging of the brow, squeezing the eyes closed, nasolabial furrowing, opening the mouth, vertically stretching the mouth, horizontally stretching the mouth, pursing the lips, holding the tongue taut, quivering of the chin, and protrusion of the tongue—was scored from the videotape in 2-second intervals for the first 20 seconds of each phase of the circumcision or for the entire duration of the phase if it lasted less than 20 seconds. The research assistant could stop and start the videotape as many times as needed to score each facial action. The recorded image had a running time clock.

A summary score of facial activity was devised to compare facial expressions between groups. First, raw scores of each facial action were expressed as the proportion of the time each action was observed during each phase of the circumcision. Then facial actions correlating poorly with other actions (r<0.15) were removed in a stepwise fashion with principal-components analysis, leaving six facial actions (bulging of the brow, squeezing the eyes closed, nasolabial furrowing, vertically stretching the mouth, holding the tongue taut, and opening the mouth) that
accounted for 64 percent of the variability in infants’ responses. These six scores were weighted according to the raw coefficients from the factor analysis and totaled to arrive at an overall facial-activity score ranging from 0 to 1.226.

Secondary outcome measures included the duration of crying during each phase of the procedure and and physiologic (heart rate and blood pressure) changes. The duration of crying was calculated from the videotape as the percentage of time an infant cried during each phase of the circumcision, with scores ranging from 0 to 100 percent. The mean heart rate was calculated for each phase from raw data recorded directly onto a laptop computer with the Datex AS3 Datacollect program (Puritan Bennett, Pickering, ON). Systolic and diastolic blood pressures were measured in each infant at base line and during lysis of adhesions.

**Laboratory Analyses**

Blood methemoglobin was measured with a co-oximeter (model 482, Instrumentation Laboratory, Lexington, Mass.) and expressed as a percentage of total hemoglobin. All samples were stored on ice for six hours or less before analysis. The 95 percent confidence interval reported by the manufacturer is ±1 percent, and the SD was 0.5%. The remainder of the blood sample was centrifuged, and the plasma was separated and frozen at -20°C. Plasma lidocaine, prilocaine and o-toluidine were measured simultaneously by high-performance liquid chromatography (Klein et al., 1994). For each compound, the limit of detection was 20 ng per milliliter and the within-day coefficient of variation varied from 3.1 to 8.3 percent.

**Statistical Analysis**

Facial activity scores, the duration of crying, and heart rate were compared between groups with repeated measures analysis of co-variance, in which seven of the circumcision phases
(application of forceps, lysis of adhesions, dorsal incision, application of the clamp, pulling of skin through the clamp, tightening of the clamp, and cutting foreskin) were included and the base line value was the covariate. Univariate and multivariate effects were examined. Methemoglobin concentrations were compared using Student's t-test. Blood pressure was analyzed by analysis of covariance. The characteristics of the infants in the two groups were compared with the chi-square test or Student's t-test. Analyses were performed with SAS computer software. Statistical tests were two-tailed.

Results

The characteristics of the 68 neonates, 38 in the lidocaine-prilocaine group and 30 in the placebo group, were similar (Table 9). Eight neonates were treated with lidocaine-prilocaine cream in an unblinded fashion and were only included in the safety analysis. Fifty-nine neonates were included in the efficacy analysis; 29 in the lidocaine-prilocaine group and 30 in the placebo group. One neonate in the lidocaine-prilocaine group was excluded because he was not circumcised on the day the cream was applied. Fifty-five of the infants were circumcised by the same pediatrician. The mean (±SD) interval between the removal of the cream and circumcision was 13±16 minutes in the lidocaine-prilocaine group and 10±11 minutes in the placebo group (P=0.35). The duration of the procedure (i.e., from the application of forceps to cutting of the foreskin) was 9±1 minutes in the lidocaine-prilocaine group and 9±2 minutes in the placebo group (P=0.34).
Table 9. Demographic Characteristics of the Neonates in the Lidocaine-Prilocaine and Placebo Groups and Their Mothers

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Lidocaine-prilocaine (N=38)</th>
<th>Placebo (N=30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age- days</td>
<td>278±9</td>
<td>276±9</td>
<td>0.36</td>
</tr>
<tr>
<td>Birthweight- g</td>
<td>3604±401</td>
<td>3548±442</td>
<td>0.59</td>
</tr>
<tr>
<td>Postnatal age- days</td>
<td>1.3±0.8</td>
<td>1.6±0.9</td>
<td>0.13</td>
</tr>
<tr>
<td>5-Minute Apgar score</td>
<td>9.1±0.3</td>
<td>9.1±0.3</td>
<td>0.54</td>
</tr>
<tr>
<td>Vaginal delivery- no. (%)</td>
<td>29 (76)</td>
<td>19 (63)</td>
<td>0.24</td>
</tr>
<tr>
<td>Mothers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>32±4</td>
<td>33±4</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Plus-minus values are means ±SD.

**Infant's Response to Pain**

The facial activity scores recorded during circumcision are shown in Figure 5. The scores increased in both groups and remained high in the placebo group throughout the procedure.

During surgery, the scores were lower in the lidocaine-prilocaine group than the placebo group (P=0.01). The lidocaine-prilocaine group had 12 to 49 percent less facial activity (P<0.001) than the placebo during forceps application, dorsal incision, application of the clamp, and foreskin cutting.

The lidocaine-prilocaine group spent less time crying during the procedure than the placebo group (P<0.001) (Table 10); they cried less (P<0.05) during six of the circumcision
phases. They also had a smaller increase in heart rate overall (P=0.007) (Table 10) and during five of the circumcision phases (P<0.05).

Table 10. Response to Circumcision in the Lidocaine-Prilocaine and Placebo Groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Lidocaine Prilocaine</th>
<th>Placebo</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase during procedure in percentage of time spent crying **</td>
<td>21±27</td>
<td>46±25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No. of infants</td>
<td>29</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Increase in heart rate ** (beats/min)</td>
<td>7±13</td>
<td>17±16</td>
<td>0.007</td>
</tr>
<tr>
<td>No. of infants</td>
<td>19</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Increase in systolic blood pressure ** (mm Hg)</td>
<td>11±17</td>
<td>14±21</td>
<td>0.22</td>
</tr>
<tr>
<td>No. of infants</td>
<td>22</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Increase in diastolic blood pressure ** (mm Hg)</td>
<td>19±22</td>
<td>24±33</td>
<td>0.16</td>
</tr>
<tr>
<td>No. of infants</td>
<td>22</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

Plus-minus values are means ±SD. Vigorous body movements during the procedure and errors in the storage of data by computers prevented the acquisition of physiologic data on some neonates, as indicated.

** The increase is the increase from base line.
**Adverse Effects**

None of the neonates had any clinical sign of methemoglobinemia. The mean methemoglobin concentration was $1.3 \pm 0.6$ percent in the lidocaine-prilocaine group and $1.3 \pm 0.2$ percent in the placebo group ($P=0.80$). There were no differences between groups at any time during sampling.

Twenty-three (61 percent) of the neonates in the lidocaine-prilocaine group had detectable plasma lidocaine concentrations, and 21 (55 percent) had detectable plasma prilocaine concentrations. The highest concentrations were detected within four hours after drug administration (Table 11). No neonate had detectable concentrations 18 hours after drug application. The metabolite o-toluidine was undetectable in all infants.
Table 11. Plasma Lidocaine and Prilocaine Concentrations in the Lidocaine-Prilocaine Group

<table>
<thead>
<tr>
<th>Hours after Application</th>
<th>Lidocaine Levels/Total</th>
<th>Prilocaine Levels/Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Samples with Undetectable Levels</td>
<td>No. of Samples*</td>
</tr>
<tr>
<td></td>
<td>ng/ml</td>
<td>ng/ml</td>
</tr>
<tr>
<td>1-1.5</td>
<td>46 (38-91)</td>
<td>35 (24-74)</td>
</tr>
<tr>
<td>1.75-2.25</td>
<td>50 (41-100)</td>
<td>33 (29-68)</td>
</tr>
<tr>
<td>3.75-4.25</td>
<td>96 (30-135)</td>
<td>62 (29-81)</td>
</tr>
<tr>
<td>5.75-6.25</td>
<td>48 (21-103)</td>
<td>49 (24-107)</td>
</tr>
<tr>
<td>9.75-10.25</td>
<td>21, 28</td>
<td>25, 45</td>
</tr>
<tr>
<td>17.75-18.25</td>
<td>-</td>
<td>5/5</td>
</tr>
</tbody>
</table>

Values are medians, with ranges given in parentheses; * The limit of detection was 20 ng per milliliter. Each of the 38 infants donated one blood sample.

Mild pallor at the site of application of the cream was observed in 12 (32 percent) of the neonates in the lidocaine-prilocaine group, as compared with 4 (13 percent) in the placebo group (P=0.08). One neonate in the lidocaine-prilocaine group had mild edema, and one had a local infection that was successfully treated with a topical antibiotic.

Discussion

We found that applying lidocaine-prilocaine cream to the penis reduced the pain of circumcision in neonates, as measured by facial activity, the duration of crying, and heart-rate
changes, confirming the results of a previous small trial (Benini et al., 1993). Although the use of lidocaine-prilocaine cream was associated with an overall decrease in pain, the magnitude of the effect varied during the procedure: it was less effective during phases associated with extensive tissue damage such as lysis of adhesions and tightening of the clamp. The neonates in the lidocaine-prilocaine group still had pain during the circumcision, albeit at an attenuated level. The efficacy of lidocaine-prilocaine cream is affected by the method of application and the dosage. Uneven distribution of cream may cause variations in the tissue concentrations of lidocaine and prilocaine and subtherapeutic concentrations in some regions.

Alternative methods of analgesia for circumcision include dorsal penile nerve block with lidocaine and subcutaneous infiltration of lidocaine in the foreskin. These techniques are more effective than applying lidocaine-prilocaine cream, decreasing the amount of time infants spend crying by up to 70 percent and preventing large increases (up to 60 beats per minute) in heart rate (Masciello, 1990; Holve et al., 1983; Stang et al., 1988). Both techniques, however, are rarely used because they require skills that most physicians have not acquired. Moreover, the injections themselves are considered painful and are associated with the risk of systemic toxicity due to inadvertent injection of the local anesthetic into a blood vessel.

Neonates have immature reductase enzyme pathways that predispose them to methemoglobinemia from oxidizing agents such as metabolites of prilocaine. However, we found no changes in blood methemoglobin concentrations up to 18 hours after the application of lidocaine-prilocaine cream. These results are consistent with those in previous studies of both preterm and full-term neonates that used single doses of 0.5 to 1.25 g of cream applied for 0.5 to 2 hours (Law et al., 1996; Taddio et al., 1996; Gourrier et al., 1995; Enad et al., 1995; Garcia et al., 1995). There have been only two reports of methemoglobinemia in young infants in
association with this treatment, and in both cases the doses of lidocaine-prilocaine cream were high (Nioloux et al., 1995; Jakobson et al., 1985). In our study, the plasma concentrations of lidocaine and prilocaine were considerably below those considered toxic (> 5000 ng per milliliter) (DeJong, 1994). In addition, o-toluidine, the metabolite believed to cause methemoglobinemia, was not detected in any neonate.

Our study was limited to white neonates. In a previous study investigating racial differences in the effectiveness of lidocaine-prilocaine cream, black subjects had less of a reduction in pain than whites, presumably because of the increased density of the stratum corneum (Hymes et al., 1986).

Physicians may be reluctant to use lidocaine-prilocaine cream when techniques (Mogen clamp, Plastibel) other than the one we used are used for circumcision. However, we believe that the use of lidocaine-prilocaine cream is justified given that the extent of tissue damage is similar regardless of which technique is used, that lidocaine-prilocaine cream poses little risk to the infants, and that it blocks afferent nociceptive input for several hours after administration. Other interventions that comfort the infant (e.g., the use of a pacifier and the administration of sucrose) should also be used (Blass et al., 1991; Gunnar et al., 1984). Acetaminophen may be effective for the post-operative control of pain (Howard et al., 1994).

One of the reasons commonly cited for not using analgesia during neonatal circumcision is that the pain experienced by the infants is inconsequential. However, untreated pain from circumcision has been linked to short-term alterations in sleeping, feeding and crying patterns (Marshall et al., 1982; Anders et al., 1974; Emde et al., 1971). In addition, circumcised infants have more pain during routine vaccinations at four to six months of age than uncircumcised
infants (Taddio et al., 1995a; Taddio et al., 1997a). Thus, circumcision may affect infant behavior months after the event.

In conclusion, the application of lidocaine-prilocaine cream decreased pain during circumcision and had no adverse effects. Lidocaine-prilocaine cream offers an alternative to nerve block that is relatively easy to administer. We recommend that its use be considered routinely in neonates undergoing this procedure in order to decrease their pain.

Figure 5. Mean Facial Activity Scores during Circumcision in the Lidocaine-Prilocaine and Placebo Groups.

A higher score indicates that the infant experienced more pain. The asterisks indicate a significant difference (P<0.001) between the groups. P=0.01 for the overall comparison between groups. Bars indicate 95 percent confidence intervals.
Statement of Significance

The above study expands our understanding regarding the efficacy of EMLA during the painful aspects of circumcision, and the safety of this preparation in the neonatal population. Since analgesia is not routinely administered for this procedure, the results of the above study can have significant impact on the current practice. Based on these findings, it is difficult to claim that EMLA is unsafe (i.e., that the toxicity of EMLA precludes its use), or that EMLA does not have beneficial effects when used to manage neonatal circumcision pain.
Chapter 4. Confirmation of Long-Term Sequelae of Pain

The results from Chapters 2 and 3 demonstrated that EMLA decreases neonatal circumcision pain, and that untreated neonatal pain can alter infant pain behaviour during routine vaccination at 4 to 6 months of age. No definite conclusions about the long-term effects of circumcision could be made from the results of Chapter 2, however, due to several limitations. These limitations included: the small sample size investigated, the retrospective nature of the analysis, and lack of control for all potential confounding variables. A larger prospective study of male infant vaccination pain response that controlled potential confounding variables was therefore warranted.

The current study was a prospective study of male infant pain responses during routine vaccination. The study included circumcised male infants who had participated in the study described in Chapter 3 and a group of uncircumcised males, who were undergoing routine vaccination. The objectives of the study were to determine whether circumcision was associated with alterations in infant pain behaviour at routine vaccination and if the administration of EMLA for circumcision pain could influence vaccination pain response. Three different methods were used to assess infant pain; facial activity, cry duration, and visual analogue scale scores.

Hypotheses 5 and 6

The null hypotheses that were investigated in this prospective observational study were that:

The vaccination pain response of circumcised male infants who did not receive analgesia for circumcision pain management would not differ from the vaccination pain response of uncircumcised male infants, and

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The vaccination pain response of circumcised male infants who received EMLA for circumcision pain management would not differ from the vaccination pain response of uncircumcised male infants and circumcised male infants who did not receive analgesia for circumcision pain.

Please note that there are additional notes to this chapter in Appendix 7.
Effect of Neonatal Circumcision on Pain Response during Subsequent Routine Vaccination


Abstract

Background: Preliminary studies suggested that pain experienced by infants in the neonatal period may have long-lasting effects on future infant behaviour. The objectives of this study were to determine whether neonatal circumcision altered pain response at 4-month or 6-month vaccination compared with the response in uncircumcised infants, and whether pretreatment of circumcision pain with lidocaine-prilocaine cream (EMLA) affects the subsequent vaccination response.

Methods: We used a prospective cohort design to study 87 infants. The infants formed three groups - uncircumcised infants, and infants who had been randomized assigned EMLA or placebo in a previous clinical trial to assess the efficacy of EMLA cream as pretreatment for pain in neonatal circumcision. Infants were videotaped during vaccination done at the primary care physician’s clinic. Videotapes were scored without knowledge of circumcision or treatment status by a research assistant who had been trained to measure infant facial action, cry duration and visual analogue scale pain scores.

Findings: Birth characteristics and infant characteristics at the time of vaccination, including age and temperament scores, did not differ significantly among groups. Multivariate ANOVA revealed a significant group effect (p<0.001) in difference (vaccination minus baseline) values for percentage facial action, percentage cry time, and visual analogue scale pain scores. Univariate
ANOVA results were significant for all outcome measures ($p<0.05$): infants circumcised with placebo had higher difference scores than uncircumcised infants for percentage facial action (136.9 vs 77.5%), percentage cry duration (53.8 vs 24.7%), and visual analogue scale pain scores (5.1 vs 3.1 cm). There was a significant linear trend on all outcome measures, showing increasing pain scores from uncircumcised infants, to those circumcised with EMLA, to those circumcised with placebo.

**Interpretation**: Circumcised infants showed a stronger pain response to subsequent routine vaccination than uncircumcised infants. Among the circumcised group, preoperative treatment with EMLA attenuated the pain response to vaccination. We recommend treatment to prevent neonatal circumcision pain.

**Introduction**

Neonatal circumcision is a common surgical procedure in male infants. Despite evidence that circumcision causes intense pain and short-term alterations in infant feeding, sleeping and crying behaviours (Anders et al., 1974; Emde et al., 1971; Marshall et al., 1982), analgesia is rarely given (Wellington et al., 1993; Howard et al., 1996; Toffler et al., 1990). There is a common belief that the effects of circumcision pain are short-lived and clinically insignificant, and, therefore, that the benefits of analgesic treatment do not outweigh the risks of adverse effects from currently available therapies (Schoen, 1990; American Academy of Pediatrics Task Force on Circumcision, 1989).

We looked at the foundations for the belief that the effects of circumcision pain are short-lived by examining infant behaviour several months after surgery. We analysed data from a clinical trial that studied the use of topical lidocaine-prilocaine 5% cream (EMLA, Astra Pharma,
Canada) during routine vaccination at infant 4 or 6 months (Taddio et al., 1994). Male infants showed a greater pain response than female infants. This difference may be linked with neonatal circumcision in male infants. Male infants who had been circumcised also exhibited a greater pain response than those who had not been circumcised (Taddio et al., 1995a). This initial analysis raised concerns about the possible long-term effects of untreated pain in infants, especially those who have repeated experience of pain. However, we could not draw definite conclusions because of the post-hoc nature of the analysis and the small sample size. The objectives of our study were, therefore, to investigate prospectively whether neonatal circumcision affects infant pain response to routine vaccination 4-6 months after surgery and whether vaccination response is affected by pretreatment of neonatal circumcision pain with EMLA.

Methods

We carried out a prospective cohort study of 87 healthy, full-term, male, newborn infants who had, when aged 5 days or less, participated in a clinical trial that investigated the safety and efficacy of EMLA cream for neonatal circumcision (Taddio et al., 1997b). The participants in this study included uncircumcised boys, who served as controls (n=32), and circumcised boys who had been randomly assigned treatment with EMLA (n=29) or placebo (n=26) during circumcision. All parents who had allowed their infants to participate in the circumcision trial were asked to enrol their infants in this study and sign a consent form for their participation. We recruited uncircumcised infants from the same study by the same inclusion criteria as for the circumcised infants, the difference being that their parents had chosen not to have their infants circumcised. The protocol received approval from the Research Ethics Boards of The Hospital for Sick Children and Women’s College Hospital.
The setting for this study was the clinic of the infant's primary care physician, where vaccination was done. Each infant's physician was contacted before the study commenced and informed about its purpose and procedures. One of the investigators telephoned all the parents 2-4 weeks before the anticipated date of the 4-month or 6-month vaccination to obtain details of the appointment date and time. We chose to study pain response during routine vaccination at 4 or 6 months to reduce the effects of fear and anticipation on infant pain response seen in older infants and children, and because vaccination pain responses do not vary greatly within this age range (Taddio et al., 1994).

Parents were sent copies of the revised infant temperament questionnaire for infants aged 4-8 months (Carey et al., 1978), to complete within the 2 weeks before the vaccination appointment. An investigator met one or both parents and their infant at the primary care physician’s clinic on the day of vaccination and the parents returned the completed questionnaire to the investigator at that time.

The vaccination procedure was standardized across settings. The infant was physically examined before the vaccination. If the infant was unsettled by this examination, the parents were asked to him. Immediately before the vaccination the infant was placed supine on the examination table. A physician or nurse then gave the infant an intramuscular injection of the vaccine (0.5mL DPT-Polio & Act-HIB, Connaught Laboratories, Ontario, Canada) in the left or right thigh. An investigator recorded the infants face for a minimum of 20 s with a videocamera (Panasonic, Ontario, Canada, Model #PV-S770A-K), before, during, and for up to 1 min after vaccination. Parents were instructed not to hold the infant for the first 30 s after the injection but were not discouraged from touching or speaking to him during the procedure.


**Pain Assessment**

Infant pain response was scored from the videotape by a research assistant who was unaware of both the purpose of the study and the treatment-group status of the infants. The research assistant was trained to score reliably infant pain reactions using the neonatal facial coding system (Grunau et al., 1987) and cry duration (test-retest Kappa = 0.76, p<0.001).

Three behavioural pain measures were used to assess pain; infant facial action, cry duration, and visual analogue scale scores. Infant facial action was a composite score from three specific facial actions (brow bulge, naso-labial furrow, and eyes squeezed shut) taken from the neonatal facial coding system (Grunau et al., 1987). This system is a sensitive and specific way of rating infant pain (Grunau et al., 1990; Craig et al., 1988b), and is the most extensively used behavioural pain measure in infant pain research (Johnston et al., 1993). The neonatal facial system was chosen as the primary outcome measure because it is considered to be the gold standard for pain assessment. We used these three facial actions because they are particularly sensitive for indicating pain (Stevens et al., 1996a).

The three facial actions and cry duration were coded as present or absent for each 1 s period of the 20 s before the vaccination (baseline), and the first 20 s during and after the vaccination. The data were then converted into percentages of time the infant exhibited the actions or cried (ie, % time = number of times action observed/20 x 100, where 20 was the number of assessments made during the 20 s period).

An overall facial action pain score for the procedure was calculated by adding together the facial action scores for the three specific facial actions. The overall facial action pain score ranged from 0-300%. Percentage cry duration ranged from 0-100%. Visual analogue scale pain scores were rated with a 10 cm pain ruler.
The revised infant temperament questionnaire records the relative frequency with which infants exhibit particular responses to specified situations, such as feeding or bathing. Nine temperament characteristics were derived from the questionnaire. The revised infant temperament questionnaire has favourable psychometric properties; internal consistency and test-retest reliability coefficients are reported to be 0.83, and 0.86, respectively (Carey et al., 1978). As well as being scored for the nine different categories, infants were assigned an overall temperament rating of easy (1), intermediate-low (2), slow to warm up (3), intermediate-high (4), or difficult (5). The numerical scores were used for ease of analysis.

On the day of vaccination, parents were asked questions about their infant’s last feeding and nap times, ingestion of paracetamol for vaccination-fever prophylaxis, and previous painful experiences. Information on birth characteristics and previous vaccinations was obtained from the infant’s medical records. Socio-economic status was scored by the Blishen scale based on maternal occupation (Blishen et al., 1987).

We based the calculation of necessary sample size on the difference in pain scores between circumcised and uncircumcised infants observed in our initial study of vaccination pain response (Taddio et al., 1994), in which the mean visual analogue pain score (unpublished) was 4.6 cm in the circumcised group and 2.7 cm in the uncircumcised group, and the SD was about 2.5 cm. Setting an α of 0.05 and β of 0.2, and to account for possible drop-outs such as parents who refused to let their children participate, those lost to follow-up, or those who could not be included for reasons arising after initial contact, we estimated that about 30 infants per group were needed (Altman, 1991).
**Statistical Analysis**

The main analysis compared difference scores (vaccination score minus baseline value) for percentage facial action, percentage cry duration, and visual analogue scale pain scores among the groups, by multivariate ANOVA. Univariate, one-way ANOVAs were carried out only if the multivariate ANOVA was significant (p<0.05). The pattern of significant differences between pairs of means was examined by post-hoc comparisons by Duncan’s method. Trend analysis was used to establish the significance of the rank order among the groups, with the expectation that the EMLA-treated group would have a pain response intermediate to those of the other groups. Demographic characteristics were compared among groups by ANOVA or Chi square test, as appropriate. Temperament scores were analyzed by multivariate ANOVA. The strength of linear relations between pain measures and infant variables was assessed by the Pearson or Spearman correlation coefficient as appropriate; correction for multiple correlations was made using the Bonferroni method.

**Results**

87 (77%) of the 113 eligible infants participated in the study (Table 12). Three infants in the uncircumcised group were circumcised after initial contact with the investigator. Two of the three infants were circumcised within 5 days of birth and the other at age 20 days. None of these infants received analgesia for circumcision pain and their results were added to the group circumcised with placebo for data analysis.
Table 12. Flow of Participating Infants Through Trial

<table>
<thead>
<tr>
<th></th>
<th>Uncircumcised</th>
<th>Circumcised with EMLA</th>
<th>Circumcised with Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eligible Subjects</td>
<td>45</td>
<td>37</td>
<td>31</td>
</tr>
<tr>
<td>Excluded</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refused to participate</td>
<td>2 (4%)</td>
<td>6 (16%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>4 (9%)</td>
<td>1 (3%)</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Logistic difficulties</td>
<td>4 (9%)</td>
<td>1 (3%)</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>Circumcised after</td>
<td>3 (7%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>initial contact</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Included in Study</td>
<td>32 (71%)</td>
<td>29 (78%)</td>
<td>26 (84%)*</td>
</tr>
</tbody>
</table>

* Includes 3 infants from uncircumcised group who were circumcised after initial contact.

There were no significant differences among the three groups in any demographic characteristics at the time of vaccination (Table 13). Infant temperament was similar in all groups (multivariate ANOVA main group effect; p=0.20); (Table 14), as were birth characteristics (Table 15). 64 clinics took part in the study. Five (6%) infants were held by a parent during vaccination and 76 (87%) were vaccinated with a 25-gauge needle. Eight infants were pretreated with EMLA for circumcision pain openly in the clinical trial from which they were recruited.
Table 13. Demographic Characteristics of Infants at Time of Vaccination

<table>
<thead>
<tr>
<th></th>
<th>Uncircumcised (n=32)</th>
<th>Circumcised with EMLA (n=29)</th>
<th>Circumcised with Placebo (n=26)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infants’ Characteristics:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postnatal Age (days)</td>
<td>133 (12.9)</td>
<td>140 (23.7)</td>
<td>143 (29.4)</td>
<td>0.22</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>7278 (792.5)</td>
<td>7608 (768.4)</td>
<td>7496 (762.9)</td>
<td>0.25</td>
</tr>
<tr>
<td>Time from last feed (min)</td>
<td>111 (82.7)</td>
<td>107 (71.8)</td>
<td>101 (67.7)</td>
<td>0.90</td>
</tr>
<tr>
<td>Time from last nap (min)</td>
<td>117 (73.1)</td>
<td>125 (66.4)</td>
<td>111 (72.4)</td>
<td>0.79</td>
</tr>
<tr>
<td><strong>Vaccination Procedure:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>number of infants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated with paracetamol</td>
<td>4 (13%)</td>
<td>3 (10%)</td>
<td>3 (12%)</td>
<td>0.97</td>
</tr>
<tr>
<td>Vaccinated by physician</td>
<td>24 (75%)</td>
<td>21 (72%)</td>
<td>20 (77%)</td>
<td>0.93</td>
</tr>
<tr>
<td><strong>Maternal Characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD) age (years)</td>
<td>31 (3.5)</td>
<td>31 (4.2)</td>
<td>33 (3.7)</td>
<td>0.07</td>
</tr>
<tr>
<td>Number of primiparas</td>
<td>15 (47%)</td>
<td>16 (55%)</td>
<td>10 (39%)</td>
<td>0.46</td>
</tr>
<tr>
<td>Mean (SD) Blishen * score</td>
<td>54 (17.8)</td>
<td>53 (12.3)</td>
<td>56 (13.4)</td>
<td>0.74</td>
</tr>
</tbody>
</table>

* Blishen method (1987)

a not known for 2 infants circumcised with EMLA and 1 circumcised with placebo.

b not known for 2 infants circumcised with EMLA and 2 circumcised with placebo.
<table>
<thead>
<tr>
<th>Category</th>
<th>Uncircumcised (n=32)</th>
<th>Circumcised with EMLA (n=29)</th>
<th>Circumcised with Placebo (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adaptability</td>
<td>2.3 (0.7)</td>
<td>2.0 (0.6)</td>
<td>2.1 (0.6)</td>
</tr>
<tr>
<td>Activity</td>
<td>4.1 (0.5)</td>
<td>4.2 (0.5)</td>
<td>3.9 (0.6)</td>
</tr>
<tr>
<td>Approachability</td>
<td>2.3 (0.7)</td>
<td>2.1 (0.7)</td>
<td>2.1 (0.8)</td>
</tr>
<tr>
<td>Distractability</td>
<td>2.4 (0.7)</td>
<td>2.1 (0.6)</td>
<td>2.4 (0.6)</td>
</tr>
<tr>
<td>Intensity</td>
<td>3.4 (0.8)</td>
<td>3.3 (0.7)</td>
<td>3.1 (0.7)</td>
</tr>
<tr>
<td>Mood</td>
<td>2.9 (0.8)</td>
<td>2.5 (0.6)</td>
<td>2.8 (0.6)</td>
</tr>
<tr>
<td>Persistence</td>
<td>3.3 (0.7)</td>
<td>3.2 (0.9)</td>
<td>3.5 (0.8)</td>
</tr>
<tr>
<td>Rhythm</td>
<td>2.7 (0.6)</td>
<td>2.8 (0.9)</td>
<td>3.2 (0.9)</td>
</tr>
<tr>
<td>Threshold</td>
<td>3.7 (0.8)</td>
<td>3.8 (0.8)</td>
<td>3.5 (0.9)</td>
</tr>
<tr>
<td>Overall Temperament</td>
<td>2.1 (0.8)</td>
<td>2.3 (1.2)</td>
<td>2.3 (1.1)</td>
</tr>
</tbody>
</table>
Table 15. Birth Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Uncircumcised (n=32)</th>
<th>Circumcised with EMLA (n=29)</th>
<th>Circumcised with Placebo (n=26)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational Age (days)</td>
<td>278 (8.4)</td>
<td>279 (9.6)</td>
<td>277 (9.3)</td>
<td>0.65</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>3645 (428.8)</td>
<td>3636 (426.1)</td>
<td>3530 (443.5)</td>
<td>0.55</td>
</tr>
<tr>
<td>5-min Apgar score</td>
<td>9 (0.3)</td>
<td>9 (0.3)</td>
<td>9 (0.4)</td>
<td>0.24</td>
</tr>
<tr>
<td>Number of infants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>29 (91%)</td>
<td>26 (90%)</td>
<td>23 (89%)</td>
<td>0.96</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>22 (69%)</td>
<td>22 (76%)</td>
<td>18 (69%)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Multivariate ANOVA revealed a significant group effect for difference in pain scores (p<0.001). Univariate ANOVAs (Figure 6) showed significant group effects for percentage facial action (p=0.04), percentage cry (p=0.01), and visual analogue scale pain scores (p=0.02). Post-hoc analysis showed that the group circumcised with placebo had higher difference scores (p<0.05) than the uncircumcised group for percentage facial action (136.9 vs 77.5%), percentage cry duration (53.8 vs 24.7%), and visual analogue scale pain scores (5.1 vs 3.1 cm). In addition, visual analogue scale pain scores were significantly higher in infants circumcised with placebo than in those circumcised with EMLA (5.1 vs 3.3 cm; p<0.05). There was a significant linear trend (p<0.05) in all three outcome measures, with scores increasing from the uncircumcised to the circumcised with placebo group.
The main results were similar when the analysis was repeated by univariate ANCOVAs with vaccination pain score as the outcome and baseline value as the covariate.

Infant factors such as age, weight, temperament, ingestion of paracetamol, time of last feeding and time of last sleep before vaccination, did not correlate significantly with pain response (Table 16).
Table 16. Relation Between Infant Characteristics and Pain Response

<table>
<thead>
<tr>
<th>Correlation Coefficient*</th>
<th>% Facial Action</th>
<th>% Cry Duration</th>
<th>VAS Pain Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postnatal Age (days)</td>
<td>-0.03</td>
<td>-0.11</td>
<td>-0.09</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>-0.12</td>
<td>-0.19</td>
<td>-0.17</td>
</tr>
<tr>
<td>Time from last feed (min)</td>
<td>-0.10</td>
<td>-0.15</td>
<td>-0.06</td>
</tr>
<tr>
<td>Time from last nap (min)</td>
<td>0.09</td>
<td>0.16</td>
<td>0.04</td>
</tr>
<tr>
<td>Treated with paracetamol</td>
<td>-0.10</td>
<td>-0.05</td>
<td>-0.05</td>
</tr>
</tbody>
</table>

Temperament Score

<table>
<thead>
<tr>
<th></th>
<th>% Facial Action</th>
<th>% Cry Duration</th>
<th>VAS Pain Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>-0.07</td>
<td>-0.14</td>
<td>-0.05</td>
</tr>
<tr>
<td>Activity</td>
<td>-0.04</td>
<td>-0.09</td>
<td>0.04</td>
</tr>
<tr>
<td>Adaptability</td>
<td>0.01</td>
<td>-0.07</td>
<td>-0.003</td>
</tr>
<tr>
<td>Approachability</td>
<td>0.03</td>
<td>-0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Distractability</td>
<td>-0.15</td>
<td>-0.09</td>
<td>-0.11</td>
</tr>
<tr>
<td>Intensity</td>
<td>0.02</td>
<td>-0.04</td>
<td>-0.05</td>
</tr>
<tr>
<td>Mood</td>
<td>-0.20</td>
<td>-0.19</td>
<td>-0.13</td>
</tr>
<tr>
<td>Persistence</td>
<td>0.02</td>
<td>0.03</td>
<td>-0.02</td>
</tr>
<tr>
<td>Rhythm</td>
<td>-0.01</td>
<td>-0.07</td>
<td>-0.003</td>
</tr>
<tr>
<td>Threshold</td>
<td>-0.08</td>
<td>0.02</td>
<td>-0.03</td>
</tr>
</tbody>
</table>

VAS = visual analogue scale; * Pearson's or Spearman's; p>0.05 on all variables.
Figure 6. Infant Pain Response to Vaccination for Infants in All Groups

Facial Action Score

Cry Duration

VAS Pain Scores

VAS = visual analogue scale, * p<0.05 for uncircumcised infants vs infants circumcised with placebo, ** p<0.05 for uncircumcised infants vs infants circumcised with placebo and for infants circumcised with EMLA vs infants circumcised with placebo.
Discussion

This study showed that neonatal circumcision in male infants is associated with increased pain response in vaccination 4-6 months after surgery. The results support our previous finding of a higher pain response in circumcised than uncircumcised male infants during routine vaccination (Taddio et al., 1995a).

We postulate that circumcision may induce long-lasting changes in infant pain behaviour because of alterations in the infant’s central neural processing of painful stimuli. Transmission of noxious afferent input from the periphery (eg, brought about by skin incision) to the spinal cord induces a sustained state of central neural sensitization or hyperexcitability that amplifies subsequent input from the wound and leads to increased postoperative pain. The specific mechanisms by which noxious peripheral stimulation induces long lasting central neuronal changes are not yet fully established, but the N-methyl-D-aspartic acid (NMDA) receptor ion-channel complex, excitatory aminoacids (eg, glutamate) and C-fiber neuropeptides (eg, substance P) have been implicated. Peripheral noxious stimulation leads to the release of excitatory aminoacids and neuropeptides in the dorsal horn of the spinal cord. Activation of the NMDA receptor in dorsal horn neurons produces an increase in intracellular calcium and other secondary messengers, which stimulate protein kinases and new gene expression (Coderre et al., 1993; Woolf et al., 1991).

This study was designed to investigate whether premedication with a topical local anaesthetic for circumcision pain would attenuate the pain response to vaccination in circumcised infants. We postulated that EMLA would at least partially block nociceptive afferent input originating from the surgical site at the time of circumcision and, therefore, any long-lasting consequences of this input on the central nervous system. The results of the study do not entirely support this hypothesis. Differences in vaccination pain response between infants pretreated with
EMLA versus no anaesthesia for circumcision pain were seen for visual analogue scale pain scores, but not facial action and cry duration. However, there was a significant trend of EMLA-treated infants to have an intermediate pain response across all three measures of pain (Figure 6). Although primary afferent injury discharge and subsequent noxious perioperative events contributes to enhanced postoperative pain (Katz et al., 1994), other factors, such as postoperative inflammatory inputs, may also induce a state of central sensitization (Woolf et al., 1993). Insufficient afferent blockade during circumcision and in the days that follow surgery may have contributed to central sensitization in both treated and untreated circumcision groups. Study of the vaccination pain response of infants who received more effective circumcision pain management (ie, dorsal penile nerve block and adequate post-operative pain management) would be interesting.

Although vaccination pain response displayed by the infants circumcised without analgesia was higher than the uncircumcised infants, this response may not be specific only to pain. The site of injury during vaccination differed from that during circumcision. In addition, vaccination pain measured by facial action and cry duration did not differ significantly between infants circumcised with or without EMLA. Although postsurgical central sensitization (allodynia and hyperalgesia) can extend to sites of the body distal from the wound (Dahl et al., 1992) suggesting a supraspinal effect, the long-term consequences of surgery done without anaesthesia are likely to include post-traumatic stress as well as pain (Katz, 1993). It is, therefore, possible that the greater vaccination response in the infants circumcised without anaesthesia may represent an infant analogue of a post-traumatic stress disorder triggered by a traumatic and painful event and re-experienced under similar circumstances of pain months during vaccination.
Factors other than circumcision may account for the observed differences in pain response. For example, there may be differences in genetic attributes, socio-economic status, or parent-infant interactions between people who have their sons circumcised and those who do not. However, race and socio-economic status did not differ between groups in this study and there were no observable qualitative differences in the way parents interacted with their infants during the vaccination.

Another possible explanation is that parents of infants who have undergone painful surgical procedures such as circumcision begin to interact differently with their infants compared with parents whose infants have not undergone such procedures. Parents’ patterns of behavioural reinforcement may develop so that by the age of 4 or 6 months, circumcised infants may display a heightened pain response to vaccination. Infant temperament was measured to discern differences among groups due to effects of the infants’ personalities. However, the revised infant temperament questionnaire did not show any differences in infant behaviour among the groups.

To keep potential bias during data collection to a minimum, we standardised the infants’ position before vaccination. Second, we waited for infants to calm down if they were unsettled by the physical examination. Third, each infant was videotaped in his own primary physician’s clinic. Finally, videotapes were coded by a research assistant who was not aware of the status of infants in each treatment group or the purpose of the study.

Several other investigators have studied the long-term effects of untreated pain in newborn infants. Fitzgerald and colleagues (1989) showed that repeated heel lancing may induce a state of hypersensitivity in pain response, and that this atypical response can be prevented by pretreatment with EMLA. Grunau and colleagues (Grunau et al., 1994b; Grunau et al., 1996) found that children born prematurely have a tendency to somatize and interpret pictures of pain-producing
situations differently from other children. Finally, long stays in hospital and repeated medical
procedures in the perinatal period have been proposed as factors affecting long-term cognitive and
motor deficits seen in low-birthweight infants (Als et al., 1994; Pharoah et al., 1994).

The results of this study are consistent with studies of pain response in animals and
behavioural studies in humans showing that injury and tissue damage sustained in infancy can
cause sustained changes in central neural function, which persist after the wound has healed and
influence behavioural responses to painful events months later. Pretreatment and postoperative
management of neonatal circumcision pain is recommended based on these results. Investigation
of the neurological basis of these effects is warranted.

Statement of Significance

The results of previous chapters suggested that untreated neonatal pain has clinically
important long-term effects on the pain behaviours of healthy infants. The above study confirms
these findings in a prospective design that accounted for all known or suspected confounding
variables. The results can be extrapolated to other painful procedures, and provide justification
for analgesic use in infants during painful medical procedures.

It is a logical extension to expect that hospitalized preterm infants who undergo repeated
painful procedures, as described by Barker (1995), and whose pain is not routinely treated, may
be most vulnerable to the long-term effects of untreated procedure pain. Thus, studies aimed at
investigating the effects of analgesics on preterm infants, appear to be warranted.
Chapter 5. Safety of EMLA in Preterm Infants

Since hospitalized preterm infants undergo many painful cutaneous procedures as part of their medical care, EMLA may be a useful pharmacologic intervention for managing pain in this population. There is a concern, however, regarding the potential for EMLA to cause methemoglobinemia in preterm infants. Preterm infants may be predisposed to methemoglobinemia due to immature NADH cytochrome b5 reductase activity and immature skin barrier properties. The relative immaturity of the methemoglobin reductase enzyme system in preterm infants results in a decreased capacity for these infants to handle oxidative stress. Skin immaturity increases the bioavailability of EMLA, increasing the systemic dose of prilocaine.

Before EMLA could be evaluated as an analgesic in this population, its safety would therefore have to be demonstrated. The objective of this study was to evaluate the safety of EMLA in preterm neonates. At the time this research was undertaken, only one previous small study, published in abstract form, had investigated the safety of EMLA in preterm neonates (Ramaioli et al., 1993). Methemoglobin concentrations were observed to be within normal limits. However, follow-up blood samples were obtained only 0.5 hours after EMLA was applied, which is before the time when the methemoglobin concentration is expected to peak (i.e., 8 to 10 hours). Of note, a higher dose of EMLA (1 g vs 0.5 g) and longer duration of application (60 minutes vs 30 minutes) was used in this study compared to the previous one (Ramaioli et al., 1993).

Hypothesis 6

The null hypothesis in this prospective randomized observational study was that there would be no change in methemoglobin concentration following use of EMLA in preterm infants.

Please note that there are additional notes to this chapter in Appendix 8.
Safety of Lidocaine-Prilocaine Cream in Preterm Neonates


Abstract

The safety of lidocaine-prilocaine cream (EMLA) was evaluated in an open trial of 30 preterm neonates (mean gestational age, 32.8 weeks; birthweight, 1911 gm). 0.5 gm was applied to the heel for one hour. Mean baseline and follow-up (4, 8, or 12 hours after EMLA application) methemoglobin levels were not different, ranging from 1.15% to 1.45%, and 1.13% to 1.49%, respectively.

Introduction

Preterm infants are subjected to painful procedures such as heel lancing (heel stick), lumbar puncture, venous or arterial puncture, or intramuscular injection. It has been common practice to administer minimal or no analgesia for these procedures despite accumulating evidence that the pain is clinically significant. Recently, a topical local anesthetic, lidocaine-prilocaine cream (EMLA 5% cream; Astra Pharma Inc.), has become available and has been shown to decrease pain response in infants during procedures such as venipuncture (Robieux et al., 1991) and vaccination (Taddio et al., 1994).

The main reason for lack of research with EMLA in the neonatal population is the concern that prilocaine may cause methemoglobinemia; the proposed mechanism is direct oxidation of hemoglobin by metabolites of o-toluidine, one of the metabolites of prilocaine (Hjelm et al., 1972). Two factors are believed to increase the risk of methemoglobinemia in the neonate: (1)
lower levels of methemoglobin reductase (Nilsson et al., 1990), the enzyme responsible for reducing oxidized methemoglobin, and (2) enhanced percutaneous drug absorption related to immaturity of the stratum corneum layer (Evans et al., 1986), leading to a higher systemic bioavailability. There are no data in preterm neonates on the plasma concentrations of prilocaine and o-toluidine that are achieved after application of EMLA. Moreover, the relative immaturity of the enzyme system has not been well studied for this population.

To our knowledge, only four studies have included preterm neonates. In two of them, the dosage of EMLA was not specified and methemoglobin levels were not obtained (Fitzgerald et al., 1989; McIntosh et al., 1994). The other two studies were published in abstract form and details about methemoglobin measurements are lacking (Garcia et al., 1995; Enad et al., 1995).

The primary objective of this study was to determine the safety of EMLA in preterm neonates by measuring changes in methemoglobin levels after topical application. Other objectives were to measure plasma concentrations of lidocaine, prilocaine, and o-toluidine, and to quantify the activity of methemoglobin reductase in preterm infants' erythrocytes.

Methods

The study received ethical approval from the hospital’s research ethics committee and informed written consent was obtained from all parents. The study had an open design. Neonates in the intensive care unit of a teaching hospital were eligible to participate. The study included neonates <6 days postnatal age and 30-37 weeks gestation at the time of the study, and with either intravascular blood access (e.g., umbilical artery catheter) or requiring repeated blood sampling for medical purposes. We excluded neonates with: (1) major congenital anomalies or neurologic disorders (e.g., seizures), (2) clinically significant cyanosis, (3) congenital or idiopathic
methemoglobinemia (if known), (4) metabolic acidosis, (5) culture proven sepsis, and (6) the need for methemoglobin-inducing agents such as sulfonamides or local anesthetics, either during the study or within the preceding 24 hours.

Each neonate was randomized to receive 0.5 mL of EMLA on the right or left heel. The exact dose was measured using a 1 mL tuberculin syringe. The cream was occluded on the skin using a transparent dressing (Tegaderm). The dressing was removed after 60 minutes and the residual cream was wiped away with gauze that was presoaked in warm water.

All neonates donated two blood samples (arterial or venous). The first blood sample was collected before the administration of EMLA, and was used to determine baseline methemoglobin level and methemoglobin reductase activity. The second follow-up sample was collected at either 4 hours, 8 hours, or 12 hours after application of EMLA, with an acceptable range of +/- 0.5 hours. The group assignment was based on randomization and sample acquisition was coordinated with routine blood studies. If it was not possible to coordinate the follow-up because labwork was not ordered, the sampling group was changed to one where this was possible. The follow-up sample was taken from a different site from the one where EMLA was applied and was used to measure methemoglobin, lidocaine, prilocaine, and o-toluidine concentrations.

After removal of the EMLA cream, local skin reactions (redness, pallor, edema) were recorded as present or absent on a four-point scale: none, mild, moderate, or severe. All neonates were monitored clinically for signs of methemoglobinemia every 4 hours for up to 12 hours after EMLA was applied.
Sample Size Calculations and Statistical Analyses

The main outcome measure was the change between pre- and post-EMLA methemoglobin levels for three groups of paired subjects (i.e., those randomized to 4, 8, or 12 hour post-EMLA methemoglobin determinations). Sample size was calculated assuming a mean methemoglobin level in preterm neonates of 2.3% (standard deviation = 1.26%) (Kravitz et al., 1956), and a standard deviation of the differences between pairs of methemoglobin levels after EMLA of 0.7% (unpublished data). For an increase in the methemoglobin level by 1%, with 95% power with an alpha statistic of 0.05, nine subjects were required for each sampling group. Altogether, 30 subjects (10 per group) were recruited to account for possible dropouts.

Changes in methemoglobin levels were compared within sampling groups using a paired t-test with the Bonferroni correction; the significance level was 0.02. Enzyme activities were compared between adult volunteers and preterm neonates using the student t-test for unpaired data. Demographic data were compared between groups using an ANOVA or Chi square test.

Laboratory Analyses

Methemoglobin levels were analyzed using a co-oximeter (IL CO-Oximeter 482, Instrumentation Laboratory, Lexington Mass.) and reported as a percentage of total hemoglobin. All samples were stored on ice for up to 6 hours before analysis. The 95% confidence intervals reported by the manufacturer is +/- 1%, and the imprecision (1 standard deviation) is 0.5%. Methemoglobin reductase activity was assessed by following reduction of ferricyanide by NADH (nicotinamide adenine dinucleotide, reduced) in erythrocyte hemolysates (Beutler, 1984). Oxidation of NADH was measured at 340nm (Cobas Fara II, Hoffmann-LaRoche, Montclair,
N.J.) and enzyme activity was expressed in international units (IU) of NADH-ferricyanide reductase per gram of hemoglobin at 37° C.

The plasma was separated and stored in the freezer at -20° C. Lidocaine, prilocaine, and o-toluidine were analyzed simultaneously using an high performance liquid chromatography method developed in our laboratory (Klein et al., 1994). For each species, the limit of quantitation was 20 ng/mL and the within-day coefficient of variation varied from 3.1% to 8.3% for replicate analyses.

**Results**

Thirty neonates participated in the study (Table 17). Two subjects in the 4 hour sampling time were identical twins and two subjects in the 12 hour sampling time were fraternal twins. Seventy percent were white.

<table>
<thead>
<tr>
<th>Table 17. Subject Characteristics</th>
</tr>
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<tbody>
<tr>
<td>Sampling Time*</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
</tr>
<tr>
<td>Birth weight (gm)</td>
</tr>
<tr>
<td>Postnatal age (days)</td>
</tr>
<tr>
<td>Gender (male)</td>
</tr>
<tr>
<td>Application to left heel (%)</td>
</tr>
<tr>
<td>Duration of Application (min)</td>
</tr>
</tbody>
</table>

* N=10 per group. Means are shown, with standard deviations in parentheses; no significant differences were found among groups.
No significant increases in methemoglobin levels were observed in any of the groups (Table 18). There were no clinical signs of methemoglobinemia in any subject during the study. Six (20%) of subjects had mild transient skin blanching where the cream had been applied.

Table 18. Methemoglobin Levels (%) after Application of Lidocaine-Prilocaine Cream (EMLA) to Preterm Neonates 30 to 37 Weeks of Gestational Age

<table>
<thead>
<tr>
<th>Sampling time*</th>
<th>Methemoglobin</th>
<th>Follow-up</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 4 hr</td>
<td>1.36 (0.31)</td>
<td>1.40 (0.33)</td>
<td>0.650</td>
</tr>
<tr>
<td>At 8 hr</td>
<td>1.45 (0.21)</td>
<td>1.49 (0.22)</td>
<td>0.481</td>
</tr>
<tr>
<td>At 12 hr</td>
<td>1.15 (0.30)</td>
<td>1.13 (0.38)</td>
<td>0.864</td>
</tr>
</tbody>
</table>

* N=10 per group. Means and standard deviations shown; ** Paired t test

Lidocaine, prilocaine and o-toluidine levels were obtained from 29 subjects (in one subject in the 12-hour sampling group, there was an insufficient quantity of blood to perform the analysis). Lidocaine and prilocaine plasma concentrations were below the detection limit in 18 (62%) subjects. O-toluidine levels were below the detection limit in all cases. Two cases from whom follow-up samples were obtained from heelstick were excluded because of potential contamination from the applied cream. In the 4-hour sampling group, the lidocaine concentrations ranged from 33.76 to 155.2 ng/mL (n=4). For the 8-hour sampling group, the range was 43.1 to 295.0 ng/mL (n=4). The lidocaine level detected in 1 subject in the 12-hour sampling group was 27.23 ng/mL. Prilocaine concentrations varied from a range of 57.2 to 97.7 ng/mL (n=3) for the 4-hour group to 21.1 to 38.3 ng/mL (n=2) for the 8-hour group. No prilocaine was detected for subjects in the 12-hour group.
The mean NADH reductase activity in these preterm infants was 24.7 IU/gm hemoglobin (SD=3.77), compared with 36.2 IU/gm hemoglobin (SD=4.96) in 31 adult control subjects, (p<0.001).

Discussion

We used 0.5 gm of EMLA because it covers a significant portion of the neonatal heel (surface area ≈ 5cm²). The same dose was also previously shown to be efficacious for reduction of neonatal circumcision pain (Benini et al., 1993). The heel was the site of application because it is the most common site of blood collection in neonates. Follow-up sampling times of 4, 8, and 12 hours were used because they represent the entire time interval when EMLA would be expected to increase methemoglobin levels (Nilsson et al., 1990; Engberg et al., 1987; Frayling et al., 1990).

The drug concentrations that were obtained indicate that the dose we used led to a very small systemic exposure of prilocaine (and therefore, of the toxic metabolite). There was, however, a large variability in the concentrations of lidocaine and prilocaine at the 4- and 8-hour sampling times. The small sample size prevented adequate investigation of the effect of gestational age, weight, or severity of illness on drug concentrations. Drug levels vary with dosage, extent of absorption (i.e., application time, skin thickness, surface area, condition of skin, and local circulation), and clearance; any of these factors may have accounted for the observed differences.

These results are in agreement with those of other investigators who used similar or higher doses (up to 1.25 gm for 1 hour) and measured either methemoglobin or drug concentrations or both. None of the studies to date have indicated a significant risk for toxic effects. Finally, our
toxicokinetic study in piglets showed minimal systemic effects from 1 gm of EMLA applied for 1 hour, and no changes in baseline methemoglobin levels up to 8 hours afterwards. In that study, the systemic bioavailability of lidocaine and prilocaine was only 4% and 7%, respectively (Gazarian et al., 1995).

Methemoglobinemia has been reported in neonates after parenteral administration of prilocaine, but not after topical administration of EMLA, and the risk of methemoglobinemia after EMLA may be substantially less. There is a single report of methemoglobinemia in a 12-week old infant who had EMLA applied for 5 hours, in addition to systemic therapy with sulfamethoxazole (another methemoglobin-inducing agent); the methemoglobinemia resolved after therapy with administration of methylene blue (Jakobson et al., 1985). Methemoglobinemia in this infant is thought to have been due to the effects of the combination of prilocaine and sulfamethoxazole, or of either agent alone.

The activity of NADH methemoglobin reductase in this cohort was significantly lower than that in adult volunteers. The results confirm previous reports of reduced enzyme activity in neonates. The relative enzymatic activity of preterm versus full-term neonates has not yet been adequately investigated. Since methemoglobin reductase activity in infants treated with EMLA correlates with maximal methemoglobin levels (Nilsson et al., 1990), knowledge of age-specific activity may be useful in the overall risk assessment of EMLA.

We conclude that a single dose of EMLA did not lead to measurable changes in methemoglobin levels and appears safe to use in this age group. The study is limited by the small sample size in that a low, but clinically significant reaction rate of 2% to 5% may not have been detected. More studies with EMLA in preterm neonates are warranted.
Statement of Significance

The results of this study demonstrated that single therapeutic doses of EMLA are safe to use in preterm neonates. This study has significant implications for the opportunity to treat procedural pain in preterm neonates. As previously mentioned, preterm neonates undergo painful procedures repeatedly in the course of their routine neonatal intensive care. The results of this study support the periodic use of EMLA, but its routine use for all cutaneous procedures cannot be recommended without further investigations of the safety of repeated doses. Investigations of the safety and efficacy of repeated doses of EMLA can now be justifiably undertaken in this patient population. These investigations are required by our concern for the immediate and long-term effects of untreated procedural pain and these investigations are possible because we have demonstrated single-dose safety.
Discussion

Summary of Study Results

This body of research investigated the safety and efficacy of EMLA in young infants undergoing cutaneous procedures that cause pain. In two separate double-blind randomized placebo-controlled trials, EMLA demonstrated efficacy in decreasing pain from routine vaccination and from circumcision. It was also shown to be safe in one open study and one double-blind randomized placebo-controlled trial when administered to newborn infants aged 30 to 42 weeks gestation. In a post-hoc comparison of male infant pain responses during vaccination, circumcised male infants demonstrated greater pain responses than uncircumcised male infants. This finding was confirmed in a prospective study. Moreover, this increased pain response was attenuated in circumcised infants that had been treated with EMLA for circumcision pain.

The working hypothesis of this research was based on our concern for the infant’s experience of pain. As the research developed, we produced evidence justifying our concern for both the immediate effects of pain as well as for longer term effects. We focused our interest on EMLA because it offered a reasonable probability of safety and efficacy. This product, combined with rigorous experimental design, produced evidence that confirmed our original concern for untreated pain in infants.

The research strategy generated evidence that discredits the myths associated with pain in infants. None of the findings can be interpreted as supporting the myth that infants do not feel pain. The measurement of infant pain is both possible and practical. Moreover, drug therapy can be safely administered to infants to alleviate their pain. The results of the studies have improved
both sides of the benefit to risk ratio: the risks are less uncertain and better understood with respect to EMLA, and there are more dimensions to the benefits than we expected. The benefits of pharmacologic intervention are not only immediate but also long-lasting. The failure to prevent pain may have persistent effects on the infant’s nociceptive system. The effects, if any, of untreated pain in infants on the development of chronic pain states or changes to other neural systems, however, is unknown.

**Generalizability of Findings**

Our research strategy consisted of two randomized double-blind controlled trials, one prospective, randomized open label study, one prospective observational cohort study, and a retrospective analysis of a randomized double-blind controlled trial. We used a variety of infant groups, enrolling preterm neonates, full-term neonates, and young infants. The studies took place in outpatient clinics and in hospitals. The outcome measures we employed had both research and clinical utility. Finally, we used a marketed product that is available for use by both parents and clinicians.

We chose our research methods based on thorough review and careful consideration of various approaches to infant pain measurement. Also, our expectations of our intervention were based on sound principles of clinical pharmacology and scientific investigation. Both clinicians and scientists can have confidence in the results of these studies. The results provide clinical information that is readily applicable to patient care. Finally, one of the products of this research strategy has been the generation of additional research questions that will need to be addressed in future studies.
Research Limitations

This next section gives a structured review of the limitations encountered in each study and of the limitations of the research area in general. In each study, the challenge of measuring infant pain objectively was encountered. We used the same definition of pain and the same objective measures of toxicity. We operationalized the definition of pain with slight variation: the pain measures we used most often were behavioural, namely, facial activity and crying. By sharing the same methodology, each study shared the same limitations.

Definition of Infant Pain

This body of research did not address whether infants “feel” pain. Rather, it assumed that they do, and asked whether infant pain could be objectively and reliably measured. The studies determined whether infant pain could be diminished with local analgesia. The behaviours used to measure pain have been previously demonstrated to be good correlates of pain in both adults and infants (Chapman et al., 1985; Johnston, 1989) and were used to infer the presence of infant pain. While the accuracy of these measurement tools can be scrutinized, one must be reminded that accurate measurement of pain in adults also continues to be both a clinical and research challenge.

Pain Assessment Techniques

We chose to use primarily behavioural pain measures. These measures included infant facial activity, cry duration, and visual analogue scale scores. The pain tools had both research and clinical utility. Infant facial activity and cry were used because they are reported to be good measures of infant pain. The visual analogue scale is also considered a valid pain measure, and demonstrated convergent validity with the other measures. Scores obtained for crying duration
and visual analogue scale scores also had clinical utility (meaning). These techniques also are non-invasive, non-disruptive and easily learned.

The pain assessment tools used in this research were shown to be reliable. Reliability was demonstrated by showing that when the same phenomena were measured repeatedly or when two different raters measured the same phenomena, the same scores were obtained (test-retest and interrater reliability). The pain assessment tools utilized were also shown to be valid. Literature searches of infant pain responses revealed that the tools encompassed the items that are considered important to the concept of pain (content validity). Construct validity was demonstrated by revealing that analgesia (i.e., EMLA) reduced infant pain intensity and that infant pain intensity (i.e., scores) increased with increasing tissue damage. Moreover, a multidimensional approach was used to assess efficacy of EMLA and the pain scores obtained from different measures yielded similar results, demonstrating convergent validity.

Behavioural pain assessments were made from videotapes taken of the infants during the various procedures being investigated. Videotaping the infants served several purposes. Firstly, it encouraged standardization of infant position during the procedure. Secondly, it facilitated the use of pain tools that require second-to-second scoring, such as duration of time crying. Thirdly, it enabled research assistants who scored infant pain to be at arm’s length of the study, and therefore blinded to study group. The videotapes provided a permanent record of the procedure and were used to measure inter-rater reliability. Finally, videotaping allowed research assistants to compress the time required for their assessments. This technique promotes reliability by reducing the time between scorings and reducing the impact of any alterations in assessment skills over time. Of note, videotaping the infants was not observed to interfere with either the procedure itself or the infant’s response.
Study Sample and Setting

The results are limited to the infants who participated and the settings where the studies were conducted. In all cases, only healthy and medically stable infants were included. It is therefore not known if the results can be extrapolated to other infant populations and in other settings.

Study Design

For studies of the efficacy of EMLA, double-blind randomized controlled studies were used. Randomization ensured that allocation of treatment was independent of other exposures which may affect outcome. Thus, confounding was ruled out as an explanation of the observed results. In addition, bias was minimized by using a double-blind design. However, it is possible that the observed differences could be simply due to chance. In this case, randomization may have produced two groups that were not comparable. The probability of any observed differences occurring by chance, when in fact, no differences existed between the groups, was calculated. It was accepted that the observed differences were not due to chance imbalance because the probability was 0.05 or less. This value, although arbitrary, is the usual value used to exclude chance imbalance. The evidence against the null hypothesis was further strengthened by other trials that reported similar results.

An observational study design was used to investigate whether untreated neonatal pain had long-lasting effects on infant vaccination pain behaviour. As such, it suffers from the limitations of non-experimental study designs. These limitations include the risk of confounding, bias and chance imbalance. Attempts were made to remove the effects of potential confounders on pain response before assessing the extent of chance imbalance. It is recognized, however, that
it is not possible to be sure that all confounders were measured. Attempts were also made to minimize the effects of bias in data collection due to lack of parental and observed blinding.

Although causality cannot be inferred from this single observational study, careful consideration of the possible alternatives and previous studies suggest that the observed associations may be due to pain. Firstly, the post-hoc analysis of the double-blind randomized trial showed similar results. Secondly, the results are biologically plausible, and fit in with current theories of pain. Previous animal and human studies have also shown that pain can have long-lasting effects on behaviour.

**Research Implications**

These research findings can be used to disprove myths and beliefs about infant pain that have prevented infants from routinely receiving analgesics. Firstly, it was demonstrated that infant pain can be accurately measured, and that most infants experience significant pain during routine medical procedures. Secondly, it was demonstrated that EMLA can be safely administered in this population and that EMLA has measurable desired effects. Finally, it was shown that untreated pain may have long-lasting effects on infant pain behaviour and that pre-emptive analgesia may protect against these changes. These results must be construed as evidence to de-construct the myths that infants do not experience pain (because they do), that we cannot accurately and objectively measure pain in infants (because we can and have), and that the risks of treatment outweigh possible benefits (because they do not).

**Ethics of Pain Management in Infants**

The results of this research raises ethical concerns about current pain management practices in infants. The treatment and alleviation of pain is considered a basic human right that
exists without regard to age. The assessment and treatment of infant pain, however, remains inconsistent. The results of this research support routine management of infant pain.

There are published guidelines currently available which also support the use of analgesics in infants. The Pain Management Guideline Panel of the U.S. Department of Health and Human Services developed clinical practice guidelines for the management of acute pain in infants, children, and adolescents (Pain Management Guideline Panel, 1992). In the guidelines, it is stated that “neonates and infants do experience pain...” and the provision of optimal pain management is stressed. The Committee on the Fetus and Newborn, Committee on Drugs, Section on Anesthesiology and Section on Surgery (1987) of the American Academy of Pediatrics also issued a consensus statement concerning appropriate use of neonatal anaesthesia. In that statement, they emphasized that current data do not support the practice of withholding anaesthesia on the basis of concerns regarding the risk-to-benefit ratio of these medications or the perceived degree of cortical maturity in this population.

The results of this research have contributed to the scientific basis for the treatment of pain in infants. They are also consistent with current guidelines on the management of pain in infants. Nevertheless, it is recognized that treatment decisions regarding pain management in infants will continue to be influenced by myths, biases, and value beliefs of clinicians.

**Future Research**

There are separate avenues of future research that can be followed from the results of this thesis. The first is the development of pain measurement tools specifically designed for different ages of infants. The next avenue, in logical sequence, is the determination of the risk to benefit ratios of commonly used analgesics in this very young age group. Finally, the development of
unique pharmacologic agents and analgesic regimens should be considered given the differences between infants and older children.

The accurate assessment of infant pain continues to be a challenge for researchers. Researchers and clinicians should aim at developing a definition of pain that can be used in infants of different ages. Further research on the most age-appropriate measurement tools is needed. Future investigators will want to aim at reporting on outcome measures that are comparable across studies.

Further studies on the safety and efficacy of analgesic agents are needed to determine the optimal dosing regimens for the prevention of infant pain. It is surprising to learn how little clinical research has been done to support the use of common analgesics in the neonatal population. For example, no studies have been performed to demonstrate the efficacy of acetaminophen although this is the most common analgesic used in adults. To date, no dose-ranging studies have been performed with EMLA for various cutaneous procedures. Thus, the optimal dosage is unknown. Studies of the safety and efficacy of multiple doses of EMLA are also needed. Trials are needed to investigate the pharmacology of other analgesics agents such as opioid analgesics, acetaminophen and NSAIDS in this age group.

Future studies should be performed to investigate the efficacy of combined analgesic regimens for different painful procedures and medical conditions. To date, no studies have investigated the effects of EMLA combined with other pharmacologic interventions. One may postulate that, based on the results of the research presented here, there may be cutaneous procedures where EMLA cannot be expected to provide optimal analgesia as the sole pharmacologic agent and in these cases, a combination of interventions may be more successful. Different combinations of drugs, routes and techniques can be explored. An example where
additional agents may be helpful is in the treatment of neonatal circumcision pain. Additional analgesia may be obtained by utilizing both EMLA and dorsal penile nerve block with lidocaine. Acetaminophen or ibuprofen may be investigated for the management of post-operative pain, which is currently untreated.

Infant neural development has not been well studied. Research can be directed at the neural pathways that mediate pain, and determination of their sensitivity to change from nociceptive input and susceptibility to pharmacologic modulation. There is a biological basis for the “memory” of nociception. Study of the relationship between early infant pain experiences and long-term development of the central nervous system appears warranted. Longitudinal studies of infant pain responses might yield important information regarding the relationship between past pain experiences and pain responses.

In the current fiscal climate, no clinical research is complete without addressing the possible economic implications. The financial implications of analgesic therapy in general, and of acute analgesia effectiveness in specific, requires further investigation. Given that severely ill preterm neonates have measurably better outcomes from developmental care, it is not unreasonable to expect that a smaller but still significant effect will result from better analgesic care. The most obvious outcomes would be length of hospitalization and the overall cost per case. However, the real value added may also be appreciated in terms of infant clinical status and performance.

Finally, this research has immediate policy implications. Further research is needed to determine the rate and extent to which the research findings are incorporated into current infant care practices. Some centres may quickly incorporate this evidence into their pain management policies and require the consideration of analgesia in all cutaneous procedures. Others centres
may be slower to adopt this information. The difference between these groups warrants further investigation.

**Summary of Research Discussion**

The above discussion described where each study contributed to the overall working hypothesis, and provided conclusive insight into the usefulness of EMLA in alleviating the pain of procedures in infants. To complete the discussion of this topic a systematic review of EMLA research was performed. The purpose of this section is to provide a comprehensive discussion of the current status of EMLA research in young infants, and to describe the context in which the above studies have contributed to the literature.
A Systematic Review of EMLA


Anna Taddio, Arne Ohlsson, Thomas R. Einarson, Bonnie Stevens, Gideon Koren

Abstract

Objective: To determine the efficacy and safety of EMLA as an analgesic for procedural pain management in neonates.

Methods: Systematic review techniques were used: studies were identified using manual and computer-aided searches (Medline, EMBASE, Reference Update, personal files, scientific meeting proceedings). Behavioral (e.g., facial action, crying), and physiologic (e.g., heart rate, oxygen saturation, blood pressure, respiratory rate) outcome data from cohort studies and randomized controlled trials in full-term and preterm neonates were accepted for inclusion to establish effectiveness of EMLA. The risk of systemic toxicity (i.e., methemoglobinemia) was estimated from all prospective studies.

Results: Ten prospective studies of the effectiveness of EMLA were retrieved. In 5 studies of pain from circumcision (n=3), percutaneous venous catheter placement (n=1), and venous puncture or arterial puncture (n=1), use of EMLA resulted in reduced pain response for ≥ 1 physiologic and/or behavioral outcome measures. Using meta-analytic techniques, the heart rate outcome data for 2 studies of circumcision pain were summarized. Increases in heart rate compared with baseline values were 12 to 27 beats per minute less for the EMLA group than in the placebo during various stages of the surgical procedure (p<0.05). In 5 studies of pain from heel lancing (n=4) and lumbar puncture (n=1), EMLA did not reduce pain. Meta-analytic
techniques revealed that methemoglobin concentrations did not differ between EMLA-treated and placebo-treated infants (weighted mean difference = -0.11%; 95% CI -0.31 to 0.10%). The incidence of clinically important methemoglobinemia from all prospective studies was 0% (95% CI; 0-0.2%). There were insufficient data to assess the risk with multiple doses of EMLA.

Conclusions: Based on available data, EMLA is recommended for management of pain from circumcision but not heel lance. There are insufficient data to recommend its use for other procedures. Single doses do not cause methemoglobinemia. Additional research is recommended in neonates before EMLA is used routinely for procedures other than circumcision.

Introduction

Hospitalized full-term and preterm neonates routinely undergo tissue-damaging interventions as part of their medical treatment. The skin is the site of noxious stimulation for many procedures, including heel lancing, venipuncture, arterial puncture, lumbar puncture (LP) and percutaneous venous catheter (PVC) placement. These cutaneous procedures are frequently repeated in many patients as necessitated by their clinical conditions. Analgesics are not routinely administered in clinical practice due to the relatively short duration of the intervention, perceived lack of importance of the pain, and concerns of toxicity from currently available agents. This practice is being questioned by recent evidence that neonates are capable of both perceiving and exhibiting reproducible responses to noxious stimulation. The immediate pain response is complex, involving behavioral changes such as facial grimacing and body movements, as well as physiologic, metabolic and hormonal changes. Preliminary data also suggest that pain may have long-term effects in neonates such as pain memories (Taddio et al., 1997a; Taddio et al., 1995a).
EMLA 5% cream (eutectic mixture of local anesthetics; lidocaine and prilocaine, Astra Pharma Inc.) is a topical anesthetic used in children and adults to diminish pain from cutaneous procedures. EMLA represents a therapeutic breakthrough as it is the first topical anesthetic preparation which penetrates intact skin to provide reliable anesthesia. The usual dose for children and adults is 1-2 g applied under an occlusive dressing for approximately 1 hour prior to the procedure.

The efficacy of EMLA for treatment of procedural pain in children and adults is well established. In neonates, however, there has been no systematic evaluation of its effectiveness. There has been no evaluation of the risk of serious adverse effects. In children and adults, adverse effects are limited to transient local skin reactions such as blanching and redness. There is substantial apprehension about using EMLA in neonates due to the potential risk of methemoglobinemia from prilocaine metabolites which can oxidize hemoglobin. As compared to children and adults, neonates are believed to be at increased risk of methemoglobinemia. Neonates have a deficiency in the enzyme which reduces methemoglobin (MetHb), NADH MetHb reductase (Nilsson et al., 1990). In addition, the higher body surface area to weight ratio in infants may result in higher systemic exposure from the same dose relative to adults. Preterm infants may be at even greater risk of toxicity due to immaturity in skin barrier properties (Evans et al., 1986) which enhances percutaneous absorption of drugs.

The purpose of this review was to systematically evaluate the efficacy of EMLA as an analgesic for procedural pain in neonates, to provide evidence based recommendations for clinical practice and to identify areas for future research.
Methods

**Literature search:** MEDLINE was searched for relevant articles published from January 1, 1966 to December 31, 1996; EMBASE from 1993-1996; and Reference Update from January 1, 1995 to December 1, 1996 with the following MeSH terms or text words; “infant-newborn, pain, analgesia, anesthesia, EMLA, lidocaine-prilocaine, local anesthetics”. In addition, manual searches of bibliographies, personal files, scientific meeting proceedings, and recent issues of key journals were performed. Language restrictions were not applied. Attempts were made to obtain additional data from investigators of published studies.

**Inclusion criteria:** Only reports with information on neonates (for this study defined as ≤ 1 month of age) were included. Randomized controlled trials and cohort studies that included a placebo/unexposed group were included for the determination of efficacy. The following procedures were included: circumcision, heel lancing, percutaneous venous catheter insertion, lumbar puncture, and venous/arterial puncture. Since neonates respond to noxious stimuli with behavioral, physiologic, hormonal and metabolic changes, all prospective studies that reported data on any of these variables were included. Experimental pain procedures, such as measurement of pain threshold using von Frey hairs, and case reports were excluded from the analysis. Two investigators (AT, AO) agreed through a consensus process on the inclusion of a specific study. For the determination of safety, all prospective studies were included. Clinically important methemoglobinemia, defined as a MetHb concentration > 5% and requiring medical intervention, was the main focus for adverse effects.

**Data abstraction:** Data abstracted from each report included the procedure studied, study design, gestational age (GA), sample size, dosage regimen, control group treatment, and outcomes. Abstracted data were verified by 2 investigators (AT, AO).
Statistical methods: *A priori*, a decision was made that if there were at least 2 randomized controlled trials that evaluated the efficacy of EMLA for the same procedure and using the same outcome measures, study results would be pooled using a random effects model for weighted mean differences to obtain an overall estimate of effect size. The overall difference in MetHb concentration between groups would be combined using the same method. The risk of methemoglobinemia would be estimated by determining the incidence and 95% confidence interval from the data provided in each prospective report.

Results

_Efficacy Studies_

Twelve studies that assessed the efficacy of EMLA in reducing pain in a total of 602 neonates were retrieved (Fitzgerald et al., 1989; Rubio et al., 1996; Benini et al., 1993; Taddio et al., 1997b; Lander et al., 1996; Ramaioli et al., 1993; Larsson et al., 1995; Stevens et al., 1996b; McIntosh et al., 1994; Enad et al., 1995; Garcia et al., 1995; Gourrier et al., 1995). Two studies were excluded: one because an experimental procedure was used to measure pain threshold (Fitzgerald et al., 1989), and another because it did not include a control/no treatment group (Rubio et al., 1996). Thus, 10 studies were used for the analysis. The characteristics of included studies are summarized in Table 19. The pain response of infants undergoing the following cutaneous procedures was investigated: circumcision, heel lancing, LP, PVC placement, venous puncture and arterial puncture. Eight of the studies were randomized controlled trials with sample sizes ranging from 13 to 116 subjects. GA at the time of delivery was provided in 7 reports, and ranged from 26-42 weeks. Two studies which included data from both neonates and older infants were included (McIntosh et al., 1994; Gourrier et al., 1995). The dose of EMLA
used was 0.5-2 g in 9 studies, and was not specified in one study. The duration of application varied from 10 minutes to 2 hours. Outcome measures included behavioral (facial action, body action, cry) and physiologic [heart rate, respiratory rate (RR), blood pressure (BP) and oxygen (O₂) saturation] parameters. Data from individual studies could not be combined using meta-analytic techniques due to a wide variability in procedures, dosage regimens, outcome measures, and reporting of results. There was only one exception: two studies of circumcision pain (Benini et al., 1993; Taddio et al., 1997b) (see below) that used similar outcome measures were combined. Due to the diversity among studies, however, the results were reported according to procedure investigated.
### Table 19. Efficacy Studies of EMLA for Procedural Pain in Neonates

<table>
<thead>
<tr>
<th>Reference</th>
<th>Procedure</th>
<th>Study Design</th>
<th>Sample Size</th>
<th>Dosage Regimen</th>
<th>Gestational Age (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benini (1993)</td>
<td>circumcision</td>
<td>p,r,pc</td>
<td>27</td>
<td>0.5g for 45-65min</td>
<td>37-42</td>
</tr>
<tr>
<td>Taddio (1997b)</td>
<td>circumcision</td>
<td>p,r,pc</td>
<td>59</td>
<td>1g for 60-80min</td>
<td>37-42</td>
</tr>
<tr>
<td>Lander (1996)</td>
<td>circumcision†</td>
<td>p,r,c</td>
<td>52</td>
<td>2g for 90min</td>
<td>full-term</td>
</tr>
<tr>
<td>Ramaioli (1993)</td>
<td>heel lancing†</td>
<td>p,r,pc</td>
<td>20</td>
<td>1cm (=0.5g) for 30min</td>
<td>29-36</td>
</tr>
<tr>
<td>Larsson (1995)</td>
<td>heel lancing</td>
<td>p,r,pc</td>
<td>110</td>
<td>0.5g for 10-120min</td>
<td>full-term</td>
</tr>
<tr>
<td>Stevens (1996b)</td>
<td>heel lancing†</td>
<td>p,r,pc</td>
<td>60</td>
<td>0.5g for 30min</td>
<td>30-36</td>
</tr>
<tr>
<td>McIntosh (1994)</td>
<td>heel lancing</td>
<td>p,c</td>
<td>35</td>
<td>nr‡ for 60min</td>
<td>26-34</td>
</tr>
<tr>
<td>Enad (1995)</td>
<td>lumbar puncture†</td>
<td>p,r,pc</td>
<td>49</td>
<td>1g for 60min</td>
<td>≥34</td>
</tr>
<tr>
<td>Garcia (1995)</td>
<td>percutaneous venous catheter placement†</td>
<td>p,r,pc</td>
<td>13</td>
<td>1.25g for 60min</td>
<td>very low birthweight</td>
</tr>
<tr>
<td>Gourrier (1995)</td>
<td>venous/arterial puncture</td>
<td>p,c</td>
<td>116</td>
<td>≥2kg: 1/4 tube (≈ 0.5g) &lt;2kg: nr‡ for 60-180min</td>
<td>26-41</td>
</tr>
</tbody>
</table>

* p=prospective, r=random, pc=placebo-controlled, c=controlled (no treatment group); 
† published in abstract form only; ‡ nr=not reported.
1. Circumcision

The efficacy of EMLA for the treatment of circumcision pain was investigated in three studies which included a total of 138 neonates (Table 19). Benini et al (1993) administered 0.5g of EMLA or petrolatum jelly placebo on the outside of the prepuce for 45-65 minutes prior to circumcision. For all outcome measures [HR, transcutaneous O₂ saturation, cry duration, facial action (scored using NFCS (Grunau et al., 1987))], EMLA was associated with a significantly (p<0.05) reduced response compared to placebo during the painful phases of the procedure (e.g., clamping, incision of foreskin, lysis, and application of Gomco clamp). The average HR for the EMLA group compared with the control group was 25 beats per minute less and the average O₂ saturation was 5% higher. Twenty percent less facial activity and 15% less crying was also observed in the EMLA-treated infants. Cry features such as maximum fundamental frequency, peak spectral energy, and dysphonation, however, were not significantly different between groups.

Taddio et al (1997b) randomly assigned neonates to 1 g of EMLA or a cosmetically identical placebo cream for 60-80 minutes prior to circumcision. Infants pre-treated with EMLA had lower (p<0.05) facial action pain scores (assessed using the NFCS (Grunau et al., 1987)), percent crying time, and HR during surgery as compared to placebo. Facial activity scores were 12 to 49% lower during various stages of the procedure. The average difference in percentage crying and HR between groups compared to baseline values, was 55% and 10 beats per minute, respectively. Blood pressure was lower in the EMLA group comparison with controls, but the difference did not reach significance (p>0.05).

Lander et al (1996) studied the efficacy of 2 g of EMLA applied for 90 minutes prior to circumcision. Infants were randomized to four groups: no treatment, EMLA, dorsal penile nerve
block, or penile ring block. Although the EMLA group had a lower mean HR during foreskin retraction than did the no treatment group (169 vs. 200 beats per minute), HR values were even lower for the two block groups (151 beats per minute). Investigators did not report the SD and p-values among treatment groups. All three intervention groups cried significantly less than the no treatment group (data and p-values were not provided).

Meta-analytic techniques could be used to summarize the heart rate outcome data for two studies of circumcision pain (Benini et al., 1993; Taddio et al., 1997b). The mean increase in HR (i.e., compared with baseline values) was 12 to 27 beats per minute less for the EMLA group compared to placebo during various stages of the surgical procedure (p<0.05) (Table 20).

<table>
<thead>
<tr>
<th>Stage of procedure</th>
<th>Weighted mean difference in heart rate * (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forceps application</td>
<td>-12.27 (-38.63, 14.09)</td>
</tr>
<tr>
<td>Lysis of adhesions</td>
<td>-12.42 (-20.34, -4.49)</td>
</tr>
<tr>
<td>Dorsal incision</td>
<td>-26.92 (-37.78, -16.07)</td>
</tr>
<tr>
<td>Application of clamp</td>
<td>-27.21 (-35.98, -18.45)</td>
</tr>
<tr>
<td>Foreskin cutting</td>
<td>-12.05 (-20.84, -3.26)</td>
</tr>
<tr>
<td>Removal of clamp</td>
<td>-11.67 (-19.93, -3.42)</td>
</tr>
</tbody>
</table>


* The mean increase in heart rate (+/-SD) from baseline was calculated for each treatment group. Then the weighted mean difference in heart rate between EMLA and placebo groups with 95% confidence intervals (CI) was calculated for each of the stages of the circumcision using the
statistical program included in Revman 3.0. A CI that does not include the value 0 indicates a significant finding at the < 0.05 level.

2. **Heel lancing**

   Pain from heel lancing was investigated in 4 studies involving a total of 225 neonates (Table 19). Ramaioli and colleagues (1993) randomly assigned preterm neonates to 1 cm (=0.5 g) of EMLA or placebo (glycerine) to the heel for 30 minutes prior to heel lancing. Pain was assessed using changes in HR, BP, RR and behavior (on the Prechtl scale). Five serial assessments were made for each subject. These assessments were made 3 minutes prior to the heel lance; at the start of sampling, at the end of sampling, and at 3 minutes and 8 minutes after sampling. Investigators did not report any statistically significant differences between groups for any of the outcome measures. Within the placebo group, systolic BP was higher at the end of sampling when compared to 3 and 8 minutes after sampling (p=0.01).

   Larsson et al (1995) used a randomized double-blind design and allocated 112 three-day old full-term neonates to 8 different application time groups (10, 20, 30, 40, 50, 60, 90, 120 minutes) following 0.5g of EMLA or a cosmetically identical placebo cream. The primary outcome measure was the occurrence of crying during the procedure. Each randomization group included 7 infants treated with EMLA and 7 infants treated with placebo. Fifty-four of the 56 neonates (96%) that received EMLA cried compared to 52 out of 54 neonates (96%) that received placebo, which was not significantly different.

   In a recent study (Stevens et al., 1996b) involving 60 preterm neonates 30-36 weeks GA, neonates randomly received 0.5g of EMLA or Glaxal® base placebo for 30 minutes prior to heel lancing. Pain was assessed by a blinded observer from a videotape and computerized physiologic data using the Premature Infant Pain Profile (PIPP) (Stevens et al., 1996a). The PIPP score is
derived by summing the pain scores obtained from 7 indicators: brow bulge, eye squeezed shut, nasolabial furrow, O2 saturation, HR, GA, and infant behavioral state. No statistically significant differences between groups were reported; the mean (SD) PIPP score was 10.2 (4.1) in the EMLA group and 9.5 (4.0) in the placebo group, (p=0.48).

McIntosh et al (1994) used a prospective, non-randomized, non-blinded study design to evaluate the effect of EMLA in heel lancing pain. EMLA was administered to 21 preterm neonates (7-35 days old) for 1 hour prior to heel lancing. The dose of EMLA, however, was not specified. A “dummy” period preceded the administration of EMLA in all cases which mimicked the procedure in all aspects except that the heel was not pricked. Neonatal response to the real heel lancing with EMLA was then compared to the “dummy” period. The outcome measures included HR, RR, trancutaneous O2 tension, and CO2 tension. Pre-treatment with EMLA was associated with a significant increase in HR (mean difference, 8; CI, 2-14), HR variability (mean difference, 9; CI, 4-12), and trancutaneous O2 tension variability (mean difference, 0.3; CI, 0.1-0.6). There also was a trend towards an increase in CO2 tension variability (p=0.053). Other interventions which were tested in the same trial included use of a spring-loaded heel pricking device and nursing comfort measures (stroking and vocal reassurance during the procedure). Unlike EMLA, both of these non-pharmacologic interventions did not significantly alter physiologic changes during heel lancing when compared with the dummy period. This study suggests that EMLA did not diminish the pain from heel lancing. Taken together, none of the studies evaluating EMLA for heel lancing pain showed a significant benefit from the drug on infant pain.
3. **Lumbar puncture**

The efficacy of EMLA in alleviating the pain from LP was investigated in one study (Table 19). Enad et al (1995) randomly applied 1g of EMLA or placebo for 1 hour prior to LP. Physiologic parameters (BP, HR, O2 saturation) and behavioral response (scored from 0-3) were assessed by a blinded observer prior to, during and 5 minutes after LP. Percent change from baseline values during and after LP did not differ between groups for physiologic parameters (p>0.09) and behavioral scores (p>0.25). The results suggest that EMLA is ineffective for the management of pain from LP. Of note, the nature of the behavioral pain measure and the observed values were not provided in the report.

4. **Percutaneous venous catheter placement**

EMLA was tested for decreasing the pain from PVC placement in one study (Table 19). Garcia et al (1995) randomly assigned very low birthweight infants to 1.25g of EMLA or zinc oxide placebo for 1 hour prior to PVC placement. Pain response was measured by a blinded observer using serial HR, RR, BP and O2 saturation measurements obtained prior to and 3, 5, and 60 minutes after skin puncture. HR was significantly lower for the EMLA-treated neonates compared with controls at all times during the procedure (p<0.05). RR response was attenuated in the EMLA group during skin puncture only. BP and O2 saturation were not significantly altered in either group during the procedure. EMLA was therefore shown to attenuate HR and RR increases during PVC placement but not BP and O2 saturation. Investigators did not provide values for HR, RR, BP and O2 saturation values for the two treatment groups.

5. **Venous and arterial puncture**

Gourrier et al (1995) used a cohort design to evaluate the effectiveness of EMLA for venous and arterial puncture in preterm neonates aged 1-64 days (Table 19). Neonates ≥ 2kg
received one-quarter of a tube of EMLA (equivalent to \( \approx 0.5 \) g); neonates < 2 kg received less, although the exact dose was not specified. EMLA was applied for 1-3 hours prior to the procedure. Pain was assessed using a behavioral pain scale developed by the investigators. The variables on the scale were infant arousal, expression, and agitation; the total score ranges from 0-5. Pain scores were graded by a blinded observer and compared between instances when EMLA was used and when it was not. Altogether, 116 infants who received 157 skin punctures were included. EMLA was utilized for 120 punctures and no intervention was administered for the remaining 37 punctures. Although it may have been preferable to use a rank test to analyze data, investigators divided pain scores into two categories for analysis according to severity: mild (0-2), and severe (3-5). Pre-treatment with EMLA was associated with a higher frequency of low pain scores (57%) than the controls (18%), \( p<0.001 \). Application times of greater than 90 minutes were more efficacious than shorter duration times \( (p<0.001) \). The frequency of low pain scores when venous stabs were administered was 79%, compared with 41% with arterial stabs, suggesting that EMLA was more successful for venous stabs than arterial stabs.

**Safety Studies**

MetHb concentrations were measured in 11 studies (Rubio et al., 1996; Taddio et al., 1997b; Lander et al., 1996; Ramaioli et al., 1993; Enad et al., 1995; Garcia et al., 1995; Ramaioli et al., 1991; Taddio et al., 1995c; Taddio et al., 1996; Gourrier et al., 1996c; Law et al., 1996). The characteristics of each study including sample size, GA of subjects, dose of EMLA, duration of exposure, timing of samples, and MetHb concentrations is provided in Table 21. MetHb concentrations were compared in infants before and after exposure to EMLA, or between infants exposed to EMLA and placebo or no treatment with no statistically significant differences in 7
studies (Taddio et al., 1997b; Ramaioli et al., 1993; Enad et al., 1995; Garcia et al., 1995; Ramaioli et al., 1991; Taddio et al., 1995c; Taddio et al., 1996). Meta-analytic techniques could be used to combine the data from 4 studies (Taddio et al., 1997b; Taddio et al., 1996; Ramaioli et al., 1993; Ramaioli et al., 1991). The results revealed that mean MetHb concentrations did not differ between EMLA-treated and placebo-treated infants (weighted mean difference = -0.11%; 95% CI -0.31 to 0.10%).
Table 21. Safety Studies with EMLA in Neonates

<table>
<thead>
<tr>
<th>Reference</th>
<th>Procedure</th>
<th>Gestational age (weeks)</th>
<th>Number exposed</th>
<th>Dosage Regimen</th>
<th>Sampling Time (h)</th>
<th>% MetHb* (mean, SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Law (1996)</td>
<td>circumcision</td>
<td>full-term</td>
<td>10</td>
<td>1.0g for 60min</td>
<td>8</td>
<td>0.44 (0.53)</td>
</tr>
<tr>
<td>Taddio (1997b)</td>
<td>circumcision</td>
<td>37-42</td>
<td>38</td>
<td>1.0g for 60min</td>
<td>1-18</td>
<td>1.3 (0.6)</td>
</tr>
<tr>
<td>Lander (1996)</td>
<td>circumcision†</td>
<td>full-term</td>
<td>13</td>
<td>2.0g for 90min</td>
<td>nr‡</td>
<td>(range. 0-4.5)</td>
</tr>
<tr>
<td>Ramaioli (1993)</td>
<td>heel lancing†</td>
<td>29-36</td>
<td>10</td>
<td>0.5g (=1cm) for 30min</td>
<td>0.5</td>
<td>0.6 (0.2)</td>
</tr>
<tr>
<td>Ramaioli (1991)</td>
<td>heel lancing†</td>
<td>full-term</td>
<td>15</td>
<td>1mL (=1g) for 30min</td>
<td>0.5</td>
<td>0.85 (0.2)</td>
</tr>
<tr>
<td>Taddio (1996)</td>
<td>heel lancing†</td>
<td>30-37</td>
<td>26</td>
<td>0.5g for 30min</td>
<td>8</td>
<td>1.3 (0.5)</td>
</tr>
<tr>
<td>Taddio (1995c)</td>
<td>heel lancing</td>
<td>30-37</td>
<td>30</td>
<td>0.5g for 60min</td>
<td>4-12</td>
<td>1.3 (0.3)</td>
</tr>
<tr>
<td>Enad (1995)</td>
<td>lumbar puncture†</td>
<td>≥34</td>
<td>nr</td>
<td>1.0g for 60min</td>
<td>4</td>
<td>0.85 (0.84)</td>
</tr>
<tr>
<td>Garcia (1995)</td>
<td>percutaneous venous catheter placement†</td>
<td>very low birthweight</td>
<td>7</td>
<td>1.25g for 60min</td>
<td>nr‡</td>
<td>(range. 0.3-2.0)</td>
</tr>
<tr>
<td>Rubio (1996)</td>
<td>needle insertion†</td>
<td>29 +/- 2.5</td>
<td>48</td>
<td>0.5-1g for 30-40min</td>
<td>8</td>
<td>0.68 (0.55)</td>
</tr>
<tr>
<td>Gourrier (1996a, 1996c)</td>
<td>‡nr</td>
<td>26-41</td>
<td>158</td>
<td>≥2kg: 1/4 tube (=0.5g)</td>
<td>nr‡</td>
<td>(range. 0.4-6.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;2kg: nr‡ for 60-180min</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† published in abstract form only. ‡ nr = not reported.

* MetHb levels were reported as a percentage of hemoglobin in all studies. The mean (SD) or range, is reported.
The lack of clinically important methemoglobinemia following administration of repeated doses of EMLA was reported in 2 studies. Over a two year study period, Gourrier and colleagues (1996a, 1996c) administered a mean of 3.2 doses of EMLA to 500 infants (GA 26-41 weeks, postnatal age ≤ 3 months). One hundred and fifty-eight follow-up MetHb concentrations were obtained. In 119 cases, the sample was obtained anytime after a delay of 24 hours from the first dose, and in the remaining 39 cases, the sample was obtained 2 hours after the first dose of EMLA. The maximum daily dose of EMLA was reported to be 1 application of 0.5 g for 1.5 to 3 hours in full-term newborns and a smaller quantity (unspecified) in preterm infants. Earlier reports by the same investigators revealed indicate that one-quarter of a tube of EMLA cream (equivalent to 1.25 g) was used per dose in full-term infants (Gourrier et al., 1995). Clarification of the dose with investigators revealed that 0.5 g is closer to the actual dose used. The MetHb concentrations were ≤ 5% for 97.5% of cases. Concentrations were > 5% on 3 occasions; the maximum observed concentration was 6.2%. No clinically important cases of methemoglobinemia were observed, that is, none required medical intervention. Elevated MetHb concentrations were believed to have been due to the influence of repeated administration of EMLA, although the interval between doses was not provided, and/or the influence of anemia as MetHb concentrations were expressed as a percentage of hemoglobin. In the cases where anemia was present, MetHb concentrations decreased after administration of blood transfusions.

Fitzgerald et al (1989) studied 7 neonates 27-32 weeks post-menstrual age. An unspecified 'small amount' of cream was rubbed onto the heel (without an occlusive dressing) four-hourly for a period of 1 to 4 weeks. Although the dose used was not specified, the tube of cream (5 g) was reported to last two weeks. Thus, a daily dose of approximately 0.36 g was
used, or 0.06 g per dose. No clinical observations of methemoglobinemia or other adverse effects were reported, although MetHb concentrations were not measured.

The ratio of cases of clinically important methemoglobinemia (MetHb > 5% and clinical signs requiring treatment with methylene blue) to total number of exposures from all published reports (including multiple exposures) was computed to calculate the overall incidence of clinically important methemoglobinemia from EMLA. For the study by Fitzgerald (1989), each neonate was included only once even though repeated applications of EMLA were administered since the number of applications was not specified. In the study by Enad (1995), the number of subjects treated with EMLA was not provided and it was assumed that 50% were treated with EMLA. The incidence of clinically significant methemoglobinemia from all exposures to EMLA, whether single dose or multiple dose, was 0% (95% CI; 0-0.2%). If the analysis was repeated including only those cases where MetHb concentrations were measured and found to be > 5% and clinical signs of methemoglobinemia were present, then the incidence was still 0% (95% CI; 0-1.00%).

The analysis was repeated using MetHb concentration > 5% to define methemoglobinemia. The calculated overall incidence was 0.79% (95% CI; 0.27-2.30%) for all neonates. The risk was 0% (95% CI; 0-3.21%) for full-term neonates and 1.14% (95% CI; 0.39-3.29%) for preterm neonates. There were insufficient data to calculate the risk of methemoglobinemia following repeated administration.

Two case reports of methemoglobinemia following application of EMLA in neonates were retrieved. In the first case, a 34 week GA, 1385 g, five-day-old neonate with sepsis had been treated with two simultaneous applications of EMLA, one for central line placement and one for lumbar puncture (Nioloux et al., 1995). The total application time was three hours. The amount
of cream applied was not provided in report. The observed MetHb concentration was 12.6%.
Methemoglobinemia was reversed with methylene blue and no long-term sequelae were reported.
In the second case, a full-term, two-day-old neonate received 3.5 g of EMLA for 60 minutes on
the outside of the prepuce prior to circumcision (Kumar et al., 1997). The infant was treated with
100% oxygen until the following day. A follow-up MetHb concentration was < 2.1%.

The incidence of minor skin reactions following EMLA was reported in five studies.
Taddio et al (1995c; 1997b) reported blanching in 20% (6/30) of neonates who received EMLA
on the heel, and 30% of those who received it on the penis and abdomen. Larsson et al (1995)
observed blanching and redness on the heels in 70% and 5% of infants, respectively. Ramaioli et
al (1991) reported no local adverse effects in 15 full-term neonates who also received EMLA on
the heel. Gourrier et al (1995) encountered erythema in 3% (3/116) of neonates due to the
occlusive (Tegaderm®) dressing. After two years of clinical use of EMLA and approximately
1500 applications, Gourrier et al (1996b, 1996c) reported the occurrence of purpuric lesions on
the site of application in five instances. Four neonates < 32 weeks gestation and less than three
days postnatal age experienced five episodes of rash (one neonate had a second reaction when
exposed to EMLA at a different skin site) after receiving doses of 1/8-1/6th of a 5 g tube of
EMLA for 90 to 120 minutes. In all cases, the rash resolved without sequelae. Rechallenge some
weeks later on two infants revealed no complications.

Four groups measured concentrations of local anesthetics in neonatal blood. Taddio et al
(1995c) measured lidocaine, prilocaine and o-toluidine (the toxic metabolite believed to lead to
methemoglobinemia) concentrations at 4, 8, or 12 hours after the dose in preterm neonates. In all
cases, the observed concentrations were <0.3 mcg/mL, <0.1 mcg/mL and <0.02 mcg/mL for
lidocaine, prilocaine, and o-toluidine, respectively. Of note, the limit of detection was 0.02
mcg/mL for all drugs. Enad et al. (1995) measured lidocaine concentrations 4 hours following administration of EMLA in neonates ≥ 34 weeks GA. The mean concentration was 0.07 mcg/mL (range, 0.0-0.1 mcg/mL).

In full-term neonates, Taddio et al (1997b) measured lidocaine, prilocaine, and o-toluidine concentrations 1 to 18 hours after administration of EMLA to the penis for circumcision. The highest observed concentrations of lidocaine and prilocaine were 0.14 and 0.11 mcg/mL, respectively. O-toluidine concentrations were below the limit of detection (<0.02 mcg/mL) in all cases. Ramaioli et al (1991) measured lidocaine and prilocaine 0.5 hours following EMLA application on the heel. In all cases, concentrations were below the limit of detection (<0.04 mcg/mL).

Discussion

There are currently few therapeutic classes of drugs available for the management of acute procedural pain in neonates. The severity of potentially adverse effects from opioid analgesics have discouraged clinicians from using them in neonates, and until recently, no commercially available local anesthetic preparation has been available that was suitable for use on intact skin. EMLA cream is considered a breakthrough in topical analgesia. This systematic review shows that EMLA’s efficacy may be related to the type of cutaneous procedure. Several studies have demonstrated that EMLA diminishes pain response during circumcision, and single studies have demonstrated some efficacy for PVC placement, venous puncture and arterial puncture. EMLA has not been shown to diminish pain from heel lancing and LP.

The observed inconsistency in EMLA’s efficacy may be due to study design issues including the sample size, procedure site, dosage regimen, outcome measures, and co-
interventions. Studies in adults have revealed that the onset and duration of action of EMLA is related to the skin thickness at the site of application and local blood flow. Characteristics of the stratum corneum, epidermis, dermis and local blood flow determine both the rate and the extent of absorption into tissues and systemic circulation. The length of analgesia depends on redistribution of the local anesthetic into the systemic circulation, and appears to be shortest for mucous membranes, the face, and diseased skin (Nielsen et al., 1992; Arendt-Nielsen et al., 1990). The analgesic effect of EMLA also varies with duration of application and duration between time of cream removal and the initiation of the procedure. For the dorsum of the forearm, the sensory and pain thresholds have been found to increase linearly for increased application times (from 30 to 120 minutes). Thresholds are significantly increased for up to 240 minutes following cream removal (Bjerring et al., 1990). There are currently insufficient data in neonates to compare with adult data, but it appears likely that differences in either procedure site or dosage regimen can significantly impact on the time-efficacy response of EMLA.

The lack of clinical efficacy of EMLA in heel lancing pain for preterm and full-term infants may be due to differences in skin and blood perfusion in the heel compared to other cutaneous sites. In a study comparing skin thickness and skin blood perfusion in full-term infants, Larsson et al (1996) found that skin perfusion was significantly enhanced in the heel compared with the other cutaneous regions (forehead, dorsum of hand). Although investigators did not investigate qualitative differences in the skin of the different regions, they speculated that the lack of efficacy of EMLA is due to rapid clearance from the site of action.

Another factor that may influence the observed effectiveness of EMLA is the outcome measure used to assess pain. Although there is no consensus regarding the most suitable way to measure neonatal pain, there are many ‘accepted’ methods. Validated behavioral pain scales such
as the Neonatal Facial Coding System (NFCS) (Grunau et al., 1987) and cry duration have been used. In addition to behavioral approaches, physiologic indicators such as HR, BP and RR, and biochemical markers such as stress hormone concentrations have also been used. Finally, composite measures are also currently available. Some composite measures are the Premature Infant Pain Profile (PIPP) (Stevens et al., 1996a), Barrier and Attia (1989) post-operative clinical scoring system, Pain Assessment Tool (PAT) (Hodgkinson et al., 1994), Neonatal Infant Pain Scale (NIPS) (Lawrence et al., 1993) and the Crying, Requires oxygen, Increased vital signs, Expression and Sleepless tool (CRIES) (Krechel et al., 1995).

With the abundance of choice in outcome measures, it is no wonder that investigators who have evaluated the efficacy of EMLA in neonates have utilized very diverse pain indicators in their studies. These differences prevent direct comparisons between studies and the use of meta-analytic techniques. Moreover, the sensitivity and specificity of these measures as indicators of neonatal pain are not clear. Neonatal ‘pain response’ varies with GA (Johnston et al., 1993), infant state (Grunau et al., 1987; Stevens et al., 1994b), severity of illness (Stevens et al., 1994b), and use of concomitant medications such as opioid analgesics or neuromuscular blockers. Preterm neonates may also respond to non-painful stimuli in a similar way as they do to painful stimuli (Craig et al., 1993) due to a limited repertoire of responses or conditioning. The use of developmentally insensitive pain indicators such as presence or absence of infant crying (Larsson et al., 1995) may have obscured differences between groups.
Co-interventions may have also contributed to the variability in the results. For example, heel warming prior to heel lancing may have increased blood flow to the region and increased uptake of EMLA into the bloodstream. Details regarding co-interventions such as heel warming were not consistently described by investigators. In addition, information about other comfort measures that were used were not described.

This systematic review demonstrated that the risk of methemoglobinemia is low following single dose application of EMLA. In full-term neonates, single doses ranging from 0.5 to 2 g applied for 10 to 180 minutes have not been reported to cause methemoglobinemia. In preterm neonates, single doses ranging from 0.5 to 1.25 g applied for 30 to 180 minutes have not been reported to cause methemoglobinemia. Concentrations of lidocaine and prilocaine are considerably lower than those considered toxic (> 5 mcg/mL) (DeJong, 1994) using these dosage regimens. There are currently insufficient data to determine the safety of repeated EMLA administration.

**Conclusions to Systematic Review**

In summary, the current data provide sufficient evidence to recommend routine use of EMLA for neonatal circumcision pain management in settings where no analgesics are routinely administered. EMLA cannot be recommended over other analgesic techniques with proven efficacy, such as regional nerve block with lidocaine. Further research is necessary to determine the relative and combined efficacy of different analgesic techniques and the most appropriate dosage regimens.

There may be some benefit from EMLA for neonates undergoing venous or arterial puncture and PVC placement, however, efficacy data for these procedures are limited. EMLA
appears to be ineffective for management of heel lancing pain.

Single doses of EMLA are safe for application to the skin of neonates. Additional research is needed before EMLA can be recommended for repeated administration. In order to facilitate systematic evaluations, investigators are encouraged to devise their research studies with similar outcomes, and to provide results in a consistent fashion (as described by The Standards of Reporting Trials Group) (Standards of Reporting Trials Group, 1994).
Conclusions

Myths continue to be propagated about infant pain which have prevented adequate pain management in this population. Examples of these myths are: infants do not feel pain, pain cannot be measured accurately, medications are associated with adverse effects, and pain does not require treatment because it is not remembered. It has been previously shown that all of the neurophysiological components required for pain perception are present by late gestation in the human fetus. It has also been previously shown that infant pain can be measured using behavioural and physiological responses. The pharmacology of analgesics in young infants is currently being studied so that age-appropriate dosage regimens can be developed, and investigators are also beginning to study the long-term effects of untreated pain.

The results of this body of research have demonstrated that EMLA is safe and efficacious for the management of pain from vaccination and circumcision. In addition, untreated neonatal circumcision pain was shown to be associated with alterations in infant pain behaviour at routine vaccination. These long-term changes were attenuated by pre-treatment of circumcision pain with EMLA.

It is recommended that the alleviation of infant pain is a recognized standard of care in health care institutions. Since infants rely on others to recognize and manage their pain, many factors can potentially interfere with consistent pain management strategies. It is therefore recommended that clinical practice pain management guidelines be developed at all institutions, and that the current research findings be incorporated into existing policies. Education should be provided for parents and health care professionals about objective pain assessment tools and pain management guidelines.
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Appendix 1. EMLA in Subcutaneous Injection

Originally published in “Effect of Lidocaine-Prilocaine Cream on Pain from Subcutaneous Injection” by Anna Taddio, Isabelle Robieux, Gideon Koren. CLINICAL PHARMACY 11:347-349. Copyright 1992. American Society of Hospital Pharmacists, Inc. All rights reserved.

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EMLA 5% cream (Astra Pharma Inc.) is a eutectic mixture of equal parts of lidocaine and prilocaine that is applied topically to induce skin anesthesia. It is currently marketed as a nonprescription medication in Canada but is not available in the United States.

The anesthesia produced by EMLA may be apparent at a depth of up to 5 mm, and the effect persists after the cream's removal (Bjerring et al., 1990). EMLA has been used effectively as an anesthetic for needle puncture (Hallen et al., 1982; Maunuksela et al., 1986; Manner et al., 1987; Hopkins et al., 1988; Halperin et al., 1989), superficial skin surgery (Juhlin et al., 1980), and removal of molluscum contagiosum lesions (Risdahl et al., 1988; de Waard-van der Spek et al., 1990). However, its effectiveness in reducing the pain associated with subcutaneous injection has not been tested.

The objective of this study was to evaluate EMLA's ability to make subcutaneous injection less painful.

Methods

Healthy adult volunteers participated in a randomized, double-blind, prospective trial. Exclusion criteria for the study were a history of sensitivity or allergy to amide-type anesthetics and receipt of any anesthetic or sedative within two hours of the study. The study was approved
by the human subject review committee of our hospital, and all subjects gave written informed consent. All participants were given an information sheet that described EMLA and its uses, as well as the purpose of the study and its blinded and placebo-controlled design. The sheet indicated how EMLA was to be applied, that it would take one hour to become affective, that 1 mL of sterile 0.9% sodium chloride solution would be injected into each arm, and that subjects would be asked to record the amount of pain they experienced for each injection. The sheet noted that redness or itching could occur.

Subjects received one 2.5-g application of either EMLA or placebo cream to the left arm over the middle of the deltoid muscle, and an occlusive dressing was applied immediately. The other cream was applied in a similar fashion to the right arm and covered. The order of cream application was random for each subject. Both placebo and EMLA creams contained the same ingredients, except that the active ingredients in EMLA were replaced with coconut oil (Miglyol 812, Dynamit Nobel, Sweden) in the placebo cream. Both formulations were visually and cosmetically identical. The creams were applied in a circular pattern, with a radius of approximately 1.5 cm. After 60-75 minutes, the dressings were removed and the creams were wiped off with a tissue. Within five minutes of cream removal, investigators rated local skin reactions (i.e., pallor, edema, or redness) as being not present, mild, moderate, or severe. The investigators were blinded to site of drug and placebo. After the area was wiped with an alcohol swab, each subject received one subcutaneous injection of 1.0 mL of room-temperature 0.9% sodium chloride injection (Astra Pharma Inc.) in each site, starting with the left arm. A 25-gauge, 5/8-inch needle was used. For each injection, the needle was inserted in the middle of the deltoid area at a 90° angle to the skin. The solution was injected over five seconds, as counted by the investigator. All injections were performed by one investigator. The area on the skin where the
cream was applied was not identified with a marker because the application area was consistent and also because the dressing left markings on the skin.

The pain associated with the procedure was scored by each subject by using an ungraded 100-mm visual analog scale (VAS) with zero denoting "no pain" and 100 mm denoting "worst possible pain." All subjects were pretested for understanding of the VAS. In this comprehension test, subjects scored the pain they would feel in the following situations: receiving a mosquito bite, falling in the snow, falling on the pavement, and slamming a door on their fingers. For each injection, subjects used one VAS to score the pain felt from the needle insertion and another to score the pain felt from the actual injection of solution. The pretest and pain evaluations were administered by the same investigator, who did not give the injections. All subjects filled out a short questionnaire about EMLA cream after receiving both injections.

The differences between the pain scores were evaluated using the Wilcoxon signed-rank test. The effect of the side of injection and the location of EMLA cream was studied using the Mann-Whitney U test. The a priori level of significance was $< 0.05$.

Results

Twenty subjects participated in the study. The mean age was 30 years (range, 19-46 years). Sixteen subjects (80%) were women. Eighteen subjects (90%) were white, one was Asian and one was black. Fifteen subjects (75%) reported no history of skin allergies. Five others (25%) reported allergies to skin cleaners, perfumes, mosquito bites, animals, or plants. Eleven subjects (55%) received EMLA on the left arm, and the remainder received it on the right arm.
Table 22 shows the pain scores from the VAS comprehension test and injections. Needle insertion caused significantly less pain in EMLA-treated arms than in placebo-treated arms ($p < 0.01$). There was also a significant difference between the pain associated with needle entry and the pain of actual injection in EMLA-treated arms ($p < 0.01$). There was no significant difference between these variables in the placebo group. Pain caused by the injection was not significantly different between EMLA- and placebo-treated arms. When other variables were held constant, there was no difference in pain between left and right arms or between left and right EMLA-treated arms.
Table 22. Ratings of Pain from Imagined Sources and from Subcutaneous Injection (n = 20)

<table>
<thead>
<tr>
<th>Pain Source</th>
<th>Scores on Visual Analog Scale (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Imagined Pain</td>
<td></td>
</tr>
<tr>
<td>Mosquito bite</td>
<td>7.9 ± 10.0</td>
</tr>
<tr>
<td>Falling in the snow</td>
<td>10.9 ± 10.0</td>
</tr>
<tr>
<td>Falling on pavement</td>
<td>31.3 ± 13.9</td>
</tr>
<tr>
<td>Slamming door on finger</td>
<td>74.6 ± 10.9</td>
</tr>
<tr>
<td>Needle Insertion</td>
<td></td>
</tr>
<tr>
<td>EMLA-treated arm</td>
<td>3.9 ± 8.5</td>
</tr>
<tr>
<td>Placebo-treated arm</td>
<td>9.1 ± 11.2</td>
</tr>
<tr>
<td>Injection of 0.9% sodium chloride</td>
<td></td>
</tr>
<tr>
<td>EMLA-treated arm</td>
<td>13.9 ± 15.8</td>
</tr>
<tr>
<td>Placebo-treated arm</td>
<td>18.3 ± 21.2</td>
</tr>
</tbody>
</table>

a 100-mm scale, where 0 mm = no pain and 100 mm = worst possible pain.

b Significantly different from scores for needle insertion into placebo-treated arm and injection of 0.9% sodium chloride solution into the EMLA-treated arm (p < 0.01, Wilcoxon signed-rank test).

All subjects reported that they believed they would be able to properly apply EMLA at home one hour before having their next injection. Eighty percent reported that they thought the one-hour application time was not difficult to fit into their schedule, and 50% stated that they
would be willing to purchase EMLA for their next injection. (The wholesale price of a 5-g tube is approximately $6 Canadian, but subjects were not given this information.)

Table 23 lists the frequency of adverse effects. No serious adverse effects were noted. EMLA and placebo were associated with adverse effects in 85% and 35% of subjects, respectively. Skin pallor was the most frequent reaction, occurring in 13 subjects (65%) on the EMLA-treated arm, compared with three subjects (15%) on the placebo-treated arm.
Table 23. Adverse Effects Associated with EMLA and Placebo Administration (n = 20)

<table>
<thead>
<tr>
<th>Effect and Severity</th>
<th>EMLA</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pallor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>12 (60)</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Moderate</td>
<td>1 (5)</td>
<td>0</td>
</tr>
<tr>
<td>Redness, mild</td>
<td>2 (10)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Other(^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>6 (30)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Moderate</td>
<td>1 (5)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Total(^c)</td>
<td>17 (85)</td>
<td>7 (35)</td>
</tr>
</tbody>
</table>

\(^a\) Adverse effects were rated by investigators as nonexistent, mild, moderate, or severe.

\(^b\) Includes heat sensation, itching, soreness, aching sensation, blotchiness or variation in skin color, redness where adhesive tape was applied, numbness, or heaviness.

\(^c\) Three subjects had two adverse effects each when treated with EMLA, one subject had three adverse effects when treated with EMLA, and one subject had two adverse effects when treated with placebo.

Discussion

Our study showed that needle insertion causes less pain if the injection site has been pretreated with EMLA. However, the pain of solution injection was not lessened by EMLA pretreatment. Since subcutaneous injection is commonly used in children for administration of
routine vaccinations, it seems reasonable to test EMLA in children during vaccination. Because needles are perceived as painful by children and parents, administration of EMLA cream may alleviate anxiety by decreasing pain.

Bjerring and Arendt-Nielsen (1990) used an 18-gauge needle in their study to determine that the depth of analgesia after topical application of EMLA cream was 5 mm during the 30 minutes after a 90-minute application time and during the 60 minutes after a 120-minute application time. Our study, which employed a 25-gauge needle inserted to a depth of 5/8 inch (16 mm), suggests that the depth of analgesia provided by EMLA may extend into the subcutaneous tissue. This is supported by the fact that EMLA provides analgesia for venous cannulation (Halperin et al., 1989).

In our study, subjects scored pain intensity on a VAS. This method has been used successfully in evaluating EMLA's efficacy in other trials as well. Halperin et al. (1989) found that children reported significantly less pain from venipuncture, subcutaneous drug reservoir puncture, and lumbar puncture after treatment with EMLA. Manner et al. (1987) also used the VAS in their evaluation of pain associated with venous cannulation in children. DeWaard-van der Spek et al. (1990) found that children, evaluating their own pain by using a VAS, reported less pain from curettage of molluscum contagiosum after treatment with EMLA cream, compared with placebo.

Our study did not address differences in pain perception resulting from the properties of the injected substance, such as pH, temperature, and osmolality. Therefore, the perception of pain in this study may not reflect the perception of pain caused by routine vaccinations. Nevertheless, this does not mean that EMLA is not useful under other conditions. In fact, it may have a greater impact on pain perception when less physiologic substances are injected. Also, the
quality of pain associated with insertion of the needle versus that of solution injection may be different, and we are uncertain of the contribution of each to the pain from the entire procedure.

EMLA cream was associated with more adverse reactions than placebo, but the reactions were mild and consisted mainly of skin pallor. Pallor is a recognized effect of EMLA cream (Astra Pharma Inc. 1990).

Conclusion

Subcutaneous needle insertion in adult volunteers was less painful after EMLA, rather than placebo, was used to pretreat the injection site.
Appendix 2. EMLA in Intramuscular Injection


Abstract

The efficacy of lidocaine-prilocaine cream (EMLA® Eutectic mixture of Local Anesthetics) in alleviating the pain of intramuscular injections was investigated in a randomized, double-blind, placebo-controlled, parallel group trial. EMLA® or placebo cream was applied to the arms of 60 adult volunteers before receiving influenza virus vaccine (Fluzone®). Twenty-nine subjects received approximately 2.5 g of EMLA® cream and 31 subjects received approximately 2.5 g of an inert placebo cream under occlusion for 60-90 minutes. The cream was then removed and each subject received one 0.5 mL intramuscular injection of influenza virus vaccine using a 22 gauge one inch needle. Pain of needle puncture and pain of injection were both assessed by the subjects using a visual analog scale. EMLA® was associated with decreased needle puncture pain (p<0.0002) and decreased pain of injection when compared to placebo (p=0.0139). There was a significant correlation between scores of needle puncture pain and injection pain. Mild skin pallor was a common skin reaction from EMLA®. While the efficacy of EMLA® to alleviate pain of venipuncture is well documented, this is the first study to show the efficacy of EMLA® for intramuscular injections.
Introduction

EMLA® 5% cream (Astra Pharma Inc., Canada) is a eutectic mixture of equal parts of lidocaine and prilocaine. It is currently available in Europe and in Canada while it is not yet available in the U.S. EMLA®, used topically to produce surface anaesthesia, has been studied for many clinical indications, including the pain associated with needle puncture, (Hallen et al., 1982; Halperin et al., 1989; Hopkins et al., 1988; Manner et al., 1987; Maunuksela et al., 1986), superficial skin surgery (Juhlin et al., 1980), and removal of molluscum contagiosum lesions (de Waard-van der Spek et al., 1990; Rosdahl et al., 1988). The depth of its effect may be up to 5 mm and persists after its removal (Bjerring et al., 1990).

We have recently studied EMLA®'s effectiveness in reducing the pain associated with subcutaneous injections of normal saline in adult volunteers, showing decreased needle pain compared with placebo (Taddio et al., 1992b). As injections are commonly utilized for administering vaccinations, EMLA® may be useful in alleviating some of the pain associated with this procedure. The objective of the present study was to evaluate EMLA®'s ability to alleviate pain associated with intramuscular injections of influenza virus vaccine in adult volunteers.

Methods

After approval by our Human Subjects Review Committee, and obtaining written informed consent, 60 healthy adult volunteers participated in a randomized, double-blind, prospective, trial. The study was held on the day when the influenza virus vaccine was being offered to hospital employees. All study subjects were informed of the study objectives and design through an information study summary sheet which was distributed to them. Exclusion criteria for the study included: subjects with a history of sensitivity or allergy to amide
anaesthetics; any contraindication to influenza vaccine including allergy to eggs, neurological disorders, concurrent upper respiratory tract infection, and pregnancy; or receipt of any anaesthetic or sedative within two hours of the study. Each subject received either one application of approximately 2.5 g of EMLA® cream or approximately 2.5 g of placebo cream (Miglyol 812 oil, Dynamit Nobel, Sweden) covered by a transparent occlusive dressing on the arm to be vaccinated, in the middle of the deltoid muscle. The placebo cream contained the same ingredients as the active cream, except that the active ingredients were substituted with coconut oil. Both formulations are cosmetically and visually identical.

After 60-90 minutes, the dressing was removed and creams were wiped off using a paper tissue. Any local skin reactions, (i.e., pallor, edema or redness at the treated site), were recorded by one investigator blinded to the treatment within two minutes of the removal of the cream, using a four point rating score of none, mild, moderate or severe. The area was then wiped with an alcohol swab and each subject then received one 0.5 mL intramuscular injection of influenza vaccine (Fluzone® subvirion vaccine, Connaught Laboratories, Canada) a two to eight degrees centigrade using a 22 gauge, one inch needle by a registered nurse in the Occupational Health Unit of our hospital. For each injection, the needle was inserted in the middle of the deltoid area at a 90 degree angle to the skin. The vaccine was injected over five seconds, as counted by the nurse. All injections were performed by two nurses familiar with the study protocol. The nurses were blinded to the treatments.

The pain associated with the procedure was scored by each subject by drawing a perpendicular line through a 100 mm ungraded tine (Visual Analogue Scale (VAS)) where zero denoted "no pain" and 100 mm denoted "worst possible pain". All patients were pretested for understanding of the VAS, by scoring the pain they would feel during the following situations:
mosquito bite; falling in the snow; falling on the pavement; and slamming the door on their fingers. A trend toward increasing pain constituted adequate understanding of this test. Each subject was instructed to score the pain felt by the needle entering the skin and the pain felt by the injection of the vaccine on two separate VAS. All subjects also participated in a short questionnaire after receiving their vaccinations. The investigator administering both the test scores and questionnaire was blinded to the treatments.

Differences in patient characteristics between the two groups were analyzed using Chi square and t-test for unpaired data whenever appropriate. The differences between the pain scores in the two groups were calculated using the nonparametric Mann-Whitney U test for unpaired data. Differences in pain scores within each group were calculated using the Wilcoxon signed rank test. A two tailed p value of \( \leq 0.05 \) was considered significant. Correlation between the puncture and injection scores was studied by the nonparametric Spearman method.

Results

Patient characteristics are listed in Table 24; no statistically significant differences between the groups were observed. Twenty-seven subjects (93%) in the EMLA® group and 26 subjects (84%) in the placebo group reported no history of skin allergies. Seven out of sixty subjects (12%) reported allergies to perfumes, metal, acrylic, formaldehyde and one specific brand of adhesive tape. Four other patients in the placebo group reported having general skin sensitivity or allergies which resulted in skin manifestations, but without identifying the causative agent or agents.
Table 24. Patient Characteristics

<table>
<thead>
<tr>
<th></th>
<th>EMLA® (N=29)</th>
<th>PLACEBO (N=31)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (% female)</td>
<td>20 (69.0)</td>
<td>26 (83.9)</td>
<td>0.2697</td>
</tr>
<tr>
<td>Mean Age (yr) (SD)</td>
<td>34 (10.3)</td>
<td>37 (11.6)</td>
<td>0.2783</td>
</tr>
<tr>
<td>(range)</td>
<td>(23-62)</td>
<td>(22-65)</td>
<td></td>
</tr>
<tr>
<td>Weight (kg) (SD)</td>
<td>64 (10.4)</td>
<td>67 (17.2)</td>
<td>0.4928</td>
</tr>
<tr>
<td>Race (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>caucasian</td>
<td>25 (86.2)</td>
<td>28 (90.3)</td>
<td>0.6211</td>
</tr>
<tr>
<td>negroid</td>
<td>2 (6.9)</td>
<td>1 (3.2)</td>
<td></td>
</tr>
<tr>
<td>oriental</td>
<td>2 (6.9)</td>
<td>1 (3.2)</td>
<td></td>
</tr>
<tr>
<td>asian</td>
<td>0 (0.0)</td>
<td>1 (3.2)</td>
<td></td>
</tr>
<tr>
<td>Vaccination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>left arm (%)</td>
<td>24 (82.8)</td>
<td>27 (87.1)</td>
<td>0.9136</td>
</tr>
</tbody>
</table>

* Chi square test

* t-test for unpaired data
Table 25 shows the pain scores from the intramuscular injection with EMLA® versus placebo. EMLA® was associated with significantly lower pain scores during needle prick when compared to placebo \((p < 0.0002)\). EMLA® was also associated with significantly lower pain scores during injection with the vaccine compared with placebo \((p = 0.0139)\). The pain scores associated with the needle prick were significantly lower than the pain from the injection of the vaccine for both the EMLA® and placebo group \((p < 0.01\) and \(p = 0.046\), respectively).

**Table 25. Visual Analog Scale (VAS) Pain Scores for EMLA® (N=29) and Placebo (N=31) after Intramuscular Injections of Influenza Virus Vaccine (9=no pain; 100=worst possible pain)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mean VAS pain score (mm) ± SD (median, range)</th>
<th>p value (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EMLA® Needle</strong></td>
<td>(4.62 \pm 7.77) (1, 0-29)</td>
<td>(p &lt; 0.0002)</td>
</tr>
<tr>
<td><strong>Placebo Needle</strong></td>
<td>(15.19 \pm 16.49) (8, 1-64)</td>
<td></td>
</tr>
<tr>
<td><strong>EMLA® Injection</strong></td>
<td>(9.52 \pm 13.65) (3, 0-50)</td>
<td>(p = 0.0139)</td>
</tr>
<tr>
<td><strong>Placebo Injection</strong></td>
<td>(18.45 \pm 19.64) (13, 0-92)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Mann-Whitney test

\(^b\) refers to assessment of pain from needle prick

\(^c\) refers to assessment of pain from influenza virus vaccine injection
Analysis of the correlation between the pain scores from the needle prick and the pain scores from injection from the vaccine revealed that in the EMLA® group, the correlation coefficient was 0.543 (0.002<p<0.01). In the placebo group, the correlation coefficient was 0.815 (p<0.001). Overall, the correlation coefficient was 0.746 (p<0.001), (Figure 7).

Figure 7. Correlation between VAS pain scores from needle prick and injection. EMLA and placebo groups combined. (Spearman correlation coefficient = 0.746, p<0.001)

All patients reported that they would be able to properly apply EMLA® at home one hour prior to having their next injection. Eighty-seven percent reported that the one hour application time would not be difficult to fit into their schedule.

No serious adverse events were reported (Table 26). Fifteen subjects (48%) in the placebo group and 28 (97%) in the EMLA® group experienced local skin reactions (p<0.0002). Mild pallor of the skin was the most frequent reaction in the EMLA® group, occurring in 68% of subjects with skin reactions. Other adverse effects included reactions such as heat or burning
sensation, numbness, skin blotchiness (i.e., red spots), goosebumps, or mild redness at site of adhesive tape. Seven patients experienced more than one adverse effect.

**Table 26. Frequency of Adverse Effects**

<table>
<thead>
<tr>
<th>Signs</th>
<th>Severity</th>
<th>EMLA® (N=29)</th>
<th>PLACEBO (N=31)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. subjects with adverse effects (%)</td>
<td></td>
<td>28 (96.6)</td>
<td>15 (48.4)</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Type of reaction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pallor</td>
<td>mild</td>
<td>19</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>moderate</td>
<td>2</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Edema</td>
<td></td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Redness</td>
<td></td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Other b</td>
<td></td>
<td>6</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Total No. Reactions</td>
<td></td>
<td>34</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

* Chi square test  
 b see text

**Discussion**

This is the first blinded, placebo controlled study to show the efficacy of EMLA® in alleviating pain associated with vaccination. We chose to score the pain from the procedures using a visual analogue scale. This method has been used successfully in similar settings where EMLA® has been studied (Halperin et al., 1989; Manner et al., 1987; de Waard-van der Spek et al., 1990; Rosdahl et al., 1988; Taddio et al., 1992b; Robieux et al., 1991). When compared to
placebo, EMLA® decreased the pain of needle penetration into the skin. These results are consistent with our previous study where twenty adult volunteers were administered 1.0 mL of normal saline subcutaneously in both arms after receiving EMLA® and placebo creams in a randomized, double-blind fashion (Taddio et al., 1992b). EMLA® was associated with statistically lower pain scores from needle prick.

When compared to placebo, EMLA® also decreased the pain of injection from influenza virus vaccine as well. This was not shown in our previous study, where EMLA® was associated with statistically lower pain scores associated with skin penetration compared to placebo, but not during the injection of normal saline (Taddio et al., 1992b). This difference between the two studies is not surprising as there are many variables that affect the amount of pain caused by various solutions. Differences may be due to the properties of the solutions used such as: temperature, volume, pH, and osmolality; method of administration; and setting. For example, in our current study, 0.5 mL of plasma was administered using a 22 gauge needle, whereas the preliminary study involved administration of 1.0 mL of saline using a 25 gauge needle.

In the present study, the pain elicited by the needle prick was less than from injecting the vaccine. The quality of pain associated with needle insertion, however, may be different from that caused by the injection of vaccine. The needle elicits anxiety and is commonly described as causing a sharp pain. The injection, however, is described as causing a dull pain. The observed correlation between the needle and injection pains suggests that the two events are not totally independent, and the pain elicited by the needle may affect the pain perception from the injection. This observation may be of high clinical relevance, because the use of skin anaesthesia may thus modulate pain perception of much deeper procedures.
Our subjects were confident they could administer EMLA® at home, and that based on their experience, it would not interfere with their schedules. This study suggests that adults perceive needles as painful and they would be willing to accommodate their routines to avoid or attenuate this pain.

EMLA® cream was associated with more local skin reactions than the placebo, but the reactions were mild, and consisted mainly of skin pallor, consistent with previous studies (Hallen et al., 1982; Halperin et al., 1989; Manner et al., 1987; Rosdahl et al., 1988; Taddio et al., 1992b).

In summary, we have shown that EMLA® cream decreases pain associated with intramuscular vaccinations in adults. Since intramuscular injections are commonly used in children for administration of routine vaccinations and since children and parents often perceive needles as painful, EMLA® may be also useful in this setting. These results support continuing research into the usefulness of EMLA® for vaccination pain in children.
Appendix 3. Pain Assessment Tool for Vaccination


Abstract

Acute pain in infants is not assessed or managed optimally. The objectives of the study were: a) to adapt a behavioral pain assessment measure (Children’s Hospital of Eastern Ontario Pain Scale, CHEOPS) for use with infants, and b) to establish the reliability and validity of the measure in a study of infants undergoing immunization. Ninety-six healthy 4 to 6 month-old infants were randomized to receive either the local anesthetic cream Eutectic Mixture of Local Anesthetics (EMLA) (n=49), or a placebo (n=47) prior to immunization. The infant’s behavioral response was videotaped immediately before and following the immunization. Postprocedural pain scores were assessed from the videotape and were significantly lower in infants who received EMLA (p=0.01). Pain scores were also significantly correlated with visual analogue scale (VAS) scores assessed during vaccination. Five independent raters also independently rated 10 infants to determine interrater reliability. Agreement between raters’ scores was high (intraclass correlation coefficient=0.95). Results from this study suggest that this measure has beginning construct and concurrent validity and interrater reliability when used in a research study. Further testing of the measure in the clinical setting is required.
Introduction

The assessment of acute iatrogenic pain in infants is problematic because pain is difficult to identify and quantify. In the case of older children, assessment tools such as the Oucher (Beyer et al., 1992), Analogue Chromatic Continuous Scale (ACCS)(Beyer et al., 1990), Poker Chip Tool (Hester, 1979), or Visual Analog Scale (VAS) can be directly administered, so that the child can provide information about his/her own pain. In infants, however, one must rely solely on the assessment made by others who have interpreted the painful event. The objective of this study was to develop a clinically applicable pain measure for young infants. To accomplish this objective, we modified an existing pain measure, and tested its reliability and validity in infants receiving routine immunizations. This study was part of a larger clinical trial, in which we tested the efficacy of a local anesthetic compared to a placebo in decreasing infant pain from vaccination (Taddio et al., 1994).

Review of Pain Assessment in Young Infants

Infant facial expressions have been extensively investigated as a measure of pain. Izard (1982) developed a coding system that classifies observed reactions according to emotional state (Maximally Discriminative Facial Movement Coding System, MAX). Videotapes of infants are taken and subsequently reviewed by trained raters for the presence or absence of designated facial movements in three areas: brow, eyes and mouth. Facial expressions are then categorized according to emotional state (Izard et al., 1987). The characteristics of pain include; lowered and drawn together brow, nasal root widening and bulging, eyes tightly closed, and angular, squarish mouth.
Grunau and Craig developed the Neonatal Facial Coding System (NFCS) (Grunau et al., 1987). Like the MAX, the NFCS uses facial actions to describe infant responses. Unlike the MAX, however, there is no attempt to describe emotional states. Infants are scored on the presence or absence of the following facial actions: brow bulge, eye squeeze, naso-labial furrow, open lips, vertical stretch mouth, horizontal stretch mouth, lip purse, taut tongue, chin quiver, and tongue protrusion. The amount of infant facial action has been shown to vary with the nature of the medical procedure (i.e., invasive or noninvasive) (Grunau et al., 1990). In addition, facial activity scores have been shown to relate to parental pain ratings, providing external validation of this method to characterize neonatal pain (Craig et al., 1988a). Despite these attributes, like the MAX, the NFCS is a labor intensive scoring system. Infant facial actions are scored on a per-second basis by trained coders who view videotapes of painful situations.

Infant vocalizations have also been used to describe pain (Grunau et al., 1990; Owens et al., 1984; Fuller et al., 1988; Porter et al., 1986). Cry characteristics included in pain assessments are: latency to cry, duration of first cry cycle, frequency of cry, intensity, melody, jitter, and dysphonia. Spectrographic analyses have demonstrated differences between pain cries and other cries, although the cry sounds from painful situations are not uniquely different from those of other types of cries (Zeskind et al., 1985; Fuller, 1991; Johnston et al., 1988). As a single measure of pain, cry is insufficient because it is not consistently observed in all infants.

Observations of infant body movements have also been used by investigators to determine pain. Infants react to acute noxious stimuli by thrashing, jerking, wiggling, withdrawing, kicking or exhibiting torso rigidity (Johnston et al., 1986; Craig et al., 1984; Mills, 1989; Dale, 1986; Maikler, 1991; Bozzette, 1993). Craig and colleagues (1993) developed the Infant Body Coding System (IBCS) in order to capture neonatal body activity. The IBCS is used in a similar fashion
as the NFCS; trained coders score the presence or absence of hand, foot, arm, leg, head and torso movements.

In addition to the behavioral approaches described above, physiological indicators have also been observed during painful situations (Owens et al., 1984; Johnston et al., 1986; Craig et al., 1993; Mudge et al., 1989; Rawlings et al., 1980; Maxwell et al., 1987; Marchette et al., 1991; Stang et al., 1988; Holve et al., 1983; Williamson et al., 1983; Marchette et al., 1989). Marked increases in respiration rate, heart rate, blood pressure, and cortisol levels have been demonstrated after infant exposure to a noxious stimulus. Conversely, oxygen saturation has been observed to decrease. Levels of adrenaline, beta-endorphin, insulin, and glucagon are particularly useful as indices of surgical stress (Anand et al., 1988; Anand et al., 1992; Anand et al., 1990). Physiologic changes provide objective pain measures, however, they are not specific to pain (Chapman et al., 1985). Observations are difficult to interpret in situations where there are technical problems (Grunau et al., 1987). In fact, application of the monitor on the very young infant may modulate the infant’s response.

To summarize, infant pain responses have been demonstrated to encompass a variety of dimensions including facial action, crying, body movements and physiologic changes. The specificity of each dimension to pain is not known, but appears to be highest for facial reactions and lowest for physiologic measures. At present, it is uncertain whether any one dimension sufficiently approximates the infant’s pain. As a consequence, currently available infant pain assessment scales include more than one dimension to facilitate a more complete description. The multidimensional approach is believed to improve the validity of the pain assessment. There is a risk, however, of including redundant variables in the measure. The inter-relationships between constituent items should therefore be carefully scrutinized during the development stages of the
tool. In general, infant pain assessment scales have concentrated on behavioral responses; namely, facial, cry and body behaviors (Rich et al., 1974; Craig et al., 1988b) due to the frequency of occurrence of these reactions and the ease with which they are observed. A review of some pain measures developed for use with infants is provided below.

Barrier and colleagues (1989) developed a post-operative pain scale to demonstrate the efficacy of an analgesic in infants. The pain scale utilized assessments of infant sleep, facial expression, cry quality, motor activity, responsiveness to stimulation, limb flexion activity, sucking, tone, consolability and sociability. Although simpler to use than facial coding systems such as the NFCS, this scoring system requires observation of the infant over the preceding hour, which is not always feasible. Recently, Buchholz and colleagues (1994) compared the scores obtained with the Barrier scale with those of VAS in infants undergoing surgery. The observation of the infant for the preceding hour was omitted. There was a statistically significant correlation in pain scores between the two measures. Pokela (1994) developed a behavioral pain measure for measuring the pain in mechanically ventilated neonates. The instrument measured pain from four behaviors; infant facial expression, movements, response to handling, and rigidity of the limbs. The measure successfully demonstrated that opioid therapy resulted in less pain during a medical procedure than treatment with saline.

Fradet and colleagues (1990) and McGrath and colleagues (1985) developed the Children's Hospital of Eastern Ontario Pain Scale (CHEOPS), which has been used to measure post-operative, venipuncture or fingerprick pain in children greater than 3 years of age. The CHEOPS scores pain across six categories; cry, facial expression, verbal expression, torso activity, touch and leg activity. A numerical score is assigned to each activity such that the total score is a sum of the individual scores. The CHEOPS has been internally validated, and
correlated with other pain measurement scores. The CHEOPS was adapted by Robieux (1991) in a clinical trial to measure pain from venipuncture in infants aged 3 to 36 months. Infants were pre-medicated with either the local anesthetic Eutectic Mixture of Local Anesthetics; lidocaine, and prilocaine (EMLA) or placebo prior to venipuncture. The tool, herein entitled the Behavioral Pain Score (BPS), scores pain from facial expression, cry and body movements. Congruent with the expectation that analgesia reduces pain intensity, the BPS pain scores in infants treated with EMLA were lower than those of infants given placebo. The limitation of the BPS is that it was developed for use in older infants and may not adequately describe the variability of the responses in younger infants.

Lawrence and colleagues (1993) recently modified the CHEOPS for use in neonates. The Neonatal Infant Pain Scale (NIPS) was designed to measure pain in the neonatal intensive care unit (NICU). Pain is scored from six items; facial expression, cry, breathing patterns, arm and leg activity, and state of arousal. The NIPS was shown have good inter-rater reliability and beginning construct validity. To date, it has only been validated on a small number of neonates and only for research purposes. Other pain measures have recently been developed and are in the process of being validated (Stevens et al., 1994; Murphy et al., 1994; Krechel et al., 1994).

Objectives

During the time we undertook this trial, a validated and clinically useful measure was not available to assess pain in infants. The objectives of this study were to: (a) modify a pain tool (i.e., the BPS), for use in infants aged 2 to 6 months; (b) establish concurrent validity, construct validity, and internal consistency of the new measure; and (c) demonstrate inter-rater agreement and test-retest reliability of the new measure.
Methods

Development of the Pain Assessment Measure

The study was approved by our hospital's Research Ethics Committee. Consent was obtained from a convenience sample of parent(s) to videotape their infant during routine vaccination in a metropolitan outpatient pediatric clinic. These videotapes were used to develop and validate the pain measure (herein called the Modified Behavioral Pain Scale, or MBPS). During a pilot study, five raters (neurologist, pediatrician, pharmacist, physiotherapist, and lay person), simultaneously viewed 11 videotaped immunizations in infants aged 9 weeks to 5 months, and collectively assessed infants' pain using the BPS scale (Table 27). Raters made two pain assessments for each vaccination session; the first was a baseline BPS pain assessment within 5 seconds before the immunization, and the second was a post-vaccination BPS assessment, made 15 seconds after the immunization. The baseline pain score was defined as the reaction observed in the infant for the majority of the time during the 5 second observation period. The post-vaccination pain score was defined as the maximum reaction observed in the infant during the 15 second observation period. The BPS assessments made after each vaccination were discussed by the raters, and the new tool (MBPS, Table 28) was developed. The changes made to the pain scale were reviewed by a psychologist familiar with pediatric pain assessment tool development. The scoring system of the tool was altered to maximize changes between baseline and post-vaccination pain scores. The minimum score that could be obtained on the MBPS was 0 and the maximum 10.
<table>
<thead>
<tr>
<th>Observed Behavior</th>
<th>Score (total 0-8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Facial expression</strong></td>
<td></td>
</tr>
<tr>
<td>positive (smiling)</td>
<td>0</td>
</tr>
<tr>
<td>neutral</td>
<td>1</td>
</tr>
<tr>
<td>negative (grimace)</td>
<td>2</td>
</tr>
<tr>
<td><strong>Cry</strong></td>
<td></td>
</tr>
<tr>
<td>Laughing or giggling</td>
<td>0</td>
</tr>
<tr>
<td>Not crying</td>
<td>1</td>
</tr>
<tr>
<td>Moaning</td>
<td>2</td>
</tr>
<tr>
<td>Full lunged cry or sobbing</td>
<td>3</td>
</tr>
<tr>
<td><strong>Movements</strong></td>
<td></td>
</tr>
<tr>
<td>Usual activities (i.e., playing)</td>
<td>0</td>
</tr>
<tr>
<td>Neutral, not moving</td>
<td>1</td>
</tr>
<tr>
<td>Attempt to withdraw limb</td>
<td>2</td>
</tr>
<tr>
<td>Complex agitation involving head, other limbs</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Observed Behavior</th>
<th>Score (0-10)</th>
<th>Operational Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Facial expression</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definite positive expression</td>
<td>0</td>
<td>smiling</td>
</tr>
<tr>
<td>Neutral expression</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Slightly negative expression: for example, rimace</td>
<td>2</td>
<td>brow bulge, naso-labial furrow</td>
</tr>
<tr>
<td>Definite negative expression: that is, furrowed brows, eyes closed tightly</td>
<td>3</td>
<td>brow bulge, naso-labial furrow, eyes closed tight, open lips, with or without reddened face</td>
</tr>
<tr>
<td><strong>Cry</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laughing or giggling</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Not crying</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Moaning, quiet vocalizing, gentle or whimpering cry</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Full lunged cry or sobbing</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Full lunged cry, more than baseline cry</td>
<td>4</td>
<td>to be scored only if infant crying during baseline</td>
</tr>
<tr>
<td><strong>Movements</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Usual movements/activity, or resting/relaxed</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Partial movement or attempt to avoid pain by withdrawing the limb where puncture is done</td>
<td>2</td>
<td>squirming, arching, limb tensing/clenching</td>
</tr>
<tr>
<td>Agitation with complex movements involving the head, torso or the other limbs, or rigidity</td>
<td>3</td>
<td>generalized limb and/or body movements, or rigidity</td>
</tr>
</tbody>
</table>
Subjects

The reliability and validity of the MBPS was tested on 4 to 6 month-old infants who participated in a randomized, double-blind clinical trial in which we assessed the efficacy of EMLA in decreasing pain from diphtheria-pertussis-tetanus (DPT) vaccine. Infants were premedicated with either EMLA or placebo for 60-90 minutes prior to vaccination, and immunizations were administered by one of two pediatricians who participated in the study. Injections were performed in a similar fashion (i.e., infants were placed in a supine position on the examining table, and 0.5 mL of injectate was administered intramuscularly in the lateral upper thigh region). The full body of the infant was videotaped by a videographer who stood approximately 3 feet away from the infant, prior to, during, and after the vaccination until the baby settled. A mirror was mounted on the wall adjacent to the examining table to enable the videographer to tape the baby's reaction as observed through the mirror image as well as face on. This method of videotaping was used because prior observations revealed that infants often turned their heads from side to side during immunization. Altogether, 96 infants were evaluable for analyses; 49 received EMLA, and 47 placebo. The mean age of the infants was 5 months, and the male to female ratio was 1:1. There were no statistically significant differences between the treatment groups in demographic characteristics of age, sex, or weight.

Validity of the MBPS

During the clinical trial, a trained observer rated infant pain immediately after the vaccination using a 100 mm ungraded Visual Analogue Scale (VAS) ruler, where 0 mm denoted no pain and 100 mm denoted worst possible pain. The pediatrician who administered the vaccine also rated infant pain using a VAS. Since two pediatricians participated in the study, each rated
approximately half of the sample. One coder viewed the videotapes at a later time and assessed infant pain using the MBPS. The MBPS was scored in a similar fashion as the BPS. Post-vaccination MBPS pain scores were correlated with the VAS pain scores to establish concurrent validity.

To establish construct validity of the MBPS, scores were compared between infants who received the anesthetic EMLA with those who received placebo. A lower pain score in the EMLA group would be congruent with our assumption that analgesia reduces pain (Erickson, 1990).

**Reliability of the MBPS**

In order to test internal consistency of the tool, the post-vaccination MBPS scores obtained on the different items of the tool were correlated. Item-total correlations were also performed between the individual items with the scale total after omitting that item.

Interrater agreement was tested with the scores from 5 raters who viewed the immunizations of a subsample of 10 infants who participated in the clinical trial. The mean infant age was 5.3 months; 5 were male. The raters independently scored baseline pain (5 seconds before the injection) and post-vaccination pain (15 seconds after the injection) for each vaccination. Thus, each rater made 20 pain assessments which were used in the analysis of interrater reliability.

To assess whether MBPS scores obtained from the raters were stable, one of the raters assessed pain from the same infants after 12 months (test-retest reliability).
Data Analysis

The agreement between the raters' scores were assessed using the intraclass correlation coefficient (ICC) as described by Streiner (1989) using a one-factor ANOVA analysis (Cicchetti et al., 1976). An acceptable level of inter-observer agreement was deemed to be >0.75 (Burdock et al., 1963). Correlations between scores were measured using Pearson's correlation coefficient. The a-priori significance level was p<0.05.

Results

Validity

Concurrent validity. Post-vaccination MBPS scores were positively correlated with observer and pediatricians’ VAS scores. The correlation coefficient between MBPS and observer VAS scores was 0.68, (p<0.001). The correlation coefficient between MBPS and pediatricians’ VAS scores was 0.74, (p<0.001).

Construct validity. Baseline MBPS scores (mean, 1.9; SD=0.8) were significantly lower than post-vaccination scores (mean, 7.3; SD=1.8), (p=0.01).

When infants were stratified by group assignment (i.e., EMLA or placebo), baseline MBPS scores were not different between groups (p=0.72). Post-vaccination MBPS scores, however, were statistically significantly lower for the EMLA group (mean, 6.8; SD=1.9) compared to the placebo group (mean, 8; SD=1.5), (p=0.01).

Reliability

Internal consistency of items. The post-vaccination pain scores obtained from each category of the MBPS (i.e., face, cry and movement) were correlated. Facial action scores
significantly correlated with cry ($r=0.67$, $p<0.001$). Body movement scores also correlated with cry ($r=0.48$, $p<0.001$) and facial action ($r=0.54$, $p<0.001$). Item-total correlations between each item and post-vaccination MBPS scores without that item produced coefficients of 0.66 ($p<0.001$) for face, 0.55 ($p<0.001$) for body movement, and 0.60 ($p<0.001$) for cry.

**Inter-rater agreement.** The ANOVA analysis of the 5 raters' assessments showed very high agreement in the scores (ICC=0.95, $p<0.001$). Systematic bias was not observed between raters ($p=0.13$), indicating that no rater was scoring consistently higher or lower than the others. The ICCs calculated on the three categories of the MBPS also showed high agreement: 0.89 ($p<0.001$) for facial movement, 0.96 ($p<0.001$) for infant cry, and 0.83 ($p<0.001$) for body movements.

**Test-retest reliability.** The correlation between the rater's scores of the same cases revealed consistency in assessments over time ($r=0.95$, $p<0.001$).

**Discussion**

Immunizations are the most common source of acute pain in healthy infants. Nevertheless, analgesics have not been routinely administered. The lack of vaccination pain management may partly be due to inadequately tested and/or feasible pain assessment tools. We modified an existing behavioral pain scale to; measure pain in young infants receiving routine immunizations; and to test the effect of a local anesthetic on infant immunization pain. The pain scores derived from the modified measure (that is, MBPS) correlated with observer and pediatricians' VAS scores, providing convergent validity for the scale. The MBPS scores were significantly higher in infants given placebo compared to EMLA, providing evidence for construct validity.
The results are congruent with the pharmacology of EMLA; namely, that it causes skin anesthesia. It is interesting to note that infants in both groups scored near the high end of the scale, suggesting that there was pain in both groups. As EMLA only penetrates up to approximately 5mm under the skin (Bjerring et al., 1990) and the vaccination is administered into the infant's muscle, it follows that although EMLA modulates the pain response from the needle prick, it probably does not prevent the pain from the administration of the vaccine, nor the vaccine itself. Future research should investigate the effect of EMLA in combination with other pain-reducing interventions.

The MBPS was developed as a practical measure that could be easily utilized to assess acute pain in a clinical setting. A multi-item scoring system was used, as no single behavior is believed to adequately reflect infant pain (McGrath, 1989). Internal consistency in scoring of the elements of the scale revealed significant, but only modest correlation coefficients. These results substantiate the notion that the scale is tapping different aspects of the same attribute, presumably pain. Each item correlated with the total pain score minus that item with coefficients greater than 0.50. A generally accepted value is a correlation coefficient greater than 0.20.

The MBPS was designed and used to measure pain in infants of varying ages (i.e., 2 to 6 months). Previous researchers have demonstrated that behavioral responses are relatively consistent in infants 4 to 6 months of age (Johnston et al., 1993; Izard et al., 1983). Older infants, (Maikler, 1991; Izard et al., 1987) exhibit a shorter duration of pain expression and less symmetrical movement.

Reliability testing demonstrated very high agreement between the raters' scores of the same vaccination episodes. Interrater agreement, however, was assessed using the same five raters that developed the scale. Further investigations of scoring consistency with different raters
will be necessary to confirm these results. Since the measure was used in a research setting, it requires validation in clinical practice. Finally, as the data are limited to healthy young infants and routine DPT vaccination, the MBPS requires testing in other age groups and in other procedures to demonstrate generalizability.
Appendix 4. Additional Notes to Chapter 1

Methods

The sample size calculation was based on a standardized difference $= 0.5$, $\alpha = 0.05$, and $\beta = 0.2$. To account for protocol deviations and drop-outs, a total sample size of 112 was recruited. Pain scores were compared between groups using non-parametric methods due to non-normal distribution of data.

Discussion

The effects of EMLA on vaccine efficacy were not assessed in this study. Before general introduction of EMLA cream for the alleviation of pain from vaccination, investigations should be carried out to assess if EMLA affects vaccine efficacy.

We monitored parental behaviours during the procedure because previous research showed that interventions such as picking up the infant could influence infant crying behaviour (Bell et al., 1972). We also measured infant temperament because we postulated that it might exert some influence on infant pain responses. It has previously been shown that temperament exerts some influence on child and adult pain behaviours (Lee et al., 1996; Schechter et al., 1991; Wallace, 1989; Young et al., 1988; Pate et al., 1996).

Temperament is a construct that describes a pattern of behaviour observed across time and contexts (Rothbart et al., 1985). Temperament has been attributed to the interaction between genetic and environmental influences (Johnson, 1992). Specific temperament patterns are present and measurable by as early as 3 to 4 months of age (Tomlinson et al., 1996). Infant temperament was measured using a parental questionnaire. Other less commonly utilized methods include home observations and laboratory studies. We chose a questionnaire technique because of the
following advantages with this method: parents are the most informed and accessible sources of data regarding their infant's behaviour, observations of infant behaviours are made under situations of daily living, internal reliability is high, and it is the most inexpensive method (Rothbart et al., 1985).

The Revised Infant Temperament Questionnaire (RITQ) (Carey et al., 1978) for use in infants aged four to eight months was used to measure infant temperament. The RITQ was developed from previous work that identified nine infant temperament categories from clinical observations of children and interviews with their parents (Thomas et al., 1963). Parents answer 95 questions regarding infant behaviour to different situations such as feeding and bathing. Each item is rated on a six-option frequency rating scale from "almost never" to "almost always". Answers are scored according to the nine temperament categories. These categories are: infant activity, adaptability, approachability, distractability, intensity, mood, persistence, rhythmicity, and threshold. A subset of the nine temperament categories is then used to assign the infant to one of five possible global infant temperament classifications: easy, intermediate low, slow to warm up, intermediate high, or difficult.

The RITQ is the most widely used measure of temperament for infants four to eight months of age (Medoff-Cooper, 1995). The RITQ has favourable psychometric properties. The internal consistency of the entire tool is 0.83; it ranges from 0.49 to 0.71 for the individual categories (Carey et al., 1978). Overall test-retest reliability for intervals of 16 to 47 days, is 0.86 (Carey et al., 1978). Interparent percent agreement in global temperament is high: 0.75-0.85 for infants of mean age, 24 weeks (Simons et al., 1985). Mothers' general ratings of their infants are highly correlated with the nine temperament factors (Carey et al., 1978). Moreover, mothers'
ratings of temperament correlate with infant crying behaviour during stressful situations (Kemp, 1984) and with the presence of colic (Jacobson et al., 1995).

A general criticism of temperament questionnaires is that they rely on parental perceptions of infant behaviours which may co-vary with parental characteristics (Vaughn et al., 1987). The fact that parental psychological characteristics may influence assessments of infant behaviour does not, however, invalidate the assessments. While it has been postulated that mothers may exaggerate infant behaviours due to stress, it is also possible that stressed mothers may parent their children differently leading to differences in infant behaviours, or that the infants perceive their mother’s distress and behave differently, or that both maternal and infant behaviours are related/due to external factors such as genetics or the family environment (Sanger et al., 1992).
Appendix 5. Safety of EMLA in Piglets


Abstract

EMLA® (eutectic mixture of lidocaine and prilocaine) cream is currently not recommended for use in infants <1 month of age because of the potential risk of methemoglobinemia as a result of the o-toluidine metabolite of prilocaine. We studied bioavailability and changes in methemoglobin levels following topical penile exposure to 1 g of EMLA cream for 1 hour in piglets. Lidocaine, prilocaine, and o-toluidine concentrations were measured simultaneously using a high-performance liquid chromatography method. The systemic bioavailability of EMLA was low: 4.0 ± (SD) 4.7% for lidocaine (range 0-13.6; n = 8) and 7.2 ± 5.7% for prilocaine (range 0-14.5; n = 8). The ratio between exposure to o-toluidine with EMLA versus intravenous administration (i.e., \( \frac{\text{AUC}_{\text{EMLA}}}{\text{AUC}_{\text{IV}}} \); see text) was also low: 4.2 ± 9.3% (range 0-28.6; n = 9). The mean maximum methemoglobin value after intravenous administration was 1.23 ± 0.64% (range 0.5-3.0; n = 12) and after penile application 0.99 ± 0.36% (range 0.5-2.0; n = 12). The methemoglobin value was elevated significantly above baseline after intravenous administration (p = 0.03), but not after penile application of EMLA. These findings suggest that penile administration of 1 g of EMLA may be safe for neonatal circumcision, but further study is required.
Introduction

Neonatal circumcision is a common procedure in countries such as the USA and Canada despite the medical controversy that surrounds it (Wiswell et al., 1988; Poland, 1990). While ample evidence exists to indicate that neonates are capable of experiencing pain and that failure to provide adequate relief has adverse effects (Anand et al., 1987a), the majority of circumcisions continue to be performed without any form of analgesia (Wellington et al., 1993). The techniques currently available include general anesthesia and dorsal penile nerve block. The first has an unacceptable risk:benefit ratio in the neonate, while the second requires technical skill and is also not without its attendant problems (Schoen et al., 1991). In either case, the added costs and potential risk from adverse effects have deterred physicians from using them.

During the last decade EMLA® 5% cream (eutectic mixture of local anesthetics; lidocaine and prilocaine) has emerged as an effective method of skin anesthesia for painful superficial skin procedures in infants, children, and adults. It has been used successfully for the painless separation of preputial adhesions in older boys (MacKinlay, 1988) and has recently been shown to be an effective anesthetic agent for circumcisions in newborns (Benini et al., 1993). EMLA has also been used in preterm neonates receiving heelstick (Fitzgerald et al., 1989; McIntosh et al., 1994), but no study has so far investigated its safety in this age group.

The main potential problem with the use of EMLA during the newborn period is the risk of methemoglobinemia. The biotransformation of prilocaine results in the formation of the metabolite o-toluidine which is then oxidized to form aminophenols. These metabolites can oxidize hemoglobin to methemoglobin (DeJong, 1994). Methemoglobin can accumulate in infants <3 months of age due to the immaturity of the methemoglobin reductase enzyme system in this age group (Nilsson et al., 1990). There is only a single case report describing a 12-week-old infant
with significant methemoglobinemia after concurrent use of EMLA and oral sulfamethoxazole (another methemoglobin-inducing agent) (Jakobson et al., 1985).

At present, the use of EMLA in infants <6 months of age is not recommended in Canada, and in the USA it is only licensed for use in infants greater than 1 month of age. There are no existing studies that have investigated either the bioavailability of EMLA or the levels of o-toluidine and methemoglobin that are produced after application to the penile region. Thus, we undertook to examine this question in the piglet model.

Ideally, an animal model should meet the following criteria: be easily obtained, of sufficient size for manipulations, survive long enough to be functional, and accurately mimic the condition being studied. Swine meet all of these criteria and, because of their physiological similarities to humans, have been recognized as useful models for various types of research, including cardiovascular, pulmonary, gastrointestinal, renal, immunologic, metabolic, embryologic, and neonatal (Phillips et al., 1985). In addition, the skin of pigs has been shown to serve as a useful anatomic and metabolic approximation to human skin (Bronaugh et al., 1982; Klain et al., 1985; Reifenrath et al., 1985), and this is an important consideration for the purposes of this study. Using this model, our aims were (1) to determine the systemic bioavailability of EMLA and the extent of formation of o-toluidine; (2) to measure changes in methemoglobin levels following topical application of EMLA to the penile region and after intravenous bolus, and (3) to describe the pharmacokinetic profiles of lidocaine and prilocaine after an intravenous bolus.

Materials and Methods

This was a randomized, crossover, open-label trial. Fifteen healthy, full-term male newborn piglets (<18 days of age) were studied. Their weight ranged from 2.2 to 6.6 kg. They
were premedicated with atropine and anesthetized with intraperitoneal pentobarbitone or inhalational halothane. The piglets were intubated and mechanically ventilated throughout the study period. They were cannulated through the jugular vein for blood sampling, and a suprapubic bladder catheter was placed for urine collection to avoid contamination of the area under study. Each piglet then had either 1 g of EMLA (containing 25 mg of lidocaine and 25 mg of prilocaine), measured with a syringe, applied to the penile surface and covered by an occlusive dressing for 1 h or a single intravenous injection over 1 min of lidocaine hydrochloride (25 mg) and prilocaine hydrochloride (25 mg) through a superficial vein. This dose of EMLA was chosen as it would be the anticipated amount that may be clinically useful in neonatal circumcision. The EMLA application was terminated by removal of the remaining cream by wiping the skin surface with cotton swabs until clear. Blood samples were collected at time 0 (prior to drug administration) and 0.5, 1, 2, 4, 6, and 8 h afterwards. Following an average washout period of 4 (range 3-6) days, the procedure was repeated with the alternative treatment. The protocol received approval from the Animal Care Committee of The Hospital for Sick Children, Toronto. Ethical considerations prevented the collection of more than seven blood samples per piglet per study day.

Laboratory Measurements

Blood samples (1 ml) were collected in heparinized syringes. Methemoglobin was analyzed using a 0.2-ml aliquot within 2 h of sample collection using a co-oximeter and was reported as percent of total hemoglobin. The lower limit of detection of methemoglobin was 0.5%. Levels <0.5% were assumed to be 0.5% for statistical analyses. The remainder of the blood was centrifuged for 10 min at room temperature and separated. The plasma was stored at -20°C.
until analysis. High-performance liquid chromatography was used to measure the lidocaine, prilocaine, and o-toluidine concentrations (Klein et al., 1994). The limit of quantitation of this assay was 20 ng/ml for lidocaine, prilocaine, and o-toluidine. The intraday coefficient of variation for the determination of all three analytes was 3.1-8.3% for six consecutive determinations, while the between-day coefficient of variation was 5.4-8.3% for 6 analytical days. The correlation between peak height ratios of the analytes and internal standard versus the concentration of the respective analyte was linear with r >0.998 for all three compounds.

**Pharmacokinetic Calculations**

The Macintosh computer program Kinfit® was used to fit the intravenous bolus data. Due to the small number of samples collected, a one-compartmental model was used to fit the data and calculate volume of distribution (Vd) and half-life (tv). The clearance rate (Cl) was calculated using the formula

\[ Cl = \frac{DOSE}{AUC} \]

where the AUC (area under the plasma concentration-time curve) was calculated using the trapezoidal method from time 0 to infinity. The elimination tv of o-toluidine was calculated from the terminal log-linear portion of the curve for the intravenous data. For some piglets, there was a rapid disappearance of drug and so only intravenous data consisting of a minimum of three measurable drug concentration-time points were analyzed for pharmacokinetic parameters.

The AUC values for intravenous (IV) administration were determined after extrapolation to time zero. The bioavailability of EMLA over the 8-hour sampling time was calculated using the formula

\[ F = \frac{AUC_{EMLA}}{AUC_{IV}} \times \frac{DOSE_{IV}}{DOSE_{EMLA}}. \]
For this calculation, levels below the limit of quantitation were assumed to be zero. For o-toluidine, the relative systemic exposure from topical EMLA was calculated using the same equation with the dose being equivalent to the administered prilocaine dose.

The plasma levels were normalized for piglet doses of lidocaine and prilocaine on a microgram per kilogram basis for graphical representation and analysis of mean values for Cl and Vd. The plasma levels were multiplied by the fraction of the dose administered to that piglet relative to the piglet with the lowest dose (i.e., minimum μg/kg dose/present piglet dose) to calculate a comparable dose for all piglets.

**Statistical Analysis**

The maximum and minimum methemoglobin levels obtained after intravenous or topical treatment were compared within groups using the Wilcoxon matched-pair signed-rank test; p<0.05 was considered statistically significant.

**Results**

A total of 15 piglets were studied; 6 piglets could not be resuscitated after the 1st study day and so complete data are not available for both treatments. The piglets who died on day 1 did not have any evidence of cardiac arrhythmia, and none had toxic levels of lidocaine (> 5 μg/ml) (Bigger et al., 1990) or methemoglobin. They died from respiratory failure following extubation which was probably due to respiratory depression from the pentobarbitone used for anesthesia. This phenomenon has been previously reported in pigs (Reibold et al., 1985). The first 9 piglets received pentobarbitone and the next 6 piglets received halothane anesthesia. There were no further deaths after changing to halothane anesthesia. Altogether, 3 piglets received only the
EMLA, 3 received only the intravenous bolus, and 9 received both EMLA and intravenous bolus. Four piglets received intravenous bolus on the 1st study day.

Figure 8 shows the dose-normalized concentrations of lidocaine, prilocaine, and o-toluidine after intravenous bolus injection of lidocaine and prilocaine. The pharmacokinetic parameters of lidocaine and prilocaine are given in table 29. The $t_{1/2}$ of o-toluidine was $5.4 \pm 4.7$ h.
Figure 8. Dose normalized concentrations (mean ± SD) of o-toluidine (a), prilocaine (b), and lidocaine (c) after intravenous bolus injection of lidocaine hydrochloride (25 mg) and prilocaine hydrochloride (25 mg; n = 12 piglets)
Table 29. Pharmacokinetics of Lidocaine and Prilocaine after Intravenous Bolus Administration (mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lidocaine (n=12)</th>
<th>Prilocaine (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vd, l/kg</td>
<td>4.1 ± 2.3</td>
<td>10.3 ± 6.1</td>
</tr>
<tr>
<td>Terminal t½, h</td>
<td>1.9 ± 0.7</td>
<td>1.4 ± 1.0</td>
</tr>
<tr>
<td>Cl, ml/min·kg</td>
<td>26.6 ± 15.5</td>
<td>111.4 ± 75.0</td>
</tr>
</tbody>
</table>

The bioavailabilities (AUC) of lidocaine and prilocaine (mean ± SD) after topical application of EMLA to the penile region were calculated in 8 piglets and were 4.0 ± 4.7% (range 0-13.6) and 7.2 ± 5.7% (range 0-14.5), respectively. The mean relative systemic exposure to o-toluidine following penile application (i.e., AUC\text{EMLA}/AUC\text{IV} ratio) was 4.2 ± 9.3% (range 0-28.6; n = 9). The bioavailability was 0% for lidocaine in 1 piglet, for prilocaine in 2 piglets, and for o-toluidine in 5 piglets. After topical administration of EMLA, the mean peak levels of drug were measured at 0.5 h for lidocaine (28.09 ng/ml) and prilocaine (54.27 ng/ml), while mean peak o-toluidine levels (13.23 ng/ml) were achieved 1.0 h after application. However, there was large interindividual variability, and, in many cases, lidocaine and prilocaine levels were below the quantitation limit after 1 h.

Figure 9 shows the mean methemoglobin levels in piglets after intravenous bolus and EMLA. The methemoglobin level peaked, on average, 6 h after the intravenous bolus dose of prilocaine and was different from the baseline methemoglobin level in the same piglets (1.23 ± 0.64 vs. 0.95 ± 0.41%; p=0.03). The baseline methemoglobin levels in the EMLA-exposed piglets
(0.98 ± 0.37%) did not differ significantly from those taken in the same piglets at the other sampling times.

Figure 9. Methemoglobin levels (mean ± SD) after intravenous bolus administration of lidocaine hydrochloride (25 mg) and prilocaine hydrochloride (25 mg) and topical EMLA (1 g; n = 12 piglets). p < 0.05 between time 0 and 6 h for intravenous bolus injection.

Discussion

Several investigators have examined the potential toxicity of EMLA in pediatric patients. Engberg et al. (1987) measured lidocaine, prilocaine, and methemoglobin levels in infants aged 3–12 months. The dose of EMLA was 2 g applied topically under occlusion for 4 h. In that study, the maximum lidocaine and prilocaine levels were <200 μg/l, and the highest methemoglobin level was 2%. Using a similar study protocol, Nilsson et al. (1990) studied infants <3 months of age.
The maximum methemoglobin level was 3.37% which was statistically higher than the baseline level, but did not lead to clinical toxicity. None of the previous reports have measured the o-toluidine levels achieved in the plasma, although o-toluidine is implicated as the metabolite that leads to methemoglobinemia (Onji et al., 1965). In addition, the youngest infant studied to date was 6 weeks of age. There is a direct relationship between age and methemoglobin reductase activity, with levels not reaching maturity until the age of 3 months (Nilsson et al., 1990). Therefore, the neonate is even more susceptible to the toxicity of prilocaine, and its safety in this age group has not been previously investigated. Our study is the first to measure lidocaine, prilocaine, o-toluidine, and methemoglobin levels simultaneously after intravenous bolus administration of lidocaine and prilocaine and topical penile administration of EMLA in a neonatal model.

We chose to evaluate the pharmacokinetics of EMLA and the production of methemoglobin in the piglet model because of its similarities to the neonate in terms of size and weight (allowing the use of a comparable dose of drug) as well as in physiology (Book et al., 1974; Glauser, 1966) and metabolic handling of drugs such as local anesthetics (Lim et al., 1992). Mets et al. (1993) investigated the pharmacokinetics of lidocaine in pigs and found similar metabolites as have been identified in humans. The reported Cl was 24 ml/min·kg, similar to the Cl obtained in this study.

The production of both o-toluidine and methemoglobin in the piglets in this study as well as the finding of similar pharmacokinetic parameters for lidocaine and prilocaine as have previously been reported in humans further support the use of this model: Arthur et al. (1979) reported an elimination \( t_{1/2} \) of approximately 1.5 h and a Cl of 2.84 l/min for prilocaine in adult volunteers. Tucker (1986) reported a \( t_{1/2} \) of 1.6 h for both lidocaine and prilocaine and Cl values
of 0.95 l/min for lidocaine and of 2.37 l/min for prilocaine. Assuming an average adult weight of approximately 70kg, this is equivalent to a Cl of 13.6 ml/min•kg for lidocaine and of 34 ml/min•kg for prilocaine. Mihaly et al. (1978) studied the pharmacokinetics of lidocaine in neonates (gestational age 26-38 weeks; postnatal age 5-42 days) after subcutaneous administration. They found that the total plasma Cl normalized on body weight for the neonates (mean 10.2 ml/min•kg) was not significantly different from the reported adult values. The neonates had a prolonged $t_\text{d}$ (mean 3.16 h) and an increased total $V_d$ (neonate mean 2.75 l/kg; adult mean 1.11 l/kg). Neonates have higher $V_d$ values for many drugs, and this, coupled with the fact that they were all recovering from respiratory illnesses in the intensive care nursery, may account for the observed differences in drug handling. The calculated value for $t_\text{d}$ of lidocaine in this study (1.9 h) is intermediate between that reported for neonates and adult volunteers. The $V_d$ of 4.1 l/kg is only slightly higher than that in neonates, but the Cl of 26.6 ml/min•kg is about twofold greater than that of adults and neonates. As with human data, the Cl values for prilocaine were found to be higher than for lidocaine.

Our pharmacokinetic analysis was limited by the use of only three data points in some instances. Since our sampling frequency was restricted, we accepted a priori that we would not be able to characterize the distribution phases of lidocaine and prilocaine. While other of investigators (Arthur et al., 1979; Tucker et al., 1975; Benowitz et al., 1978) have demonstrated that the pharmacokinetics of lidocaine and prilocaine fit a multicompartment model, a one-compartment model was an acceptable alternative for the purposes of this study. The data fit the model well, and we were able to characterize the elimination phase of lidocaine and prilocaine from the piglet. The main limitation with the use of this model is that the $V_d$ may be
overestimated. Another limitation due to our infrequent sampling is that the maximum measured levels of lidocaine and prilocaine may not actually represent the true peak levels achieved.

The mean bioavailability of lidocaine in this study was 4% and that of prilocaine 7.2% after penile application of 1 g of EMLA for 60 min. The calculated bioavailability for lidocaine and prilocaine, however, may underestimate to some extent the true bioavailability because many determinations following EMLA application were below the quantitation limit (20 ng/ml). The mean AUC of o-toluidine after topical administration of prilocaine was 4% of that after the same dose of intravenously administered prilocaine, consistent with low systemic exposure to this metabolite. Data were recalculated using values above the detection limit (4 ng/ml) for the high-performance liquid chromatography assay. The mean bioavailability increased to only 4.4 and 7.5% for lidocaine and prilocaine, respectively.

In our study, the percent methemoglobin levels did not change after EMLA. The levels following intravenous bolus administration of prilocaine were statistically different as compared with baseline, but did not reach clinical significance. Although there are no published guidelines for the normal range of methemoglobin levels in the piglet, the levels measured by us are comparable to those reported in the healthy term human neonate where the mean level is 1.5% (range 0-2.8); methemoglobin levels are slightly higher (mean 2.2, range 0.08-4.7%) in the preterm infant (Kravitz et al., 1956). Thus, the mean maximum methemoglobin level of 1.23% achieved in our study is well within the normal range reported for human neonates.

In conclusion, the bioavailabilities of lidocaine and prilocaine and the relative exposure to o-toluidine from topical application of EMLA to the penile region of the neonatal piglet were low. The methemoglobin levels were not significantly altered, supporting the lack of significant amounts of drug absorption following EMLA application. These findings suggest that 1 g of
EMLA applied to the penile region for 1 h may be safe for neonates. A preliminary study of 14 term neonates treated with EMLA prior to circumcision showed no adverse effects, but the methemoglobin levels were not measured (Benini et al., 1993). While neonatal circumcision is one possible indication for the use of EMLA (Gazarian, 1995), there are many other procedures during the newborn period for which it may be potentially useful. However, further study is required to determine the safety of EMLA in human neonates prior to advocating more widespread usage. In particular, preterm neonates, whose skin has been shown to be more permeable to xenobiotics (Nachman et al., 1971; Harpin et al., 1983a), warrant a cautious approach.
Appendix 6. Additional Notes to Chapter 3

Methods

Inclusion Criteria

Caucasian infants were defined according to geographical origin and included infants of parents that were from Europe, India, Pakistan, Afghanistan, Arabia, North Africa, the Middle East and Asia Minor. Infants of parents from Asia (except as previously stated), Greenland, and Africa (except North Africa) were excluded. Infants born of parents of different origins (e.g., Asia and Europe) were included. Black infants were excluded from the study due to concerns that methemoglobinemia would be more difficult to diagnose in this population. It is recognized that exclusion of black infants may affect the generalizability of the results due to racial differences in skin properties (e.g., stratum corneum, melanocytes, skin hydration) that may affect the efficacy of EMLA.

Sample Size Calculation

Previous research with EMLA for circumcision pain revealed an effect size of approximately 0.8 (Benini et al., 1993). Setting a two-tailed significance level at 0.05, power of 90%, and accounting for possible drop-outs, it was estimated that 30 subjects per group were needed.

Treatment of Missing Data

Occasionally, infants moved their heads or study personnel blocked visibility of the baby and there were missing data. The percentage of missing data was very low, with only 1.98% of data missing for all facial actions across all phases. When facial actions were individually analyzed, the results were as follows: brow bulge 0.43%, naso-labial furrow 0.27%, eye squeeze
0.071%, open lips 0.03%, horizontal mouth stretch 0.004%, vertical mouth stretch 0.004%, purse lip 0.01%, chin quiver 0.011%, tongue protrusion 0.01%, and taut tongue 21.9%. The higher percentage of missing data for taut tongue was due to poor visibility of the inside of the baby's mouth because of inadequate illumination during videotaping.

All missing facial action data were replaced using two different methods. First, if 21-50% (i.e., 10 seconds) of data from a phase of the circumcision was missing, then it was assigned the mean value of the remaining data for that phase in that infant. When the majority of data were missing from a phase, then the mean value of the infants in that treatment group for that phase was used.

**Factor Analysis**

Previous research studies demonstrated that a subset of the original 10 facial actions on the NFCS were more specific to pain than others (Grunau et al., 1989; Benini et al., 1993; Stevens et al., 1996a; Rushforth et al., 1994). Accordingly, factor analysis was used to identify the facial actions that best described infant circumcision pain response. Table 30 shows the results of the factor analysis. Applying the rule of retaining factors with eigen-values of greater than 1, one factor was identified (with a value of 3.86). This factor accounted for 64.3% of the variability in infant response and included 6 of the 10 facial actions.
Table 30. Factor Analysis Results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Factor Loading</th>
<th>Factor Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brow bulge</td>
<td>0.900</td>
<td>0.233</td>
</tr>
<tr>
<td>Taut tongue</td>
<td>0.842</td>
<td>0.218</td>
</tr>
<tr>
<td>Eye squeeze</td>
<td>0.893</td>
<td>0.231</td>
</tr>
<tr>
<td>Naso-labial furrow</td>
<td>0.909</td>
<td>0.235</td>
</tr>
<tr>
<td>Open lips</td>
<td>0.658</td>
<td>0.171</td>
</tr>
<tr>
<td>Vertical mouth stretch</td>
<td>0.531</td>
<td>0.138</td>
</tr>
</tbody>
</table>

A composite measure of infant facial activity was also derived from the analysis. The 6 facial action proportion scores were weighted by factor coefficients obtained in the model (see table above). Then the weighted proportion scores were summed together for an overall facial action pain score. This method of summarizing NFCS variables has been used by the original investigators of this tool in previous studies (Craig et al., 1994; Craig et al., 1993).

**Results**

RM MANCOVA revealed a significant treatment effect ($F[1,55]=6.98$, $p=0.01$); within subjects, circumcision stage effects ($F[6,50]=5.63$, $p<0.001$) and treatment-by-stage effects ($F[6,50]=4.24$, $p=0.002$) were observed. Univariate analyses revealed that EMLA-treated infants had lower facial action pain scores ($p<0.001$) during four of the circumcision stages: forceps application, dorsal incision, application of clamp, and foreskin cutting.

RM MANCOVA revealed a significant treatment effect for percent cry ($F[1,55]=18.14$, $p<0.001$). Circumcision stage effects ($F[6,50]=37.63$, $p<0.001$) and treatment-by-stage effects
(F[650]=5.80, p<0.001) were also observed. Univariated F-tests revealed that EMLA-treated infants cried less during six of the circumcision phases.

RM MANCOVA revealed a significant treatment effect for heart rate (F[1,35]=8.09, p=0.007). Circumcision phase effects (F[6,30]=51.19, p<0.001) and treatment-by-phase effects (F[6,30]=4.37, p=0.003) were also observed. Univariate analysis revealed that infants treated with EMLA had lower heart rate changes during dorsal incision, application of clamp, pulling skin through clamp, tightening clamp, and cutting foreskin.

We also investigated whether infant delivery characteristics were important factors of pain response because a previous study suggested that obstetric medications and mode of delivery may influence infant pain response during routine heel lancing (Grunau et al., 1989). We demonstrated no significant effects from delivery characteristics such as mode of delivery, gestational age, and birthweight, or time of last feeding, on circumcision pain responses.
Appendix 7. Additional Notes to Chapter 4

Results

The mean difference vaccination pain scores in infants circumcised with EMLA were; 115.3% for facial action, 40.7% for cry duration, and 3.3 cm for VAS scores.
Appendix 8. Additional Notes to Chapter 5

Methods

Laboratory Analysis

Cytochrome b5 is responsible for reducing methemoglobin and is maintained in a reduced state by NADH through the activity of methemoglobin reductase. Since it is difficult to obtain cytochrome b5, the activity of methemoglobin reductase was measured by following reduction of ferricyanide by NADH in erythrocyte hemolysates (Beutler, 1984). This is a well established method of determining NADH methemoglobin reductase activity. The procedure described by Beutler (1984) was used for this study. Blood samples anticoagulated with EDTA or heparin were collected for the analysis. White blood cells and plasma were separated from red blood cells using filter paper, normal saline washing, and cold centrifugation. The red blood cells were stabilized using a beta-mercaptoethanol-EDTA stabilizing solution and then lysed in dry ice. NADH and potassium ferricyanide were added to the hemolysates and the oxidation of NADH was measured at 340 nm.

The oxidation of NADH was measured over the first 10 minutes at 37 degrees centigrade using the Cobas Fara II (Hoffmann-LaRoche, Montclair, NJ). The reaction was observed to be linear under these conditions. The enzyme activity was expressed in international units (IU) of NADH-ferricyanide reductase per gram of hemoglobin. The within-day coefficient of variation varied from 1.7% to 9.3% for replicate analyses.