DRUG RESEARCH IN PEDIATRICS: OVERCOMING THE ETHICAL CHALLENGES

by

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A thesis submitted in conformity with the requirements for the degree of MSc.

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ABSTRACT

Drug Research in Pediatrics: Overcoming the Ethical Challenges

by

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The American Academy of Pediatrics recognized that failure to conduct pediatric drug research may deprive children of significant therapeutic advances. However, several ethical questions need to be addressed if we continue to undertake pediatric drug research. In studies requiring repeated blood sampling, issues of potential risk, invasiveness, and pain management strategies need to be addressed. To characterize the pharmacokinetic parameters of amlodipine in children, a population pharmacokinetic design was utilized in order to address the issue of repeated blood sampling. Subsequently, a comparative bioavailability study of two formulations of amlodipine was completed in adult volunteers instead of a pediatric population due to ethical concerns. In order to offer additional pain treatment options for children before blood sampling procedures, the topical local anaesthetic amethocaine was studied in adults. This thesis attempts to show that research in children is ethically defensible, but adults must sometimes be used to answer pediatric research questions.
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ABBREVIATIONS

AAP: American Academy of Pediatrics
ACE inhibitor: Angiotensin Converting Enzyme Inhibitor
ANOVA: Analysis of Variance
AUC: Area Under the plasma drug concentration-time Curve
CCB: Calcium Channel Blocker
Clapp.: Apparent Clearance
Cmax: Concentration at Maximum
CYP: Cytochrome P450
DBP: Diastolic Blood Pressure
EMLA: Eutectic Mixture of Local Anaesthetics
FDAMA: Federal Drug Administration Modernization Act
HSC: Hospital for Sick Children
ICH: International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
k: Terminal Elimination Rate Constant
NIH: National Institute of Health
PI: Principal Investigator
PK: Pharmacokinetic
PPK: Population Pharmacokinetic
PPRU: Pediatric Pharmacology Research Units
REB: Research Ethics Board
SBP: Systolic Blood Pressure
$t_{1/2}$: Elimination Half-life
Tmax: Time to Maximum
VAS: Visual Analog Scale
Vdapp.: Apparent Volume of Distribution
VRS: Verbal Rating Scale
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SECTION 1: BACKGROUND

1.1 The Need for Research in Children

Failure to conduct pediatric drug testing may deprive children of significant therapeutic advances.

As a consequence, there is now a moral imperative to formally study drugs in children so that they can enjoy equal access to existing as well as new therapeutic agents.(1)

"Children as a special population must be included in the developmental phases of therapeutic products so that potential beneficial or detrimental effects of therapeutic interventions are determined, that age-appropriate dosing regimens are developed, that the impact of physiological and maturational changes on drug pharmacokinetics are assessed, and that the potential drug interactions, which may occur with drug regimens specific to the pediatric population are explored."(2)

Such a statement has stemmed from the realization that about 80% of approved drugs lack dosage information for children or contain pediatric disclaimers. This situation exposes children to greater risks, deprives them of potential benefits, and creates unfair differences compared to adults in terms of information guiding the use of a drug.(3) As such, children have been labelled “therapeutic orphans”, a term coined by Dr. Harry Shirkey in 1968 because they are being experimented on outside formal clinical trials by being administered medicines for which there are no adequate studies in pediatrics.(4) Despite the recognition of these facts, many drugs continue to be approved without being evaluated in children.

The exclusion of children from clinical trials partly resulted from the 1962 Harris-Kefauver Act following the thalidomide tragedy. This Act mandated all investigational drugs to proceed through various phases of clinical testing before regulatory approval. The Act also excluded pregnant women and children from participation in clinical trials.(5) Other reasons for the exclusion of children from drug research are the small size of the pediatric patient population, and the increased cost of pediatric drug studies, partly because drugs need to be tested across the developmental age
span. The consequence is that pediatric health care providers are forced to use most drugs "off-label" based on experience, estimated calculations, or case studies. Off-label use of a drug refers to the use of an approved drug for either a non-approved indication or in a population for whom the drug is not specifically approved.(6) A main reason why off-label prescribing is acceptable in children, is that by failing to prescribe the medication, the physician may be denying the child an effective intervention.(7)

Another reason why drugs lack pediatric indications is the difficulty in recruiting pediatric subjects. Many parents are not willing to have their children participate in biomedical research, mostly because of ethical constraints, such as the duty to protect children's vulnerability, and a general lack of societal acceptance. However, this view is relatively new, considering that prior to the 20th century, children were used as research subjects in experiments that carried considerable risk.

1.2 Historical Perspective of Research in Children

Probably the first significant recorded event of childhood experimentation involved smallpox vaccination by Edward Jenner in the 1700's. Other significant events that followed include the use of incapacitated or mentally challenged children in studies that were conducted to test the new germ theory, and consisted of infecting these children with syphilis, gonorrhea, and scarlet fever. Children in hospitals or orphanages were also infected with tuberculosis, measles, mumps and diptheria in an attempt to develop vaccines for these diseases. With the invention of the X-ray in the mid-1900's, children were used in serial x-ray experiments where radioactive iron and calcium were administered. During World War II, many children were brutally experimented upon by Nazi doctors. More recently, from 1955 through to the 1970’s, institutionalized children at Willowbrook State School in New York were deliberately infected with hepatitis virus in order to determine the effects of a vaccine.(8-10) Over time, the criticism that grew among society regarding the use of
children as guinea pigs led to the protection of children from abuse and exploitation through formal guidelines that outlined the practice of ethical biomedical research in humans.

1.3 Summary of Guidelines Governing Research in Children

The first legal document containing guidelines for research in human subjects was the Nuremberg Code, written in 1947. This code laid down 10 standards to which physicians must conform when carrying out experiments on human subjects, and established a new standard of medical behaviour. The code explicitly states that the person involved in the research must have the legal capacity to give consent, and “should have sufficient knowledge and comprehension of the elements of the subject matter involved as to enable him to make an understanding and enlightened decision.”(11) This statement does not support pediatric research, since children are not able to provide legal informed consent. In 1964, the Declaration of Helsinki remedied this oversight in the 11th basic principle: “when the subject is a minor, permission from the responsible relative replaces that of the subjects in accordance with national legislation.”(12)

More recently, the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), developed guidelines for the clinical investigation of medicinal products in the pediatric population, explicitly stating that pediatric patients should be given medicines that have been appropriately evaluated for their use.(13) With these new guidelines in place, the conduct of clinical drug trials in children became ethically feasible, and could be carried out if appropriate steps were taken to ensure that children were protected from unnecessary harm.

In October 1992, the FDA took steps to improve the quantity and quality of pediatric information available on drug labels. New regulations were designed to promote the inclusion of clinical trials,
published studies, and case reports in children, in an effort to provide basic dosing and monitoring information. In 1993, the National Institute of Health (NIH) helped fund a network of Pediatric Pharmacology Research Units (PPRU’s) in university teaching hospitals to conduct studies in children, and to serve as a resource for pediatric investigators.(4;14)

Further progress was made in December 1994, when the FDA mandated pediatric labelling information to be included in all pertinent New Drug Applications.(15) However, this rule only encouraged drug manufacturers to submit pediatric data voluntarily, and did not provide incentives to conduct such studies. As a result, manufacturers perceived this ruling as a roadblock to drug approval, and many drugs continued to be approved without pediatric data. To gain more support for this rule, the FDA Modernization Act (FDAMA) was enacted in November 1997, and included new provisions for improved information about drug use in pediatrics. These provisions would extend by 6 months any existing exclusivity or patent protection on a drug for which the FDA has requested pediatric studies to the manufacturer, in exchange for pediatric clinical trials. This incentive aims to offset the economic imbalance of a limited market versus development.(4) In addition, the development of a special formulation for some drug products such as a liquid or chewable tablet was required to enable children to take the drug as prescribed.(16)

The FDA can waive the requirement for pediatric information if it is deemed that the product is likely to be unsafe or ineffective in pediatric patients, if pediatric studies are impossible or highly impractical, or if reasonable efforts to develop a pediatric formulation fail.(17) Unfortunately, the rule provides no incentive to conduct studies on drugs that are off-patent. This will undoubtedly force manufacturers to conduct pediatric studies on only those drugs for which the incentives are most valuable, such as drugs with large sales.(18) Moreover, the approval of a drug will not be
delayed if pediatric studies have not been completed. However, this potential drawback is being recognized. In August 2001, the Health, Education, Labor and Pensions Committee announced that it wants to create a fund to help underwrite pediatric clinical trials for drugs already gone off-patent.(19)

Since 1997, the pediatric exclusivity program, which will expire in 2002 if legislation is not renewed, has become very active in initiating new medicinal research in children. Supporters of the program maintain that it encourages pediatric research for which there is little other incentive. Opponents of the program, mainly generic drug companies and groups of senior citizens, argue that it secures huge profits for brand name manufacturers, locks generic firms out of the market for longer periods of time, and provides little benefit to the public. This is especially true for seniors who will continue to pay prime rates for another 6 months.(20;21) However, it is evident that both children and physicians are reaping benefits. Since the program’s inception, the FDA has received 218 proposals from companies hoping to take advantage of the program, and 18 new labels with pediatric indications have already been approved. Several of those labels include significant changes in dose recommendations for children.(22)

This thesis will address major ethical dilemmas that have been a barrier to clinical drug research in pediatrics, and show that drug research in children is ethically defensible. It will also attempt to show that pediatric research questions can sometimes be answered by conducting research in adults.
1.4 Should Children be Research Subjects?

This question has always been a major ethical dilemma for scientists and bio-ethicists because of the inability of a child to provide fully informed consent. Conditions under which children can be allowed to participate in research include: 1) cases where there is minimal risk of harm 2) if benefit to the child or society is anticipated, and 3) if the research procedures are invasive, there cannot be more than a minor increment over minimal risk.(23) In order to understand and satisfy these conditions, it is important to determine whether the research is therapeutic or non-therapeutic, and to assess the level of risk that will be imposed on the child.

1.4.1 Therapeutic and Non-Therapeutic Research: Which is Acceptable in Children?

Research is considered therapeutic if it produces benefits to the participating subject, as well as contributes to general knowledge of the disease or condition. Non-therapeutic research does not promote the welfare of subjects, but the research results should secure generalizable knowledge.(24;25) In the case of children, non-therapeutic research procedures raise distinctive moral issues since there is no benefit to the child for participating.

Some argue that non-therapeutic research teaches children to be altruistic, and contributes to their moral education.(8) It has also been stipulated that children have a social obligation to participate in research, thus justifying potential risk to the individual child because of the possible contribution to the good of all children.(26) In contrast, Part III, No. 4 of The Declaration of Helsinki states “in research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject,” suggesting that societal obligations cannot be considered.(12) Despite this, non-therapeutic research in children has been accepted if it was
demonstrated that the research would produce no more than *minimal* risk, defined as being equal to what the child experiences in everyday life, or in a routine medical, dental, psychological, social or educational situation. Thus, minimal risk is considered “baseline risk”, because it is assumed to be equal to the normal course of a child’s everyday life.(8;27-29) But even here, problems emerge regarding the limits of risk and discomfort to which children may be exposed for reasons unrelated to their own welfare.(30) To address this issue, the terms ‘therapeutic’ and ‘non-therapeutic’ have been replaced with a conceptualization of levels of risk to the child.

### 1.4.2 Assessment of Research Risk in Children

An assessment of research risk in children differs from an assessment of such risk in adults. One should not only consider the risks associated with the clinical procedures of the study, but also the level of discomfort, inconvenience, pain, fright, separation from parents or familiar surroundings, and the size and volume of biological samples.(1) Children react differently to experiences of being in a medical setting, perceive pain differently, and are more anxious of common medical procedures. The risk in any pharmacological research study will primarily depend on whether the child has a medical condition that needs to be treated or whether the child is healthy.

Children with a specific disease may benefit from participating in research that has been designed to benefit their illness, or may contribute directly to the benefit of other children with the same condition. For this reason, pediatric clinical drug research that is being conducted in Canada uses a population of sick children who have a need for the drug in question. Guidelines from the Royal College of Paediatrics state that such research procedures in sick children are not necessarily unethical.(31) On the other hand, using healthy children has created a fundamental moral dispute of whether they should be used in research at all. This is because subjecting a child to a drug where a medical need has not been established, may present an increase over minimal risk because of the
potential for adverse events. (32) According to the Therapeutic Products Directorate Guidelines published by Health Canada, “trials in healthy pediatric subjects are not usually recommended, with the exception of vaccines and prophylactic products”. (2) Since healthy children will not receive any direct benefit from the experiment, the only incentive to participate would be either financial rewards or the contribution to society as a whole. However, children are not allowed to receive monetary awards for research participation. (33) Compensating children with gifts that are acceptable to them and not necessarily to the parents has become standard practice and is considered appropriate by Research Ethics Boards (REB). (34)

In any study, potential research risk cannot be assessed in advance for all child subjects. Once a child has been identified as a possible research participant, a separate assessment of the likely reactions of the individual child to the proposed procedures should always be made. For example, even though a sick child may be used to certain medical procedures, the reactions of some sick children may be intensified by their debilitated condition. In most cases, the parent is a good judge of the mental condition of the child and may decide whether the child is appropriate for the study.

1.5 Invasiveness of Repeated Blood Sampling in Children

The number of blood samples required in order to complete a drug study has always been a crucial determinant of whether the study will be approved by REB’s or not, and formal drug trials have been refused on ethical grounds because of blood sampling requirements. (35) For the purposes of this thesis, the number of blood samples taken to collect pharmacokinetic (PK) data will be the focus, however, the recommendations made could be applied to other studies in children.
In order to collect PK data, repeated blood sampling has to be performed. This is difficult to justify in most instances due to restrictions on the amount of blood that can be taken from a child and the level of risk and discomfort. This dilemma has greatly contributed to the lack of drug studies in children.(6) Currently, two approaches exist for collecting blood for PK trials: the traditional PK method, and the population pharmacokinetic (PPK) method. The subjects of traditional PK studies are usually healthy volunteers, and the research involves administering either single or multiple doses of a drug to a relatively small group of subjects. Subsequently, subjects are sampled repeatedly over specified time intervals. In contrast, PPK’s is the study of the sources and correlates of variability in drug concentrations among individuals who are representative of the target population to be treated with clinically relevant doses of the drug. It also treats individuals represented in the study as a random sample from a large population, therefore a relatively large number of patients are needed, but only a few plasma concentration-time points are required per individual.(36;37) Another difference between the PPK and the traditional PK design, is that when a PPK study is proposed, certain preliminary PK information and the drug’s major elimination pathways should already have been completed in adults. This is because a PPK analysis requires previous assumptions about the individual PK parameters for a drug from a known population, usually adults. Only then can it provide an estimate of PK parameters based on limited data. (38)

In pediatrics, the PPK approach is preferable if enough patients are available, because it poses fewer issues concerning the volume and frequency of blood withdrawal. The possibility of being able to collect sparse data is more ethical, and samples may be taken during routine bloodwork or clinic visits, which do not rely on explicitly defined time intervals.(39) However, when repeated blood sampling is required, insertion of an indwelling venous line is necessary to minimize repeated
needle insertions. A local anaesthetic cream should also be applied beforehand to reduce the pain of needle-stick.

1.6 Ethics of Pain Management in Children

Ethical issues surrounding pain management in children began to be seriously considered only in 1985 when the mother of a premature infant discovered that her baby had not received anaesthesia for the repair of a patent ductus arteriosus. This case and many others are the result of basing pain management in children on myths rather than scientific evidence. The biggest myth was that infants were unable to feel pain because of an immature or incompletely myelinated nervous system that did not allow for transmission of noxious stimuli from the site of injury to the central nervous system. Secondly, it was thought that if infants and children were exposed to injurious stimuli, they would not remember it. However, both term and preterm infants have the anatomic and functional capacity for mounting a response to a noxious stimulus and do possess pain perception even at birth.

In this thesis, the focus of pain management in children will be on the administration of local pain relief from venipuncture by a topical anaesthetic. Topical anaesthetics work by blocking nerve conduction, therefore have the ability to stop the transmission of pain completely. This makes them ideal for reducing pain associated with many local procedures. When a topical anaesthetic is applied, it must penetrate the intact skin to produce anaesthesia, and this process is generally slow. Because of this, inadequate pain management associated with blood draws has mostly been blamed on lack of time. Many nurses claim that they do not have the time to prepare children for venipuncture, and in most cases, blood is taken without a local anaesthetic.
Another reason for the lack of adequate pain management in children is the difficulty of assessing pain in a child. Various pain rating scales have been developed and are used in clinical practice in order to facilitate pain diagnosis, define the impact of procedures on the individual, and determine the efficacy of pain management interventions. However, limited cognitive and language capabilities of children hinder their ability to express pain in a way that is meaningful, and caregivers are not always certain if child behaviours during medical procedures are signs of pain.(40) In fact, children mostly depend on their parents to infer pain from their behavioural and physiologic indicators.(43)

One example of a scale used frequently in pain research is the Visual Analog Scale (VAS). It is a horizontal 10cm line with word anchors at the extremes, usually “no pain” on the left end and “the worst pain you can imagine” on the right end. The patient is asked to make a mark along the line to represent pain intensity. This scale is easy to administer and has documented validity. A pain score is obtained by measuring in millimeters up to the point the patient has indicated.(43) However, self-reported pain is not always correlated with behavioural distress in children. This is because distress responses may not only indicate pain, but also fear, anxiety, separation from parents or some other phenomena, with the result that pain assessment in children becomes confused with these factors.(45)

In research with novel pain treatment interventions such as local anaesthetics, conducting the research in adults first is more acceptable ethically. This is because if it is unknown whether a new agent is effective, it is unethical to subject children to unnecessary pain if other pain treatment strategies are available.(40) Further, because a novel drug would be administered, the incidence of adverse reactions must first be assessed in order to avoid exposing the child to an increase over
minimal risk. Lastly, the difficulty in assessing pain responses in children may result in not being able to adequately assess the efficacy of the anaesthetic.

The efficacy of new anaesthetic agents is also often assessed by administering placebos. Usually, this is done to determine between organic or psychologic pain.(46) In these circumstances, the patient must be told that a placebo will be used in the informed consent form, but it is not necessary to tell the patient exactly when the placebo will be administered, which incorporates a component of deception and secrecy into the study protocol.(43) In children, it is very difficult to justify the use of a placebo, because it is generally assumed that a child is not able to comprehend its purpose.

Secondly, the researcher would be deliberately administering an inert or sub-therapeutic substance, possibly causing harm.(46) Further, the AAP has listed conditions under which a placebo may be used in a child. Briefly, these conditions state that a placebo may be used in a child when there is no other commonly accepted therapy, and/or the agent under study is either of questionable efficacy or carries with it a high frequency of undesirable side effects. (1)

Pain has been labelled a research priority, and research endeavours have focused on the development of valid assessment tools to measure pain in children, testing of effective interventions, as well as a comparison of costs and benefits.(41)

The study in section 4 focuses on the testing a new topical anaesthetic agent in adults for future application in children.
SECTION 2: THE PEDIATRIC USE OF AMLODIPINE IN THE TREATMENT OF HYPERTENSION: A POPULATION PHARMACOKINETIC TRIAL

This clinical trial was sponsored by Pfizer Central Research, Groton, CT

1.7 BACKGROUND

1.7.1 Pediatric Hypertension

The incidence of hypertension in the pediatric population varies from 0.5% to 11%, depending upon the age group and the criteria used to define hypertension, however, the causes differ significantly from those in adults. Hypertension in children has been defined as average systolic blood pressure (SBP) and/or diastolic blood pressure (DBP) that is between the 90th and 95th percentile for age, sex and corrected to height on at least three different examinations. Significant hypertension is defined as average SBP and/or DBP equal to or greater than the 95th percentile. The correction for height allows consideration of different growth rates in children, and permits a more precise classification of blood pressure according to body size.

Pediatric hypertension is often due to an identifiable disease process. Approximately 70% of children with elevated blood pressure have a renal abnormality, thus antihypertensive therapy has become an important component in the care of most pediatric nephrology patients. Other causes of hypertension include coarctation of the aorta, cardiovascular diseases, endocrine diseases or drugs.

1.7.2 Pharmacologic Treatment in Childhood Hypertension

In most cases of childhood hypertension, pharmacologic intervention needs to be carefully weighed with non-pharmacologic approaches and should only be initiated if absolutely necessary. In cases of significant hypertension, therapy is usually begun with a single agent, and if blood pressure
does not decrease to the 90\textsuperscript{th} percentile for age and sex, a second agent is usually added after the maximum dose is given.(53)

In the past, diuretics and beta-blockers were the initial steps of therapy for the hypertensive child, but angiotensin converting enzyme inhibitors (ACE inhibitors) and calcium channel blockers (CCB) have recently become first-line therapy due to the lower incidence of side effects.(54) However, their use is limited in the pediatric population mostly because of the tablet or capsule formulation.(55) Other reasons are a short half-life and duration of action necessitating multiple daily dosing, and low absolute bioavailability.(51)

Agents such as nifedipine, felodipine, amlodipine and nicardipine are the most commonly prescribed CCB's in children. However, nifedipine is only given to children in acute crises due to its rapid absorption and immediate mode of action, limiting long-term treatment. Extended-release CCB's are available, but the dosage form cannot be broken down for pediatric use because it disrupts the delivery system.(55;56) Other classes of CCB's such as the phenylalkylamine (verapamil) or benzothiazepine (diltiazem) derivatives are rarely used in children due to the high incidence of adverse effects.(57) Amlodipine is a novel oral CCB worth considering in the pediatric population since it has a long elimination half-life ($t_{1/2}$) allowing once-daily dosing, and can be prepared in an alternate formulation such as a liquid or suspension.(58)

1.7.3 Amlodipine

Amlodipine was designed in an effort to make a compound that produced typical dihydropyridine effects, such as nifedipine, but that would also exhibit an increased bioavailability and a prolonged duration of action.(59) Hence, amlodipine is a structural analog of nifedipine and has a similar mechanism of action (Figure 1). In contrast to nifedipine, amlodipine is not photolabile because it
does not contain a nitro substitution, resulting in greater stability. Amlodipine has also been formulated as a non-sustained release product, meaning that it can be divided into smaller doses without affecting its pharmacokinetics. It is considered an oral second generation 1,4-dihydropyridine CCB that is approved for use in adults for the treatment of hypertension or angina pectoris in doses of 5 to 10 mg daily.

Figure 1: Chemical structures of amlodipine and nifedipine

Amlodipine inhibits calcium (Ca\(^{2+}\)) ion flux into cardiac and vascular smooth muscle cells through voltage-dependent L-type Ca\(^{2+}\) channels, so-called because when activated, these channels have a large ion carrying capacity. They also inactivate relatively slowly, thus remain open for long periods of time. The L-type channel complex is an oligomeric structure with a total molecular weight of around 400kD, and contains 5 subunits (\(\alpha_1\), \(\alpha_2\), \(\beta\), \(\gamma\), and \(\delta\)). The \(\alpha_1\) subunit provides the central pore of the channel, and also contains high affinity binding sites for the dihydropyridine-based CCB's (Figures 2 and 3).
The dihydropyridines are unique in that they block vascular smooth muscle Ca\textsuperscript{2+} channels at concentrations below those required for cardiac Ca\textsuperscript{2+} channel blockade, and may be more effective in their smooth muscle action than their cardiac action.\textsuperscript{(60)} The blood pressure lowering effect is therefore the result of the vascular smooth muscle relaxing effects.\textsuperscript{(59)}

Amlodipine also has high aqueous solubility and a pKa value of 8.6 due to the presence of the amino side chain in the 2-position of the dihydropyridine ring, meaning that more than 90\% of molecules are ionized at physiological pH. The other dihydropyridines are neutral. This may account for amlodipine’s unique binding to the Ca\textsuperscript{2+} channel since it cannot penetrate through the hydrophobic regions of membrane lipid bilayers as an ionized molecule.\textsuperscript{(59)} In addition, amlodipine has a slow onset and offset from the channel due to charge pairing at the receptor site, which is also channel state independent. This is in contrast to other dihydropyridines, where there is a preferential interaction with depolarized states of the channel, resulting in diffusion and partitioning of the ligand into the membrane lipid phase.\textsuperscript{(61)}
1.7.4 Pharmacokinetics and Metabolism

The advantages of using amlodipine result from its unique pharmacokinetic properties, which are believed to be important when considering its use in the pediatric hypertensive population. A major advantage is the long $t_{1/2}$ of about 34 hours after a single oral dose and 45 hours after repeated daily dosing, which does not depend upon the use of a slow release formulation. The long $t_{1/2}$ is attributed to amlodipine's high degree of ionization and slow onset of action at the L-type Ca$^{2+}$ channel, hindering approach to binding sites.

After oral administration, amlodipine is slowly but completely absorbed from the gut, and has an oral bioavailability of 64%, indicating some first-pass metabolism. It accumulates in the liver and is redistributed into the circulation at a relatively slow rate. Peak drug concentrations are not achieved until after about 8 hours post-dose, ranging from 7-9 hours, and rise slowly due to its slow absorption. This property eliminates some of the adverse events such as reflex tachycardia and extracellular fluid volume retention, commonly seen with other antihypertensives when peak drug concentrations are reached too quickly. Amlodipine does not disturb atrioventricular
conduction, and this property contributes to its improved safety profile. Limitations such as an increased risk for cardiovascular events seen with the CCB's are not apparent in long-acting agents such as amlodipine.\(^{(59)}\)

The volume of distribution (Vd) is 21 L/kg, indicating that most of the drug goes into body reservoirs and is slowly released upon equilibration.\(^{(60)}\) Meredith and Elliott (1992) hypothesized that the high Vd is probably associated with the positively charged basic side-chain on the dihydropyridine ring, allowing a strong electrostatic interaction between the drug and phospholipid molecules of the lipid bilayer.\(^{(67)}\)

When given once-daily, amlodipine shows minimal fluctuations between peak and trough plasma concentrations, suggesting the maintenance of a sustained antihypertensive effect over a 24-hour period.\(^{(64)}\) Its low plasma clearance (Cl) of 7ml/min/kg contributes to the minimal fluctuations, and also accounts for its accumulation in plasma after steady-state dosing. Steady-state drug concentrations are reached after about 7 days. As well, amlodipine’s antihypertensive effect is well correlated with plasma concentrations, and it has been shown to achieve similar pharmacodynamic responses at all dosage levels.\(^{(63;68)}\)

Elimination is by the cytochrome P450 3A4 enzyme (CYP3A4) in the liver, but this process is slow. This is an advantage to patients who have secondary hypertension due to renal dysfunction since the dose does not need to be adjusted.\(^{(55)}\) In addition, the pharmacokinetics of amlodipine are also not affected by clinically significant states of renal impairment, making it the preferred agent in patients with impaired renal function.\(^{(58)}\) However, in patients with liver disease, the t\(_{1/2}\) is
significantly prolonged.\(^{(68)}\) None of the metabolites have any significant calcium antagonist activity relative to amlodipine.\(^{(69)}\)

Drug interactions are a possibility, considering that oxidation of 1,4-dihydropyridine into pyridine by CYP3A4 is the main metabolic pathway of amlodipine, and it is 97%-99% protein bound. Yet, studies with many drugs such as beta-blockers, cimetidine, thiazide diuretics, ACE inhibitors, other CCB's, sublingual nitroglycerin, warfarin, non-steroidal anti-inflammatory drugs, antibiotics, digoxin, and oral hypoglycemics have found no drug-drug interactions.\(^{(59)}\) An explanation may be that amlodipine's rate of oxidation may be slower than that of other CCB's. This is suggested by its less extensive first-pass metabolism.\(^{(54)}\) Secondly, amlodipine is probably not biotransformed in the intestinal wall compared to other CCB’s.\(^{(70)}\) Thirdly, CCB’s such as verapamil also inhibit P-glycoprotein mediated drug transport, which may alter intestinal absorption of several drugs. \textit{In vitro} data suggest that amlodipine is not transported by and does not inhibit P-glycoprotein.\(^{(71)}\) Ma et al. (2000) conducted a study that characterized the \textit{in vitro} inhibition profiles of six CCB’s including amlodipine on the cytochromes P450 enzymes 3A4, 2D6 and 2C9 in human liver microsomes. The study commented that the relatively fewer cases of drug interactions with amlodipine are possibly because the IC50 values obtained in the presence of NADPH were >200-fold higher than reported amlodipine plasma concentrations after a therapeutic dose.\(^{(72)}\)

There has been some controversy regarding the levels of cyclosporine in pediatric transplant patients on concomitant therapy with amlodipine. Some studies have reported no changes in cyclosporine blood levels, while others have reported an increase, suggesting that amlodipine may be inhibiting the CYP3A4 enzyme.\(^{(56;57)}\)
Administration of amlodipine with food does not alter its pharmacokinetics. (73) One study demonstrated that co-administration of amlodipine with grapefruit juice inhibits its first-pass metabolism thus increasing plasma drug levels. However, this study also claimed that the effect was much smaller compared to other dihydropyridine class compounds. (74) In contrast, a recent study by Vincent et al. (2000) concluded that grapefruit juice had no effect on amlodipine pharmacokinetics. (75)

Figure 4: Schematic representation of the main factors involved in the slow onset and sustained action of amlodipine

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1.7.5 Adverse Effects

Amlodipine is generally very well tolerated in both adult and pediatric populations, with a lower adverse event profile compared to other CCB's. (56;57) Many of the serious side effects seen with other CCB's such as reflex tachycardia, peripheral edema, adverse effects on atrioventricular conduction and myocardial contractility are not reported with amlodipine, probably because it takes about 8 hours to reach peak plasma levels. (63) Adverse events such as peripheral edema, headache,
fatigue, flushing and dizziness have been reported with amlodipine use. These side effects are attributed to its property as a vasodilator and are due to a reduction in peripheral resistance. (66)

Peripheral edema is probably the most serious adverse effect, however it is usually mild or moderate and rarely requires treatment withdrawal. Haria & Wagstaff analyzed 40 double-blind placebo controlled studies and found that adverse events were reported by 30% and 22% of patients receiving amlodipine and placebo, respectively. Treatment withdrawals were also similar in both groups. (60)

Data from pediatric studies report that the adverse event profile is similar to adults, although edema linked with flushing and headache are the most common. Specific adverse events from recent pediatric studies include fatigue, headache, facial flushing, dizziness, peripheral edema, vomiting, abdominal pain, and constipation. However, these were reported to be mild, and most resolved once blood pressure control was achieved. (55-57)

1.7.6 Use of Amlodipine in Children

Amlodipine has the most widespread use in children out of all the CCB's, despite the fact that there have only been 5 studies to date investigating the use of amlodipine in children. These studies used a sample size between 15 and 55 patients ranging from 15 months to 19 years. All reported a reduction in blood pressure to below the 95th percentile with amlodipine use, and concluded that amlodipine was safe and effective in the pediatric population. (55-57; 76; 77)

The first study to demonstrate the antihypertensive properties of amlodipine in children was conducted by Pfammater et al. in 1998. The study assessed the effects of amlodipine in 28 pediatric hypertensive patients between the ages of 3 and 19 years. Out of the 28 patients, 12 were being
treated with other antihypertensive medications, and their medication schedule continued during treatment with amlodipine. Amlodipine was started once-daily at a dose of 5mg. The dose was adjusted to 10mg if blood pressure did not decrease below the 95th percentile after 3 weeks. Amlodipine was withdrawn in 5 patients who experienced edema and flushing. Blood pressure was significantly reduced in the remaining patients. (56)

A retrospective analysis of 15 pediatric bone marrow transplant patients who had amlodipine incorporated into their antihypertensive drug regimen revealed significantly lower blood pressure values after amlodipine therapy compared to baseline; 123.5 ±2.1mmHg and 117.2 ±2.2mmHg, SBP before and during amlodipine, p<0.05: 81.5 ±1.8mmHg and 75.5 ±2.6mmHg DBP before and during amlodipine, P<0.05. The average initial dose of amlodipine was 0.12mg/kg/day with a mean maximum dose of 0.16mg/kg/day. Four patients had their amlodipine dose increased. Two patients experienced ankle edema during amlodipine treatment, which led to discontinuation of the drug. (55)

Tallian et al. (1999) determined the efficacy of amlodipine in 21 pediatric patients, with a mean age of 13.1 years. Amlodipine was administered once-daily at a starting mean dose of 0.07 ±0.04mg/kg/day in newly diagnosed patients, or was substituted for, or added to poorly controlled or intolerant existing therapy. The amlodipine dose was increased 25% to 50% every 5 to 7 days if the mean blood pressure measurements remained above the 95th percentile. The mean titrated dose required to control blood pressure was 0.29 ±0.11mg/kg/day for children under 13 years of age, and 0.16 ±0.11mg/kg/day for children over the age of 13 years, suggesting that higher doses of amlodipine were required for younger patients. (57)
Rogan et al. (2000) compared the efficacy of amlodipine to felodipine or nifedipine in 11 hypertensive pediatric patients between the ages of 9 to 17 years. Ten children were renal transplant patients. Each patient was randomized to receive amlodipine in an oral liquid formulation, or the CCB they had been taking prior to the study (nifedipine or felodipine) in two separate 30-day treatment periods. The approximate initial dose of amlodipine was 0.10mg/kg. If the patient’s blood pressure did not decrease below the 95th percentile after 1 week of therapy, the dose was increased by 50-100% to a maximum of 10mg. The study found no significant differences in blood pressure between each treatment period, suggesting that amlodipine is as effective as nifedipine or felodipine. However, amlodipine may be optimal because it can be delivered in a liquid formulation.(76)

A retrospective study by Flynn et al. (2000) studied the efficacy of amlodipine in 55 hypertensive children (mean age of 11.5 ±5.4 years), 49 of whom had secondary hypertension. Efficacy was assessed by comparing pretreatment blood pressure to follow-up blood pressure in an outpatient Nephrology clinic. Thirty-two patients achieved blood pressure control with amlodipine as monotherapy, and 31 patients received amlodipine twice-daily. The mean amlodipine dose was 0.16 ±0.12mg/kg/day, and the follow-up blood pressure was significantly lower than pretreatment blood pressure (p=0.003). The study also suggested that younger children required significantly higher doses than older children, and that twice-daily dosing may be required. (77)

Specific studies on the PK profile of amlodipine in children have not been attempted, but are required to further assess the efficacy and safety of amlodipine in the pediatric hypertensive population.
1.8 RATIONALE: The Need for Pharmacokinetic Studies in Children

The term *pharmacokinetics* refers to the way a drug is handled by the body, and reflects the absorption, distribution, metabolism and elimination of the drug, which ultimately controls systemic drug exposure. These parameters in adults often do not accurately predict pharmacokinetics in children due to growth and developmental changes, and there are significant problems with the extrapolation of findings from PK studies involving adults to children.(78) To characterize these parameters in children, the pharmacokinetics over the entire pediatric age range in which the drug will be used need to be assessed.

One of the major arguments for conducting pediatric PK studies is that treating children with untested drugs may place them more at risk than including them in controlled studies of the drug in the first place. A classic clinical example of this risk is the "grey-baby syndrome" caused by inappropriate chloramphenicol dosing. In the 1950's, chloramphenicol was given to premature infants to treat bacterial sepsis in doses that were analogous to adults. Results from a randomized clinical trial found that the antibiotic was a cause of death rather than a cure, since babies suffered from cardiovascular collapse due to toxic drug concentrations.(24;32) More recently, results of a randomized trial showed an increase in the number of deaths in pediatric intensive care unit patients treated with propofol off-label for sedation instead of anaesthesia. The results have prompted the manufacturer to issue a warning.(79)

These devastating consequences are due to the fact that physicians have to estimate pediatric doses, and face this dilemma every time when deciding to treat a child with a drug that has not been approved for use in this population. The consequence is that children are being treated with sub-optimal drug regimens, or are given the drug based on safety assumptions from adult studies and
previous observations of drug use in children. This non-validated treatment approach places the child at a potential risk of adverse drug reactions or therapeutic failures. According to the FDA lead deputy commissioner:

“When drug labels do not include adequate pediatric information, health care providers are forced to play a guessing game that may compromise the care of their patients. As a result, not only does this mean that sick kids sometimes don’t get better, but they also have the potential to get worse as a result of unexpected adverse events.” (17)

Pediatric PK studies determine how the dosage regimen in the pediatric population should be adjusted to achieve approximately the same level of systemic exposure that is safe and effective in adults, and should be performed in all pediatric age groups to allow dose adjustment within an individual over time.(39) If PK studies are to be conducted for the many drugs that are used off-label in children, the degree of risk that is inflicted upon the child participating in such research must be determined. The design of the study may largely influence whether or not the child or the child’s parents will agree to participate. Generally, PK studies that are performed in the pediatric population use children receiving treatment for a specific medical condition, and in whom a medical need for the drug in question has been established.(80) This way, the child’s routine medical care remains unchanged, but crucial data is collected to establish pediatric PK parameters for the drug in question.

1.9 Objectives

To characterize the pharmacokinetics of amlodipine in children between the ages of 6 months to 17 years, in whom the medical need for daily amlodipine has previously been established, and in whom routine daily administration of amlodipine precedes entry to the trial. Secondary objectives are to describe the safety of amlodipine in hypertensive children.
1.10 Hypothesis

The results of this trial will provide estimates of PK parameters in a real-world pediatric hypertensive population.

1.11 Ethics and Patient Consent

This study was approved by the REB of the Hospital for Sick Children (HSC) on February 16, 2000. A copy of the approval is included in Appendix 1. Proxy consent was obtained from the parent(s) and assent was obtained from the child if they were 7 years of age or older. Copies of the informed consent and assent forms are included in Appendix 2. Both patients and parents also received a summary of the study design and procedures for easy reference (Appendix 3).

1.12 Patient Enrollment and Eligibility Criteria

Patients between the ages of 6 months to 17 years who had a confirmed diagnosis of hypertension and who had a medical need for daily amlodipine were considered. All patients had to be on a stable dose of amlodipine for at least 30 days before entry into the trial. Patients were identified using clinic charts from the hypertension, renal transplant and bone marrow transplant clinics, and were approached by the principal investigator (PI), clinic nurse or co-ordinator (myself) during their regularly scheduled clinic visit. Inclusion criteria are listed in Table 1, and exclusion criteria are listed in Table 2.

Table 1: Inclusion criteria

| **Males and females between the ages of 6 months to 17 years, with a history of hypertension.** |
| **Witnessed written parental or legal guardian’s consent and assent from the child if above the age of 7 years.** |
| **Daily administration of any stable dose of amlodipine that has remained constant over the 4 week period prior to enrollment and intention to continue the same dose during the trial interval.** |
Table 2: Exclusion criteria

- Concomitant therapy with other investigational drugs or use within one month prior to entry. Subjects will not receive any other investigational drug until the study is completed.

- Transient, unstable, malignant or accelerated hypertension.

- Factors that may interfere with the conduct or interpretation of the trial such as: Subjects with poor vascular access making blood sampling difficult, subjects with drug dependence, alcohol dependence or other conditions that will impact study drug compliance, safety, and reliable follow-up. Subjects with a history of poor medication compliance, or a history of repeatedly missing clinic visits.

1.13 METHODOLOGY: Clinical Trial Design and Clinical Procedures

The study design is an open-label, multi-centre trial, whereby children continued to receive the same daily amlodipine dose that had been prescribed prior to entry into the trial. Ten sites within the United States and Canada were involved, with HSC being the only Canadian site. This study consisted of a screening period of up to 4 weeks duration followed by a PK sampling phase consisting of 4 visits (Figure 5). In order to ensure adequate representation across age groups, each site was required to limit enrollment of children ages 15 through 17 to no more than 2 or 3. Patients were recruited mainly from the outpatient Pediatric Nephrology Clinic since the majority of children who were prescribed amlodipine were renal transplant patients.

Figure 5: Clinical trial design

<table>
<thead>
<tr>
<th>Screening Phase</th>
<th>Pharmacokinetic Sampling Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCREEN VISIT</td>
<td>VISIT 2</td>
</tr>
<tr>
<td>Enrollment visit</td>
<td>PK Clinic Day</td>
</tr>
<tr>
<td>Begin dosing diary</td>
<td>Multiple samples</td>
</tr>
<tr>
<td></td>
<td>VISIT 3</td>
</tr>
<tr>
<td></td>
<td>PK Sample Trough</td>
</tr>
<tr>
<td></td>
<td>VISIT 4</td>
</tr>
<tr>
<td></td>
<td>PK Sample Trough</td>
</tr>
</tbody>
</table>
After appropriate consent was obtained, the screening visit was usually performed on the same day if time permitted. Otherwise, patients were screened during their next clinic visit, which was usually 1 week later. The screening visit consisted of a general physical exam including height, weight, race, blood pressure, heart rate and Tanner staging, and a medical history that was performed by the PI. Medical history details were verified using the patient’s health record at a later time. In addition, any medications that the patient was taking 30 days prior to entry into the study, and any concomitant medications were recorded from the health record. Measurement of blood pressure was performed using a Dinemap monitor (Critikon Inc.). A blood sample was also obtained usually in conjunction with a routine blood sampling in order to obtain baseline blood chemistry values, and a hepatitis A, B and C panel. After the sample was taken, the blood was allowed to clot for at least 30 to 60 minutes, after which it was centrifuged for 10 minutes at room temperature at 3500 rpm. Blood chemistry was repeated during the PK sampling phase at Visit 2. The patient was allowed to proceed to the PK sampling phase if blood chemistry values did not indicate any unknown major disease. All laboratory value abnormalities were flagged and commented on by the PI.

During the enrollment and screening visit, patients were also given a daily dosing and meal diary (Table 3 and 4, respectively). The patient discontinued their current amlodipine prescription and was prescribed a new supply of amlodipine at their current dose range, which was provided by Pfizer for the duration of the study, and was stored and dispensed by the Hospital Pharmacy. This ensured that all patients received the same study drug. The patient or the parent was asked to record the date and exact time of study drug taken in order to monitor compliance for a period of at least 1 week before the PK sampling phase (Visit 2). The meal diary had to be completed at least 2 days prior to Visit 2.
On the day of Visit 2, an indwelling venous catheter (Angiocath, Becton Dickinson) was inserted. Blood samples were obtained at 3, 5, 8 and 10 hours post-dose for children maintained on an AM and BID dosing schedule, and at 15, 18, 20 and 24 hours post-dose for children maintained on a PM dosing schedule. Approximately 3 cc. of blood was obtained each time in lithium-heparin tubes (Vacutainer™ Sodium Heparin, Becton Dickinson). Blood was centrifuged at 4° at 3500rpm for 10 minutes, and stored at -20°C in 5ml screw-capped polypropylene or polystyrene tubes within 1 hour of collection until analysis. The exact time of each blood sample taken was recorded. A seated blood pressure was also obtained during this visit. Patients or parents were asked to maintain the dosing diary until the completion of the study.
Visits 3 and 4 were conducted from 24 hours to 3 weeks after Visit 2, and consisted of 1 blood sample that was taken at approximately 24 hours post-dose for children on an AM and PM dosing schedule, and at 12 hours post-dose for children on a BID dosing schedule. Patients were instructed to withhold their amlodipine dose until the trough sample was obtained. To monitor for safety, both the patients and the PI were asked to report any adverse events to the co-ordinator. Any adverse events were reported to the REB. A copy of the adverse event report form used throughout the study is included in Appendix 4.

All sites were required to store the blood samples no more than 4 weeks after collection, after which all samples were shipped on dry ice to CRL Medinet in Lenexa, Kansas, USA. Samples were subsequently analyzed by PPD Development in Richmond, Virginia, USA. The analytical method used was liquid chromatography followed by mass spectrometry (LC/MS/MS). The assay is both sensitive and specific for amlodipine, however details of the assay could not be released by Pfizer at this time.

1.14 Results

Overall, 74 patients completed the study across 10 centers. At HSC, 8 patients were recruited, 4 boys and 4 girls with an average age of 12.4 ± 3.3 years, and a mean weight of 39.4 ± 14.5 kg. Six patients were from the renal transplant clinic, 1 patient was from the bone marrow transplant clinic, and 1 patient was from the hypertension clinic. All patients had a medical need for daily amlodipine, and had been taking amlodipine as monotherapy for hypertension at least 30 days prior to entry into the trial. The average dose of amlodipine was 7.5 ± 3.8 mg/day, and no patients had their amlodipine dose increased during the duration of the trial (Table 5). Patient #6 had a requirement for twice-daily amlodipine. All patients were taking amlodipine in tablet form. It is evident from Table 6 that blood pressure in all patients was adequately controlled by amlodipine.
Table 5: Patient demographics and amlodipine regimen

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Amlodipine Dose (mg)</th>
<th>Dosing Schedule</th>
<th>Hypertension Etiology</th>
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<td>7</td>
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<td>10</td>
<td>30.6</td>
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</tr>
<tr>
<td>8</td>
<td>F</td>
<td>8</td>
<td>30.4</td>
<td>12.5</td>
<td>PM</td>
<td>Post-bone marrow transplant</td>
</tr>
</tbody>
</table>

| Mean ±sd | 12.4 ±3.3 | 39.4 ±14.5 | 7.5 ±3.8 |
| Min.     | 8         | 20.7       | 2.5      |
| Max.     | 17        | 67.1       | 12.5     |

Table 6: Patient blood pressure readings

<table>
<thead>
<tr>
<th>Patient #</th>
<th>SBP at Screening</th>
<th>DBP at Screening</th>
<th>SBP at Visit 2</th>
<th>DBP at Visit 2</th>
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<td>105</td>
<td>75</td>
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</tr>
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</table>

| Mean ±sd | 122 ±15.6 | 68.9 ±7.5 | 126.6 ±14.5 | 71.3 ±4.4 |
| Min.     | 105        | 61        | 106          | 67          |
| Max.     | 129        | 80        | 151          | 80          |

1.15 Pharmacokinetic Evaluation

Amlodipine concentrations at specific time-points were graphed on Microsoft Excel. Since results were only available for 8 patients, it was not possible to use a PPK analytical approach. For our data set, PK parameters were manually calculated using standard non-compartmental methods because of the small number of patients.
In order to obtain a $t_{1/2}$ from the graph, the slope was first obtained using log-transformed values from at least the last two plasma concentrations using the formula: $y_2-y_1/x_2-x_1$. Subsequently, the elimination rate constant (k) was obtained from the slope using the formula: $-2.303 \times$ slope. The $t_{1/2}$ was obtained from the formula: $\ln2/k$. Since pharmacokinetics were being measured on steady-state levels, there was no value for the concentration at time zero (y-intercept) in order to obtain a starting point to calculate the Area Under the Curve (AUC). Therefore, the y-intercept had to be extrapolated by regression analysis. Subsequently, using the few time-points that were available for each patient, the linear trapezoidal method was applied to calculate the AUC.

For patients whose trough sample was not taken at 24 hours post-dose, their concentration at the 24-hour time-point had to be estimated. This is because AUC can only be calculated if the curve is generated using time-points from the same dose, thus trough concentrations that were obtained from patients from different doses could not be used in this analysis. In these cases, we had to assume that the extrapolated concentration of amlodipine at the 24 hour time-point was equal to the extrapolated concentration of amlodipine at time zero. In all cases, the residual area of the AUC did not need to be calculated since the study was performed under steady-state concentrations, and did not reach zero concentrations. Apparent clearance ($Cl_{app}$) was calculated using the formula: Dose (mg/kg)/AUC. This weight-adjusted clearance value was further used to calculate the apparent volume of distribution ($Vd_{app}$) using the formula: $Cl_{app}/k$. Peak plasma concentration ($C_{max}$) and time to reach $C_{max}$ ($T_{max}$) were obtained directly from the data. Refer to section 3.9.1 for an example PK analysis. See Table 7 for individual amlodipine plasma concentrations. See Appendix 5 for concentration-time curves for all 8 patients.
### Table 7: Plasma concentrations of amlodipine (ng/ml) after steady-state dosing

<table>
<thead>
<tr>
<th>Patient #</th>
<th>3</th>
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<th>8</th>
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<td>39.8</td>
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<td></td>
<td></td>
<td></td>
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</table>

*N/A means the sample was not obtained
* A blank space indicates that because of the patient’s dosing schedule, they were not required to provide a blood sample at that time-point
* Except for patient #1, 24-hour time-points were not used to calculate PK parameters because they were not taken during the same dosing interval
1.15.1 Sample PK Analysis

Patient #4 is a 17-year old 67.1 kg male taking 5mg amlodipine once daily every morning. The patient has been on amlodipine therapy for 5 years since his renal transplant. Since the patient is on an AM dosing schedule, blood samples were taken at 3, 5, 8, and 10 hours (approximately) post-dose (Figure 6).

Figure 6: Plasma amlodipine concentration-time curve for patient #4
Example Calculation of PK Parameters:

1. A C_{max} of 10.2 ng/ml occurring at a T_{max} of 5.0 hours was obtained directly from the graph.

2. Calculation of t_{1/2}:

\[ \text{Slope} = \log 8.21 - \log 9.48/10-8 = -0.062/2 = -0.031 \]

\[ k = -2.303 \times -0.031 = 0.072 \]

\[ t_{1/2} = \ln 2/k = \ln 2/0.072 = 9.6 \text{ hr} \]

3. AUC calculation:

Total AUC = Sum of the individual areas under each time-point at each dosing interval.

Concentration at 24 hours (C_{24}) needed to be extrapolated using the following method:

\[ C_{24} = \text{Concentration at any time-point} (e^{-kt}) \]

\[ C_{24} = C(10\text{hr}) \frac{e^{-0.072(24\text{hr} - 10\text{hr})}}{} = 3.0 \text{ ng/ml} \]

C_{24} is assumed to be equal to C_0 because the patient is on a 24-hour dosing schedule.

\[
\begin{align*}
\text{AUC1} &= \text{base (b) x height (h)} = 2.75 \times 3.0 = 8.25 \\
\text{AUC1.5} &= \frac{1}{2} \text{ bh} = \frac{1}{2} [(2.75 \times 9.75) - 3.0] = 9.28 \\
\text{AUC2} &= \text{bh} = (5-2.75)(9.75) = 21.94 \\
\text{AUC2.5} &= \frac{1}{2} \text{ bh} = \frac{1}{2} [(2.25 \times 10.2) - 9.75] = 0.51 \\
\text{AUC3} &= \text{bh} = (8-5)(9.48) = 28.44 \\
\text{AUC3.5} &= \frac{1}{2} \text{ bh} = \frac{1}{2} [(3 \times 10.2) - 9.48] = 1.08 \\
\text{AUC4} &= \text{bh} = (10-8)(8.21) = 16.42 \\
\text{AUC4.5} &= \frac{1}{2} \text{ bh} = \frac{1}{2} [(2 \times 9.48) - 8.21] = 1.27 \\
\text{AUC5} &= \text{bh} = (24-10)(3.0) = 42 \\
\text{AUC5.5} &= \frac{1}{2} \text{ bh} = \frac{1}{2} [(14 \times 8.21) - 3.0] = 36.47 \\
\end{align*}
\]

Total AUC = \sum \text{AUC1} + \text{AUC1.5} + \text{AUC2} + \text{AUC2.5} + \text{AUC3} + \text{AUC3.5} + \text{AUC4} + \text{AUC4.5} + \text{AUC5} + \text{AUC5.5} = 165.7 \text{ ng/ml hr}

4. Calculation of apparent clearance (Cl_{app}):

\[ \text{Cl}_{\text{app}} = \frac{\text{Dose (mg/kg)/AUC}}{} = \frac{5\text{mg/67.1kg/165.7 ng/ml x hr}}{} = 0.075 \text{ mg/kg/165.7 x 10^{-6} = 452.6/60 min} \cdot \frac{1}{7.5 \text{ ml/min/kg}} \]

5. Calculation of the apparent volume of distribution (Vd_{app}):

\[ \text{Vd}_{\text{app}} = \frac{\text{Cl}_{\text{app}}}{k} = 7.5\text{ml/min/kg}/0.072/60 \text{ min} = 7.5/1.2 \times 10^{-3}/1000 = 6.3 \text{ L/kg} \]

Analyses for the remaining 7 patients were performed in the same way as for Patient #4 (Table 8)
Table 8: PK parameters of amlodipine after steady-state dosing

<table>
<thead>
<tr>
<th>PK parameters</th>
<th>Patient #</th>
<th>Mean ±sd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>k (hr⁻¹)</td>
<td>0.033</td>
<td>0.034</td>
</tr>
<tr>
<td>t½ (h)</td>
<td>21.0</td>
<td>20.4</td>
</tr>
<tr>
<td>AUC (ng/ml x hr)</td>
<td>668.7</td>
<td>935.3</td>
</tr>
<tr>
<td>Cl_app. (ml/min/kg)</td>
<td>5.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Vd_app. (L/kg)</td>
<td>9.6</td>
<td>4.7</td>
</tr>
<tr>
<td>C_max (ng/ml)</td>
<td>39.6</td>
<td>47.5</td>
</tr>
<tr>
<td>T_max (hr)</td>
<td>10.0</td>
<td>7.7</td>
</tr>
</tbody>
</table>

* N/A means that accurate C_max or T_max values could not be obtained from the data
Comparing the mean values of the PK parameters in our 8 patients to reported adult values, Cl_{app} is increased in children (mean of 9.9 ±5.9ml/min/kg, compared to 7ml/min/kg in adults), probably due to the increased clearance. In addition, t_{1/2} is reduced (13.7 ±6.3hr compared to 34 hours for adults). The Vd_{app} in children is also greatly decreased (9.8 ±4.3L/kg compared to 21L/kg in adults).

1.15.2 Adverse Events

No patients experienced any adverse events related to amlodipine during the study period. Patient #2 experienced bruising on the right arm after catheter withdrawal that lasted for about 1 week. Patient #3 experienced a small hematoma on the right arm during catheter insertion. Patient #5 experienced a generalized seizure during the study period, which was not considered to be due to study drug by the PI since the patient had a previous history of seizures. This patient also developed a urinary tract infection during the study period and was admitted to hospital due to highly elevated creatinine. The diagnosis was end stage renal disease, which was consistent with the patient’s history of chronic organ rejection.

1.16 Discussion

This clinical trial characterized the pharmacokinetics of amlodipine in 8 pediatric patients for whom a medical need for daily amlodipine has been established. The patient’s physician had predetermined the medical requirement for amlodipine, and study risks were limited to phlebotomy. A recent report from the PPRU stated that the testing of antihypertensive medications in children is timely because physicians caring for hypertensive children as well as clinical pharmacologists have almost no approved drugs for children as therapeutic options, and somewhat outdated official recommendations.
It was assumed that because our study population consisted of children with hypertension associated with a medical condition, they accepted medical care as part of their routine, were more comfortable with healthcare staff, and were used to providing blood samples. Conversely, conducting such a study in healthy children would have been unethical. Chesney stated that “there exists little or no benefit for the testing of an antihypertensive agent in a healthy child, and the concern of more than minimal harm is real in this instance”. (81) It is also highly probable that parents would not subject their healthy child to take a medication for which the child has no need. Therefore, using healthy children in the context of biomedical research involving a drug presents a risk that outweighs the benefit, even if the purpose of the research is to benefit society as a whole. (8)

Since the requirement for the collection of blood samples was in order to obtain PK data, it was not part of the child's routine medical care, and by definition the study was non-therapeutic. However, venipuncture is considered to be a minimal risk procedure since it is no greater than a similar risk from minor cuts, bruises, or transient pain, and is an acceptable risk in drug research. (1) Rowell & Zlotkin support this statement by considering venipuncture, urine collection and even the collection of data from health records as equal risk. (32) Ackerman (1994) claims that venipuncture satisfies the standard of minimal risk in a child because they are not being exposed to any increment of risk beyond that inherent in their daily activities, thus the duty to protect their welfare is not violated. (82) The Royal College of Paediatrics also stated that such procedures are deemed to have the least possible risk. (31)

Some psychological risks of blood sampling in children may include emotional distress caused by pain and discomfort of the needle, and an increased fear of doctors and/or hospitals or clinics in
general. Such emotional risks may be substantially lessened if the child is informed of the procedures and is given the opportunity to assent. However, questions can still be raised about the appropriate role of children's wishes in deciding about these interventions. Thus a child's assent or dissent must take precedence over proxy consent in a situation where the welfare of the child will not be compromised by not participating. (82)

The objective was to obtain multiple drug levels per patient at different time-points to permit an estimation of amlodipine's PK parameters in children aged 6 months to 17 years. According to the U.S Department of Health and Human Services, the pharmacokinetics of a drug in children 16 years of age and older are expected to be similar to that of adults. (39) Therefore, the number of children in the 15 to 17 age group was limited by Pfizer to only 2 or 3 patients per study site.

The barrier that we needed to overcome in terms of recruitment was the fact that these children had to provide additional blood samples in order to participate in the study. We assumed that parents would be willing to have their child participate because the only risk involved would be the blood sampling procedure, which we believed was routine for all children involved. However, we found that many parents did not want to put their children through the stress of additional blood sampling. We also found that the level of inconvenience played a large role in the decision of the family to consent to the research. Parents who decided not to have their child participate usually did not live close to the hospital, or only came to the hospital a few times a year, and were not willing to make the extra trip for study visits.

In the end, our study population consisted of 4 adolescents between the ages of 14 to 17 years, and 4 children between the ages of 8 and 11. We were unable to recruit any infants since there were no
patients in this age group that were being seen in clinic during the recruitment phase. The small number of younger subjects that were identified as potential candidates did not qualify for the study. Our patient population was not unusual, as previous experience with pediatric PK trials with antihypertensive medications demonstrated that conducting such studies in children under 6 years old is difficult. The main reason is that the prevalence of hypertension in children under the age of 6 is extremely low.(81)

Our study population was also considered ideal because all children had a medical need for the drug under investigation due to their medical condition (mostly renal transplant patients). Hypertension is a common problem following renal transplantation, occurring in approximately 50 to 60% of pediatric recipients.(83) Sorof et al. (1999) recently reported that post-transplant antihypertensive medication use in children remains at 58% at 5 years post-transplant.(84) The etiology of the hypertension is multifactorial, and is comprised of acute and chronic rejection, steroid therapy, cyclosporine therapy, or transplant renal artery stenosis.(85) Post-transplant hypertension correlates negatively with renal allograft survival, and is a major risk factor for accelerated cardiovascular disease and graft dysfunction or failure and mortality. Immunosuppressive treatment with cyclosporine improves the survival of renal allografts, but is associated with renal vasoconstriction and hypertension.(86)

CCB’s such as amlodipine are considered excellent agents to treat post-transplant hypertension. This is because pharmacologically, CCB’s such as amlodipine counteract the vasoconstriction that is produced by cyclosporine on the afferent arteriole of the kidney and increase the glomerular filtration rate, probably by increasing renal plasma flow and decreasing renal vascular resistance.(54;87;88)
From the results, children were found to have higher $Cl_{app}$ for amlodipine than adults. Accordingly, the $t_{1/2}$ was reduced. This phenomena may explain why children require higher amlodipine doses than adults, or even have a requirement for twice-daily dosing. A study of carbamazepine and its metabolite, which is a CYP3A4 product, demonstrated an age dependent decrease in the ratio of the metabolite to the parent drug in serum in children between the ages of 2 weeks and 15 years. These data suggest a higher CYP3A4 activity in children and a gradual maturation to adult levels during adolescence. In a study of single-dose pharmacokinetics of piperacillin and tazobactam in infants and children, the elimination parameters $t_{1/2}$ and $Cl_{app}$ decreased and increased, respectively, with increasing age.

In our study group, there was a visible trend in $Cl_{app}$ rates, as values for the adolescents were similar to adults (patient #1, #4, #5, #8). The younger children had increased $Cl_{app}$ values (patient #3, #6, #7). However, these trends were not consistent with the $t_{1/2}$, as we would have expected to see larger $t_{1/2}$ values for the adolescents. It was not possible to stratify the results according to various age groups in order to confirm these trends due to the limited patient population.

A limitation was that we did not have results for all 74 patients. Therefore, it was not possible to perform a PPK analysis with only 8 patients. Calculation of PK parameters using non-compartmental methods was also difficult because only few points were available per subject, and had to be performed manually. Further, all of our 8 patients were prescribed the tablet formulation of amlodipine, thus it was not possible to assess PK parameters of children taking the suspension. Once the results of the PPK analysis are obtained, our results will be compared in order to determine whether a small number of patients and a small number of blood samples can provide PK parameters that are comparable to the PPK analysis.
Lastly, PK parameters were mostly assessed in renal transplant patients. Although it has been shown that renal impairment has little or no effect on the pharmacokinetics of amlodipine, it is not known whether the pharmacokinetics of amlodipine would differ in children with essential hypertension, having no other medical problems, and not taking other medications.(59;93)

1.17 Conclusion and Future Studies

PK studies are more difficult to perform in children, mostly as a result of limited patient populations and ethical concerns. This study assessed the pharmacokinetics of amlodipine in a clinically relevant, non-artificial setting, and placed an emphasis on a low level of inconvenience to the both the child and family by recommending that bloods be collected from routine procedures as much as possible.

Results from 8 patients demonstrated that children had higher clearance values than adults, with a corresponding decrease in the t½ in some cases. However, these results are only preliminary, and may not be representative of the entire patient population that participated in the clinical trial.

Ethically, conducting such a study in children for whom a medical need for amlodipine has been established, outweighed the risks, since the drug was being given as part of the child's routine medical care, and the results of the study were aimed at providing generalizable knowledge. The risk of the child receiving amlodipine, which at the time was an un-tested medication in the pediatric population, was only a minor consideration, as this risk was present from the day the child was prescribed the drug off-label.

Future studies need to assess amlodipine pharmacokinetics in neonates. Further, this study design should be applied as a model to perform other PK studies on drugs for which children have a
medical need. However, if we find that our results compare to the PPK analysis, investigators may be able to conduct minimally invasive pediatric PK studies in a single center.
SECTION 3: A COMPARATIVE BIOAVAILABILITY STUDY OF A TABLET AND SUSPENSION FORMULATION OF AMLODIPINE

1.18 RATIONALE

The PATH-2 clinical trial was performed in order to characterize PK parameters of amlodipine in children. Although there were no patients in the study at our site that required the amlodipine suspension, other centers had recruited patients who were taking this formulation. Since appropriate formulations for administration to children exist for only a minority of commercially available drugs, we felt that it was important to consider this new formulation as an alternative for children who require amlodipine. This is because in many cases, parents are forced to crush or divide tablets in order to provide a suitable way of delivering drug to their child. Although tablets and capsules may be more palatable than a liquid, they are not practical for young children. Unfortunately, the bioavailability of any product in a crushed or divided form may be highly variable, and the quantity of drug administered may be inexact.

A stability study of amlodipine in two liquid dosage forms by Nahata et al. (1999) determined that amlodipine can be easily made into a suspension, and demonstrated that amlodipine in this liquid form was stable for up to 3 months under refrigeration. (94) Many children receive amlodipine in a liquid, however the pharmacokinetics of the suspension are unknown and cannot be assumed to have a rate and extent of absorption comparable to the tablet. It is not known why Pfizer did not choose to perform a comparative bioavailability study between the tablet and suspension formulation of amlodipine before commencing the PATH-2 clinical trial.

Bioequivalence is defined as the absence of a significant difference in the rate and extent to which the active ingredient becomes available at the site of drug action when administered at the same
molar dose under similar conditions. (95) The term bioequivalence applied to different formulations of the same drug refers to equivalence with respect to rate and extent of absorption. (96;97)

The finding of bioequivalence between the amlodipine tablet and suspension would imply that the products are therapeutically equivalent, and would ensure that patients will be able to use either product interchangeably. The study is expected to produce positive results since the absorption of amlodipine is independent of its formulation. Faulkner et al. (1986) stated that the slow absorption of amlodipine is a property of the drug itself as tablets and solution formulations are absorbed equally slowly. (64)

1.19 Study Objectives

To compare relative bioavailability between a tablet and suspension formulation of amlodipine by measuring the concentration of amlodipine in plasma over time in a group of adult volunteers.

1.20 Hypothesis

The mean $\text{AUC}_0-\infty$, $\text{AUC}_{0-\infty}$, and $C_{\text{max}}$, which represent the rate and extent of absorption of a single dose of a tablet and suspension formulation of amlodipine are within $\pm 20\%$ of each other based on 90% confidence limit testing.

1.21 Ethics and Patient Consent

This study was approved by the REB on June 5, 2000. A copy of the approval is included in Appendix 6. Healthy adult volunteers were either students or employees of HSC, and were either approached directly by the study co-ordinator or recommended to participate through friends. The nature of the study was explained to all potential candidates. A copy of the informed consent form is included in Appendix 7. All potential candidates were also given a summary of the study design (Appendix 8).
1.22 Subject Enrollment and Eligibility Criteria

According to the sample size calculation, 24 subjects needed to complete the study (Appendix 9). Accounting for an attrition rate of about 20%, we needed to recruit 28 subjects in total. Subjects over the age of 18 who agreed a priori to commit to the time required to complete the study were considered, and were assessed for eligibility based on the inclusion and exclusion criteria, shown in Table 9 and 10, respectively.

Table 9: Inclusion criteria

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<thead>
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<th>Criteria</th>
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<tr>
<td>• Over 18 years of age</td>
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<td>• Normotensive subjects (subjects with SBP &lt;140mmHg, and DBP &lt;90mmHg)</td>
</tr>
<tr>
<td>• Signed informed consent</td>
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<td>• Willing and able to comply with both phases of the study</td>
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Table 10: Exclusion criteria

<table>
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<tr>
<td>• Abnormal liver function tests (AST, ALT, total bilirubin, alkaline phosphatase)</td>
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<tr>
<td>• History of drug allergy to amlodipine or other CCB’s</td>
</tr>
<tr>
<td>• History of a gastrointestinal condition that could affect drug absorption</td>
</tr>
<tr>
<td>• Cardiovascular contraindications obtained from the individual’s medical history: Conditions such as heart disease, cardiomyopathy, endocarditis, and other heart related problems</td>
</tr>
<tr>
<td>• Pregnant and lactating women</td>
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</table>

1.23 METHODOLOGY: Clinical Trial Design and Clinical Procedures

This open-label, randomized study used a two-period, single oral dose, crossover design in which both the test (suspension) and reference (tablet) product were administered to the same group of individuals under fasting conditions on two separate occasions (Figure 7). Both Health Canada and the FDA have recommended that such a design be used to demonstrate bioequivalence because it allows intra-subject comparisons between formulations and removes inter-subject variability.

(97;98)
The study consisted of a screening period followed by two phases of five days each. After signing the informed consent form, subjects completed a screening visit (Visit 1), which involved a physical examination performed by a fellow from the Division of Clinical Pharmacology. One blood sample was obtained in a heparanized syringe for the liver function test, and all female subjects provided a urine sample for a pregnancy screen. All blood and urine samples were analyzed by the Department of Pediatric and Laboratory Medicine at HSC. Prescription drugs other than oral contraceptives were not permitted. All subjects except for subject #17 were non-smokers. If the subject was determined to be eligible, they proceeded to Phase I.

1.23.1 Phase I

Subjects were divided into 3 groups of approximately 7 subjects each because it was not possible to have all subjects come in at the same time. See Table 11 for a description of the study timeline.
The amlodipine suspension was compounded by HSC Pharmacy according to the method described by Nahata et al.(94) Amlodipine tablets were provided by Pfizer Central Research (Groton, CT), and were stored at the hospital pharmacy. The appropriate amlodipine formulation was dispensed for each subject according to a prescription that was written by the study co-ordinator and signed by the PI. A copy of a sample prescription is in Appendix 10. Amlodipine was given to the subjects during the study visit by the co-ordinator, making compliance a non-issue.

Visit 2: Patients were randomly assigned to receive either a 5mg amlodipine tablet with water or 5mg of amlodipine prepared as an oral suspension after an overnight fast. A seated blood pressure measurement was taken before drug administration (Table 13). An indwelling venous catheter was inserted, and a baseline (pre-dose) blood sample was collected. The exact time of the dose was recorded for each patient, and serial 3ml blood samples were drawn at 0.5, 1, 2, 3, 4, 6, 8, and 10 hours post-dose in lithium-heparin tubes (Vacutainer™ Sodium Heparin, Becton Dickinson). Subjects were then given a standard breakfast consisting of orange or apple juice, tea or coffee, fruit and a muffin after the 0.5hr blood sample. A pizza lunch was served approximately 3 hours after drug administration, and a snack (tea, coffee and cookies) was served approximately 6 hours after drug administration. Samples were centrifuged at 4° at 3500rpm for 10 minutes, and stored at -20°C in polypropylene or polystyrene tubes within 1 hour of collection until analysis. The local

<table>
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<th>Phase</th>
<th>Timeline</th>
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<td>Week 1: May 7-May 11, 2001</td>
</tr>
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<td>I</td>
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<td>Week 4: June 4-June 8, 2001</td>
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<td>2</td>
<td>II</td>
<td>Week 5: June 11-June 15, 2001</td>
</tr>
<tr>
<td>3</td>
<td>II</td>
<td>Week 6: June 18-June 22, 2001</td>
</tr>
</tbody>
</table>

Table 11: Study timeline
anaesthetic amethocaine 4% gel (Ametop Gel, Smith & Nephew Healthcare Ltd.) or EMLA (Eutectic Mixture of Local Anaesthetics containing lidocaine 5% and prilocaine 5%) was used to reduce the pain caused by venous catheter insertion, and will be the focus of the 4th section of the thesis.

**Visit 3, 4, 5, 6 and 7:** Subjects returned to the hospital for the next consecutive 4 days for 1 blood sample: 24 hour post-dose for Visit 3, 48 hours post-dose for Visit 4, 72 hours post-dose for Visit 5, and 96 hours post-dose for Visit 6. The blood sample for Visit 7 was performed on the same day as Visit 6, except that it was taken approximately 8 hours later. A 23-gauge winged needle infusion set (butterfly) was used for these venipunctures to withdraw 3cc. of blood into lithium-heparin tubes. All subjects completed Phase I before proceeding to Phase II.

### 1.23.2 Phase II

After 1 to 4 weeks, subjects were switched to receive the drug formulation that they did not receive previously. The same procedure of blood drawing was followed as in Phase I. It was necessary to wait at least 1 week in between phases since the drug had to be completely eliminated from the body. Since amlodipine has a t1/2 of 34 hours, and 3 to 5 half-lives are required to clear a drug from the body, between 102 and 170 hours (4 to 7 days) were needed in a wash-out period so that there was no drug accumulation from the previous dose.

#### 1.24 Subject Compensation

Since participation in the study was voluntary, out-of-pocket expenses were reimbursed at the end of the study.
1.25 Determination of Amlodipine in Plasma

The determination of amlodipine in plasma was performed according to a liquid chromatography/mass spectrometry (LC/MS/MS) assay that was developed and validated by PPD Development, Richmond, VA. Amlodipine and a spiked internal standard, amlodipine-d4, were extracted from human plasma containing sodium heparin using ion exchange solid phase extraction. Chromatographic separation was achieved on a BDS Hypersil C8, 5μm (2.0x100mm) column with a flow rate of 0.300ml/min. The mobile phase was comprised of a gradient mixture of 0.05% formic acid in acetonitrile and 35:65 (v/v) 0.05% formic acid in 5mM ammonium acetate/acetonitrile. Extracts were analyzed by LC/MS/MS using positive ion electrospray ionization (Turbo IonSpray, MDS Sciex) using multiple reactions monitoring (MRM). The validated lower limit of quantitation (LOQ) for this method is 0.100ng/ml, and the upper LOQ is 50ng/ml using a 0.200ml aliquot of human plasma. Values outside this calibration range were not reported. The coefficient of variation for inter-assay variability ranged from 3.5-6%. Samples were stored at -20°C prior to analysis. Quantification was based on peak area ratios from the response of amlodipine to the internal standard, amlodipine-d4, referenced to a nine-point standard curve. All sample concentrations were calculated using a linear (1/concentration^2) weighted regression curve.

We would like to thank Pfizer Central Research for providing funding for sample analysis.

1.26 Pharmacokinetic Analysis

The sample data was assessed according to Health Canada’s “Drugs Directorate Guidelines for the Conduct and Analysis of Bioavailability and Bioequivalence Studies, Part A: Oral Dosage Formulations Used for Systemic Effects”, published by Health Canada in 1992.(98)

Amlodipine pharmacokinetics were calculated using non-compartmental methods on the Windows based program WINNONLIN Version 1.0. See Tables 14 and 15 for individual amlodipine plasma
concentrations at each sampling interval. It has been stipulated that data from bioequivalence studies must be analyzed using non-compartmental methods because it is a better reflection of experimental data in all subjects. Compartmental models assume that absorption occurs by a specific rate process, which cannot be assumed to be the same in all individuals.\(^{(99;100)}\)

Key parameters used to determine bioequivalence were \(\text{AUC}_{0-t}, \text{AUC}_{0-\infty}, C_{\text{max}}\) and \(T_{\text{max}}\).\(^{(97;98)}\) AUC is a function of the amount of product absorbed, and the AUC from zero to the last measured time point (0-\(t\), was calculated using the linear trapezoidal method. AUC from zero to infinity (0-\(\infty\)) was calculated using the formula: \(\text{AUC}_{0-t} + C_t/k\), where \(C_t\) is the last measurable plasma concentration, and \(k\) is the terminal elimination rate constant, calculated by least squares regression of the natural logarithms of at least the last five plasma concentrations. This extrapolated fraction should not exceed 20% of the total AUC.\(^{(101)}\)

\(C_{\text{max}}\) and \(T_{\text{max}}\) were obtained directly from the data. \(T_{\text{max}}\) is a function of the rate of absorption, and \(C_{\text{max}}\) is a function of both the rate and extent of absorption, however \(T_{\text{max}}\) is limited by sampling times and is too variable, thus is not considered an important criterion for judging bioequivalence.\(^{(97)}\) Other parameters such as \(t_{1/2}\), elimination rate constant \((k)\), \(C_{\text{app}}\), and \(V_{d_{\text{app}}}\) were calculated using the appropriate formula as mentioned in the Pharmacokinetic Evaluation section (section 3.9) of the PATH-2 clinical trial, using Microsoft Excel. PK parameters for each subject are in Tables 16 and 17.

### 1.26.1 Statistical Analysis

According to both Health Canada and FDA guidelines, bioequivalence is achieved when the difference between the test and reference formulation is no more than ±20% based on a 90% confidence limit of the relative log-transformed mean of \(\text{AUC}_{0-t}, \text{AUC}_{0-\infty},\) and \(C_{\text{max}}\).\(^{(97;98)}\) Two
one-sided statistical tests were carried out using log-transformed measured data from the study to
test whether the 90% confidence interval for the ratio of the test to reference formulation is within
the limits of 80% to 125%. The asymmetry about 100% is due to transforming the data back into
relative percentage. (102) This criterion does not imply that there can be a 20% to 25% difference
between the mean of the two products. (95;96;103) The 20% difference is arbitrary and has been set
as the clinically meaningful parameter, and means that therapy would not be compromised if the
two formulations differed by less than 20%.(104)

In order to calculate the 90% confidence interval, a repeated measures two-way analysis of variance
(ANOVA) was performed on the log-transformed AUC_{0-t}, AUC_{0-∞}, and C_{max} data. Log-transformed
data are used in view of the frequently skewed distribution of time-concentration data. (103) The
ANOVA gives the appropriate intra-subject variance estimate, and a Mean Square Residual
(MSResidual) for the calculation of the 90% confidence interval. The following formulas were
applied to calculate the upper and lower 90% confidence limits:

1. **Difference** = Test mean - Reference mean (log values)
2. **SE Difference** = \( (2 \times MS\text{Residual}/n)^{0.5} \)
3. **AUC or C_{max} Ratio** = 100 x e^{(difference)}

**Formula for the 90% confidence limits:**

\[
100 \times e^{\left( \text{Difference} \pm \text{(critical value for } t \text{ at } \alpha=0.05 \text{ and } df=n-2) \times \text{SE Difference} \right)}
\]

4. **Lower confidence interval** = 100 x e^{\left( \text{Difference} - \text{(critical value for } t \text{ at } \alpha=0.05 \text{ and } df=n-2) \times \text{SE Difference} \right)}

5. **Upper confidence interval** = 100 x e^{\left( \text{Difference} + \text{(critical value for } t \text{ at } \alpha=0.05 \text{ and } df=n-2) \times \text{SE Difference} \right)}

To calculate intra-subject and inter-subject variability, the following formulas were used:

1. **Intra-subject variability** =100 (MSResidual)^{0.5}
2. **Inter-subject variability** = 100 (MS\text{Subject (seq)} - MS\text{Residual}/2)^{0.5}
$T_{\text{max}}$ values were compared by the non-parametric Wilcoxon Rank Sum Test using raw data, since data were not normally distributed. Other PK parameters such as $t_{1/2}$, $k$, $C_{\text{app}}$, and $V_{d_{\text{app}}}$ were compared using the paired t-test. All statistical calculations were performed with the statistical software package SigmaStat for Windows, version 2.03.

1.27 Results

Twenty-seven adult subjects were screened to participate (12 males and 15 females), and all were randomized to receive either the tablet or the suspension formulation of amlodipine. Twenty-one subjects completed both phases of the study (13 females and 8 males). The age range of the patients was 20-58 years, with an average age of 27.5 ±9.0 years, mean height of 164.7 ±9.0cm, and a mean weight of 64.4 ±13.0kg. Table 12 presents subject demographics and the randomization code.

Twelve patients were Caucasian, 6 patients were Oriental, and 3 patients were of East Indian origin.

Two subjects withdrew consent before entering phase I, 3 subjects withdrew consent during phase I, and 1 subject withdrew consent before entering phase II. Of the 6 patients who withdrew consent, all discontinued due to reasons associated with traumatic phlebotomy, and were not included in the analysis. All subjects had normal liver function tests and physical exams, and no females were pregnant. All subjects were normotensive (Table 13).

All of the blood samples for subject #27 were erroneously labeled as belonging to Phase II, therefore these results could not be included in the analysis. The analysis was therefore completed on 20 subjects, 13 females and 7 males. Amlodipine plasma concentration-time curves for each subject are included in Appendix 11.
Table 12: Subject demographic data and treatment randomization

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Gender (M/F)</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Randomization</th>
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<td>F</td>
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<td>F</td>
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</tr>
<tr>
<td><strong>Mean ±sd</strong></td>
<td><strong>27.5 ±9.0</strong></td>
<td><strong>164.7 ±9.0</strong></td>
<td><strong>64.4 ±13.0</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Min.</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Max.</strong></td>
<td></td>
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</tr>
</tbody>
</table>

TS = tablet, ST = suspension
Table 13: Blood pressure measurements before amlodipine administration in phase I

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<th>Subject #</th>
<th>SBP</th>
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<th>Heart Rate</th>
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<td>74</td>
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<tr>
<td>02</td>
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<td>133</td>
<td>64</td>
<td>83</td>
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<tr>
<td>04</td>
<td>132</td>
<td>66</td>
<td>73</td>
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<tr>
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<td>110</td>
<td>80</td>
<td>87</td>
</tr>
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<td>106</td>
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<td>62</td>
<td>80</td>
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<td>65</td>
</tr>
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<td>123</td>
<td>66</td>
<td>91</td>
</tr>
<tr>
<td>12</td>
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<td>26</td>
<td>107</td>
<td>62</td>
<td>64</td>
</tr>
</tbody>
</table>

Mean ±sd 114.7 ±10.9 66.1 ±6.3 73.9 ±11.2
Min. 98 62 56
Max. 133 80 91

The mean C<sub>max</sub> levels were 3.91±0.77 ng/ml for the tablet formulation and 3.41±0.98 ng/ml for the suspension formulation (P=0.013), and were reached at a median T<sub>max</sub> of 4 hours for the tablet and 6 hours for the suspension (P=0.8). The mean values for AUC<sub>0-t</sub> were 153.58±30.19 ng/ml.hr for the tablet and 136.42±29.88 ng/ml.hr for the suspension (P=0.026). The mean values for AUC<sub>0-∞</sub> were 179.97±41.35 for the tablet formulation, and 161.70±41.84 for the suspension (P=0.046). The ratio of AUC<sub>0-t</sub>/AUC<sub>0-∞</sub> did not exceed 80% in all individuals, but the average ratio was 86.14% ±5.95% for the tablet AUC ratio, and 85.23% ±8.4% for the suspension AUC ratio (P=0.29). This means that that sampling period covered an average of 85.7% of total AUC's for both the test and reference formulation. The mean t<sub>1/2</sub> was 34.4±8.52 hours for the tablet and 35.0±7.80 hours for the
suspension (P=0.54). Plasma amlodipine concentrations were measurable at the last concentration
time point in all volunteers. Results for individual PK parameters are given in Table 16 and 17.
Plasma amlodipine concentration-time curves for the tablet and suspension formulation are in
Figure 8 and 9, respectively. A comparative mean plasma-concentration time curve for both the
tablet and suspension formulation is presented in Figure 10.
### Table 14: Plasma concentrations of amlodipine (ng/ml) following a single oral dose of the TABLET formulation of 5mg amlodipine

<table>
<thead>
<tr>
<th>Subject #</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;0.10</td>
<td>0.615</td>
<td>3.18</td>
<td>2.52</td>
<td>4.02</td>
<td>4.37</td>
<td>2.94</td>
<td>3.21</td>
<td>2.87</td>
<td>1.85</td>
<td>1.15</td>
<td>0.810</td>
<td>0.655</td>
<td>0.520</td>
</tr>
<tr>
<td>2</td>
<td>&lt;0.10</td>
<td>0.348</td>
<td>0.721</td>
<td>3.76</td>
<td>4.24</td>
<td>4.95</td>
<td>3.77</td>
<td>4.26</td>
<td>3.77</td>
<td>3.06</td>
<td>2.25</td>
<td>1.56</td>
<td>1.12</td>
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</tr>
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<td>3</td>
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<td>0.282</td>
<td>1.24</td>
<td>2.91</td>
<td>3.99</td>
<td>4.05</td>
<td>3.26</td>
<td>2.85</td>
<td>2.24</td>
<td>1.38</td>
<td>1.26</td>
<td>0.586</td>
<td>0.311</td>
<td>0.401</td>
</tr>
<tr>
<td>4</td>
<td>&lt;0.10</td>
<td>0.199</td>
<td>0.484</td>
<td>1.45</td>
<td>2.11</td>
<td>2.89</td>
<td>2.92</td>
<td>3.21</td>
<td>2.38</td>
<td>1.66</td>
<td>1.81</td>
<td>1.16</td>
<td>1.05</td>
<td>0.910</td>
</tr>
<tr>
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<td>0.172</td>
<td>1.38</td>
<td>2.04</td>
<td>2.94</td>
<td>3.57</td>
<td>3.05</td>
<td>3.38</td>
<td>3.47</td>
<td>2.07</td>
<td>1.40</td>
<td>0.871</td>
<td>0.578</td>
<td>0.440</td>
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<td>1.01</td>
<td>2.00</td>
<td>2.85</td>
<td>2.95</td>
<td>3.15</td>
<td>3.17</td>
<td>3.51</td>
<td>2.92</td>
<td>1.44</td>
<td>0.987</td>
<td>0.462</td>
<td>NSR</td>
</tr>
<tr>
<td>7</td>
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<td>0.414</td>
<td>1.50</td>
<td>4.33</td>
<td>5.03</td>
<td>5.00</td>
<td>4.22</td>
<td>3.47</td>
<td>2.97</td>
<td>2.44</td>
<td>1.47</td>
<td>1.14</td>
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<td>0.460</td>
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<td>&lt;0.10</td>
<td>0.687</td>
<td>0.844</td>
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<td>2.40</td>
<td>2.34</td>
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<td>2.34</td>
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<td>1.84</td>
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<td>3.95</td>
<td>2.57</td>
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<td>2.92</td>
<td>3.25</td>
<td>2.96</td>
<td>2.93</td>
<td>2.01</td>
<td>1.53</td>
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<td>&lt;0.10</td>
<td>&lt;0.10</td>
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<td>0.802</td>
<td>1.73</td>
<td>2.31</td>
<td>2.90</td>
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<td>2.46</td>
<td>1.43</td>
<td>0.822</td>
<td>0.686</td>
<td>0.608</td>
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</table>

| Mean     | 0.533 | 1.415 | 2.306 | 3.037 | 3.619 | 3.296 | 3.201 | 2.996 | 2.175 | 1.362 | 0.858 | 0.567 | 0.506 |
| +/−sd    | ±0.39 | ±0.89 | ±1.28 | ±1.38 | ±1.11 | ±0.70 | ±0.56 | ±0.63 | ±0.43 | ±0.39 | ±0.26 | ±0.23 | ±0.18 |
| Min.     | 0.172 | 0.454 | 0.514 | 0.802 | 0.976 | 2.310 | 2.140 | 1.740 | 1.380 | 0.593 | 0.406 | 0.192 | 0.157 |
| Max.     | 1.530 | 3.180 | 4.430 | 5.030 | 5.210 | 4.680 | 4.260 | 3.950 | 3.060 | 2.250 | 1.560 | 1.120 | 0.910 |

* NSR indicates that No Sample was Received for analysis
Table 15: Plasma concentrations of amlodipine (ng/ml) following a single oral dose of the SUSPENSION formulation of 5mg amlodipine

<table>
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<tr>
<th>Subject #</th>
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<th>3</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>100</th>
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<td>1.26</td>
<td>2.75</td>
<td>2.88</td>
<td>3.02</td>
<td>3.04</td>
<td>2.80</td>
<td>2.81</td>
<td>1.50</td>
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<td>0.642</td>
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<td>2.33</td>
<td>2.46</td>
<td>2.57</td>
<td>1.83</td>
<td>1.11</td>
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<td>&lt;0.10</td>
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<td>3.92</td>
<td>3.97</td>
<td>2.95</td>
<td>2.57</td>
<td>2.33</td>
<td>1.89</td>
<td>1.22</td>
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<td>0.512</td>
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<td>3.47</td>
<td>3.24</td>
<td>3.28</td>
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<td>1.92</td>
<td>1.86</td>
<td>1.45</td>
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<td>5</td>
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<td>&lt;0.10</td>
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<td>0.959</td>
<td>2.16</td>
<td>2.71</td>
<td>3.55</td>
<td>3.04</td>
<td>2.66</td>
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<td>0.659</td>
<td>0.406</td>
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<td>2.03</td>
<td>2.65</td>
<td>2.35</td>
<td>2.08</td>
<td>2.52</td>
<td>1.66</td>
<td>0.949</td>
<td>0.652</td>
<td>0.459</td>
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<td>2.03</td>
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<td>1.23</td>
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<td>1.50</td>
<td>1.87</td>
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<td>0.977</td>
<td>0.650</td>
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<td>5.36</td>
<td>4.72</td>
<td>3.84</td>
<td>NSR</td>
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<td>2.77</td>
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<td>1.60</td>
<td>1.07</td>
<td>0.523</td>
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</tr>
<tr>
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<td>&lt;0.10</td>
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<td>1.55</td>
<td>2.94</td>
<td>3.11</td>
<td>2.95</td>
<td>2.09</td>
<td>2.25</td>
<td>1.75</td>
<td>0.941</td>
<td>0.740</td>
<td>0.416</td>
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<td>1.93</td>
<td>2.74</td>
<td>3.67</td>
<td>3.59</td>
<td>2.80</td>
<td>2.77</td>
<td>2.16</td>
<td>1.00</td>
<td>0.687</td>
<td>0.429</td>
<td>0.343</td>
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<tr>
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<td>0.177</td>
<td>1.48</td>
<td>2.03</td>
<td>2.27</td>
<td>2.49</td>
<td>2.81</td>
<td>2.46</td>
<td>2.50</td>
<td>1.36</td>
<td>0.800</td>
<td>0.510</td>
<td>0.301</td>
<td>0.337</td>
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<tr>
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<td>0.957</td>
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<td>5.12</td>
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<td>3.97</td>
<td>4.21</td>
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<td>0.338</td>
<td>0.737</td>
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<td>2.57</td>
<td>3.31</td>
<td>2.66</td>
<td>2.68</td>
<td>1.57</td>
<td>0.870</td>
<td>0.434</td>
<td>0.229</td>
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<td>&lt;0.10</td>
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<td>1.93</td>
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<td>2.65</td>
<td>2.25</td>
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<td>3.82</td>
<td>3.75</td>
<td>4.03</td>
<td>3.64</td>
<td>3.52</td>
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<td>0.715</td>
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<td>2.29</td>
<td>2.51</td>
<td>2.19</td>
<td>2.34</td>
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<td>2.52</td>
<td>2.44</td>
<td>2.31</td>
<td>1.91</td>
<td>1.40</td>
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<td>0.585</td>
<td>0.338</td>
<td>0.315</td>
</tr>
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<td>&lt;0.10</td>
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<td>0.757</td>
<td>1.40</td>
<td>1.76</td>
<td>2.45</td>
<td>2.46</td>
<td>2.76</td>
<td>1.85</td>
<td>1.19</td>
<td>0.777</td>
<td>0.529</td>
<td>0.457</td>
</tr>
</tbody>
</table>

**Mean**

| 0.390 | 1.211 | 2.186 | 2.761 | 2.931 | 2.950 | 2.682 | 2.644 | 1.836 | 1.108 | 0.753 | 0.482 | 0.467|

**±sd**

| ±0.29 | ±0.68 | ±1.10 | ±1.21 | ±0.89 | ±0.62 | ±0.57 | ±0.56 | ±0.35 | ±0.30 | ±0.25 | ±0.19 | ±0.20|

**Min.**

| 0.123 | 0.237 | 0.757 | 1.300 | 0.230 | 0.490 | 1.500 | 1.870 | 1.360 | 0.794 | 0.434 | 0.229 | 0.178|

**Max.**

| 1.200 | 2.330 | 5.360 | 6.130 | 5.120 | 4.030 | 3.970 | 4.210 | 2.870 | 1.860 | 1.450 | 1.060 | 0.976|

* NSR indicates that No Sample was Received for analysis
Table 16: \( C_{\text{max}}, T_{\text{max}}, \text{AUC}_{0+1}, \text{AUC}_{0-\infty} \) and AUC ratios of amlodipine after oral administration of a 5mg dose of the tablet and suspension formulation

<table>
<thead>
<tr>
<th>Subject # (rand.)</th>
<th>( C_{\text{max}} ) (ng/ml)</th>
<th>( T_{\text{max}} ) (hr)</th>
<th>( \text{AUC}_{0-100h} ) (ng/ml/hr)</th>
<th>( \text{AUC}_{0-\infty} ) (ng/ml/hr)</th>
<th>( \text{AUC}<em>{0-100h}/\text{AUC}</em>{0-\infty} \times 100 = \text{AUC Ratio} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>01 (TS)</td>
<td>4.37</td>
<td>3.04</td>
<td>4.00</td>
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<td>143.38</td>
</tr>
<tr>
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<td>4.95</td>
<td>2.57</td>
<td>4.00</td>
<td>10.00</td>
<td>232.72</td>
</tr>
<tr>
<td>03 (ST)</td>
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<td>3.97</td>
<td>4.00</td>
<td>4.00</td>
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<td>04 (ST)</td>
<td>3.21</td>
<td>3.48</td>
<td>8.00</td>
<td>10.00</td>
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</tr>
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<td>05 (TS)</td>
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<td>3.55</td>
<td>4.00</td>
<td>6.00</td>
<td>159.40</td>
</tr>
<tr>
<td>06 (ST)</td>
<td>3.51</td>
<td>3.73</td>
<td>10.00</td>
<td>6.00</td>
<td>175.00</td>
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<td>07 (TS)</td>
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<td>3.31</td>
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<td>3.00</td>
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<td>4.00</td>
<td>2.00</td>
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<td>6.00</td>
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<td>3.00</td>
<td>177.79</td>
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<td>4.00</td>
<td>2.00</td>
<td>103.28</td>
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<td>6.00</td>
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<td>6.00</td>
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<td>2.76</td>
<td>10.25</td>
<td>10.00</td>
<td>161.01</td>
</tr>
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<td>3.91</td>
<td>3.41</td>
<td>5.26</td>
<td>5.31</td>
<td>153.58</td>
</tr>
<tr>
<td><strong>±sd</strong></td>
<td>±0.77</td>
<td>±0.98</td>
<td>±2.41</td>
<td>±2.51</td>
<td>±30.19</td>
</tr>
<tr>
<td><strong>Min.</strong></td>
<td>2.34</td>
<td>1.87</td>
<td>3.00</td>
<td>2.00</td>
<td>103.28</td>
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<td><strong>Max.</strong></td>
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<td>6.13</td>
<td>10.25</td>
<td>10.00</td>
<td>232.72</td>
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<tr>
<td><strong>Median</strong></td>
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<td>3.36</td>
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<td>157.89</td>
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<td>28.7</td>
<td>45.8</td>
<td>47.3</td>
<td>19.7</td>
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<td><strong>P:</strong></td>
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<td></td>
<td></td>
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</table>

Paired t-test
Figure 8: Plasma concentration-time curves of amlodipine measured after oral administration of a 5mg dose of the tablet formulation.

Figure 9: Plasma concentration-time curves of amlodipine measured after oral administration of a 5mg dose of the suspension formulation.
Figure 10: Mean plasma concentration-time curve of amlodipine after oral administration of a 5mg dose of the tablet and suspension formulation to 20 subjects

Results of the ANOVA on log-transformed data revealed that the 90% confidence interval for AUC$_{0-t}$ was between 83% and 94%, which was entirely within the bioequivalence range of 80% to 125%. Similarly, the 90% confidence interval for AUC$_{0-\infty}$ was between 83% and 96%, and the 90% confidence interval for C$_{\text{max}}$ was between 80% and 93%. See Table 18, 19 and 20 for the ANOVA table for the AUC$_{0-t}$, AUC$_{0-\infty}$, and C$_{\text{max}}$ data respectively. Table 21 contains results of the bioequivalence analysis.
Table 18: Analysis of variance of AUCₘₙ data on log-transformed data

<table>
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<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
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<td>0.0330</td>
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<td>0.166</td>
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<td>0.148</td>
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<td>Total</td>
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</table>

Table 19: Analysis of variance of AUCₖₙₙ data on log-transformed data

<table>
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<tr>
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<th>Degrees of freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
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<td>0.124</td>
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</tr>
</tbody>
</table>

Table 20: Analysis of variance of Cₘₚₙ data on log-transformed data

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence</td>
<td>1</td>
<td>0.415</td>
<td>0.415</td>
<td>5.695</td>
<td>0.028</td>
</tr>
<tr>
<td>Subject (seq)</td>
<td>18</td>
<td>1.312</td>
<td>0.0729</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period</td>
<td>1</td>
<td>0.0754</td>
<td>0.0754</td>
<td>3.784</td>
<td>0.068</td>
</tr>
<tr>
<td>Formulation</td>
<td>1</td>
<td>0.229</td>
<td>0.229</td>
<td>11.492</td>
<td>0.003</td>
</tr>
<tr>
<td>Residual</td>
<td>18</td>
<td>0.359</td>
<td>0.0199</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>2.390</td>
<td>0.0613</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For Tₘₚₙ, there was no statistically significant difference based on the Wilcoxon Rank Sum Test (P=0.8). There was also no difference of t₁/₂ between the tablet and suspension based on the results of a paired t-test (P=0.54). Both Clₘₚₚ and Vₙₘₚₚ were statistically significant (P=0.03 and P=0.002, respectively).
Table 21: Statistical evaluation of logarithmically transformed data for AUC_{0-t}, AUC_{0-\infty}, and C_{\text{max}} of two amlodipine formulations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC_{0-t}</th>
<th>AUC_{0-\infty}</th>
<th>C_{\text{max}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio (100 \times e^{\text{difference}})</td>
<td>88.5%</td>
<td>89.4%</td>
<td>86.0%</td>
</tr>
<tr>
<td>90% confidence intervals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower limit</td>
<td>83%</td>
<td>83%</td>
<td>80%</td>
</tr>
<tr>
<td>Upper limit</td>
<td>94%</td>
<td>96%</td>
<td>93%</td>
</tr>
<tr>
<td>Intra-subject variability</td>
<td>12%</td>
<td>13%</td>
<td>14%</td>
</tr>
<tr>
<td>Inter-subject variability</td>
<td>16%</td>
<td>19%</td>
<td>16%</td>
</tr>
</tbody>
</table>

1.28 Adverse events

No subjects experienced any adverse reactions to amlodipine.

1.29 Discussion

Although the bioequivalence study was conducted to generate results that can be extrapolated to the pediatric population, it was not ethical to conduct such a study in healthy children. The ICH guidelines state: "relative bioavailability comparisons of pediatric formulations with the adult oral formulation typically should be done in adults". (13) If healthy children were recruited for the study, amlodipine would have to be administered to a healthy child. This presents a major increase over minimal risk since the drug is something that the child would not normally be exposed to.

Another issue would be that if we recruited children who were already taking amlodipine, we would find mostly the younger children taking the suspension and the older children taking the tablet. In order to eliminate this bias, we would have to randomize children to either the tablet or the suspension, which is unethical because we do not know if the suspension is equivalent to the tablet. Thus if a child who was receiving a tablet was randomized to the suspension, and the suspension produced clinically different drug-plasma levels, this would be compromising medical
care in the child. Another reason is that traditional bioequivalence studies require repeated blood sampling over specified intervals of time, thus in children, a PPK study would have to be designed. (105) We concluded that a bioequivalence study in children would not be ethically defensible in this case, but that results of the study in adults could be extrapolated to children if developmental differences were accounted for.

The aim of this study was calculate the relative bioavailability of the amlodipine suspension compared to the tablet, since both of these formulations were recently utilized in a clinical trial (PATH-2). The rate and extent of absorption of amlodipine is believed to be similar regardless of the formulation, because its slow rate of absorption is thought to be an inherent property of the molecule. Faulkner et al. (1989) conducted a study to determine whether the rate or extent of absorption of amlodipine is dependent on its dissolution from a solid dosage form, and found that a gelatin capsule formulation was bioequivalent to an amlodipine tablet crushed in water. The study concluded that the slow transfer of drug from the gut to the venous circulation is a physiochemical property of amlodipine, and not a consequence of delayed or slow dissolution from the capsule formulation. (73) This theory led us to formulate the hypothesis that both formulations of amlodipine will be bioequivalent.

The randomized, single-dose, two-period crossover design in healthy volunteers that was used is recommended by regulatory agencies to evaluate the bioequivalence of two formulations of the same drug product, and is yet another reason why this study could not be conducted in children. (98;106) This design has more statistical power than a parallel design because subjects serve as their own controls. (107) To minimize variability, all subjects received the amlodipine formulations on the same day of the week, and at approximately the same time.
Concerning the sampling intervals, regulatory agencies recommend that samples be taken until at least 3 half-lives in order to be able to adequately represent AUC and calculate PK parameters. Therefore, the requirement to collect blood samples up to at least 3 half-lives justified the measurement at up to 100 hours post-dose. Slight variations of these time points have been used by other studies assessing the pharmacokinetics of amlodipine in healthy human subjects.\textsuperscript{(64;66;108)} However, this requirement is another reason why the study could not be performed in children. Since amlodipine has a $t_{1/2}$ of 34 hours, at least 5 days of blood sampling were required in order to adequately characterize the AUC. Such an extended sampling period requires multiple needle pokes and increases the potential risk of the study to more than minimal. Previous bioequivalence studies that have been conducted in children in the literature used venous catheters and were completed within 6 to 24 hours. This indicates that the child was poked only once, when the venous catheter was inserted. A PK study with amlodipine is not ethical because of the drug's long $t_{1/2}$. In order to accurately characterize the AUC of amlodipine up to at least 3 half-lives, a sampling interval of at least 5 days is required, necessitating multiple needle punctures, and substantially increasing the invasiveness of the study. The results indicate that the duration of blood sampling was sufficient to account for at least 80% of the AUC. A washout period of 1 to 4 weeks ensured that there were no carry-over effects from Phase I into Phase II.

Further, a total of 14 blood samples were collected from each subject per dose. This amount was sufficient in order to calculate relevant PK parameters because generally 12 to 18 samples are recommended by regulatory agencies. Also, from 5 to 10 time-points were used to determine the terminal log-linear phase of the curve. This gave an accurate estimation of the terminal phase of the curve and $k$, as it is recommended that at least 4 points be used.\textsuperscript{(98)}
Our sample size of 20 subjects was sufficient to determine bioequivalence. Even though our original n-value of 24 subjects was not met, this value was calculated based on a 30% inter-subject variability for AUC from previous studies. In theory, the required number should be based on an estimate of intra-subject variability, but this is not available in studies investigating the pharmacokinetics of amlodipine because studies of this type are not crossover designs.(109) Therefore, our initial sample size calculation was an overestimate. In the study, we found an intra-subject variability of 12% to 14%, which allowed us to make conclusions with sufficient power based on only 20 subjects. These intra- and inter-subject variabilities were also consistent with Health Canada guidelines, which state that for drugs with uncomplicated characteristics, the intra-subject variability should generally be less than 20%.(98)

Our results indicate that a single 5mg dose of the tablet and suspension formulation of amlodipine exhibits similar PK behaviours, and can be considered to have the same rate and extent of absorption. This is evidenced by the fact that the 90% confidence interval for all PK parameters that are used to demonstrate bioequivalence fell within the 80% to 125% limit.

Although the ANOVA analysis found a statistically significant difference between the formulations, a statistically significant difference in the comparison of bioavailability between drug products does not necessarily imply that there is a clinically significant difference in terms of therapeutic effect. Regulatory agencies have accepted that although the results may be statistically significant, a difference in $AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{\text{max}}$ of less than ±20% between formulations is taken as being not clinically significant. (Table 21).(106)
Nevertheless, these significant results were further investigated, and it was found that when all the values for AUC$_{0-t}$ for the tablet were compared between period 1 and period 2, the means were comparable (159.07 ±32.47 in period 1, and 150.03 ±34.06 in period 2, P=0.55). In contrast, when the same calculation was performed for the suspension formulation, the means were statistically different (148.09 ±28.33 in period 1 and 122.81 ±17.67 in period 2, P=0.03). Secondly, the differences in the mean AUC$_{0-t}$ values of the tablet and suspension formulation in Period 1 were not statistically different (P=0.43), whereas the mean differences in AUC$_{0-t}$ values of the tablet and suspension formulation in Period 2 were statistically different (P=0.04). This is reflected in a significant period effect, meaning that there is a difference between drug formulations that varies with the order of administration.(107) Hence, subjects who received the suspension second had significantly lower AUC values compared to subjects who received the suspension first. A potential explanation is that there was decreased stability over time with the suspension formulation.

The hospital pharmacy made only one batch of the suspension in Ora Plus/OraSweet which was stored at room temperature. It was reported by Nahata et al. (1999) that amlodipine in suspension is stable for up to 56 days at room temperature, and for up to 91 days under refrigeration at 4°. The suspension was made by the hospital pharmacy on May 1, 2001, and our study was completed in 43 days (from May 7 to June 18, 2001), which is a total of 50 days for the suspension, quite close to the expiry date. To provide further proof for the decreased stability over time of the suspension, the AUC$_{0-t}$ suspension to tablet (S/T) ratio for each subject was plotted over time (Appendix 12). Linear regression analysis revealed a downward trend in ratios over time, which was statistically significant ($r^2 = 0.25$, df = n-2, P<0.05).
In order to extrapolate these results to children, it is important to account for age-appropriate developmental factors that may influence drug pharmacokinetics and bioavailability. Rate of absorption from the gastrointestinal tract is a major determinant of bioavailability, and both gastric pH and gastric emptying are factors that determine the amount of drug that is found in the systemic circulation. Gastric pH is less acidic at birth and in infants, however it drops to adult values in the first 2 years of life. This phenomenon can hinder absorption of acidic drugs and enhance absorption of basic drugs in very young children. Gastric emptying is slow and erratic in the neonate, however normal adult rates are reached at about 6 to 8 months. The intestinal surface area and biliary function are already near adult values in infants. Further, the metabolic capacity and activity of the gastrointestinal microflora is only highly variable at birth. Therefore, oral absorption of amlodipine should equal adult rates before about 2 years of age.

Secondly, body composition changes with the physical growth and development of the child, and plays a major role in drug distribution. Children have a greater proportion of body fluid, contributing to a higher Vd for drugs that are water soluble. This total body water decreases from 80% at birth to adult levels (60%) at 1 year due to a reduction in extracellular fluid. However, adult proportions of intra- and extracellular water are not reached until early adolescence, thus polar drugs such as amlodipine would require higher doses to reach the same plasma levels as in adults. Fat content also increases rapidly after birth, and represents 30% of a child's bodyweight at 1 year. Muscle mass also increases quite rapidly. Therefore, for lipophilic drugs, there should be a higher Vd in young children. Other factors that may contribute to changes in Vd are relative organ size, and the binding affinity for plasma and tissue proteins. Infants can experience changes in protein binding due to reduced albumin concentrations and reduced albumin binding affinity, however
these altered binding capacities are only important in the very young. In addition, the reduced plasma protein concentrations do not directly affect free drug concentrations at steady state.(35;78)

In terms of metabolism, it has been documented that most of the enzymatic microsomal systems for biotransformation are present at birth. However, in neonates and infants, hepatic metabolic processes are immature, resulting in reduced clearance rates. Rapid maturation has been reported to occur in the first week of life, and approaches adult levels by 6 to 12 months of post-natal age.(78) Adult activity may be exceeded between 1 to 4 years of age, but progressively declines to normal adult levels at the conclusion of puberty. (Leeder JS 1997)

In general, the most dramatic PK changes occur only during the first 12 months of life, indicating that for a drug such as amlodipine, the bioequivalence of the two formulations, and the corresponding PK parameters should not differ between adults and children above the age of 1 or 2. Salmun et al. (2000) assessed the pharmacokinetics of loratadine syrup and its metabolite desloratadine in 18 healthy children aged 2 to 5 years. The study found that the PK parameters AUC, Cmax and Tmax, were of the same order of magnitude as the exposure after administration of a 10mg loratidine tablet in adults.(111) A study by Gan et al. (1992) assessed the pharmacokinetics (AUC, Cmax and Tmax) of a clarithromycin suspension in 24 infants and children aged 6 months to 10 years for whom an orally administered antibiotic had been prescribed. Results indicated that the clarithromycin suspension was well absorbed, with PK values that were comparable to those reported in adults given a clarithromycin tablet.(112) Guay et al. (1993) assessed the bioavailability of a clarithromycin suspension and tablet in 22 healthy adult volunteers, and compared the results to the bioavailability of a clarithromycin suspension given to 24 infants and children. Both formulations were found to have comparable bioavailability in both adults and children.(113) A
comparison of the pharmacokinetics of naproxen tablets and suspension in 23 children aged 8 to 4 years found that the two formulations were bioequivalent, and were comparable to adult PK values. However, it was found that children exhibited a higher Cl_{app}. rate.\textsuperscript{114} \textsuperscript{115}

The main limitation of this study was that due to ethical constraints, the study could not be conducted in children, even though the results will be applied to the pediatric population. However, there should be no difference in the bioavailability of either formulation in children compared to adults. This is because if the factors that determine bioavailability such as gastric pH, gastric emptying and biotransformation are accounted for in terms of the physiochemical properties of amlodipine, then it can be assumed that children above the age of two can be expected to exhibit similar bioavailability. In addition, previous studies have shown that different formulations of various drugs in children have comparable bioavailability to adults.

Lastly, performing such a single-dose study in healthy volunteers does not represent actual clinical use of the drug. However, it does minimize variability so there is a greater chance of proving equivalence.\textsuperscript{116} Lastly, because an intravenous dose of amlodipine was not administered, absolute oral bioavailability cannot be calculated.

1.30 Conclusions and Future Studies

Results of this study can be extrapolated to the pediatric population if age-dependent developmental characteristics are accounted for. This demonstrates that such a study, which is ethically questionable in children can be performed in adults in order to yield generalizable knowledge for the pediatric population. It is evident from the results that performing such a study in healthy adults generates a low subject variability, contributing to the conclusion of equivalence between the two formulations.
Our data show that the suspension formulation of amlodipine is bioequivalent to the commercial tablet with respect to the rate and extent of absorption according to regulatory standards. The statistical significant differences found in the analysis of variance are not clinically relevant. However, the previous assumption of stability of the suspension formulation for 56 days at room temperature is not supported, and the suspension needs to be frozen or used within a shorter period of time. These results support the use of this suspension in children who cannot take the tablet, or for those in whom the needed dose cannot be given based on the tablet form.

Future studies utilizing the suspension must assay stability over time in order to ensure that correct doses are administered. This becomes important if the suspension is prescribed to the pediatric hypertensive population, since an unstable or improperly stored or prepared suspension may yield sub-therapeutic drug levels.
SECTION 4: THE EFFICACY OF AMETHOCAIN GEL USING DIFFERENT APPLICATION TIMES

Parts of this section have been prepared as a manuscript for publication entitled: “Amethocaine: A topical anaesthetic for procedural pain,” authored by Dorothy A. Lyszkiewicz, Lisa O’Brien, Anna Taddio and Gideon Koren, for the journal Pediatric Drugs.

1.31 RATIONALE: New Topical Local Anaesthetic

Before approval of the comparative bioavailability study, the REB suggested using a local anaesthetic prior to each venipuncture to ease any discomfort, and recommended the use of EMLA (Eutectic Mixture of Local Anaesthetics containing lidocaine 5% and prilocaine 5%). EMLA is the current gold standard for topical anaesthesia in clinical practice. However, the new local anaesthetic amethocaine 4% gel (Ametop®) which is currently on the market, has begun to emerge as an alternative and perhaps superior local anaesthetic to EMLA (Figure 1). The requirement for repeated blood sampling in the comparative bioavailability study gave us an opportunity to study the efficacy of amethocaine. Our goal for conducting this study was to offer healthcare providers with a larger repertoire of pain control methods in children before procedures such as venipuncture or venous cannulation.

Similar to EMLA, amethocaine’s main indication is topical use for dermal anaesthesia/analgesia, particularly in pediatric populations undergoing invasive procedures. Previous studies comparing EMLA and amethocaine have concluded that amethocaine is superior, since it provides a more rapid onset of action and longer duration of anaesthesia, making it more feasible in busy hospital wards or clinics. (117-121) What is not clear from the literature is the efficacy of amethocaine at different application times.
1.32 Amethocaine

Amethocaine is a local anaesthetic manufactured by Smith & Nephew Healthcare Ltd., and consists of 4% amethocaine base in a white semi-transparent gel (Table 22). In its uncharged form, amethocaine is highly lipophilic with a high affinity for neuronal receptors (Figure 12). Consequently, a small dose can produce rapid and long-lasting effects.

According to the manufacturer, the minimum recommended application time is 30-45 minutes, producing anaesthesia that lasts 4 to 6 hours after a single application. Amethocaine’s extended duration of action is clinically important, as the anaesthetic can be applied at home prior to the procedure, reducing waiting time in the clinic. In addition, amethocaine can be applied without the analgesic effects wearing off before the procedure is performed.

For minor procedures such as venipuncture or intravenous cannulation, generally one tube of amethocaine (1.5g) is used, which covers an area of 30-cm$^2$. A 6cm x 7cm OpSite Flexigrid transparent dressing is applied on top of the gel mound. The gel should not be left on for more than 1 hour, presumably because of the incidence of skin reactions.

Figure 11: Structural formula of amethocaine

\[
\text{H}_9\text{C}_4\text{N} - \text{COOCH}_2\text{CH}_2\text{N} - \text{CH}_3 \\
\text{H} - \text{CH}_3
\]
1.32.1 **Previous Studies**

Numerous studies have been conducted with amethocaine, both in children and in adults. However, studies evaluating the efficacy of amethocaine at different application times are lacking. O'Connor et al. (1995) assessed the efficacy and safety of amethocaine before venous cannulation in a placebo-controlled, double-blind trial of amethocaine (1g) in 42 adult volunteers. The study demonstrated that a 40-minute application time was sufficient to produce clinically acceptable anaesthesia when applied to the dorsum of the hand. Subjects rated their pain using a 10cm VAS and a verbal rating scale (VRS), where 0 indicated no sensation of pain, 1 indicated some sensation of pain but no discomfort, and 2 indicated significant pain. In the amethocaine group, complete anaesthesia (verbal score of 0) was experienced by 67% of subjects compared to 24% in the placebo group (p<0.05). Clinically acceptable anaesthesia (verbal score of 0 or 1) was achieved in 90% of subjects in the amethocaine treatment group as opposed to 52% in the placebo group (p<0.05). (122)

A clinical study of infants and children undergoing venipuncture found that when amethocaine was applied for a minimum time of 30 minutes, it produced clinically acceptable anaesthesia in 1100 of 1241 (88.7%) topical applications. Subjects were asked to rate the intensity of pain resulting from venipuncture using the following 4-point scale: 1 = completely pain free procedure, 2 = minimal

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**Table 22: Constituents of Ametop gel 4%**

<table>
<thead>
<tr>
<th>Constituent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amethocaine 40 mg</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>Sodium methyl-p-hydroxybenzoate</td>
</tr>
<tr>
<td>Sodium propyl-p-hydroxybenzoate</td>
</tr>
<tr>
<td>Monobasic potassium phosphate</td>
</tr>
<tr>
<td>Xanthan gum</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Purified water</td>
</tr>
</tbody>
</table>
sensation but no discomfort, 3 = moderate pain, 4 = no apparent anaesthesia. Scores of 1 and 2 were regarded as successful anaesthesia. The results after 30 minutes were summarized as follows: 70.8% (879 cases) experienced no pain, 17.9% (222 cases) experienced minimal sensation, 6.9% (86 cases) perceived moderate pain and 4.4% (54 cases) had no pain relief.(123)

When a topical anaesthetic preparation is being evaluated, it is important to consider the rate of systemic absorption of the active ingredient and the subsequent plasma concentrations. In order to ascertain plasma concentrations of amethocaine, Mazumdar et al. (1991) studied the absorption of amethocaine 4% (2g) when applied to a 6-cm² area of skin on the dorsum of the hand for 4 hours (240 minutes) in 10 healthy adult volunteers. Serial blood samples were obtained from each volunteer at 0, 30, 60, 120 and 240 minutes, and analyzed for amethocaine and its metabolite n-butyl-p-aminobenzoic acid using high-pressure liquid chromatography (HPLC). In 7 out of 10 subjects, amethocaine was not detected at any of the testing times. Amethocaine concentrations ranging from 0.02 mg/L – 0.18 mg/L were detected in the plasma of three volunteers. The metabolite was only detected in the plasma of one volunteer, with an initial detectable concentration of 0.82 mg/L after 30 min, and a final detectable concentration of 0.24 mg/L after 240 minutes. No toxic reactions occurred in any of the volunteers.(124)

In children, most clinical data on the efficacy of amethocaine has been generated by comparing it to EMLA. The main reason is probably because use of a placebo cannot be ethically justified in a randomized trial in children when effective standard treatment such as EMLA is available and currently used in practice. However, Jain & Rutter (2000) conducted two placebo-controlled, randomized, double-blind trial in infants and neonates to assess the efficacy of amethocaine. In one study, amethocaine (1.5g) or placebo (1.5g) was applied to the dorsum of one foot and covered with
an occlusive dressing for one hour.(125) In the other study, the amethocaine or placebo was applied for either 30 or 60 minutes.(126) Anaesthesia was assessed by eliciting the cutaneous withdrawal reflex in response to stimulation with a series of 20 graded nylon filaments (von Frey hairs). Nylon filaments of increasing thickness were placed on the dorsum of the foot and deformed by downward pressure to elicit a withdrawal reflex. A difference in thickness of the filament required to elicit the cutaneous withdrawal reflex from both feet was taken as evidence of local anaesthetic action. In the first study, 17/31 infants showed evidence of local anaesthetic action compared to 5/29 in the placebo group (P=0.003). In the second study, 23/36 infants showed evidence of local anaesthetic action on the amethocaine treated foot after 30 minutes and 26/36 after 60 minutes. There was no significant difference between the different application times.

An open-label non-comparative study of 148 children aged 3-12 years was conducted to determine if amethocaine produced clinically acceptable anaesthesia during venous cannulation. Amethocaine (1g) was applied to the dorsum of the hand and covered with an occlusive dressing for 40 to 60 minutes. Pain was assessed using the following scale: 0 = no sensation of pain, 1 = some sensation/no obvious discomfort, 2 = painful, including withdrawal of the hand. Pain was perceived in 8% of the patients, 31% experienced some sensation, and 61% experienced no pain. Overall, clinically acceptable anaesthesia (pain scores of 0 and 1) was achieved in 92% of patients.(127)

1.32.2 Tolerability

The most common side effect reported with amethocaine use is localized erythema, which is primarily due to the vasodilator property of the anaesthetic. In most cases, erythema resolves spontaneously upon removal of the preparation.(123;128-130) Edema and itching at the site of application are less common side effects.(122;128) There has been no evidence of sensitization upon repeated exposure to amethocaine.(123;129)
Amethocaine has low systemic bioavailability, as it is rapidly metabolized by non-specific esterases in the tissue and blood. This property significantly limits the risk of developing systemic toxicity. However, the drug is contraindicated in persons with known hypersensitivity to ester-type local anaesthetics, and should not be applied to mucous membranes or broken skin.

1.33 Objective

To determine the relative efficacy of amethocaine 4% gel using different application times, and compare results to a placebo.

1.34 Study Hypothesis

The relative efficacy of amethocaine is directly proportional to application time.

1.35 Ethics and Patient Consent

This study was approved by the REB on March 15, 2001 as an amendment to the comparative bioavailability study protocol. A copy of the approval is included in Appendix 13. The nature of the study was explained to all candidates who were participating in the comparative bioavailability study, and a choice was given of whether subjects wanted to participate in this extra procedure. A copy of the informed consent form is included in Appendix 14.

1.36 Subject Enrollment and Eligibility Criteria

We planned to include all subjects who were participating in the comparative bioavailability study, thus the eligibility criteria for this study are identical, except for some minor additions in the exclusion criteria (Table 23). Our sample size is limited by the number of patients participating in the comparative bioavailability study.
Table 23: Exclusion criteria

- Known hypersensitivity to local anaesthetics
- Subjects with broken skin on the arm cubital fossa area
- Subjects who had used analgesic preparations within the previous 24 hours

1.37 METHODOLOGY: Study Design and Clinical Procedures

Subjects were randomized by the co-investigator to receive amethocaine for either 20 or 40 minutes prior to venous cannulation by drawing numbers out of a hat (Table 24). Subjects were also randomized to receive either amethocaine or placebo for either 20, 30 or 40 minutes using a Latin Square design (Table 25). These times have been chosen based on manufacturer recommendations and previous studies.

Amethocaine was provided by Smith and Nephew Healthcare Ltd. The placebo “Aquatain Moisturizing Lotion” was purchased at Shopper’s Drug Mart, and had identical texture and colour to amethocaine. In order to maintain blinding, a 1 g dose of amethocaine or placebo were transferred into 3cc. plastic syringes (Exacta-Med Dispenser) provided by HSC pharmacy. Each syringe was labeled with the subject’s initials and randomized application time by the co-investigator.

All subjects had a 1 g dose of amethocaine or placebo administered by the co-ordinator as a gel mound, which was covered by the OpSite FlexiGrid as specified by the product monograph. It was also noted on which arm the cream was being applied (left or right), and in what area (ex., cubital fossa). The appearance of the skin was noted after cream removal. In all cases, venipuncture was completed immediately after cream removal.
1.37.1 Phase I

Subjects who met eligibility criteria were accepted into Phase I. Pain was measured using a 10cm VAS. Refer to Appendix 15 for an example of a VAS. The concept of the VAS was explained to all subjects, and all were tested on their ability to understand the scale using a VAS Comprehension Test before the procedure. A copy of the test is included in Appendix 16. All subjects provided a VAS score immediately after venipuncture.

Visit 2: Subjects were randomized to receive amethocaine for 20 or 40 minutes prior to insertion of the venous catheter. Pain was assessed by asking the subjects to make a mark on the line at a point representing the severity of pain using the VAS immediately after catheter insertion.

Visit 3, 4, 5, and 6: At each of these visits, one blood sample was obtained from each subject for the comparative bioavailability study. Subjects were randomized to have either amethocaine or placebo applied for either 20, 30 or 40 minutes before venipuncture. Pain was assessed on the VAS as previously described.
Table 24: Subject randomization to treatment to amethocaine before venous cannulation

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Visit 2</th>
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<tbody>
<tr>
<td>1</td>
<td>A</td>
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<tr>
<td>2</td>
<td>A</td>
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</tr>
<tr>
<td>21</td>
<td>A</td>
</tr>
</tbody>
</table>

*A=40 minutes, B=20 minutes

Table 25: Latin-Square subject randomization scheme to amethocaine before venipuncture

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Visit 3</th>
<th>Visit 4</th>
<th>Visit 5</th>
<th>Visit 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>D</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
</tbody>
</table>

Patient #’s continued… Repeat Latin Square

*A=placebo, B=20 minutes, C=30 minutes, D=40 minutes
1.38 Results

All subjects who agreed to participate in the comparative bioavailability study participated in this study except for subject #27. Therefore, 21 subjects completed the study. After 12 subjects had amethocaine applied, we noticed that most of them developed local skin reactions that seemed to worsen with each subsequent application of amethocaine. We therefore performed an interim safety analysis to look for patterns in the incidence of these adverse effects, and reported these events to the REB. Refer to Appendix 17 for a copy of the adverse event report.

The skin reactions were coded in order to identify patterns, where 0=no adverse reactions or erythema, 1=mild itchiness, 2=presence of small, red, raised papules, and 3=vesicular rash and skin blistering. We also took note of whether the right or left arm was being used, and on what location on the arm the anaesthetic was applied, to determine whether the reactions were the result of sensitization or contact dermatitis. (Table 26). For example, there is strong evidence for a systemic effect in subject #9, as in the first 3 visits there are no adverse reactions when the anaesthetic is applied on the right arm, but when the anaesthetic is applied on the fourth day to the left arm, there is evidence of a vesicular rash after anaesthetic removal. Further, when the anaesthetic is applied on the right arm the subsequent day, the vesicular rash re-appears.

Some of the adverse events were consistent with the literature, such as erythema, however, there were several instances of small, red, raised papules, persistent itchiness and redness, and blistering (vesicular rash), that have not been previously reported. Skin reactions were transient in subjects #4, #7, #17 and #18. Subjects #1 and #8 seemed to have been already sensitized to amethocaine from previous exposure because of the presence of a skin reaction on the first day. We did not re-expose subject #08 to amethocaine because she discontinued the study after this visit. However, she
did complete the study 2 weeks later. In the remaining 6 patients (#2, #3, #5, #9, #11, #23), it appears that the reactions may have intensified over time, although the results are inconsistent.

Due to the incidence of these skin reactions to amethocaine, we decided to discontinue the study, as we could not justify exposing other subjects to the drug. Instead, we decided to use the local anaesthetic EMLA. Since EMLA is the current gold standard in clinical practice, there was no need to test different application times, and the manufacturer recommended application time of 60 minutes was used before each venipuncture. We continued to assess pain in subjects receiving EMLA using the VAS, and we were also interested in seeing whether local skin reactions would develop to EMLA upon repeated exposure. The same method for assessing pain and noting skin reactions was performed as was done for amethocaine. The REB supported the revised methodology, and approved an amendment to the protocol on June 5, 2001. A copy of the approval letter is included in Appendix 18. A copy of the amended informed consent form, which includes EMLA as the local anaesthetic is in Appendix 19.

Subsequent to this decision, group #3 received only EMLA in both Phase I and Phase II, whereas Group #1 and Group #2 received amethocaine in Phase I, and EMLA in Phase II. Subjects who received amethocaine in Phase I were required to receive EMLA in Phase II to look for the incidence of skin reactions. However, subjects who received EMLA in Phase I were not required to receive EMLA again in Phase II, and only received it upon request.

Out of a total of 12 subjects who had amethocaine applied, 9 (75%) experienced some degree of adverse skin reaction. Six reported mild itchiness, and 4 had evidence of small, red, raised papules. Subject #9 developed a vesicular rash. In contrast, only 1/10 (10%) subjects who were exposed to
EMLA exhibited a local skin reaction. A Fischer-Exact test revealed that these results were highly significant (P=0.004). Subject #14 developed a mild rash in response to EMLA after the 4th day of exposure. This rash resolved after 24 hours and reappeared in a more severe form after EMLA exposure on the 5th day. This subject was not exposed to amethocaine in Phase I. In addition, subjects who developed skin reactions to amethocaine in phase I did not develop any reactions to EMLA in phase II.
Table 26: Adverse local skin reactions to amethocaine or placebo following application times of 20, 30 or 40 minutes

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>Visit 4</th>
<th>Visit 5</th>
<th>Visit 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40 min AM L,2</td>
<td>20 min PL R,0</td>
<td>20 min AM R,0</td>
<td>30 min AM R,2</td>
<td>40 min AM R,0</td>
</tr>
<tr>
<td>2</td>
<td>42 min AM L,0</td>
<td>20 min AM L,0</td>
<td>30 min AM L,1</td>
<td>40 min AM L,0</td>
<td>30 min PL L,0</td>
</tr>
<tr>
<td>3</td>
<td>20 min AM L,0</td>
<td>30 min AM L,1</td>
<td>40 min AM R,2</td>
<td>40 min PL R,0</td>
<td>20 min AM R,0</td>
</tr>
<tr>
<td>4</td>
<td>20 min AM R,0</td>
<td>40 min AM L,0</td>
<td>20 min PL R,0</td>
<td>25 min AM L,0</td>
<td>30 min AM L,0</td>
</tr>
<tr>
<td>5</td>
<td>20 min AM R,0</td>
<td>30 min PL L,0</td>
<td>20 min AM L,2</td>
<td>30 min AM R,1</td>
<td>40 min AM L,1</td>
</tr>
<tr>
<td>7</td>
<td>40 min AM L,0</td>
<td>30 min AM R,0</td>
<td>40 min AM R,0</td>
<td>20 min PL R,0</td>
<td>20 min AM L,1</td>
</tr>
<tr>
<td>8</td>
<td>40 min AM R,1</td>
<td>No cream 1</td>
<td>No cream 1</td>
<td>No cream 1</td>
<td>No cream 1</td>
</tr>
<tr>
<td>9</td>
<td>20 min AM R wrist,0</td>
<td>40 min PL R,0</td>
<td>25 min AM R,0</td>
<td>25 min AM L,2</td>
<td>40 min AM R,3</td>
</tr>
<tr>
<td>11</td>
<td>45 min AM L,0</td>
<td>40 min AM L,0</td>
<td>40 min AM R,0</td>
<td>20 min PL R,0</td>
<td>20 min AM R,0</td>
</tr>
<tr>
<td>17</td>
<td>20 min AM L wrist,0</td>
<td>30 min PL R,0</td>
<td>19 min AM R,1</td>
<td>30 min AM R,1</td>
<td>40 min AM R,1</td>
</tr>
<tr>
<td>18</td>
<td>40 min AM L wrist,0</td>
<td>21 min AM R,0</td>
<td>30 min AM R,0</td>
<td>40 min AM R,0</td>
<td>60 min PL L,0</td>
</tr>
<tr>
<td>23</td>
<td>40 min AM R,0</td>
<td>40 min AM R,0</td>
<td>40 min AM L,0</td>
<td>30 min PL R,0</td>
<td>20 min AM L,2</td>
</tr>
</tbody>
</table>

Legend:

AM: Amethocaine
PL: Placebo
R: Right arm cubital fossa
L: Left arm cubital fossa

0: Redness, or no adverse reaction
1: Mild itchiness or skin dryness
2: Small, red, raised papules
3: Vesicular rash, blistering
These adverse events were unexpected, as before the study began, the co-investigator contacted the manufacturer to inquire about the incidence of adverse reactions upon repeated application of amethocaine, to which the manufacturer responded that they had no data. In addition, there are two studies in the literature reporting that repeated use of amethocaine does not cause local skin reactions after repeated exposure.(123;129)

VAS scores for both amethocaine and EMLA were measured with a ruler, and scores were recorded and analyzed on the statistical program Sigma Stat. See table 27 for raw VAS scores for Phase I, and table 28 for raw VAS scores for Phase II.
<table>
<thead>
<tr>
<th>Subject</th>
<th>Drug</th>
<th>Time</th>
<th>Visit 2: catheter</th>
<th>Time</th>
<th>Visit 3</th>
<th>Time</th>
<th>Visit 4</th>
<th>Time</th>
<th>Visit 5</th>
<th>Time</th>
<th>Visit 6</th>
<th>Average EMLA score</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>AM</td>
<td>40</td>
<td>0.00</td>
<td>20</td>
<td>3.00</td>
<td>20</td>
<td>3.30</td>
<td>30</td>
<td>0.00</td>
<td>40</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>AM</td>
<td>42</td>
<td>0.00</td>
<td>20</td>
<td>0.30</td>
<td>30</td>
<td>0.10</td>
<td>40</td>
<td>0.10</td>
<td>30</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>03</td>
<td>AM</td>
<td>20</td>
<td>0.10</td>
<td>30</td>
<td>1.40</td>
<td>40</td>
<td>0.25</td>
<td>40</td>
<td>0.10</td>
<td>20</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>04</td>
<td>AM</td>
<td>20</td>
<td>1.80</td>
<td>40</td>
<td>2.20</td>
<td>20</td>
<td>2.45</td>
<td>25</td>
<td>0.40</td>
<td>30</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>05</td>
<td>AM</td>
<td>20</td>
<td>1.65</td>
<td>30</td>
<td>2.20</td>
<td>20</td>
<td>0.00</td>
<td>30</td>
<td>0.25</td>
<td>40</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>06</td>
<td>EMLA</td>
<td>60</td>
<td>2.90</td>
<td>60</td>
<td>0.40</td>
<td>60</td>
<td>0.10</td>
<td>60</td>
<td>0.10</td>
<td>60</td>
<td>0.10</td>
<td>0.18</td>
</tr>
<tr>
<td>07</td>
<td>AM</td>
<td>40</td>
<td>0.35</td>
<td>30</td>
<td>0.60</td>
<td>40</td>
<td>0.15</td>
<td>20</td>
<td>1.70</td>
<td>20</td>
<td>5.10</td>
<td></td>
</tr>
<tr>
<td>08</td>
<td>EMLA</td>
<td>65</td>
<td>0.00</td>
<td>60</td>
<td>0.00</td>
<td>62</td>
<td>0.00</td>
<td>55</td>
<td>0.00</td>
<td>60</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>09</td>
<td>AM</td>
<td>20</td>
<td>2.50</td>
<td>39</td>
<td>0.00</td>
<td>25</td>
<td>0.20</td>
<td>25</td>
<td>0.00</td>
<td>40</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>AM</td>
<td>40</td>
<td>2.90</td>
<td>30</td>
<td>0.30</td>
<td>40</td>
<td>4.90</td>
<td>20</td>
<td>6.20</td>
<td>20</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>EMLA</td>
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<td>0.65</td>
<td>60</td>
<td>0.00</td>
<td>60</td>
<td>0.40</td>
<td>60</td>
<td>0.00</td>
<td>60</td>
<td>1.00</td>
<td>0.35</td>
</tr>
<tr>
<td>13</td>
<td>EMLA</td>
<td>55</td>
<td>0.25</td>
<td>55</td>
<td>1.35</td>
<td>55</td>
<td>0.50</td>
<td>55</td>
<td>0.35</td>
<td>60</td>
<td>0.40</td>
<td>0.65</td>
</tr>
<tr>
<td>14</td>
<td>EMLA</td>
<td>55</td>
<td>0.60</td>
<td>60</td>
<td>0.00</td>
<td>60</td>
<td>0.40</td>
<td>70</td>
<td>0.40</td>
<td>60</td>
<td>0.25</td>
<td>0.26</td>
</tr>
<tr>
<td>15</td>
<td>EMLA</td>
<td>70</td>
<td>1.30</td>
<td>55</td>
<td>2.40</td>
<td>60</td>
<td>0.95</td>
<td>60</td>
<td>0.00</td>
<td>65</td>
<td>3.00</td>
<td>1.59</td>
</tr>
<tr>
<td>16</td>
<td>EMLA</td>
<td>50</td>
<td>0.70</td>
<td>70</td>
<td>0.00</td>
<td>60</td>
<td>0.00</td>
<td>63</td>
<td>0.85</td>
<td>60</td>
<td>0.00</td>
<td>0.21</td>
</tr>
<tr>
<td>17</td>
<td>AM</td>
<td>20</td>
<td>4.90</td>
<td>30</td>
<td>2.00</td>
<td>19</td>
<td>0.90</td>
<td>30</td>
<td>1.25</td>
<td>40</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>AM</td>
<td>40</td>
<td>5.25</td>
<td>21</td>
<td>0.50</td>
<td>30</td>
<td>0.80</td>
<td>40</td>
<td>0.00</td>
<td>60</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>AM</td>
<td>40</td>
<td>N/A</td>
<td>30</td>
<td>0.10</td>
<td>40</td>
<td>0.00</td>
<td>30</td>
<td>1.30</td>
<td>20</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>EMLA</td>
<td>55</td>
<td>0.75</td>
<td>60</td>
<td>0.60</td>
<td>60</td>
<td>0.00</td>
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<td>0.00</td>
<td>60</td>
<td>0.40</td>
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</tr>
<tr>
<td>25</td>
<td>EMLA</td>
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<td>0.10</td>
<td>55</td>
<td>0.10</td>
<td>60</td>
<td>0.10</td>
<td>60</td>
<td>0.10</td>
<td>60</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>26</td>
<td>EMLA</td>
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<td>60</td>
<td>0.00</td>
<td>60</td>
<td>0.00</td>
<td>60</td>
<td>0.30</td>
<td>60</td>
<td>0.30</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* AM = Amethocaine
* EMLA = Eutectic mixture of local anaesthetics
* The time and score in bold indicates placebo treatment
* N/A means either the subject did not receive anaesthetic or did not give a pain score
### Table 28: Visual Analog Scores for phase II

<table>
<thead>
<tr>
<th>Subject</th>
<th>Drug</th>
<th>Time</th>
<th>Visit 2: catheter</th>
<th>Time</th>
<th>Visit 3</th>
<th>Time</th>
<th>Visit 4</th>
<th>Time</th>
<th>Visit 5</th>
<th>Time</th>
<th>Visit 6</th>
<th>Average EMLA score</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>EMLA</td>
<td>60</td>
<td>1.75</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>0.05</td>
</tr>
<tr>
<td>02</td>
<td>EMLA</td>
<td>60</td>
<td>0.00</td>
<td>60</td>
<td>0.00</td>
<td>55</td>
<td>0.10</td>
<td>45</td>
<td>0.00</td>
<td>55</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>03</td>
<td>EMLA</td>
<td>60</td>
<td>0.10</td>
<td>65</td>
<td>0.10</td>
<td>90</td>
<td>0.10</td>
<td>60</td>
<td>0.10</td>
<td>55</td>
<td>0.10</td>
<td>0.23</td>
</tr>
<tr>
<td>04</td>
<td>EMLA</td>
<td>65</td>
<td>1.10</td>
<td>55</td>
<td>0.70</td>
<td>60</td>
<td>0.10</td>
<td>60</td>
<td>0.00</td>
<td>60</td>
<td>0.10</td>
<td>0.40</td>
</tr>
<tr>
<td>05</td>
<td>EMLA</td>
<td>60</td>
<td>5.00</td>
<td>55</td>
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<td>0.00</td>
<td>45</td>
<td>1.60</td>
<td>60</td>
<td>0.00</td>
<td>0.15</td>
</tr>
<tr>
<td>06</td>
<td>EMLA</td>
<td>60</td>
<td>0.10</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>0.20</td>
</tr>
<tr>
<td>07</td>
<td>EMLA</td>
<td>60</td>
<td>3.60</td>
<td>65</td>
<td>0.10</td>
<td>60</td>
<td>0.40</td>
<td>60</td>
<td>0.00</td>
<td>65</td>
<td>0.10</td>
<td>0.15</td>
</tr>
<tr>
<td>08</td>
<td>EMLA</td>
<td>60</td>
<td>0.00</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>0.15</td>
</tr>
<tr>
<td>09</td>
<td>EMLA</td>
<td>n/a</td>
<td>n/a</td>
<td>75</td>
<td>0.10</td>
<td>80</td>
<td>0.30</td>
<td>60</td>
<td>0.00</td>
<td>55</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>11</td>
<td>EMLA</td>
<td>65</td>
<td>8.10</td>
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<td>0.00</td>
<td>55</td>
<td>0.80</td>
<td>45</td>
<td>0.00</td>
<td>55</td>
<td>0.20</td>
<td>0.25</td>
</tr>
<tr>
<td>18</td>
<td>EMLA</td>
<td>50</td>
<td>2.10</td>
<td>55</td>
<td>0.30</td>
<td>60</td>
<td>0.00</td>
<td>65</td>
<td>0.00</td>
<td>60</td>
<td>2.20</td>
<td>0.63</td>
</tr>
<tr>
<td>23</td>
<td>EMLA</td>
<td>60</td>
<td>0.10</td>
<td>50</td>
<td>0.10</td>
<td>55</td>
<td>0.15</td>
<td>60</td>
<td>0.00</td>
<td>60</td>
<td>0.20</td>
<td>0.11</td>
</tr>
</tbody>
</table>

* AM = Amethocaine
* EMLA = Eutectic mixture of local anaesthetics
* N/A means either the subject did not receive anaesthetic or did not give a pain score
Descriptive statistics for amethocaine VAS scores before venous cannulation are in table 29, and before venipuncture are in table 30. Mean VAS scores for EMLA are in table 31.

**Table 29: Mean VAS scores for amethocaine before venous cannulation**

<table>
<thead>
<tr>
<th>Application time</th>
<th>Mean score ±sd</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 min.</td>
<td>2.19 ±1.75</td>
<td>1.80 (0.10-4.90)</td>
</tr>
<tr>
<td>40 min.</td>
<td>1.70 ±2.33</td>
<td>0.35 (0.00-5.25)</td>
</tr>
</tbody>
</table>

**Table 30: Mean VAS scores for amethocaine before venipuncture**

<table>
<thead>
<tr>
<th>Application time</th>
<th>Mean score ±sd</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>1.82 ±1.76</td>
<td>1.70 (0.00-6.20)</td>
</tr>
<tr>
<td>20 min.</td>
<td>1.10 ±1.61</td>
<td>0.45 (0.00-5.10)</td>
</tr>
<tr>
<td>30 min.</td>
<td>0.45 ±0.50</td>
<td>0.25 (0.00-1.40)</td>
</tr>
<tr>
<td>40 min.</td>
<td>0.75 ±0.90</td>
<td>0.25 (0.00-2.60)</td>
</tr>
</tbody>
</table>

**Table 31: Mean VAS scores for EMLA**

<table>
<thead>
<tr>
<th>Visit #</th>
<th>Mean score ±sd</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit 2 (cannulation)</td>
<td>1.53 ±2.04</td>
<td>0.70 (0.00-8.10)</td>
</tr>
<tr>
<td>Visit 3</td>
<td>0.33 ±0.61</td>
<td>0.10 (0.00-2.40)</td>
</tr>
<tr>
<td>Visit 4</td>
<td>0.23 ±0.28</td>
<td>0.10 (0.00-0.95)</td>
</tr>
<tr>
<td>Visit 5</td>
<td>0.18 ±0.40</td>
<td>0.00 (0.00-1.60)</td>
</tr>
<tr>
<td>Visit 6</td>
<td>0.46 ±0.80</td>
<td>0.20 (0.00-3.00)</td>
</tr>
</tbody>
</table>

An unpaired t-test demonstrated that there was no statistically significant difference between the 20 minute and the 40 minute amethocaine application time before venous cannulation (P=0.72).

A Friedman Repeated Measures ANOVA on Ranks was performed on the different application times of amethocaine or placebo before venipuncture because data were not normally distributed, and revealed no statistically significant difference (P=0.15).
For the EMLA portion of the study, a paired t-test was conducted to determine if there were any differences between scores of subjects who received EMLA for 60 minutes compared to the same subjects who received amethocaine for 20 or 40 minutes prior to venous cannulation. However, only 4 subjects had both the 20 minute amethocaine score along with an EMLA score and only 4 subjects had the 40 minute amethocaine score along with an EMLA score. The results of the test showed no statistically significant differences ($P=0.98$ and $P=0.10$, respectively), but these results may be due to low power.

To compare the scores of subjects who received EMLA to subjects who received amethocaine or placebo during venipuncture, an average pain score was first obtained from each subject’s EMLA VAS score from Visit 3, 4, 5 and 6 in both Phase I and Phase II. This was possible because each subject received exactly the same treatment every day. Subsequently, these averaged scores were compared to placebo or amethocaine after each application time using the Wilcoxon Rank Sum Test because data were not normally distributed. There were no statistically significant differences between EMLA scores and the 20, 30 and 40 minutes amethocaine scores ($P=0.06$, $P=0.86$ and $P=0.41$, respectively). There was a statistically significant difference between the average EMLA scores and placebo ($P=0.006$).

1.39 Discussion

This study, which was initially designed to compare the efficacy of analgesia using various application times of amethocaine in order to provide more evidence for its use in pediatrics, allowed us to uncover a high incidence of local skin reactions upon repeated exposure to amethocaine, a phenomenon which was never tested for previously. The local anaesthetic EMLA had no such effect, even in subjects who reacted to amethocaine in Phase I and were later exposed
to EMLA. The incidence of local skin reactions in 9 out of 12 (75%) subjects to amethocaine was unexpected, and forced us to discontinue the study.

In the literature, there is 1 case report of local skin reactions developing with amethocaine use at the Royal Children’s Hospital in Melbourne. Within an 18 month time period, amethocaine was used for over 20,000 procedures at the Melbourne hospital. One case involved the use of amethocaine gel on a 4-year-old boy prior to venous cannulation. Within 5 minutes, the boy complained of intense pain at the site of application. The cream was removed after 15 minutes as the pain had not subsided, and revealed red, inflamed skin that blistered and peeled 48 hours later. Another case was reported by a staff anesthetist at the same hospital, who developed a localized erythematous reaction to amethocaine that lasted several days. An audit was conducted by the hospital to determine the prevalence of these adverse reactions. Data was collected on the reactions of 272 day-surgery patients exposed to amethocaine. Amethocaine was applied to a 1.5 x 1.5 cm area of skin and covered with an occlusive dressing for a minimum time of 30 minutes. The appearance of the skin following removal of the preparation was noted and classified as follows: normal, erythema, raised erythema (reddening and edema of the skin), urticaria and other. Follow-up was conducted 24-hours later by telephone to detect any delayed reactions. Sixty-eight percent of patients were classified as “normal” upon immediate removal of the anaesthetic, and this rose to 83% by the time they were discharged. Erythema was noted in 26% of patients upon immediate removal of the preparation, but this number fell to 15% by the time they were discharged. Other reactions such as paleness were reported in 3.7% of subjects. All adverse reactions resolved in all patients. No statistical association was found between the duration of amethocaine application and the incidence of skin reactions. The hospital concluded that amethocaine is a safe and effective topical analgesic. (129) However, the incidence of adverse reactions after repeated exposure was
not tested. We believe that the skin reactions seen in our subjects were most likely amethocaine sensitization or delayed hypersensitivity reactions, which have not been previously reported.

Our primary objective was to determine whether there were any differences between placebo and various application times of amethocaine. A Latin Square Design for randomizing subjects ensured that all subjects received each application time. Our results demonstrated that there were no statistically significant differences between the placebo and 20, 30 and 40 minutes of amethocaine prior to both venous cannulation and venipuncture. However, because this study had to be stopped prematurely, we did not have enough power to be able to detect this difference.

There was a trend among the VAS scores, suggesting that lower pain scores were achieved when amethocaine was applied for a longer period of time for both venous cannulation and venipuncture. (Tables 29 and 30). Similarly for EMLA, the VAS score after cannulation was higher than for all venipuncture scores (Table 31).

Limitations of this study were that the pain that was reported in adults may not be of the same intensity as would be reported by children, reflected by a very small range of pain scores seen in our results. This may be because with venipuncture, adults generally experience less fear and anxiety with such a medical procedure. Another explanation for the small range in pain scores could be that subjects did not understand the pain rating scale. The VAS has been criticized in being too subjective, since there are no specific numbers arranged along the 10cm VAS line, making it difficult to use.(43) However, this explanation does not seem likely, as all subjects were administered the VAS Comprehension Test prior to submitting pain scores. Further, in this type of study, subjects who are anxious or fearful of blood sampling procedures and who may score higher
than average on the VAS, would probably not agree to participate. In fact, the 6 subjects who withdrew consent discontinued due to traumatic phlebotomy procedures, or were not comfortable with the blood sampling requirements. Lastly, blood samples were not always drawn by the same person for the duration of the study. However, individual subjects had their blood taken by the same person in each phase.

Another limitation with conducting such a study in adults is that adults tend to be more aware of the study procedures. This was reflected by the fact that subjects were trying to guess whether they had the anaesthetic or the placebo administered, potentially leading to inaccurate pain scores.

From an ethical perspective, results of this study can be generalized to the pediatric population. The finding of a high incidence of local skin reactions with repeated amethocaine exposure may preclude use of this anaesthetic in children, especially if a safer alternative such as EMLA is available.

A final limitation, is that because of insufficient power, we were not able to detect differences in efficacy between different application times of amethocaine. We were also not able to detect if there were any differences in efficacy between amethocaine and EMLA due to the small number of subjects that received each treatment.

1.4.0 Conclusions and Future studies

There has been increased interest in the topic of pain relief in children, and new pharmacologic agents are being considered. Data from clinical trials of both adult and pediatric patients undergoing venipuncture and venous cannulation have established amethocaine to be an effective topical local anaesthetic. The lipophilic nature of amethocaine allows for the use of smaller concentrations of
drug, producing rapid and long lasting effects compared to the relatively more hydrophilic anaesthetics such as lidocaine and prilocaine contained in EMLA.

As evident from our study, the assessment of amethocaine in an adult population first, has provided important data that can be applied to pediatrics. In general, no statistically significant differences were found between the various application times of amethocaine or placebo, and no differences were found among the efficacy of EMLA compared to amethocaine, except for a statistically significant difference between EMLA and placebo. This is probably because we did not have enough power to detect this significance among groups. What is of significance, is that this study allowed us to discover a high incidence of local skin reactions upon repeated exposure to amethocaine, and that these skin reactions do not occur upon repeated exposure to EMLA. These results can now be used when considering the application of local anaesthetics in children, with the possibility that amethocaine may not be advocated.

Future studies are required to make more careful assessments of the skin reactions to both amethocaine and EMLA. This can be accomplished by following up all subjects who experienced local skin reactions by a dermatologist. Secondly, a study that specifically documents the incidence of adverse reactions to amethocaine should be completed.
SUMMARY

Pediatric research has been conducted for hundreds of years, however it is only recently that an ethical framework within which to conduct pediatric studies has been in existence. From a historical perspective, many advances in pediatric medicine resulted from unethical research protocols. Since then, the importance of involving children in clinical drug research has begun to materialize. The FDA has taken enormous steps to address this issue, and it is widely agreed that there is a need to conduct PK studies in children.

It became clear that research studies had to move away from the 'therapeutic' and 'non-therapeutic' distinction and adopt a careful assessment of the risks instead. Currently, if a non-therapeutic research study involves only minimal risk to the child, it can be conducted in order to obtain useful scientific knowledge, providing that both the parents and child agree to such procedures. Since the objective of the PATH-2 clinical trial was to obtain PK parameters in the pediatric population, a strategy had to be developed as to how the research was to be conducted on ethical grounds. Applying the PPK design was the most ethical way to obtain such data in children because of the requirement for only sparse blood samples. Further, the risk of exposing a child to a drug for which they had no need was not an issue because of the pediatric population that was chosen to participate.

The comparative bioavailability study attempted to overcome the ethical limitation of conducting such a study in children by using adult volunteers to answer the research question of whether the two formulations of amlodipine exhibited a similar rate and extent of absorption. Results from this study can now be applied to the pediatric situation.
The amethocaine study clearly demonstrated that it is crucial to conduct a study in adults first when the effects of a drug are unknown. Our study found that repeated use of amethocaine produced sensitization and local skin reactions. This previously unknown adverse effect can now be tested formally, in order to be able to accurately estimate the incidence of this event. Before this is accomplished, caution should be exercised by health care practitioners when children require the drug repeatedly.

The recognition of the rights of children to have access to safe and effective drugs by regulatory agencies has made a significant contribution to pediatric biomedical research. This recognition and support will continue to play a crucial role in providing both manufacturers and clinical investigators with the motivation to study both new and established drug therapies in children, and will hopefully pave the way for a new age in pediatric health care.
REFERENCES


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Appendix A: List of publications


February 23, 2000

Dr. Gerald Arbus
Division of Nephrology
The Hospital for Sick Children

Dear Dr. Arbus

REB File No. 99/277

On behalf of the Research Ethics Board, I am writing to provide confirmation that your study "Pediatric Use of Amlodipine in Treatment of Hypertension: A Population Pharmacokinetic Trial (PATH 2) Version date 4 October 1999 and consent and assent forms version date 16 January 2000 are approved for a 1 year period commencing February 16, 2000.

Yours sincerely

Max Perlman
Chair, Research Ethics Board
THE HOSPITAL FOR SICK CHILDREN

INFORMED CONSENT FOR PARTICIPANTS 16 YEARS OF AGE OR OLDER

TITLE OF STUDY: Pediatric Use of Amlodipine in the Treatment of Hypertension: A Population Pharmacokinetics Trial (PATH-2)

Protocol Number A0531023  Issue date: October 4, 1999
Hospital or Institution: The Hospital for Sick Children, Toronto, Canada
Patient Name: ________________________________

Primary Investigator
Dr. Gerald Arbus
Nephrology
Hospital for Sick Children, Toronto
(416) 813-4463

Study Co-ordinator
Dorothy Lyszkiewicz
Division of Clinical Pharmacology
Hospital for Sick Children, Toronto
(416) 813-7284 x 9

PURPOSE OF THE RESEARCH

You are being asked to participate in a research/drug study sponsored by Pfizer Inc. You are currently being treated with amlodipine and your doctor has indicated that you will continue to receive amlodipine in the future. Amlodipine has been used for the treatment of high blood pressure and angina (chest pain) in adults for seven years and is commonly used in adults. There is a large amount of information about the safety and actions of amlodipine in adults but there is no information about how children and young adults absorb and eliminate amlodipine. There is also no known information about how amlodipine moves through a young person’s body (known as distribution).

The purpose of this study is to obtain a few blood samples from you in order to characterize its absorption, movement and elimination in your body. Participation in this study in no way changes your routine treatment regimen.

EXPLANATION OF PROCEDURES TO BE FOLLOWED:

DESCRIPTION OF RESEARCH

You will undergo a physical examination and a medical history will be collected. A routine part of your examination will consist of assigning a category (number) that describes your sexual development (for girls, breast development and pubic hair, for boys, penile development and pubic hair). The number that is assigned is called the “Tanner stage.” No tests are required in order to assign a number. Also, a routine blood sample will be collected for clinical laboratory testing. If all of the test results are satisfactory, you will be eligible for participation in this study.
By agreeing to participate in this study, you will agree to return to this medical facility for the following procedures:

One blood sample (less than one teaspoonful of blood) will be collected at the screening visit. The nurse will put a cream on your arm each time before blood is taken so that you hardly feel the needle go into your vein. You will be informed whether you are appropriate for participating in this study. If you are appropriate you will be asked to return to the clinic for Visit 1.

Visit 1: With the help of your study co-ordinator, you will be asked to maintain a daily diary to record the date and time when you take amlodipine. This diary should be maintained for one week. On the last 2 days of the diary you are also asked to maintain a meal diary. At the end of one week of making entries to the diary, you will be asked to return to the clinic for Visit 2.

Visit 2: You will receive a telephone call from the study co-ordinator to tell you exactly when you should take your routine dose of amlodipine. The study co-ordinator will instruct you on what time to come to the clinic for 4 blood samples (total less than 3 teaspoonfuls). The nurse will put a small plastic I.V tube into your arm for these blood samples so that a needle does not have to be put into your vein each time. This is done for your comfort. The blood samples are spaced apart over most of the day, where you will remain in the clinic under clinical supervision.

Visit 3: One day following Visit 2 one blood sample (less than one teaspoonful) will be obtained just prior to your routine dose of amlodipine. Your doctor may ask you to wear a blood pressure monitor for a full day so that a 24-hour record of your blood pressure can be obtained. Your doctor will tell you whether you are appropriate for the day-long blood pressure monitoring.

Visit 4: One final blood sample (less than one teaspoonful) will be obtained just prior to your routine dose of amlodipine. The study co-ordinator will provide instruction on when you should take your dose of amlodipine.

EXPECTED DURATION OF THE STUDY AND NUMBER OF PATIENTS EXPECTED TO PARTICIPATE.

This study consists of a screening period (up to 4 weeks duration) followed by a series of 4 visits that can take place over 2 to 4 weeks. Approximately 40 children and young adults will be enrolled in this study at about 6 clinics.

POTENTIAL HARMs, INJURIES, DISCOMFORTS OR INCONVENIENCE

During the collection of blood samples, you may experience pain and/or bruising at the needle site.
Although rare, localized clot formation and infections may occur from blood sampling. Lightheadedness and/or fainting may also occur during or shortly after blood draw. If, during the course of the study there are any changes in the way that the study is being done, or if any previously unknown risks become known, you will be informed by your doctor or study co-ordinator.

POTENTIAL BENEFITS

All tests, examinations and medical care required as part of this study are provided at no cost to you. Participation in this study is purely for research purposes and you will not gain benefits from participating in this study. Your participation in this trial may benefit others through the medical information that will be gained in the treatment of childhood hypertension.

IF YOU DO NOT WANT TO PARTICIPATE IN THIS STUDY:

There will be no change in your healthcare management.

REMOVAL FROM THE STUDY

The doctor in charge of this study or the sponsor (Pfizer Central Research) can remove you from the study without your consent. This would be based on the need to adjust your medical management or because you have failed to follow the study schedule.

OFFER TO ANSWER QUESTIONS ABOUT THE STUDY

If you have any questions about the conduct of this study, you should contact:

Dr. Gerald Arbus at (416) 813-6288
Dorothy Lyszkiewicz at (416) 813-7284 x 9

If you have any questions concerning your rights as a patient in this study, you should contact:

Dr. Max Perlman at (416) 813-6340

If you have any questions concerning adverse drug effects (possible side effects), you should contact:

Gerald Arbus, MD (416) 813-6288
If you are injured as a direct result of taking part in this study, medical treatment shall be made available to you primarily through Dr. Arbus and the Hospital for Sick Children.

Pfizer Central Research will pay for the costs of this care. Financial compensation for such things as lost wages, disability or discomfort due to this type of injury is not routinely available. You understand however, that you have not waived any of your legal rights by signing this form. Participation acknowledgement of nominal value (less than $30.00) will be given to you. In addition, the family of each participant will be provided up to $50.00 per visit for travel expenses (gas, parking, tolls etc...)

RIGHT TO WITHDRAW FROM THE STUDY

You are free to leave this study at any time after telling the doctor in charge of the study. Either declining to participate or leaving the study will not affect your future medical care, and will not penalize you in any way. The following steps must be followed if you participate in the study and then decide to withdraw:

1. Let your doctor know that you will not be continuing the study.
2. Arrange to discuss stopping the study medication with your doctor.
3. You will be asked to return for a clinic visit to complete any paperwork necessary and to complete a standard physical exam.
4. You must return all amiodipine that was provided specifically for the study.

CONFIDENTIALITY

Your family doctor (general practitioner or other) will be told that you will be taking part in this study. Your records obtained while you are in this study as well as related health records will remain strictly confidential at all times. However, you understand that these will need to be made available to Pfizer Central Research, others working on Pfizer’s behalf, the independent review board/independent ethics committee members and medical regulatory agencies such as the Food and Drug Administration (FDA) or the Health Protection Branch (HPB).

By signing the consent form you agree to this access for the current study and any further research that may be conducted in relation to it (even if you withdraw). The information disclosed will remain confidential.

Your identity will not otherwise be released except as may be required by law.

The results of your treatment, including laboratory tests, may be published for scientific purposes provided your identity is not revealed.
DATA PROTECTION: USE OF DATA COLLECTED FROM THIS STUDY

Your personal data, which may be sensitive (such as date of birth) will be collected and processed but only for research purposes in connection with this study. The study data will be sent around the world, but you will not be referred to by name or identified in any report or publication nor could the data be traced back to you. By taking part in this study, you agree not to restrict the use of any data even if you withdraw. Pfizer Inc. (who will control the use of the data) will take steps to ensure your personal data is protected.

CONSENT

"I acknowledge that the research procedures described above have been explained to me and that any questions that I have asked have been answered to my satisfaction. I have been informed of the alternatives to participation in this study, including the right not to participate and the right to withdraw without compromising the quality of medical care at The Hospital for Sick Children for me and for other members of my family. The potential harms and discomforts have been explained to me and I also understand the benefits (if any) of participating in the research study. I know that I may ask now, or in the future, any questions I have about the study or the research procedures. I have been assured that records relating to me and my care will be kept confidential and that no information will be released or printed that would disclose personal identity without my permission unless required by law."

I hereby consent to participate:

_________________________________________  ________________________________
Name of patient                                 Name of person obtaining consent

_________________________________________  ________________________________
Signature of patient                            Signature of person obtaining consent

__________________________  __________________________
Date                                     Date
THE HOSPITAL FOR SICK CHILDREN

ASSENT TO PARTICIPATE IN RESEARCH

TITLE OF STUDY: Pediatric Use of Amlodipine in the Treatment of Hypertension: A Population Pharmacokinetic Trial (PATH-2)

Protocol Number A0531023 Issue date: October 4, 1999
Hospital of Institution: Hospital for Sick Children, Toronto, Canada
Patient Name:____________________________________________

Investigator
Dr. Gerald Arbus
Nephrology
Hospital for Sick Children, Toronto

Study Co-ordinator
Dorothy Lyszkiewicz
Division of Clinical Pharmacology
Hospital for Sick Children, Toronto

Why are we doing this study?

We are asking you to take part in a research study because we are trying to learn more about high blood pressure in children. The name of the medicine that you take every day is called amlodipine. When you take your medicine, most of it goes into your blood. We are doing this study because we want to measure how much of your medicine is found in your blood. We would also like to know how your medicine travels inside your body at different times of the day.

What will happen during the study?

If you agree to be in this study, you will come to the hospital for your first visit. During this visit, the study doctor or the study co-ordinator will ask you questions about your health. They will also ask you questions about what medicines you are taking and why you are taking them. The doctor or study co-ordinator will also take your blood pressure. Blood pressure is 2 numbers that tell how strongly and how often your heart pushes blood around your body. To take your blood pressure, the doctor or study co-ordinator will put a band around your arm. They will pump air into the band and you will feel a squeezing pressure around your arm. This will be done at 3 different times. The doctor or study co-ordinator will also take a blood sample from you which will be about 1 teaspoon of blood. This is to help them decide if you can be in the study. If the doctor or study co-ordinator decide that you can be in the study you will come back for another visit to the hospital.

The reason for the next visit is that we would like to measure the amount of medicine that is found in your blood. In order to do this we need to take blood from your arm. The nurse will put a cream on your arm so that you hardly feel the needle in your vein. The amount of blood that we will take from your arm each time will be less than one teaspoon.
We can only take blood from you if you come for a visit to the hospital so your mom or dad will bring you here. You will only have to come to the hospital 6 times and we will take blood from you 7 times in the whole study. You will be in this study for 4 weeks or 1 month. What we would also like you to do is to write down the date and time when you take your medicine at home. You will only have to come to the hospital 6 times and we will make blood from you 7 times in the whole study. You will be in this study for 4 weeks or 1 month.

Are there good things about the study?

The good thing about the study is that we will be able to see how much of your medicine is found in your blood. This will help us give the correct amount of medicine to other children in the future that may help other kids like you.

Are there bad things about the study?

The one bad thing about the study is that when we take blood from your arm it may bleed and also hurt a little. You may also see that the area gets red or bruised, but this will go away in a couple of days.

Who will know about what I did in the study?

Your family doctor will be told that you will be taking part in this study. Your health records obtained while in the study will be kept private at all times. You have to understand that your health records will need to be looked at by the company that is paying for the study and other people and companies who are involved with that company. By signing this form you are allowing these people to look at your personal information even if later you decide to not participate in the study. The information that they see will always remain private and your name will never be told to anyone, except as may be required by law. The results of your treatment may also be published, but we will not mention your name.

No one will see the papers with your name and results except for the people doing the study. The completed study will be sent around the world, but your name will not be anywhere on it.

The company that is responsible for this study will make sure that your name is protected.

You can ask any questions that you have about the study. If you have any questions later or during the study you can call anytime to:

Dr. Arbus at (416) 813-6288 or Dorothy Lyszkiewicz at (416) 813-7284 x 9
Can I decide if I want to be in the study?

Please talk this over with your parents before you decide whether or not to participate. We will also ask your parents to give their permission for you to take part in this study.

But even if your parents say "yes", you can still decide if you want to be in the study or not. It is also okay if you decide to be in the study and then change your mind. Nobody will get mad at you if you do not want to be in the study. You can also ask questions at any time.

CONSENT:

By signing your name at the bottom means that you want to be in this study. Even if you do not want to be in this study, you will still receive the same medical treatment.

The person doing this study has explained to me what will happen if I take part in this activity. I know that no one will get mad at me if I say no. I agree to be in this study.

__________________________________________
Name of patient

__________________________________________
Date

"I was present when ________________________ read this form and gave his/her verbal assent."

__________________________________________
Name of person who obtained assent

__________________________________________
Signature Date
THE HOSPITAL FOR SICK CHILDREN

STUDY DESIGN for PATH-2:

"The Pediatric Use of Amlodipine in the Treatment of Hypertension: A Population Pharmacokinetic Trial"

This describes what will happen at each visit when you come to the clinic. Please keep this for your information.

Patients who CAN be included in the study:

- Patients who have agreed to participate in the study and have signed their names on a consent form.
- Males and females up to 17 years of age.
- Patients who are currently receiving daily administration of amlodipine over the 4 week period prior to enrollment.
- Patients who have liver or kidney impairment will be included in this study if specific laboratory tests that need to be performed show acceptable results.

Patients who CANNOT be included in the study:

- Patients who are receiving other medications or who have received other medications one month prior to participating in the study.
- Patients who are chosen to participate cannot receive any other drug until the study is complete. If the patient requires another medication, they may be removed from the study by the study investigator.
- Patients who have high blood pressure that is not stable and well controlled.
- Factors that may interfere with the conduct or interpretation of the trial: Subjects with drug dependence, alcohol dependence or other conditions which will impact study drug compliance, safety, and reliable long-term follow-up.
- Patients with a history of poor medication compliance, or a history of repeatedly missing clinic/office visits are excluded, as this will interfere with the interpretation of the results.

There will be 5 visits in total for the duration of the study:

Screening visit
Visit 1: Start of daily dosing and meal diary
Visit 2: Blood sampling day
Visit 3: Additional blood sampling
Visit 4: Additional blood sampling
The study co-ordinator has asked you to come to the clinic on this day to evaluate you as a possible participant in the study.

The study co-ordinator will explain the study to you and ask you to sign a consent form to participate in the study. The reason that you are being asked to participate is because you are receiving the blood pressure medication amlodipine (Norvasc) and your doctor is expecting you to remain on this medication in the future.

A doctor will obtain your medical history, complete a physical exam and take your blood pressure and pulse.

If you are interested in participating in the study and you have signed the informed consent form, you will have the following tests done:

1. Medical history
2. General physical examination including height, weight and Tanner stage (this is done to assess your stage of puberty)
3. A blood sample will be taken in order to determine your liver and kidney function.
4. A blood test will be done to exclude the possibility of hepatitis (a liver disease).

The amount of blood that will be taken from you is less than one teaspoonful. We need this blood test in order to determine if you are eligible to participate in the study.

The laboratory data that becomes available to the study investigators upon screening will be used to determine your health. If your health changes, these tests may have to be repeated.
Screening visit - Page 2

The study co-ordinator will provide you with a special supply of amlodipine (Norvasc) at the same dose that you normally take.

This special supply has been counted and must be returned at the next visit.

- Only take this supply of amlodipine each day
- Stop taking your usual supply of amlodipine

This is the completion of your screening visit.

Within 4 weeks of this screening visit, you will enter the blood sampling phase if the study co-ordinator decides that you are appropriate to participate in the study.

The study co-ordinator will phone you when the results of your blood studies have been determined, and schedule you for Visit 1.
PATH-2: Patient and Parent Instructions

Visit 1 → Start of daily dosing and meal diary

No blood sampling will be done at this visit.

The study co-ordinator has asked you to come to the clinic today because you have been taking the same dose of amlodipine each day for the past month.

Today you will be instructed on how to use your dosing and meal diary. You will be required to record the date and time of each dose of amlodipine that you take and also the date, time, and description of your meal.

The study co-ordinator will call you each day between this visit and your next visit to tell you exactly what time to take your medication. You will be asked to record this exact time in your dosing diary.

The study co-ordinator will also review your meal diary with you each day to be sure that you are recording all your meals and snacks in the diary correctly.

You will also be asked if you have experienced any side effects to your medication between the screening visit and visit 1.

Two days before your next visit, the study co-ordinator will ask you to pay particular attention to the recording of all your meals and snacks in your meal diary.

The study co-ordinator will schedule Visit 2 with you.

Don’t forget to bring both your meal diary and your dosing diary with you to Visit 2. Bring all of your unused amlodipine (Norvasc) with you.
PATH-2: Patient and Parent Instructions

Visit 2 → Blood sample collection - Page 1

If you do not hear from your study co-ordinator by 7:00 this morning, please phone her at (416) 813-7284 or page her at (416) 589-6727.

Last night or early this morning your study co-ordinator called you at home to remind you to take your amlodipine (Norvasc) at a particular time. You then recorded this exact time in your dosing diary.

DO NOT take any more amlodipine today until after the blood draws are completed.

Your study co-ordinator will also tell you what time to come to the clinic this morning, and remind you to bring your dosing and meal diary with you. Bring all of your unused amlodipine with you.

An appointment will be made to obtain 4 blood samples from you and to measure your blood pressure and pulse. The amount of blood that will be taken with each blood draw will be less than one teaspoonful. As well, the nurse will use a cream on the arm before blood is taken so that the needle is hardly felt. A small plastic tube (slightly bigger than a needle) will be inserted into your arm so that blood can be easily taken four times during the day without puncturing your skin with a needle each time. With this needle you will be free to move around the clinic.

You will be at the clinic all day today for about 10 hours. The following things will make the day easier for you:

- wear comfortable clothes
- bring your favourite things with you, like a stuffed animal or walkman.
- Bring some snacks with you that you may not be able to get at the clinic.
- Your mom or dad can stay with you all day if you wish.
At the end of the day, the study co-ordinator will schedule a time for you to come to the clinic tomorrow for your next visit. This next visit will be Visit 3.

If you take amlodipine either once a day (in the evening) or twice a day, and you have not had your evening dose today, the study co-ordinator will give you this dose prior to your leaving the clinic today.

Remember: DO NOT take another dose of amlodipine until AFTER your blood is taken at Visit 3 tomorrow.

You are instructed to continue your daily dosing and meal diary.
PATH-2: Patient and Parent Instructions

Visit 3 → Additional blood sampling

Today you will only need to come to the clinic for a short time.

Bring your dosing diary with you.

The study co-ordinator will contact you and instruct you to take your medication at a specific time of day BEFORE your visit. This time will be recorded by you in your diary and by the co-ordinator.

You will NOT take any amlodipine on this day until AFTER your blood has been drawn. The amount of blood that will be taken is less than one teaspoonful.

After this blood sample is obtained, you can continue taking your daily dose of amlodipine.

Some patients will be selected by the doctor to participate in a 24-hour "Blood Pressure Monitoring" at this visit. This is where we may ask you to wear a special device on your arm that will measure your blood pressure every 30 minutes for 24 hours.

We will also ask you if you are experiencing any side effects to your medication.

At this visit, the study co-ordinator will schedule your last visit which will be Visit 4.

Please continue your daily dose and meal diary until you come in for Visit 4.
PATH-2: Patient and Parent Instructions

Visit 4 ➔ Additional blood sampling

Today, you will only come to the clinic for a short period of time.

Last night or early this morning, the study co-ordinator called you at home to remind you to take your amlodipine at a particular time. You then recorded that exact time in your dosing diary.

DO NOT take any more amlodipine today until after your blood is taken. The amount of blood that will be taken will be less than one teaspoonful.

Bring your dosing diary with you and all of your unused supply of amlodipine.

After this blood sample is obtained, you can continue taking your daily dose of amlodipine.

You will be instructed to continue your daily dose and meal diary.

We will also ask you if you are experiencing any side effects to your medication.

This is your last visit.

THANK YOU FOR PARTICIPATING IN THE STUDY!
A summary of what will happen at each visit:

<table>
<thead>
<tr>
<th></th>
<th>Screening</th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>Visit 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical history</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical exam</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood pressure measurement</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24-hour blood pressure monitoring</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Blood test</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily dose and meal diary</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>You will be asked if you are on other medications</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>You will be asked if you are experiencing side effects</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Blood sampling</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
Appendix 4: Adverse event report form

**Amiodipine**

**Visit**: End of Study

**DATE OF VISIT** (dd-MMM-yyyy)

Investigator: [Name]

Please print all details, and INITIAL and DATE all corrections. Indicate [ ] where applicable.

**□ NO ADVERSE EVENT**

**ADVERSE EVENT (AE) REPORT**

Record all adverse events regardless of suspected causality to study drug.

**ADVERSE EVENT**

(specify diagnosis, not individual symptoms, if possible):

Date of onset (dd-MMM-yyyy)

(Enter an approximate date if actual not known)

**SEVERITY** - Check ONE only

<table>
<thead>
<tr>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
</table>

**ACTION**: Study drug dose was:

- [ ] Increased
- [ ] Reduced
- [ ] Stopped temporarily
- [ ] Permanently discontinued

Withdrawn from the study

Treatment given

(specify details in concomitant treatment section)

Other

(specify):

**DO SERIOUS CRITERIA APPLY?**

Fatal; Life-threatening; Inpatient hospitalization or prolongation of hospitalization; Persistent or significant disability/incapacity; Congenital anomaly/birth defect; Important medical event (i.e. may jeopardize subject and may require medical/surgical intervention to prevent above outcomes).

- [ ] Yes
- [ ] No

**IF "YES" NOTIFY PFIZER IMMEDIATELY**

**OUTCOME OF AE TO DATE**

Still present?

- [ ] Yes
- [ ] Unknown
- [ ] No - resolved

Date (dd-MMM-yyyy):

**COMPLETE THE FOLLOWING SECTION AT THE TIME THE EVENT RESOLVES OR AT THE END OF THE STUDY**

**CAUSALITY**

In the Investigator's judgement was study drug the most likely cause of the AE?

- [ ] Yes/Unknown
- [ ] No*

*If "No" what was the most likely cause of the AE? (Check ONE only)

- [ ] Disease under study
- [ ] Other illness (specify):
- [ ] Concomitant Treatment - drug or non-drug

(specify):

- [ ] Other (specify):

**FOR PFIZER USE ONLY** - Case ID #

*Unknown is not acceptable
Appendix 5: Amlodipine plasma concentration-time curves

Patient #1

![Graph showing plasma concentration-time curve for Patient #1.]

Patient #2

![Graph showing plasma concentration-time curve for Patient #2.]

THE HOSPITAL FOR SICK CHILDREN
RESEARCH ETHICS BOARD

Approval & Terms of Agreement

APPLICANTS: Dr. Shinya Ito, Ms. D. Lyszkiwicz

PROJECT TITLE: A Bioequivalence Study Comparing the Pharmacokinetics of a Tablet and Suspension Formulation of Amlodipine (Consent Form version date May 9, 2000; Study Design version date May 9, 2000)

FILE NUMBER: 2000/078

MEMBERS OF THE BOARD*: Dr. Max Perlman, Chair Ms. J. Clarkson Dr. M. Rossi Dr. A. Taddio Dr. D. Bagli Ms. S. Serena Dr. B. McCrindle Dr. M. Crawford Ms. S. Doyle Dr. M. McGuigan Dr. M. Dennis Dr. P. Mckeever Mrs. B. Benoliel Ms. M. Rowell Dr. C. Fandino-Cirilli Dr. M. Freedman Dr. A. Feigenbaum Dr. L. Komar

*Meeting may not have been attended by all members.

I agree to carry out the proposed research involving human subjects in accordance with the protocol approved by the Research Ethics Board using the approved consent form/s. I shall notify the department/division chief and the Research Ethics Board prior to implementing any modifications in the protocol and of any adverse or unexpected events as soon as possible.

SIGNATURE(INVESTIGATOR) May 19, 2000

I agree to monitor the protocol on an ongoing basis, and to notify the Research Ethics Board as appropriate.

SIGNATURE (DEPARTMENT/DIVISION HEAD) Date

The Research Ethics Board of the Hospital for Sick Children has reviewed and approved the above-named project.

Chair, Research Ethics Board

DATE OF APPROVAL JUN - 5 2000

EXPIRY DATE JUN -- 2001

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THE HOSPITAL FOR SICK CHILDREN
INFORMED CONSENT

TITLE: “A Bioequivalence Study Comparing a Tablet and Suspension Formulation of Amlodipine.”

This consent form describes a clinical research study and your role in it if you decide to participate. Please read this information carefully and do not hesitate to ask any questions at any time.

Primary Investigator
Dr. Gideon Koren
Division of Clinical Pharmacology
Hospital for Sick Children, Toronto
(416) 813-5781

Study Co-ordinator
Dorothy Lyszkiewicz
Division of Clinical Pharmacology
Hospital for Sick Children, Toronto
(416) 813-7283

PURPOSE OF THE STUDY

You are being asked to participate in a research study that will take place at the Hospital for Sick Children. The main purpose is to determine how the drug amlodipine (Norvasc ®) is handled by the body. Primarily, we are interested in how the drug is absorbed and how it is eliminated. Amlodipine in tablet form is currently approved by Health Canada for use for high blood pressure and angina, and has been used in adults for seven years. It is available as a prescription drug in Canada. In this study, we are interested in seeing how your body treats amlodipine when it is given to you either as a tablet or crushed and prepared in a liquid. The amlodipine liquid is a new formulation of the drug. The importance of this study is that if your body treats the two forms of the medication in the same way, the liquid may become widely used in the future for people with high blood pressure who are unable to take their medication in tablet form.

DESCRIPTION OF THE STUDY

Before you begin, we will determine whether you are appropriate to participate. This will be a screening period where we will ask you about your medical history and determine if you are on any other medications. A blood sample will be taken for a pregnancy test if you are a female of childbearing age. All participants will also have a blood sample taken for a liver function test.

Your participation will be about 3 weeks and you will need to come to this medical facility for evaluations of your response to the medication. This study will be conducted in a “randomized crossover” design and will consist of 2 phases. This means that you will be randomly assigned (like the toss of a coin) to one of two groups, Phase I or Phase II at the beginning of the study:
Phase I: You will receive either a 5mg amlodipine tablet with 100 ml water or the 5 mg amlodipine tablet crushed in water. 5mg is the standard dose for this medication. We will take blood from your forearm before you receive your dose of the drug and then at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48, 72, 96 and 120 hours after your dose. For the first 12 hours you will be required to stay in the hospital for periodical blood sampling. We will attach a small plastic tube to your arm so that we can draw blood from you without putting a needle into your vein each time. This is done for your convenience and comfort. During this day, 15 blood samples will be taken from you. The amount of blood that will be taken in total for all the blood samples, will be approximately 125 ml or less than ¼ a cup. For the next 5 days you will return to the hospital every morning for one blood draw. The amount of blood that will be taken each day will be about 1 teaspoon (5ml). The total number of blood draws for phase 1 will be 20.

After 1 week you will start phase 2: (the “crossover” part of the study) It is called “crossover” because we are using one person to try both forms of the medication.

Phase II: You will receive the dosage form that you did not receive before. So if you received the tablet first, after two weeks you will receive the liquid. We will again take blood from your arm in the same way as in Phase I. The total number of blood draws for this phase will also be 20.

For the entire duration of the study you will receive two amlodipine doses, once in a tablet and another time in a liquid.

POTENTIAL RISKS

During the study, we will need to draw blood samples from you. Unforeseen harmful consequences may occur such as an allergic reaction to the medication. However, the chances of this happening are rare. Potential side effects that you may experience are swelling of the ankles, headache, flushing (blushing or face redness), fatigue and dizziness. There may also be a small amount of bleeding at the needle site and slight discomfort and bruising or redness that will usually disappear in a few days. If, during the course of the study any previously unknown risks become known, you will be notified of these changes.

POTENTIAL BENEFITS

All tests, examinations and medical care required as part of this study are provided at no cost to you. Participation in this study is purely for research purposes. Your participation in this study may benefit others through the medical information that will be gained.
PARTICIPATION

Your participation in this study is completely voluntary. If you agree to participate and then decide to stop, you may do so without any penalty. By signing this consent form you are agreeing to follow the study schedule where you will be required to spend one full day (12 hours) at the hospital where we will obtain 15 blood samples from you, and 1 blood sample for the next 5 days. You also agree to participate in BOTH phases of the study.

Travel expenses as well as your time are considered and you will be reimbursed for travel expenses, gas, parking, food etc. The amount offered will be $50 for the full day that you spend at the hospital and $30 for each subsequent visit. You are entitled to $430 at the completion of the study.

As well, please be aware that the investigator can withdraw you from the study without your consent if it is in your best interest, or if you fail to follow the study schedule. Your participation may contribute to the creation of a new amlodipine liquid. However, your participation in this study will not entitle you to a share in future economic benefits of any kind.

SPONSORSHIP

This study is sponsored by the Motherisk Research Fund and Pfizer Inc.

REIMBURSEMENT

If you are injured as a direct result of taking part in this study, medical treatment shall be made available to you.

Financial compensation for such things as lost wages, disability or discomfort due to this type of injury is not routinely available. You understand however, that you have not waived any of your legal rights by signing this form.

CONFIDENTIALITY

Confidentiality will be respected and no information that discloses your identity will be released or published without consent unless required by law. Your personal data, such as date of birth will be collected and used for research purposes in connection with this study. The study data will be sent around the world, but you will not be referred to by name or identified in any report or publication, nor could the data be traced back to you.

The results of your treatment may be published for scientific purposes provided your identity is not revealed. You agree not to restrict the use of any data, even if you withdraw.
CONSENT

"I acknowledge that the research procedures described above have been explained to me and that any questions that I have asked have been answered to my satisfaction. I have been informed of the alternatives to participation in this study, including the right not to participate and the right to withdraw without compromising the quality of medical care at The Hospital for Sick Children for me and for other members of my family. As well, the potential harms and discomforts have been explained to me and I also understand the benefits (if any) of participating in the research study. I know that I may ask now, or in the future, any questions I have about the study or the research procedures. I have been assured that records relating to me and my care will be kept confidential and that no information will be released or printed that would disclose personal identity without my permission unless required by law."

I hereby consent to participate:

Name: ____________________________________________________________

Signature: _______________ Date: _______________

Witness: __________________________________________________________

Signature: _______________ Date: _______________
Appendix 8: Summary of the study design

**STUDY DESIGN:** "A Bioequivalence Study Comparing a Tablet and Suspension Formulation of Amlodipine."

*This describes what will happen at each visit. Please keep this for your information.*

Volunteers who CAN be included in the study

- Over 18 years of age
- Healthy male and female adults
- You must sign the informed consent form and be willing and able to comply with the study schedule

Volunteers who CANNOT be included in the study

- A diagnosed liver disease such as cirrhosis or hepatitis or any other liver disease
- History of drug allergy to amlodipine or other heart medications
- History of a gastrointestinal condition
- A diagnosed heart complication such as heart disease or any other heart problems
- High blood pressure
- Pregnant women

There will be 7 visits for Phase I of the study:

VISIT 1 → Screening visit
VISIT 2 → Phase I: Blood sampling from 0-12 hours.
VISIT 3 → Blood sampling at 24 hours
VISIT 4 → Blood sampling at 48 hours
VISIT 5 → Blood sampling at 72 hours
VISIT 6 → Blood sampling at 96 hours
VISIT 7 → Blood sampling at 120 hours
VISIT 1 → Screening Visit: Participant Instructions

You have been asked to come to the hospital today to evaluate you as a possible participant in the study. The study co-ordinator will explain the study to you and ask you to sign a consent form stating your willingness to participate in the study.

A doctor or nurse will obtain your medical history and take your blood pressure and pulse. A blood sample will also be taken from you for a liver function test and a pregnancy test if you are a female of childbearing age.

If you are eligible to participate, you will be scheduled by the study co-ordinator for Visit 2 within 1 week of the screening visit.

Visit 2 → Phase I: Participant Instructions

You have been asked to come to the hospital today because the study co-ordinator has determined that you are eligible to participate in the study.

You will be randomly assigned to receive either a tablet form of the study drug amlodipine (Norvasc) with some water or the syrup. The dose that you will receive is 5mg, which is the lowest standard dose for this medication.

Before you receive your first dose, a small plastic tube will be attached to your arm so that blood can be taken from you frequently throughout the day without putting a needle into your vein each time. This is done for your convenience and comfort, and you are free to move around with the plastic tubing. The first blood sample will be taken just before you receive the medication.

As soon as you take the medication, your blood will be taken ½ hour after you have taken the drug, and at 1, 2, 3, 4, 6, 8, 10, and 12 hours after you have taken the drug.

You will be asked to stay in the hospital until the last blood sample is taken, which is 12 hours after you took the drug. The study co-ordinator will tell you what time to come back for your blood draw each time you have your blood taken.

If you are late for your blood draws, you may be removed from the study.

After we have taken the last blood sample at 12 hours, the study co-ordinator will tell you what time to come back to the hospital the next morning.
For Visit 3 through to Visit 7, only 1 blood sample will be taken each time. During these visits, you only have to come to the hospital for a short period of time.

Visit 3: Blood sampling at 24 hours
Visit 4: Blood sampling at 48 hours
Visit 5: Blood sampling at 72 hours
Visit 6: Blood sampling at 96 hours
Visit 7: Blood sampling at 120 hours

PHASE II

There will be 6 visits for Phase II of the study.
There is no Screening Visit for this phase.

VISIT 1 → Phase II: Blood sampling from 0-12 hours.
VISIT 2 → Blood sampling at 24 hours
VISIT 3 → Blood sampling at 48 hours
VISIT 4 → Blood sampling at 72 hours
VISIT 5 → Blood sampling at 96 hours
VISIT 6 → Blood sampling at 120 hours

The study co-ordinator will schedule you to come to the hospital for your second dose of the study drug amlodipine (Norvasc)

This time you will receive the drug in the form that you did not receive in Phase I. The dose that you will receive is 5mg which is the lowest standard dose for this medication.

For example: If you received the tablet in Phase I, you will now receive the syrup.
If you received the syrup in Phase I, you will now receive the tablet.

Visit 1 → Phase II: Participant Instructions

Phase II is a REPEAT of Phase I, except that this time you have received a different form of the medication.
Please refer to patient instructions for Phase I: Visit 2 through to Visit 7 for Phase II procedures.

THANK YOU FOR PARTICIPATING!
Appendix 9: Sample size calculation

Formula for repeated observations or equivalence trials:

\[ N = \left[ \frac{(Z\alpha + Z\beta)\sigma}{\delta} \right]^2 \]

- \( Z\alpha = 1.96 \) at an \( \alpha \)-level of 0.05
- \( Z\beta = 1.28 \) for a \( \beta \) of 0.2, for 80% power (1-0.2)
- \( \sigma = 30\% \) or 0.3, which represents inter-subject variability from previous studies
- \( \delta = 20\% \) or 0.2, which is the difference that we want to be able to detect between formulations

\[ N = \left[ \frac{(1.96 + 1.28)0.3}{0.2} \right]^2 \]

\( N = 23.6 \) or 24 subjects
THE HOSPITAL FOR SICK CHILDREN
DEPARTMENT OF PHARMACY
555 University Avenue
Toronto, Ontario
PHONE: 416/813-1500

June 1/01

Dorothy Lyszkiewicz
28 09 75 F

WEIGHT (KG)  WEIIGHT (CM)

"Amlodipine Study"

PHASE 1

Amlodipine tablet 5 mg

1 dose

Reprinted from above details by hand

PHARMACY USE

Repeats: 

A For pick-up on Sunday June 3rd at 7am.

CANCELL UNUSED SPACE

Repeats: 

SIGN

PLEASE PRINT

Name or Registry No.

FORM 32175 REV. 2/86

PRESCRIPTION REQUISITION 1487376
Appendix 11: Amlodipine plasma concentration-time curves

Subject #1

Subject #2

Subject #3
Appendix 12: Plot of the AUC_o-t ratio of suspension to tablet (S/T) over time

\[ y = -0.0422x + 1.0824 \]
\[ R^2 = 0.2483 \]
RESEARCH ETHICS BOARD

March 30, 2001

Dr. Gideon Koren  
C/o Ms. D. Lyszkiewicz  
Division of Clinical Pharmacology & Toxicology  
Hospital for Sick Children

Dear Dr. Koren

Your study “A Bioequivalence Study Comparing the Pharmacokinetics of a Tablet and Suspension Formulation of Amlodipine: Amendment to Protocol involving testing the relative efficacy of amethocaine cream”

REB File No. 2000/078

On behalf of the Research Ethics Board, I am writing in response to your request dated 15 March 2001 to provide REB approval for the study amendment and revised consent form.

Yours sincerely

[Signature]

Brian McCrindle, MD, FRCPC  
Associate Chair, Research Ethics Board
INFORMED CONSENT II: Additional procedure

TITLE: “The Efficacy of Amethocaine Gel in Venopuncture”

Primary Investigator: Dr. Gideon Koren (813-5781)
Co-investigator: Dr. Anna Taddio (813-6235)
Study Co-ordinator: Dorothy Lyszkiewicz BSc. (813-7283): Graduate student under the supervision of Dr. Gideon Koren

This consent form describes an additional procedure to the study that you will be participating in entitled “A Bioequivalence Study Comparing a Tablet and Suspension Formulation of Amlodipine.” (“Bioequivalence Study”). You are not required to participate in this section of the study.

PURPOSE OF THE STUDY

You are being asked to participate in an additional clinical procedure that will be part of the “Bioequivalence Study.” This procedure will also take place at the Hospital for Sick Children and does not involve any additional time on your part. The main purpose is to determine how well the local anaesthetic drug “amethocaine” works in adults. Amethocaine (Ametop®) is being used as a local anaesthetic, and is manufactured by the company Smith & Nephew Healthcare Ltd. It is applied to the forearm before a blood sample is taken so that a small area of the arm will feel numb. This is done so that you feel either less pain or no pain upon needle insertion.

DESCRIPTION OF THE STUDY

Before you begin the “Bioequivalence Study”, we will ask if you are willing to participate in this section. When you begin the “Bioequivalence Study”, a screening visit will be performed to determine if you are eligible to participate. One blood sample will be taken to measure your liver function. We will ask you to rate the level of pain that you feel upon needle insertion. After we receive your results from the screening visit, you will be asked to return to the hospital to begin Phase I of the “Bioequivalence Study”. Phase I is where we will give you either a tablet or a liquid form of amlodipine, and we will take blood samples from you to measure the amount of amlodipine found in your blood (please refer to the information about Phase I included in the “Bioequivalence Study” folder under the “Clinical Procedures” section).

Phase I: When you arrive for Visit 2, the local anaesthetic amethocaine will be put on your arm so that you feel less or no pain of the intravenous catheter insertion. At Visits 3, 4, 5 and 6, we will also put the local anaesthetic on your forearm so that you feel less pain of needle insertion during the blood draw.

During one time, we will put a cream on your arm that does not contain any active medicinal ingredients. This is called a “placebo”. You will not know at which time you will receive the placebo or when you will receive amethocaine. This is done so that we can test how effective amethocaine is at relieving the pain of needle insertion. After each blood draw, we will ask you to rate the severity of pain on a scale from 0 to 10. A score of 0 is no pain at all and a score of 10 is the worst pain you can imagine. We will also be looking to see how easy or difficult it is to take a blood sample from you.
After you complete Phase I, you will enter Phase II where you will receive the formulation of amlodipine that you did not receive previously. For example, if you received the tablet in Phase I, you will now receive the liquid (please refer to the information about Phase II in the “Bioequivalence Study” folder under the “Clinical Procedures” section).

Phase II: When you arrive for your first visit during this phase, the local anaesthetic amethocaine will be put on your arm so that you feel less or no pain of the intravenous catheter insertion. At Visit 3, 4, 5, 6 and 7, you will receive the local anaesthetic on your forearm so that you feel less or no pain during needle insertion. We will not be giving you a placebo at any time, so during this phase you will always have a local anaesthetic before a blood draw.

POTENTIAL BENEFITS

You will receive a local anaesthetic prior to 11/13 skin punctures by a needle during the entire duration of the study. You may receive no benefit if a placebo is used on your arm prior to the blood draw.

POTENTIAL RISKS

Potential side effects that you may experience from amethocaine are slight redness in the area of application, itching, and small bumps may appear if your skin is sensitive to the anaesthetic. If these side effects are more than mild, or if you are not comfortable receiving more anaesthetic the next time, you have the option of discontinuing this part of the study.

CONSENT

"I acknowledge that the research procedures described above have been explained to me and that any questions that I have asked have been answered to my satisfaction. I have been informed of the alternatives to participation in this study, including the right not to participate and the right to withdraw without compromising the quality of medical care at The Hospital for Sick Children for me and for other members of my family. As well, the potential harms and discomforts have been explained to me and I also understand the benefits (if any) of participating in the research study. I know that I may ask now, or in the future, any questions I have about the study or the research procedures. I have been assured that records relating to me and my care will be kept confidential and that no information will be released or printed that would disclose personal identity without my permission unless required by law."

I hereby consent to participate:

Name: ________________________________________________________________

Signature: ___________________________ Date: ___________________________

Witness: __________________________________________________________

Signature: ___________________________ Date: ___________________________

A health care teaching and research centre dedicated exclusively to children; affiliated with the
Appendix 15: Visual Analog Scale

Visual Analogue Scale - Investigator's Evaluation

No pain

Worst possible pain

Patient No.

mm
Appendix 16: Visual Analog Scale comprehension test

**VAS COMPREHENSION TEST 1**

<table>
<thead>
<tr>
<th>NO PAIN</th>
<th>THE WORST PAIN YOU CAN THINK OF</th>
</tr>
</thead>
</table>

**EXPLANATION**

This is a pain ruler.

The left end means you didn't feel any pain.

The right end means you had very bad pain.

**TEST**

Show me how bad your pain would be if you:

1. had a mosquito bite
2. fell in the snow
3. fell on the pavement (sidewalk)
4. slammed the door on your fingers
Appendix 17: Adverse event report
May 24, 2001

Re: "A bioequivalence study comparing a tablet and a suspension formulation of amlodipine" and "The efficacy of amethocaine gel using different application times"

Primary investigator: Dr. Gideon Koren
Co-investigator: Dr. Anna Taddio
Study co-ordinator: Dorothy Lyszkiewicz, graduate student under Dr. Koren

Dear Dr. Perlman,

This letter is to report several adverse events that have occurred during the study following topical application of the local anaesthetic amethocaine (Ametop Gel).

Summary of study:

The bioequivalence study requires 9 blood samples to be taken on 1 day, and then 1 blood sample to be taken every subsequent day for the next 4 days. Since the first day requires 9 blood samples, we are using an Angiocath catheter during this day. An Angiocath is not required on subsequent days since only 1 blood sample is taken, hence we have been using butterfly needles. According to a recommendation from the REB, the use of a topical local anaesthetic was to be used on the subjects before each venipuncture. We had suggested using the new topical local anaesthetic amethocaine due to its recent introduction into clinical practice, and its faster onset of action compared to the topical local anaesthetic EMLA. The use of amethocaine also gave us an opportunity to study the efficacy of anaesthesia using different application times, and a separate informed consent form was made to reflect this protocol amendment. We received approval from the REB on March 30, 2001 to conduct this local anaesthetic study within the bioequivalence study.

Adverse reactions to amethocaine in general:

Adverse reactions commonly reported with amethocaine use are local erythema (due to the drug's vasodilatory property), but this reaction is usually mild and transient, and rare cases of localized edema and itchiness which are also transient. Dr. Anna Taddio also contacted the manufacturer to ask about the incidence of adverse reactions upon repeated application of the anaesthetic, to which the manufacturer responded that they had no data. However, there are two studies in the literature reporting that repeated use of amethocaine does not cause sensitization (Woolfson AD et al. 1990, Wongprasartsuk P et al. 1998).

Adverse reactions to amethocaine in our study:

To date, 11 subjects have participated in Phase I of the study. We noticed that some subjects developed local skin reactions that were getting worse with each subsequent application of amethocaine. We therefore have performed an interim safety analysis to look for patterns in the incidence of these adverse effects. Please refer to table 1 for a complete description of adverse reactions to amethocaine during each day of application. Please note that a placebo was used on one of the days (as indicated on table).
<table>
<thead>
<tr>
<th>Subject ID#</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>40 min AM</td>
<td>20 min PLACEBO</td>
<td>20 min AM</td>
<td>30 min AM</td>
<td>40 min AM</td>
</tr>
<tr>
<td></td>
<td>- Mild redness and presence of small, red, raised bumps (moderate) that were still present 24 hours later</td>
<td>- Small, red, raised bumps still present (mild) from Day 1. Today there was only mild redness from the OpSite</td>
<td>-Mild redness from the OpSite</td>
<td>-Mild redness and mild itchiness. Also the small, red, raised bumps appeared again</td>
<td>-Mild redness that was “patchy” (only is some areas)</td>
</tr>
<tr>
<td>02</td>
<td>42 min AM</td>
<td>20 min AM</td>
<td>30 min AM</td>
<td>40 min AM</td>
<td>30 min PLACEBO</td>
</tr>
<tr>
<td></td>
<td>-Mild redness</td>
<td>-Mild redness from OpSite</td>
<td>-Moderate redness and mild itchiness. Subject reported a feeling of “raw, irritated skin” at the anaesthetic site</td>
<td>-Moderate redness</td>
<td>-Mild redness over the entire area</td>
</tr>
<tr>
<td>03</td>
<td>20 min AM</td>
<td>30 min AM</td>
<td>40 min AM</td>
<td>40 min PLACEBO</td>
<td>20 min AM</td>
</tr>
<tr>
<td></td>
<td>-No adverse effects</td>
<td>-Mild redness from OpSite and mild itchiness</td>
<td>-Mild redness, mild itchiness and moderate small, raised, red bumps. Patient reported being itchy in the area the entire previous night, and the itchiness got worse at night</td>
<td>-Very mild redness, possibly from OpSite</td>
<td>-Patchy redness</td>
</tr>
<tr>
<td>04</td>
<td>20 min AM</td>
<td>40 min AM</td>
<td>20 min PLACEBO</td>
<td>25 min AM</td>
<td>30 min AM</td>
</tr>
<tr>
<td></td>
<td>-Mild redness from OpSite</td>
<td>-Mild redness from OpSite</td>
<td>-Mild redness from OpSite</td>
<td>-Mild redness from OpSite, but very white at the site of the anaesthetic</td>
<td>-Mild redness from OpSite, but very white at the site of the anaesthetic</td>
</tr>
</tbody>
</table>

AM = Amethocaine
<table>
<thead>
<tr>
<th>05</th>
<th>20 min AM</th>
<th>30 min PLACEBO</th>
<th>20 min AM</th>
<th>30 min AM</th>
<th>40 min AM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-Mild redness from OpSite</td>
<td>-Mild redness and mild small, red, raised bumps</td>
<td>-Mild redness. Skin area is mildly dry and flaky</td>
<td>-Mild patchy redness. Skin area is dry, flaky and rough from previous application</td>
<td></td>
</tr>
<tr>
<td>07</td>
<td>40 min AM</td>
<td>30 min AM</td>
<td>40 min AM</td>
<td>20 min PLACEBO</td>
<td>20 min AM</td>
</tr>
<tr>
<td></td>
<td>-Mild redness from OpSite</td>
<td>-Mild redness</td>
<td>-Mild redness</td>
<td>-No adverse effects</td>
<td>-Mild patchy redness and mild itchiness</td>
</tr>
<tr>
<td>08</td>
<td>40 min AM</td>
<td>No anaesthetic or placebo</td>
<td>No anaesthetic or placebo</td>
<td>No anaesthetic or placebo</td>
<td>No anaesthetic or placebo</td>
</tr>
<tr>
<td></td>
<td>-Moderate redness, mildly itchy. Felt hot, like burned skin</td>
<td>Redness persisted. Mildly itchy</td>
<td>Redness persisted. Mildly itchy</td>
<td>Redness persisted. Mildly itchy</td>
<td>Less redness, but still present. Mildly itchy. Subject reported that redness was present for about 1 week after anaesthetic application.</td>
</tr>
<tr>
<td>09</td>
<td>20 min AM</td>
<td>39 min PLACEBO</td>
<td>25 min AM</td>
<td>25 min AM</td>
<td>40 min AM</td>
</tr>
<tr>
<td></td>
<td>-No adverse effects</td>
<td>-No adverse effects</td>
<td>-No adverse effects</td>
<td>-Mild redness and moderate blistering (Vesicular rash according to Eran Kozier)</td>
<td>Mild redness and moderate itchiness. More severe vesicular rash than the day before</td>
</tr>
<tr>
<td>11</td>
<td>45 min AM</td>
<td>40 min AM</td>
<td>40 min AM</td>
<td>20 min PLACEBO</td>
<td>20 min AM</td>
</tr>
<tr>
<td></td>
<td>-Mild redness from OpSite</td>
<td>-Mild redness from OpSite</td>
<td>-Mild redness from OpSite</td>
<td>-Redness persisting from previous day</td>
<td>-Mild redness all over site</td>
</tr>
<tr>
<td>17</td>
<td>20 min AM</td>
<td>30 min PLACEBO</td>
<td>19 min AM</td>
<td>30 min AM</td>
<td>40 min AM</td>
</tr>
<tr>
<td></td>
<td>-No adverse effects</td>
<td>-Very mild redness</td>
<td>-Mild itchiness, but subject has eczema at the site.</td>
<td>-Mild redness and mild itchiness</td>
<td>-Mild redness and mild itchiness</td>
</tr>
</tbody>
</table>

**AM = Amethocaine**
As evident from the table, some of the adverse events are consistent with the literature, however, the incidence of small, red, raised bumps, persistent itchiness and redness, and blistering has not been reported to date upon repeated use. In a case report from the Royal Children's Hospital in Melbourne, a 4-year old boy experienced intense pain and red, inflamed skin that blistered and peeled 48 hours later after one 15 minute application time of amethocaine. In addition, a staff anaesthetist developed a localized erythematous reaction that lasted several days after one application of amethocaine. However, when an audit was conducted by the hospital to determine the prevalence of these adverse local skin reactions, no statistical association was found between the duration of application of amethocaine, and the incidence of local reactions (Wongprasartsuk P. et al. 1998).

In our study, the adverse skin reactions were transient in subjects #04, 07, 17 and 18, which is expected. Subject #01 and #08 seemed to have been already sensitized to amethocaine from previous exposure because of the presence of a skin reaction on the first day. We did not re-expose subject #08 to amethocaine. In the remaining 6 patients (#02, 03, 05, 09, 11, 23), it appears that their reactions may have intensified over time.

We believe that these adverse local skin reactions are most likely amethocaine sensitization, and feel that this portion of the study possibly needs to be discontinued. However, since we still need to complete Phase I and Phase II of the study, we propose to use the local anaesthetic EMLA before venipuncture. EMLA is less likely to cause delayed hypersensitivity reactions. Subjects who experienced local skin reactions to amethocaine will be followed up by a dermatologist.

If you have any questions or concerns, please do not hesitate to contact one of us anytime.

Sincerely,

[Signature]

Gideon Koren, M.D
Anna Taddio, PhD
Dorothy Lyszkiewicz, Study co-ordinator
RESEARCH ETHICS BOARD

June 5, 2001

Dr. Gideon Koren
C/o Ms. Dorothy Lyszkiewicz
Division of Clinical Pharmacology & Toxicology
Hospital for Sick Children

Dear Dr. Koren

Your study "A Bioequivalence Study Comparing the Pharmacokinetics of a Tablet and Suspension Formulation of Amlodipine: The Efficacy of the topical local anaesthetic EMLA"

REB File No. 2000/078

On behalf of the Research Ethics Board, I am writing in response to your request for study amendment and revised consent forms to replace the local anaesthetic amethocaine with EMLA. I hereby provide REB approval for this amendment.

Yours sincerely

Max Perlman, MD
Chair, Research Ethics Board
INFORMED CONSENT II: Additional procedure

TITLE: “The Efficacy of the topical local anaesthetic EMLA”

Primary Investigator: Dr. Gideon Koren (813-5781)
Co-investigator: Dr. Anna Taddio (813-6235)
Study Co-ordinator: Dorothy Lyszkiewicz BSc. (813-7283), graduate student under the supervision of Dr. Gideon Koren

This consent form describes an additional procedure to the study that you will be participating in entitled “A Bioequivalence Study Comparing a Tablet and Suspension Formulation of Amlodipine.” (“Bioequivalence Study”). You are not required to participate in this section of the study.

PURPOSE OF THE STUDY

You are being asked to participate in an additional clinical procedure that will be part of the “Bioequivalence Study.” This procedure will also take place at the Hospital for Sick Children and does not involve any additional time on your part. The main purpose is to determine how well the local anaesthetic drug “EMLA” or “Eutectic Mixture of Lidocaine 2.5% and Prilocaine 2.5% anaesthetics” works in adults. EMLA is widely used in clinical practice as a topical local anaesthetic, and is manufactured by the company Astra. It is applied to the forearm before a blood sample is taken so that a small area of the arm will feel numb. This is done so that you feel either less pain or no pain upon needle insertion.

DESCRIPTION OF THE STUDY

PHASE I: We will give you either a tablet or a liquid form of amlodipine, and subsequently take blood samples from you in order to measure the amount of amlodipine found in your blood. Please refer to information about Phase I that is included in the “Bioequivalence Study” folder under the “Clinical Procedures” section.

Each day when you arrive at the hospital for your blood samples, EMLA will be put on your arm so that you feel less or no pain at the needle site. In order for the anaesthetic to work, it needs to stay on your arm for 60 minutes, therefore you will be asked to wait 60 minutes before a blood sample is taken from you. After each blood draw, we will ask you to rate the severity of pain on a visual pain scale. A score of 0 means that you felt no pain at all, and a score of 10 indicates the worst pain you can imagine.

PHASE II: You will enter Phase II several weeks after you complete Phase I. You will receive the formulation of amlodipine that you did not receive previously. For example, if you received the tablet in Phase I, you will now receive the liquid (please refer to the information about Phase II in the “Bioequivalence Study” folder under the “Clinical Procedures” section).
Once again, each day that you arrive at the hospital for your blood samples, EMLA will be put on your arm for 60 minutes so that you feel less or no pain at the needle site. In this phase of the study we will not be asking you to rate the severity of your pain.

**POTENTIAL BENEFITS**

You will receive a local anaesthetic prior to every skin puncture by a needle during the entire duration of the study. This is entirely for your benefit, as you may not feel any pain during the procedure of blood sampling.

**POTENTIAL RISKS**

Potential side effects that you may experience from EMLA are whitening or redness of the skin in the area of application, slight puffiness or initial burning or itching in the area that EMLA is applied. These are normal reactions caused by the active ingredients in the cream, and will disappear without any treatment. If these side effects are more than mild, or if you are not comfortable receiving more anaesthetic the next time, you have the option of discontinuing this part of the study.

**CONSENT**

"I acknowledge that the research procedures described above have been explained to me and that any questions that I have asked have been answered to my satisfaction. I have been informed of the alternatives to participation in this study, including the right not to participate and the right to withdraw without compromising the quality of medical care at The Hospital for Sick Children for me and for other members of my family. As well, the potential harms and discomforts have been explained to me and I also understand the benefits (if any) of participating in the research study. I know that I may ask now, or in the future, any questions I have about the study or the research procedures. I have been assured that records relating to me and my care will be kept confidential and that no information will be released or printed that would disclose personal identity without my permission unless required by law."

I hereby consent to participate:

Name: ______________________________________________________

Signature: __________________________ Date: __________________

Witness: ________________________________

Signature: __________________________ Date: __________________