COMPARISON OF THE EFFECTS OF CITALOPRAM AND FLUOXETINE ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF ALPRAZOLAM IN VIVO

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
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Comparison of the Effects of Citalopram and Fluoxetine on the Pharmacokinetics and Pharmacodynamics of Alprazolam *in vivo*

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The SSRI antidepressant fluoxetine (Prozac®) inhibits alprazolam (Xanax®) metabolism by inhibition of the cytochrome p450 3A4 (CYP3A4) isozyme. Citalopram (Celexa®) is an SSRI antidepressant that has not been fully assessed with respect to its potential for CYP3A4-mediated drug interactions *in vivo*. Building upon *in vitro* studies that suggest a minimal effect of citalopram on CYP3A4, we hypothesized that citalopram (20mg), as compared to fluoxetine (20mg), may cause less impairment in the metabolism of the CYP3A4 probe drug alprazolam (1mg) *in vivo*. Single-dose alprazolam pharmacokinetics and pharmacodynamics were compared in 21 healthy volunteers before and after three weeks of citalopram or fluoxetine administration. Fluoxetine increased alprazolam area under the curve (AUC) (0-infinity) by 32%, and elimination half life (t1/2) by 14%, but did not effect a pharmacodynamic change. Citalopram caused only a prolongation of alprazolam T_{max}. This study confirms the low potential for CYP3A4-mediated drug interactions with citalopram.
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1 Background

1.1 Cytochrome p450 Enzymes

The cytochrome p450 (CYP) enzymes are a superfamily of heme-containing monooxygenases that function in the oxidative and reductive metabolism of a large variety of endogenous substrates and xenobiotics (Hasler, 1999). Today, there are over 40 known CYP proteins in humans and more than 1000 genes for different CYPs have been cloned (Nelson et al., 1996). The number of chemicals that can serve as substrates for CYP metabolism is enormous and increasing every year. Included are reactions involved in (a) steroidogenesis: the conversion of cholesterol to androgens, estrogens, and gluco- and mineralo- corticoids; (b) the metabolism of cholesterol to bile acids; (c) the conversion of vitamins to their active form; (d) the synthesis and degradation of prostaglandins and other unsaturated fatty acids; and (e) metabolism of xenobiotics. Over 40 different types of reactions (hydroxylation, N- and O-dealkylation, \( \beta \)-scission etc.) have been identified thus far (Coon et al., 1996). Therefore, these enzymes have a great diversity of action.

CYP enzymes are found in almost all tissues in the body, including the brain, gastrointestinal tissue, and kidney, but are primarily located in liver tissue where they are bound to the endoplasmic reticulum and can make up as much as 20% of the protein in this membrane fraction. These CYPs are primarily responsible for the biotransformation of xenobiotics (Hasler, 1999).
Because of the large and growing number of CYPs being discovered and cloned, the issue of nomenclature becomes important. The recommended naming system for CYP enzymes was suggested by Nebert in 1987. The CYP "Families" group together CYP proteins that have a greater than 40% protein sequence resemblance and are denoted by an Arabic number, while "sub-Families" group together proteins with greater than 60% sequence resemblance and are denoted using a capital letter. Finally, these sub-Families are subdivided into specific genes denoted by a second Arabic number (Nebert et al., 1989). Four Families of CYPs are located within the mitochondria, where they act to synthesize bile acids and aldosterone from cholesterol and participate in the activation and degradation of vitamin D, but the greatest number of CYPs are microsomal and belong to the families CYP1 to CYP4, where they are responsible for the biotransformation of drugs, chemical pollutants, and steroids and participate in the oxidation of fatty acids (Hasler, 1999).

Several CYP enzymes are most often implicated in the metabolism of xenobiotics, and are therefore considered to be more important than other CYPs when studying the metabolism of drugs; particularly CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A, which all have different but overlapping substrate specificities. CYP3A constitutes about 30% of all CYP enzymes in the liver; CYP2C makes up about 20% and CYP2D6 makes up 2%, although these relative amounts do not necessarily reflect the number of reactions catalyzed, since different CYPs have different substrate affinities (Shimada et al., 1994; Tsunoda et al., 1999).
Mutations present in the gene that codes for a CYP enzyme can lead to inactive or partially-inactive mutant enzymes. Such genetic polymorphisms can be identified by a bimodal or polymodal enzyme activity-distribution curve in the general population, and can divide those populations into poor and extensive metabolizing phenotypes. Some polymorphisms appear to be common to all studied populations, such as the CYP2D6*5 gene deletion which has been found in Africans, Orientals and Caucasians, or can be specific for one population, such as the CYP2D6*10 mutation in Orientals (Hasler, 1999). The type and frequency of allelic variants within a population can influence the pharmacological and toxicological effects of drugs, toxins and carcinogens. CYPs that demonstrate polymorphisms include CYP2D6, where 5-10% of Caucasians are poor metabolizers, and CYP2C19, where 2-6% of Caucasians are poor metabolizers (Hasler, 1999).

1.2 Drug Interactions Involving CYP Enzymes

When two medications are administered together, drug interactions can occur. Drug interactions involving CYPs generally result from one of two processes; enzyme induction or enzyme inhibition. Enzyme induction occurs when one or both drugs stimulate the synthesis of more enzyme protein, resulting in enhanced metabolic capacity and reduced drug levels. Alternately, when coadministered drugs are biotransformed by the same CYP, inhibition of the enzyme by one or both drugs can occur, resulting in pharmacokinetic changes that can lead to impaired clearance and prolonged drug action. This latter phenomenon is common in clinical practice, especially during multi-drug therapy, due to the broad substrate specificity of many
CYP enzymes. It is important to be able to predict which medications have a high risk of causing pharmacokinetic interactions in order to avoid adverse events. Drugs for which this effect is particularly important include drugs with a narrow therapeutic range, and orally administered drugs that are subject to a high first pass metabolism.

CYP enzymes have been implicated in many drug-drug interactions, particularly involving psychotherapeutic medications. For example, the selective serotonin reuptake inhibitor (SSRI) antidepressant fluoxetine has been found to inhibit CYP2D6 and CYP3A4 in vivo, and can cause clinically significant increases in plasma concentrations of substrates of these enzymes including haloperidol, clozapine, carbamazepine, tricyclic antidepressants, diazepam and alprazolam (Sproule et al., 1997). The SSRI paroxetine, also a potent inhibitor of CYP2D6 has been shown to increase the plasma concentration area under the curve (AUC 0-8 hours) of the antipsychotic perphenazine by as much as 7-fold, leading to significantly greater sedation and psychomotor impairment as well as excessive extrapyramidal side effects (Ozdemir et al., 1997).

1.3 Cytochrome p450 3A4

Members of the CYP3A family are the most abundant, accounting for 30% of CYPs in the human liver, and it has been estimated that they are involved in the metabolism of more than 50% of drugs used in humans (Shimada, Yamazaki, Mimura, Inui, and Guengerich, 1994;Bertz and Granneman, 1997). The CYP3A subfamily consists of three known isoforms in humans—CYP3A4, CYP3A5, and
CYP3A7, although the CYP3A4 form is thought to be dominant in humans (Tsunoda, Velez, von Moltke, and Greenblatt, 1999). CYP3A4 is involved in the metabolism of a broad spectrum of drugs, including antidepressants (e.g. imipramine, amitriptyline, nefazodone), the anticonvulsant and mood stabilizing agent carbamazepine, many benzodiazepines (e.g. alprazolam, triazolam, midazolam), antiarrhythmics, calcium channel blockers, HIV drugs, antineoplastics, antibiotics and antihistamines (Nemeroff et al., 1996), and is involved in many clinically significant drug interactions. Drugs that are clinically significant inhibitors of CYP3A4 include nefazodone, fluvoxamine, fluoxetine, erythromycin, clarithromycin, ritonavir, ketoconazole, itraconazole, cimetidine and grapefruit juice (Dresser et al., 2000).

One of the best known examples of a drug interaction involves a drug metabolized by CYP3A4; terfenadine (Seldane®). Terfenadine is a prodrug that undergoes complete first-pass hepatic metabolism by CYP3A4 to form the active carboxy-metabolite, the antihistamine fexofenadine. When potent CYP3A4-inhibitors such as ketoconazole or erythromycin are coadministered with terfenadine, a build-up of this parent compound occurs and can lead to a life-threatening heart arrhythmia known as Torsades-de-Points (von Moltke et al., 1994a). Clinically significant inducers of CYP3A4 include phenytoin, carbamazepine, dexamethasone and rifampin (Villikka et al., 1997; Spina et al., 1996).

CYP3A4 is present predominantly in the liver, but is also present in the lungs, kidney, intestine, brain and placenta (Zhang et al., 1999; Hasler, 1999). Some drugs, such as cyclosporin, are metabolized extensively by gut CYP3A4, resulting in
reduced oral bioavailability, while others (diazepam, alprazolam) are relatively unaffected by gut wall metabolism (Hebert, 1997).

The CYP3A4 genes are located in chromosome 7 (7q22.1) (Brooks et al., 1988). CYP3A4 does not display a bimodal enzyme activity-distribution curve, although there are large inter-individual differences (10 to 40 fold) in CYP3A4 expression and activity due, in part, to environmental and dietary factors (smoking, exposure to grapefruit juice, chemical pollutants), genetic factors and possibly age (Bertz and Granneman, 1997; Ozdemir et al., 2000).

1.4 Probing CYP3A4 Activity

It has become increasingly important to attempt to predict drug-drug interactions, particularly drugs that are likely to be used in combination with other medications. Probe drugs are used in vitro and in vivo to directly ascertain the effects of inhibitors and inducers on CYP enzyme activity. In vitro screens are generally carried out first using either human liver tissue or recombinant enzymes to estimate the activity of an enzyme in the presence and absence of inhibitory or inducing substrates. The relationship between the rate of metabolite formation and substrate concentration is first evaluated. This data can often then be analyzed using classical Michaelis-Menten enzyme kinetic methods to yield a $V_{\text{max}}$ value (maximum rate of reaction) and a $K_m$ value (substrate concentration at $\frac{1}{2} V_{\text{max}}$). When an inhibitor is added at varying concentrations, the $K_i$ value can be determined. The $K_i$ value (measured in $\mu$mol/l) is the in vitro competitive inhibition constant, where lower values indicate
higher inhibitory potency. Thus it is possible to ascertain the inhibitory potency of several drugs on one enzyme, or of one drug on several enzymes (von Moltke et al., 1998). There are many instances where in vitro probing studies of CYP3A4 have successfully predicted clinically significant drug interactions in vivo. For example, in vitro studies using alprazolam as a probe for CYP3A4 activity predict norfluoxetine, a metabolite of the antidepressant fluoxetine, to be a stronger inhibitor of CYP3A4 than the antidepressant sertraline (von Moltke et al., 1994b). In vivo studies have confirmed that alprazolam levels are increased by up to 40% in patients taking fluoxetine, while alprazolam levels are not affected by sertraline (Greenblatt et al., 1992; Hassan et al., 2000). Likewise, in vitro studies have accurately predicted in vivo kinetic interactions between amitriptyline and fluoxetine (Schmider et al., 1999a), triazolam and ketoconazole (von Moltke, Greenblatt, Schmider, Wright, Harmatz, and Shader, 1998), desipramine and other 3A4 inhibitors (von Moltke et al., 1995), terfenadine and ketoconazole (von Moltke, Greenblatt, Duan, Harmatz, and Shader, 1994a), and other 3A4-mediated interactions (Schmider et al., 1999b).

There are, however, several limitations to in vitro studies that can make it difficult to extrapolate this data to in vivo conditions. Validity of the in vitro model is based on a series of assumptions and hypothesis, the most important of which relate to the concentration of inhibitor at the actual site of metabolic inhibition. Many studies indicate that concentrations of lipophilic psychotropic drugs in the liver considerably exceed those in the blood or plasma, suggesting that blood plasma levels of SSRIs will underestimate the intrahepatic concentrations available for inhibition of CYPs (von Moltke, Greenblatt, Court, Duan, Harmatz, and Shader, 1995). The liver:water partition ratio in humans can be estimated from studies with mice, however since
this is not a direct method there is much room for error. As well, in vitro studies do not take into account extrahepatic enzymes or the different biochemical environment found in vivo (e.g. pH, protein, phospholipid content) (Sproule, Naranjo, Bremner, and Hassan, 1997). Thus, although a quick and relatively easy method for initial estimation, there are limitations to in vitro studies. There are also many factors that must be addressed with respect to the clinical significance of potential drug interactions: (1) whether the drug concentration at the enzyme site is sufficient to cause inhibition; (2) whether multiple metabolic pathways for the drug exist, for example inhibition of one enzyme may have little clinical impact if the drug in question can be sufficiently biotransformed through a secondary enzymatic pathway; (3) whether the drug has metabolites that are pharmacologically active, since the impact of an inhibitor or inducer may then vary; (4) the therapeutic window of the substrate, since medications with smaller therapeutic windows may be at higher risk of having clinically significant effects from small changes in concentration; (5) the large interindividual variation in enzyme activity; and (6) the individual risk for adverse effects, for example the frail elderly may be at greater risk for experiencing cognitive and extrapyrimidal effects from CNS drugs (Sproule et al., 1997). Therefore it is important to carry out in vivo interaction studies.

To be effective, a probe drug must be specifically metabolized by a single CYP in order to ensure that the effect of other CYPs is negligible. Alprazolam has been used as a specific probe of CYP3A4 both in vitro (von Moltke, Greenblatt, Cotreau-Bibbo, Harmatz, and Shader, 1994b;von Moltke, Greenblatt, Court, Duan, Harmatz, and Shader, 1995) and in vivo (Nolting and Abramowitz, 2000;Hassan, Sproule,
Naranjo, and Herrmann, 2000; Yasui et al., 1998; Yasui et al., 2000; Greenblatt, Preskorn, Cotreau, Horst, and Harmatz, 1992a; Preskorn et al., 1997). Alprazolam is metabolized almost exclusively by CYP3A4 to form the major metabolite, 4-OH-alprazolam and a minor metabolite, \( \alpha \)-OH-alprazolam (Greenblatt et al., 1993). Therefore the rate of formation of 4-OH-alprazolam in the presence of an inhibiting or inducing agent can be used as an in vitro probe of CYP3A4 activity, or the pharmacokinetics of alprazolam pre- and post- inhibitor can serve to determine CYP3A4 activity in vivo.

When performing in vivo interaction studies, another important consideration is the frequency that the probe drug and CYP3A4 inhibitor are actually used together in clinical practice. This ensures that the interaction under investigation is clinically relevant. Alprazolam is often prescribed for anxiety, and anxiety is very often associated with depression, alcohol abuse, and other comorbid psychiatric conditions (Sherbourne et al., 1993; Kessler et al., 1998). Therefore, there is considerable merit in studying the clinical significance of CYP3A4-mediated interactions between alprazolam and drugs used for these disorders.

1.5 Comorbid Anxiety and Depression

A strong association has been identified between anxiety and depression. Anxiety has been estimated to be present in up to 96% of patients with depressive illness (Nutt, 1999). A study by Maier found that anxiety disorders showed an excess comorbidity which was substantially more common than expected on the basis of
their prevalence rates in both primary care and in the general population. For example, the expected 1-month prevalence of comorbid depression and generalized anxiety disorder (GAD) in primary care was expected by chance to be 1.0% based on epidemiological data on depression and GAD, however the observed rate of comorbidity was almost five times higher (4.8%) (Maier and Falkai, 1999). The prevalence of comorbid anxiety and depression in community samples has been reported to be as high as 10% to 20% (Lepine et al., 1993). The National Comorbidity Survey found a strong association between the lifetime prevalence of panic and depression (odds ratios: for panic attacks with depressive episodes, 6.2; for panic disorder with depression, 6.8) (Kessler, Stang, Wittchen, Ustun, Roy-Burne, and Walters, 1998). Approximately 35%, and up to 70%, of patients suffering from social anxiety disorder/social phobia report at least one major depressive episode (Stein et al., 1990a; Van Ameringen et al., 1991). Similarly, one study reported that 63% of patients with panic disorder had experienced at least one depressive episode (Stein et al., 1990b). The biological rationale for the association between anxiety and depression remains unclear.

In light of the high rate of comorbidity between anxiety and depression, a significant number of patients are likely to receive combination pharmacotherapy, making the issue of drug interactions of major importance.
1.6 Major Depressive Disorder and the Selective Serotonin Reuptake Inhibitors (SSRIs)

Major depression is an extremely widespread, chronic and debilitating illness, characterized by depressed mood and/or loss of interest or pleasure in activities for most of the day and on most days for a period of at least two weeks, plus at least three to four of the following symptoms during the same period; weight changes, sleep changes, psychomotor agitation or retardation, fatigue, feelings of worthlessness, diminished concentration, and suicidal ideation (DSM-IV). A typical episode of major depressive disorder, if left untreated, lasts between 4 and 12 months and nearly two-thirds of patients will suffer at least one recurrence (Hirschfeld, 2000).

There is a well-established relationship between suicide and mood disorders, and it has been estimated that 50-80% of completed suicides are associated with mood disorders (Simon and VonKorff, 1998; Kasper et al., 1996). About 15% of depressed patients will eventually commit suicide, and often tablet poisoning (especially antidepressants) is chosen as the suicide method (Henry and Rivas, 1997; Kasper, Schindler, and Neumeister, 1996). Depressed patients are 30 times more likely to commit suicide than the general population (Bertschy and Vandel, 1991).

Studies have indicated that over 17% of Americans will suffer from major depressive disorder at some point in their lives, and women are more often affected than men (Kessler et al., 1994). Furthermore, depression is often found concurrently with other
disorders such as anxiety (Maier and Falkai, 1999), substance abuse and alcoholism (Swendsen et al., 1998; Sherbourne, Hays, Wells, Rogers, and Burnam, 1993; Dhossche et al., 2000), heart disease (Sesso et al., 1998; McGann, 2000), HIV infection (Evans et al., 1998), diabetes (Grandinetti et al., 2000) and cancer (Massie et al., 1994). Comorbid depression can seriously affect the quality of life of patients with these diseases (Xuan et al., 1999), increase the burden of disease and care, and increase morbidity (Dwight and Stoudemire, 1997; Clarke, 1998; Dhossche, Meloukheia, and Chakravorty, 2000). The direct and indirect cost of depression in the United States has been estimated at $US26 billion to $US43.7 billion (in 1990) (Greenberg et al., 1993). Thus, depression is a common, debilitating and potentially life-threatening disease.

Modern treatment of Major Depression started in the 1950s with the unexpected discovery that the monoamine oxidase inhibitor (MAOI) iproniazid, being tested for anti-tubercular properties, led to psychostimulation and a reversal of reserpine-induced sedation (Pletscher, 1991). Other MAOI drugs, such as phenelzine, were subsequently developed, however the use of these drugs has declined significantly due to potentially life-threatening side effects such as potentiation of the blood-pressure elevating action of food amines, and the risk of serious drug interactions when used in combination with other sympathomimetic medications (Cooper, 1989). The tricyclic antidepressants (TCAs) were developed as a class of antihistamine drugs, and one of these, imipramine, was found by clinical observation to have antidepressant properties (Pletscher, 1991). The TCAs are still widely used today; imipramine and its metabolite desipramine, amitriptyline and its metabolite
nortriptyline are examples. The more recent classes of antidepressants include the selective serotonin reuptake inhibitors (SSRIs) fluoxetine, fluvoxamine, paroxetine, sertraline and citalopram, as well as other newer drugs such as bupropion, nefazadone, mitazapine and venlafaxine which work via several different mechanisms.

The leading theory of depression since the discovery of the monoamine oxidase inhibitors has been the 'monoamine theory' which hypothesizes that depression is caused by a deficiency in one or another of the three biogenic amines norepinephrine, serotonin and dopamine (Stahl, 1998). This theory is supported by the fact that every known antidepressant medication increases neurotransmission of one or more of these monoamines by blocking reuptake pumps and/or receptors for these chemicals, or by inhibiting their breakdown by monoamine oxidase. Side effects of these medications are due to their primary effects on monoamine neurotransmission or to unwanted blockade of other receptors; for example the tricyclic antidepressants can cause sedation, dry mouth, blurred vision and constipation due to their unwanted antihistaminergic and anticholinergic properties.

A recent British study found that over a five year period from 1991 to 1996, the prescription of TCA antidepressants increased by 40% while the prescription of SSRIs increased by 460% (Lawrenson et al., 2000). In the United States, the SSRIs account for well over half of all antidepressant prescriptions (Stahl, 1998). The SSRIs are becoming the drug of choice for depression due to their similar efficacy to TCAs, fewer side effects and greater safety in overdose (Barbey and Roose, 1998).
Studies comparing the safety and efficacy of TCAs versus SSRIs have consistently found TCAs to have similar efficacy to the SSRIs, but less tolerability (McGrath et al., 2000; Mundo et al., 2000; Hirschfeld, 2000). One study found that the rate of fatality in users of TCAs was one in 8130, while for SSRIs as a group the association was one in 411,800 (Mason et al., 2000). SSRI antidepressants are rarely fatal in overdose when taken alone. Moderate overdoses (up to 30 times the common daily dose) are associated with minor or no symptoms, while ingestion of greater amounts typically result in drowsiness, tremor, nausea, and vomiting. At very high doses (> 75 times the common daily dose), more serious adverse events, including seizures, electrocardiogram (ECG) changes, and decreased consciousness may occur (Barbey and Roose, 1998). SSRI overdoses in combination with alcohol or other drugs are associated with increased toxicity, and almost all fatalities involving SSRIs have involved coingestion of other substances.

SSRI antidepressants are effective in about 60% to 70% of patients, and require several weeks to achieve a full response (Stahl, 1998; Pletscher, 1991). When an SSRI such as fluoxetine is administered to a rat, the concentration of serotonin increases in the synaptic cleft, which is the desired dynamic effect of the medication. However, the autoregulatory 5-HT 1B autoreceptors and somatodendritic 5-HT 1A autoreceptors are also activated, which decreases the firing rate of the serotonin neurons, effectively negating the desired effect. After two to six weeks of repeated administration, however, the autoreceptors become desensitized, resulting in increased serotonin at the synaptic cleft (Paul, 1999; Stahl, 1998).
There are currently five SSRIs approved for use in Canada; fluoxetine (Prozac®), fluvoxamine (Luvox®), paroxetine (Paxil®), sertraline (Zoloft®), and most recently, citalopram (Celexa®) which was approved in March, 1999 (CPS, 2000). All SSRIs appear to have similar efficacy and side effect profiles, but differ in their chemical structure (fig 1), and kinetics (Hirschfeld, 2000; Baumann and Rochat, 1995). For example, fluoxetine is unique in its long-half life and its active metabolite with an
even longer half life, norfluoxetine (Lucas, 1992). The other SSRIs have significantly shorter half lives and metabolites that are much less potent than the parent compound. Fluoxetine and citalopram have chiral centers, the others do not (Baumann and Rochat, 1995). Sertraline and citalopram display linear dose-concentration curves, while fluvoxamine, fluoxetine and paroxetine may show greater than expected concentration increases with increased dose (Preskorn, 1997). The SSRIs also vary in their potential for causing metabolic interactions through inhibition of different species of CYP enzymes (table 1). For example, paroxetine is a potent inhibitor of CYP2D6 (Ki=0.065), dramatically increasing it’s potential for pharmacokinetic interactions with other CYP2D6-metabolised medications, as confirmed by reports in the literature (Sproule, Naranjo, Bremner, and Hassan, 1997), while citalopram is a weak inhibitor of CYP2D6 (Ki=7) and has been implicated in very few interactions with CYP2D6-metabolised drugs (Brosen et al., 1993);(Gram et al., 1993);(Jeppesen et al., 1996;Brosen, 1998;Naranjo et al., 1999). Fluoxetine and it’s metabolite norfluoxetine are moderate in vitro inhibitors of CYP3A4, and in vivo studies have shown that fluoxetine inhibits CYP3A4 in vivo, leading to significant accumulation of the CYP3A4 probe drug alprazolam (Lasher et al., 1991;Greenblatt, Preskorn, Cotreau, Horst, and Harmatz, 1992). In vitro studies suggest that citalopram does not inhibit CYP3A4, but incomplete in vivo studies have been carried out to confirm this. The purpose of this study was to compare in vivo inhibition of CYP3A4 by both citalopram and fluoxetine, using the anxiolytic alprazolam as an in vivo CYP3A4 probe.
Table 1. Relative Inhibitory potency of the SSRIs with respect to four of the most important CYPs

<table>
<thead>
<tr>
<th>SSRI</th>
<th>CYP2D6 $K_i$ (μmol/L) Dextromethorphan probe</th>
<th>CYP3A $K_i$ (μmol/L) Alprazolam probe</th>
<th>CYP2C19 $K_i$ (μmol/L) S-mephenytoin probe</th>
<th>CYP 1A2 $IC_{50}^{**}$ (μM) Paracetimol probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citalopram</td>
<td>7</td>
<td>*</td>
<td>87.3</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Desmethyl-citalopram</td>
<td>6</td>
<td>*</td>
<td>55.8</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>0.17</td>
<td>83.3</td>
<td>5.2</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Norfluoxetine</td>
<td>0.19</td>
<td>11.1</td>
<td>1.1</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td>1.8</td>
<td>10.2</td>
<td>_</td>
<td>0.2</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>0.065</td>
<td>39.4</td>
<td>7.5</td>
<td>45</td>
</tr>
<tr>
<td>Sertraline</td>
<td>1.5</td>
<td>23.8</td>
<td>2.0</td>
<td>70</td>
</tr>
</tbody>
</table>

*Minimal or no inhibition

**IC$_{50}$ = the concentration of the inhibitor which reduced the formation of a metabolite by 50% where lower values indicate higher inhibitory potency (Naranjo, Sproule, and Knoke, 1999)

1.7 Citalopram

Citalopram (Celexa®) (1-[3-(dimethylaminopropyl)-1-(4-fluorophenol)-1,3-dihydroiso-benzo-furan-5-carbonitrile]) was first synthesized in 1972 by H. Lundbeck A/S, Copenhagen (fig. 2). As a substituted phthalane derivative with a tertiary amino acid side chain, it is a highly lipophilic compound with one chiral center (Willetts et al., 1999). Released in Canada in March, 1999, it has become one of the most popular medications on the market, ranking 89th in 2000 (IMS Canada, 2000).
Of the SSRIs, citalopram is the most selective inhibitor at the serotonin reuptake pump, and has a minimal effect on the neuronal uptake of norepinephrine and dopamine, as well as little or no affinity for histamine H1, muscarinic cholinergic, benzodiazepine, gamma aminobutyric acid (GABA) and opioid receptors (CPS, 2000). However, it is less potent at the serotonin reuptake pump than paroxetine, the most potent of the SSRIs (Bezchlibnyk-Butler et al., 2000). Tolerance to the inhibition of serotonin reuptake is not induced by long-term therapy in rats (14-days) (CPS, 2000).

The absorption of citalopram is not affected by food, and oral bioavailability is approximately 80%. Peak plasma levels occur two to four hours after single or multiple doses. Citalopram is 50% protein bound, far less so than the other SSRIs, and therefore potentially less likely to be involved in drug interactions with highly bound medications such as NSAIDS, warfarin and valproic acid. Citalopram is highly distributed among peripheral tissue, with the volume of distribution ($V_d$) estimated to be between 12 and 16 L/kg (Bezchlibnyk-Butler, Aleksic, and Kennedy, 2000). At clinically relevant doses, citalopram displays linear pharmacokinetics, although there is no dose-response correlation. Interindividual variation in steady-state plasma
levels is extremely high, about 7-fold, and appears to be independent of age (up to 65) and sex. Steady state levels are reached within approximately 1 to 2 weeks, and the trough concentration is reported to be approximately 130 ± 70 nmol/L at 20mg per day. Approximately 12 to 23% of an oral dose of citalopram is excreted as unchanged citalopram in the urine, and 10% is excreted in the feces (Bezchlibnyk-Butler, Aleksic, and Kennedy, 2000). Citalopram is metabolized in the liver by two N-demethylation steps to demethylcitalopram (DCT) via CYP2C19 and CYP3A4, and subsequently to didemethylcitalopram (DDCT) via CYP2D6 (fig. 3). Oxidation occurs by monoamine oxidases A and B and aldehyde oxidase to form a propionic acid derivative and citalopram-N-oxide (Rochat et al., 1997; von Moltke et al., 1999; Bezchlibnyk-Butler, Aleksic, and Kennedy, 2000). At steady-state as well as after single doses, the ratio of metabolites in relation to parent compound, are 30 to 50% for DCT, and 5 to 10% for DDCT. In vitro studies suggest that citalopram is at least 4 times more potent than DCT, and 13 times more potent than DDCT. These metabolites do not appear to enter the brain as readily as the parent compound, and are therefore thought not to play a significant role in the clinical action of citalopram (Willetts, Lippa, and Beer, 1999; Bezchlibnyk-Butler, Aleksic, and Kennedy, 2000). Citalopram shows biphasic elimination; the distribution half life (t1/2) is about 10 hours, while the elimination t1/2 has been estimated between 30 and 35 hours for citalopram, 50 hours for DCT and 100 hours for DDCT. Dosage adjustment does not appear to be required in mild to moderate renal impairment, but lowering of the initial dose is recommended in patients with impaired hepatic function and in the elderly over 65 years of age (CPS, 2000; Willetts, Lippa, and Beer, 1999; Bezchlibnyk-Butler, Aleksic, and Kennedy, 2000; Baumann and Rochat, 1995).
Fig. 3. Biotransformation of citalopram

Therapeutic dosage of citalopram ranges from 10 to 80 mg/day, with most common dosage between 20 and 60 mg/day (Willetts, Lippa, and Beer, 1999). In vitro studies with rats have shown that S-citalopram and S-DCT are potent SSRls, whereas the corresponding R-enantiomers are not. Studies have suggested that S-(+)-citalopram plasma levels reach about one third of total citalopram in depressed patients, with a range of 24 to 49% (Baumann and Rochat, 1995).

Citalopram has demonstrated efficacy in clinical trials for major depression, and is equally as effective as the tricyclics, with fewer side effects. Citalopram is also as efficacious as equivalent doses of fluvoxamine, sertraline and fluoxetine. There is also evidence that citalopram may be useful for patients with panic disorder.
Citalopram appears, from in vitro experiments, to have a higher Ki value (denoting less inhibition of metabolism of the probe drug specific for a particular CYP) than the other SSRIs for all CYPs important in psychotropic drug metabolism (table 1) (von Moltke, Greenblatt, Grassi, Granda, Venkatakishnan, Duan, Fogelman, Harmatz, and Shader, 1999). In particular, citalopram has a minimal in vitro inhibitory effect on CYP3A4. This suggests that citalopram may have less potential for drug interactions with other medications metabolized by CYPs including CYP3A4. This hypothesis will be tested in this study.

1.8 Fluoxetine

Now available as a generic, fluoxetine is among the most commonly prescribed antidepressants in Canada (IMS Canada, 2000) and is the most popular SSRI in many states in the US, for example in New York State and Michigan fluoxetine represents almost 20% of antidepressant prescriptions (NIMH, 2000). Fluoxetine, p-trifluoro-phenoxyphenylo N-methyl-propylamine (fig. 4), was developed by Eli Lilly and was the first SSRI marketed in Canada. It is indicated not only for the treatment of depression, but also for obsessive-compulsive disorder and bulimia, and also appears to be efficacious in the treatment of anxiety disorders (CPS, 2000; Alexander, 1991). Since most SSRIs require 3-6 weeks before the therapeutic
action is noticeable, benzodiazepines such as alprazolam can be prescribed concurrently during this time.

Fig. 4. Chemical structure of fluoxetine

Fluoxetine selectively inhibits the neuronal reuptake of serotonin. It binds to muscarinic, histaminergic, dopaminergic, opioid and alpha-adrenergic receptors to a lesser degree than the tricyclic antidepressants, accounting for its more favourable side effect profile (CPS, 2000) (Lucas, 1992).

Fluoxetine is well absorbed after oral administration, and food does not appear to affect systemic bioavailability, although may delay its absorption inconsequentially. Following a single oral dose of 40mg, peak concentrations ($C_{\text{max}}$) from 43 to 159 nmol/L are observed after 6 to 8 hours. Plasma binding of fluoxetine is high, around 94% (CPS, 2000).
Fluoxetine is primarily metabolized by hepatic CYP2D6, CYP3A4, and CYP2C19 to the active metabolite norfluoxetine and a number of other unspecified metabolites (fig. 5) (Altamura et al., 1994). Norfluoxetine, formed by demethylation of fluoxetine, appears to have similar pharmacological activity to the parent compound, and this contributes to the prolonged duration of action of fluoxetine. Steady state concentrations of fluoxetine are reached after 5-6 weeks of continuous administration. After 30 days of dosing at 20mg/day, mean plasma concentrations of fluoxetine are 228.7 ± 96.6 nmol/L and norfluoxetine 373 ± 121 nmol/L have been observed (CPS, 2000). The plasma half life of fluoxetine is 1 to 3 days after acute administration, and 4 to 6 days after chronic administration, and dose does not correlate with plasma concentration. Norfluoxetine appears to have a linear dose-concentration relationship, and the half life of this metabolite is 7-15 days after acute and chronic administration (CPS, 2000) (Altamura et al., 1994; Lucas, 1992). The slow elimination of fluoxetine and norfluoxetine results in significant accumulation of
these compounds after chronic use, and it may take up to two months for the active
drug substances to disappear from the body, therefore drugs that may interact with
fluoxetine should not be administered for at least 6 weeks after discontinuation.

The S-enantiomers of fluoxetine and norfluoxetine are more potent than their
_corresponding R- enantiomers: about 1.5 times more potent for fluoxetine, and 20
times more potent for norfluoxetine (Baumann and Rochat, 1995). It is believed that
the difference in metabolism of these enantiomers is what makes it difficult to get
consistent dose-effect studies of fluoxetine. The average therapeutic dose of
fluoxetine ranges between 10 and 80mg/day (Lucas, 1992). There is some evidence
that this dose needs to be adjusted in the elderly, and patients with renal and liver
disease may show impaired clearance as well (Bergstrom et al., 1993).

Both fluoxetine and norfluoxetine are in vitro inhibitors of CYPs 2D6, CYP3A4, and
CYP2C19 (Naranjo, Sproule, and Knoke, 1999). Both are fairly strong inhibitors of
CYP2D6, although not as strong as paroxetine, the most potent of the SSRls with
respect to inhibition of CYP2D6 (Ki= 0.17µmol/L, 0.19µmol/L, 0.065µmol/L for
fluoxetine, norfluoxetine and paroxetine respectively) (table 1). Norfluoxetine is a
more potent in vitro inhibitor of CYP3A4 than fluoxetine (Ki= 11.1 µmol/L vs. 83.3
µmol/L respectively). There have been many reports of fluoxetine interacting with
drugs metabolized by CYP2D6, for example in vivo coadministration with fluoxetine
has been associated with increased serum concentrations of haloperidol, tricyclic
antidepressants, and codeine (Otton et al., 1993;Kudo and Ishizaki, 1999;Sproule,
Fluoxetine has also been associated with increased plasma levels of trazodone and clozapine associated with blockade of CYP2D6 and/or CYP3A4 (Sproule, Naranjo, Bremner, and Hassan, 1997; Ferslew et al., 1998). Diazepam and alprazolam, benzodiazepines metabolized primarily by CYP3A4, display reduced plasma clearance in the presence of fluoxetine (Lemberger et al., 1988; Greenblatt, Preskorn, Cotreau, Horst, and Harmatz, 1992). In vivo, the addition of fluoxetine to single or multiple doses of alprazolam (1mg) increased the AUC and half life of alprazolam, increasing alprazolam plasma concentrations by as much as 40%, and these kinetic changes were associated with reduced psychomotor performance (Greenblatt, Preskorn, Cotreau, Horst, and Harmatz, 1992).

1.9 Anxiety disorders and Anxiolytics

Anxiety is one of the most common and pervasive psychiatric illnesses, and was described in the writings of Hippocrates as early as the fourth century B.C. (Mendlowicz and Stein, 2000). As a group, the anxiety disorders are the second most common psychiatric illness after substance abuse; the lifetime prevalence is approximately one in four people (Kessler, McGonagle, Zhao, Nelson, Hughes, Eshleman, Wittchen, and Kendler, 1994). There are seven principal types of anxiety disorders: social phobia, simple phobia, agoraphobia, generalized anxiety disorder, panic disorder, obsessive-compulsive disorder and post-traumatic stress disorder. In 1990, the cost associated with anxiety disorders in the United States was US$46.6 billion, and accounted for over 31% of that years expenditure on mental health
(Mendlowicz and Stein, 2000). The quality of life of people with anxiety disorders is also affected. One study found that 35% of people who suffered from panic attacks felt that they were in poor physical health, and 38% of sufferers felt they were in poor emotional health, whereas controls not suffering from anxiety reported significantly lower rates (19% and 16% respectively) (Mendlowicz and Stein, 2000). Generalized anxiety disorder is diagnosed when patients emphasize the presence of excessive or unrealistic worry and somatic symptoms for at least 6 months (DSM-IV), and has a lifetime prevalence of just over 5%. Persons with generalized anxiety are more often unmarried or divorced, and a significantly higher proportion of patients with this disorder have received disability benefits at some point during their lives (Mendlowicz and Stein, 2000). Generalized anxiety is associated with decreased emotional health and lower life satisfaction and higher rates of work impairment (Mendlowicz and Stein, 2000). However the authors of these studies stress that generalized anxiety is almost always found in combination with another anxiety disorder. Indeed, anxiety disorders in general are commonly found in combination with other illnesses, particularly alcohol use disorders (Kushner et al., 2000), respiratory illness (Smoller et al., 1999) and depression (Kessler, Stang, Wittchen, Ustun, Roy-Burne, and Walters, 1998).

Currently, anxiety is thought to be caused by dysfunction of one or more neurotransmitters and their receptors. Alteration in the influx of chloride ions within the benzodiazepine-gamma-aminobutyric acid (GABA) receptor complex is associated with the development of anxiety (Doble, 1999). Other neurotransmitters have also been implicated in the etiology of anxiety. For example, drugs affecting the
noradrenergic beta receptor and the serotonin receptors have anxiolytic properties (Zohar and Westenberg, 2000).

The newer classes of benzodiazepines have replaced the traditional barbiturates in the treatment of anxiety disorders. At one time, benzodiazepines were the number one prescription drug in the United States and the world: one study found that 6.2% of the adult population purchased a benzodiazepine in 1987 (Olivier et al., 1998) (Olfson and Pincus, 1994). Although their use has declined in recent years, benzodiazepines are still one of the most commonly prescribed psychotropic medication in Canada (IMS Canada, 2000).

Benzodiazepines act at the level of the limbic, thalamic and hypothalamic regions of the CNS and can produce any level of CNS depression including sedation, hypnosis, skeletal muscle relaxation, amnesia, anticonvulsant activity and coma. The action of these drugs is mediated through the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) (Doble, 1999). Central benzodiazepine receptors interact allosterically with GABA receptors, potentiating the effects of GABA and increasing the inhibition of the ascending reticular activating system. Benzodiazepines block the cortical and limbic arousal that occurs following stimulation of the reticular pathways. Clinically, all benzodiazepines cause a dose-related central nervous system depressant activity varying from mild impairment of task performance to hypnosis. The benzodiazepine alprazolam has shown efficacy in the treatment of panic disorder (Sheikh and Swales, 1999; Rickels and Schweizer, 1998; Lydiard et al., 1997), and clonazepam was effective in 78% of patients experiencing social phobia (Davidson...
and Moroz, 1998). Many benzodiazepines have been studied for the treatment of generalized anxiety disorder, and results generally show moderate or marked improvement in 65% to 75% of patients (Ballenger, 1999). Benzodiazepines are well tolerated, the most commonly reported side effects are drowsiness, lightheadedness, fatigue, and impaired coordination (CPS, 2000). In long term-use, tolerance to the side effects of benzodiazepines occurs, but tolerance to the anxiolytic effects do not appear (Ballenger, 1999). As well, benzodiazepines have a high therapeutic index and are safer in overdose than the barbiturates. Fatalities with benzodiazepines rarely occur except when other drugs, alcohol or aggravating factors are involved (Moller, 1999; Lydiard, Ballenger, and Rickels, 1997).

The monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs) have also demonstrated efficacy in the treatment of panic disorder, and MAOIs also appear to be useful in the treatment of social anxiety (Rouillon, 1999). More recently, the SSRIs have been found to be effective in a wide spectrum of anxiety disorders including panic, obsessive compulsive disorder, generalized anxiety disorder, and social anxiety (Rouillon, 1999). Since the SSRIs require several weeks for full effectiveness, benzodiazepines can be used concurrently to treat anxiety during the initial weeks. An international study utilizing a panel of 73 experts in the pharmacotherapy of anxiety disorders were polled over a five year period between 1992 and 1997 to determine the trends in recommendations for the pharmacotherapy of anxiety disorders. The study found that experts recommending a medication in 1992 most often chose a benzodiazepine as a first line treatment for panic disorder, generalized anxiety disorder, and simple phobia. In 1997, the expert
panels' most frequent recommendation for panic disorder changed to a SSRI, however, overall there was only a small decline in recommendations for benzodiazepines (Uhlenhuth et al., 1999).

1.10 Alprazolam

Alprazolam has been among the most frequently prescribed benzodiazepines in Canada for many years, and remains in the top 150 prescription products (IMS Canada, 2000). Alprazolam was the most commonly prescribed benzodiazepine in the United States from 1988 until 1993 (Shader and Greenblatt, 1993). Alprazolam (8-Chloro-1-methyl-6-phenyl-4H-s-triazolo[4,3][1,4] benzodiazepine) is a triazolo 1,4 benzodiazepine analog that binds with high affinity to the GABA benzodiazepine receptor complex (fig. 6). Considerable evidence suggests that the central pharmacologic/therapeutic actions of alprazolam are mediated via interaction with this receptor complex (Huybrechts, 1991).

![Chemical structure of alprazolam](image)

Fig. 6. Chemical structure of alprazolam
Following oral administration, alprazolam is readily absorbed and is over 90% bioavailable. Peak concentrations in the plasma occur 1-2 hours following administration. Plasma levels are proportionate to the dose given; over the dose range of 0.5 to 3.0 mg, peak levels of 26 to 129 nmol/L have been observed. The mean plasma elimination half-life of alprazolam has been found to be about 11.2 hours (range: 6.3-26.9 hours) in healthy adults (Huybrechts, 1991). Alprazolam is often preferable to benzodiazepines such as diazepam because it has a relatively shorter half-life and does not have active metabolites that can lead to accumulation, particularly in the elderly. The drug is widely distributed and is over 90% plasma protein-bound. Alprazolam may cross the placenta and distribute into breast milk (Huybrechts, 1991). Alprazolam undergoes CYP3A4-mediated oxidative metabolism in the liver, producing two predominant metabolites; α-hydroxy-alprazolam and 4-hydroxy alprazolam (Greenblatt and Wright, 1993a) (fig. 7). The biological activity of α-hydroxy-alprazolam is approximately one-half that of alprazolam. 4-hydroxy alprazolam is essentially inactive. Plasma levels of these metabolites are extremely low, thus precluding precise pharmacokinetic description. However, their half-lives appear to be of the same order of magnitude as that of alprazolam. Both the active and inactive derivatives of the drug are excreted in the urine (Huybrechts, 1991; Greenblatt and Wright, 1993a).
Fig. 7. Biotransformation of Alprazolam

Changes in the absorption, distribution, metabolism and excretion of benzodiazepines have been reported in a variety of disease states including alcoholism, impaired hepatic function and impaired renal function (Juhl et al., 1984; Ochs et al., 1986). Changes have also been demonstrated in geriatric patients. A mean half-life of alprazolam of 16.3 hours has been observed in healthy elderly subjects compared to 11.0 hours in healthy adult subjects (Kaplan et al., 1998). The co-administration of oral contraceptives to healthy women increased the half-life of alprazolam as compared to that in healthy control women (Scavone et al., 1988; McAuley and Friedman, 1999). In patients with alcoholic liver disease the half-life of alprazolam was increased to 19.7 hours from 11.4 hours in healthy subjects.
(Juhl, Van Thiel, Dittert, and Smith, 1984). In an obese group of subjects the half-life of alprazolam was 21.8 hours (Abernethy et al., 1984).

Like all benzodiazepines, the side effect profile of alprazolam includes somnolence, fatigue, dizziness, lightheadedness, impaired motor coordination, headache, and nausea (CPS, 2000).

The recommended starting dose of alprazolam for GAD and panic disorder is 0.25 mg given 2 or 3 times daily. If required, increases may be made in 0.25 mg increments according to the severity of symptoms and patient response. It is recommended that the evening dose be increased before the daytime doses. Very severe manifestations of anxiety may require larger initial daily doses. The optimal dosage is one that permits symptomatic control of excessive anxiety without impairment of mental and motor function (CPS, 2000).
2 Rationale and Hypothesis for Study

Alprazolam (Xanax®) has been used as a specific in vivo probe for CYP3A4. Based on the existing in vitro and in vivo evidence, we hypothesize that therapeutic doses of the SSRI antidepressant citalopram (20mg/day), as compared to the SSRI fluoxetine (20mg/day), will cause less impairment in the metabolism of the probe drug alprazolam (1mg) through inhibition of the CYP3A isozymes, as measured by pharmacokinetic and pharmacodynamic parameters in vivo. This experiment will provide useful information to the clinician who must determine the most appropriate antidepressant to prescribe in patients who are on multi-drug therapy and are taking medications metabolized by CYP3A4.
3 Methods

3.1 Study design

The study was conducted in 20 healthy volunteers following a within-subject, double-blind, placebo-controlled, parallel design. Subjects attended a total of 4 study sessions. The first two study sessions occurred in the absence of SSRI medications, while the final two sessions occurred after a minimum of twenty-one days of administration of SSRI medication (Citalopram 20mg or Fluoxetine 20mg) (fig. 8). At each study session, which took place at least three days apart, subjects were randomly assigned to a single dose of alprazolam (1 mg) or placebo; i.e., one of each medication was given in a random order during the two sessions before SSRI administration and, likewise, one of each medication was given during the final two sessions after SSRI administration. Trough SSRI serum concentrations (including metabolites) were determined on five occasions: on days 1 and 7 of SSRI administration, prior to alprazolam/placebo administration at Study Sessions 3 and 4, and 7 days after discontinuation of study medications.

Briefly (details of session day procedures follow), blood samples for alprazolam concentration determination occurred during the study sessions at baseline, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 24.0 and 48.0 hours after alprazolam/placebo single dose administration. Pharmacodynamic effects were measured once at baseline and
Fig. 8. Study Design

Session 1: Alprazolam (1mg) or placebo
Session 2: Placebo or Alprazolam (1mg)

3 weeks

Citalopram (20mg/day) or Fluoxetine (20mg/day)

Session 3: Alprazolam (1mg) or placebo
Session 4: Placebo or Alprazolam (1mg)
Fig. 9. Study Session Day Procedure.

<table>
<thead>
<tr>
<th>Time</th>
<th>Activities</th>
</tr>
</thead>
</table>
| 08:00 | - Catheter inserted  
       | - Urine Screen (toxicology) and breathalyzer  
       | - Practice DSST and MTT                      |
| 08:30 | - Sedation measure  
       | - DSST                                        
       | - MTT                                         
       | - Blood drawn                                 |
| 09:00 | - Drug administered                               |
| 09:30 | - Sedation measure  
       | - DSST                                        
       | - MTT                                         
       | - Blood drawn                                 |
| 10:00 | - Breakfast                                      |
| 10:30 | - Sedation measure  
       | - DSST                                        
       | - MTT                                         
       | - Blood drawn                                 |
| 11:00 | - Sedation measure  
       | - DSST                                        
       | - MTT                                         
       | - Blood drawn                                 |
| 11:30 | - Sedation measure  
       | - DSST                                        
       | - MTT                                         
       | - Blood drawn                                 |
| 12:00 | - Sedation measure  
       | - DSST                                        
       | - MTT                                         
       | - Blood drawn                                 |
| 13:00 | - Lunch                                         |
| 15:00 | - Sedation measure  
       | - DSST                                        
       | - MTT                                         
       | - Blood drawn                                 |
| 17:00 | - Sedation measure  
       | - DSST                                        
       | - MTT                                         
       | - Blood drawn                                 |
at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, and 8.0 hours following the single dose administration of alprazolam/placebo (fig. 9). The pharmacodynamic assessments were computerized and included subjective assessment of sedation using the Sedation Scale (from the Tufts University Benzodiazepine Scale), and objective assessment of psychomotor functioning using the Digit Symbol Substitution Test and the Manual Tracking Test (details follow).

Subjects were recruited by posters on bulletin boards at the University of Toronto and Sunnybrook & Women’s College Health Sciences Centre.

3.2 Inclusion and Exclusion Criteria

Healthy subjects were included in the study if they were between 18 and 55 years of age, willing to refrain from using caffeine and alcohol for at least 24 hours before, and during, each study sessions and if they signed the study consent form, approved by the Research Ethics board at Sunnybrook (appendix 1). After giving written informed consent, a thorough medical history was taken and a brief medical examination, routine clinical biochemistry and hematological tests were conducted to screen for medical contraindications. Biochemistry tests included blood electrolytes, renal profile (urea, creatinine), liver profile (AST, ALT, ALP, bilirubin), GGT, total protein, glucose levels and a complete blood count (including differential). In addition, a urine screen for illicit drugs and pregnancy was performed. Females of child bearing age were required to use an effective form of birth control other than oral contraceptives, due to interactions with alprazolam affecting the metabolism and
bioavailability of alprazolam (Scavone et al., 1988; Stoehr et al., 1984; McAuley and Friedman, 1999).

Individuals older than 55 were excluded from the study due to evidence that alprazolam metabolism may be impaired relative to younger controls (Kaplan et al., 1998). Alprazolam metabolism may be affected by a number of other factors, which were used as exclusionary criteria. Renal disease may produce increased sensitivity to the sedative effects of alprazolam (Schmith et al., 1992) and alprazolam clearance may be reduced in subjects with histories of renal (Ochs et al., 1986) or hepatic disease (Juhl, Van Thiel, Dittert, and Smith, 1984). Liver enzymes and serum creatinine were examined to assess current renal and liver function. Cigarette smokers may have increased metabolic clearance of alprazolam (Smith et al., 1983). Obese subjects, in excess of 30% of ideal body weight, may have decreased elimination of alprazolam (Abemethy et al., 1984). Subjects who use potent CYP3A4 inhibitors such as grapefruit juice, ketoconazole, astemizole, nefazadone, or other SSRIs may already have impaired alprazolam metabolism (Greenblatt et al., 1992; Lasher et al., 1991; Fleishaker and Hulst, 1994). Likewise subjects using inducers of CYP3A4 (rifampin, carbamazepine) were excluded. Other exclusionary criteria included women who were pregnant or breast feeding, subjects with known contraindications to study medications, individuals with abnormal biochemical or hematological laboratory test results and subjects using illicit drugs, which have the potential to interfere with pharmacodynamic assessments of alprazolam.
3.3 Sample Size

Based on previous work in our lab, the $C_{\text{max}}$ for alprazolam after a single dose was expected to be $39.7 \text{ nmol/L} \pm 18.8$ (Hassan, Sproule, Naranjo, and Herrmann, 2000). In order to detect at least a 50% change in the $C_{\text{max}}$ of alprazolam (i.e. an increase to $60 \text{ nmol/L}$) within subjects (before and after SSRI administration) with a power of 80%, and an alpha of 0.05, it was calculated that we would need a total of 10 completed subjects in each group.

3.4 Randomization

Randomization was done before the start of the study by a person not in contact with the study volunteers in order to ensure successful blinding. No one else was given access to the randomization codes unless an emergency arose, and the people in daily contact with study subjects were kept blinded until the end of the experiment. All randomization was done in blocks of four to ensure even distribution of all randomized conditions (appendix 2). Randomization to citalopram or fluoxetine was done in blocks of four (2 citalopram/2 fluoxetine per block). The order of drugs (alprazolam and placebo) given in pre-SSRI sessions was randomized in the same blocks of four (2 alprazolam first/2 placebo first per block) to ensure that the effects of drug order on dynamic tests could be measured at a later time. Finally, the order of alprazolam and placebo post-SSRI was randomized to the same order as pre-SSRI (2 per block) or the opposite order (2 per block).
3.5 Study Session Procedures

Subjects attended one information session and four study sessions in the Human Psychopharmacology Laboratory at Sunnybrook and Women’s College Health Sciences Centre. Study sessions were conducted prior to SSRI treatment and at steady state concentrations of citalopram (20mg) or fluoxetine (20mg).

The first day of each study session proceeded from 8:00 am to 5:00 pm and subjects were asked to return at 9:00 am on the following two days for a short time only to obtain blood samples. Twenty-four hours before and during all 3 days of each study session, subjects were asked to abstain from caffeine and alcohol-containing foods or beverages which may interfere with the pharmacodynamic effects of alprazolam. Subjects were asked to avoid grapefruit juice throughout the study. Subjects were also asked to fast overnight from 11:00 pm to 8:00 am and to get at least 8 hours of sleep before the first day of each study session. On each full day study session, a urine sample was taken for drug screening. A breathalyzer test was performed in the morning of each full session day to ensure compliance with the request for abstinence from alcohol. One hour and four hours after drug administration, subjects received a standard light breakfast (apple juice & muffin) and light lunch, respectively.

A single dose of either alprazolam (1 mg) or placebo was randomly administered at approximately 9:00am on the first day of Study Sessions 1 and 2 and at the same time on the first day of Study Sessions 3 and 4 (fig. 9). Peak serum alprazolam
concentrations were expected between 0.7 and 3.0 hours post-administration, both before and after inhibition of CYP3A4 (Greenblatt and Wright, 1993a; Greenblatt, Preskorn, Cotreau, Horst, and Harmatz, 1992; Hassan et al., 2000). Blood collection times were scheduled at baseline, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 24.0 and 48.0 hours after alprazolam or placebo single dose administration. Immediately following each of the first 10 blood samples, subjects rated their current level of sedation and performed psychomotor tests (described below). Vital signs (temperature, pulse and blood pressure) were monitored over the course of the day and any adverse events were recorded using a symptom checklist. On the first day of each study session, an intravenous catheter (saline-lock, 20 gauge) was inserted into the subjects' forearm cephalic vein for the collection of blood samples from baseline to 8 hours, after which the catheter was removed and the subject left the laboratory. Remaining blood samples were collected by venipuncture at 9:00 am on the following two days of the study sessions. Citalopram and fluoxetine trough concentrations were determined at baseline on the first day of the final two study session and by venipuncture on Days 1 and 7 of SSRI administration to monitor compliance and document steady state conditions. SSRI concentrations were also determined 7 days after discontinuation of the medication to determine declining drug levels.

3.6 Pharmacodynamic Assessments

Subjects were evaluated for sedation and psychomotor performance at baseline, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0 and 8.0 hours after administration of
alprazolam/placebo. No clinically significant pharmacodynamic effects were expected after the 8 hour period (Hassan, Sproule, Naranjo, and Herrmann, 2000). Psychomotor performance was measured by the Digit Symbol Substitution Task and the Manual Tracking Test. The total pharmacodynamic assessment was expected to take 10 to 12 minutes (Hassan, Sproule, Naranjo, and Herrmann, 2000)

Sedation Measure: Subjectively rated sedation was assessed using a series of 100mm visual analogue scales anchored from the Tufts University Benzodiazepine Scale (Shader et al., 1986). Previous studies have indicated that these scales are reliable and very sensitive in measuring changes in sedation (Naranjo et al., 1984; Greenblatt et al., 1988; Kaplan, Greenblatt, Ehrenberg, Goddard, Harmatz, and Shader, 1998; Kaplan et al., 2000) (appendix 3).

Digit Symbol Substitution Test (DSST): This is a computerized test in which subjects are asked to make as many correct symbol-for-digit substitutions on screen as possible within 90 seconds (appendix 3). The DSST is used by many investigators as a sensitive measure of psychomotor performance, reflected in speed of motor response, recognition of sensory information and visuo-motor coordination (Shader, Dreyfuss, Gerrein, Harmatz, Allison, and Greenblatt, 1986; Kaplan, Greenblatt, Ehrenberg, Goddard, Harmatz, and Shader, 1998; Greenblatt, Harmatz, Dorsey, and Shader, 1988).

Manual Tracking Test (MTT): This computerized test measures the subjects' ability to keep a “plane” in the centre of a road moving down an 8x10cm oscilloscope
screen at a fixed rate, using a joystick. The distance in centimeters between the plane and the centre of the road and the proportion of time the plane spends over the road are calculated by the computer. Three 20 second trials are run and the results are averaged to determine a final score. Our group has extensive experience with the use of this test to assess psychomotor performance (Dorian et al., 1983; Naranjo, Sellers, Kaplan, Hamilton, and Khouw, 1984; Hassan, Sproule, Naranjo, and Herrmann, 2000).

Since a practice effect can be expected to occur over repeated administration of the DSST and MTT tests, subjects were required to practice these tests on the morning of each full day study session until a consistent score was obtained at least three times in a row.

3.7 Compliance with study medications

Subject were given a log in which they were asked to record all over-the-counter and prescription medications taken throughout the study, as well as the dose and time of day these medications were taken. Subjects were asked to take the SSRI medication every morning at 8:00am in order that the trough concentrations could be accurately measured, since blood samples were taken at 8:00am on the full-day study session mornings. Therefore subjects were also asked to record the time of day the study medication was taken every day. Pill counts were done after each subject completed the experiment to confirm compliance with the study medications. Blood samples were taken after day 1 and day 7 of SSRI treatment, and again at the
beginning of study sessions 3 and 4 to confirm that adequate levels of the medications were on board.

3.8 Drug Assays

All blood samples were drawn into 7mL glass blue-top additive-free vacutainer tubes, and allowed to rest at room temperature for 30 to 60 minutes before being spun at 3000 rpm for 15 minutes. The serum was separated and stored in either glass or polypropylene screw-top tubes for no more than 6 months. The citalopram, desmethylcitalopram (DCT) and didemethylcitalopram (DDCT) serum levels were analyzed by H.Lundbeck A/S, while alprazolam, fluoxetine and norfluoxetine were analyzed by St. Joseph’s Hospital in London, Ontario. Alprazolam was extracted using a liquid/liquid technique, and analysis was carried out on a liquid chromatograph with Mass Selective detector (HP MSD). Integration was monitored using HP Chemstation. Fluoxetine was extracted using a liquid/liquid technique, and analysis was carried out on a liquid chromatograph with ultraviolet (214nm) detection (HP Series 1100). Integration was monitored using HP Chemstation. The same method was used for norfluoxetine. The limit of detection for fluoxetine and norfluoxetine was 20 nmol/L, and the limit of detection for alprazolam was 2 nmol/L. Information regarding drug assays for citalopram are pending. The limit of detection for citalopram, DCT and DDCT was 3 nmol/L.
3.9 Ethical considerations

Safety of Drug Doses

Subjects were provided with information regarding the drugs used in the study including doses and commonly reported adverse events. Subjects were required to read this information and sign informed consent before participating in the study.

Alprazolam: Single oral doses of 1 mg alprazolam have been used in many healthy volunteer studies (Greenblatt, Preskorn, Cotreau, Horst, and Harmatz, 1992; Hassan, Sproule, Naranjo, and Herrmann, 2000; Kirkwood et al., 1991) and were well tolerated in all cases. Multiple doses of 1 mg have also been used in healthy volunteers (Fleishaker and Hulst, 1994; Lasher, Fleishaker, Steenwyk, and Antal, 1991) with only one report of dizziness (Fleishaker and Hulst, 1994). Furthermore, benzodiazepines are known for their wide margin of safety in patient populations and therefore the risk to our subjects was minimal even if alprazolam levels are increased due to inhibition of alprazolam metabolism by either SSRI. The most common side effects of alprazolam include sedation, dizziness and lack of coordination. Subjects were examined before discharge and sent home by taxi if necessary. Subjects were informed of potential side effects before consenting to participate in the study, and they were free to withdraw from the study at any time.

Citalopram: Citalopram and it’s primary metabolites of demethylcitalopram and didemethylcitalopram are highly selective serotonin reuptake inhibitors. The
metabolites are more than 10 times less potent than the parent drug, contributing negligibly to the pharmacological effect of this drug (Bezchlibnyk-Butler, Aleksic, and Kennedy, 2000). The standard starting daily dose is 20 mg, with expected trough plasma citalopram levels of 130 ± 70 nmol/L (Bezchlibnyk-Butler, Aleksic, and Kennedy, 2000). Citalopram has a wide margin of safety. The most commonly reported adverse events during premarketing clinical trials were dry mouth, increased sweating, somnolence, insomnia, nausea and asthenia (Bezchlibnyk-Butler, Aleksic, and Kennedy, 2000). These side effects are generally transient and tend to disappear after 1 to 2 weeks. In premarketing clinical trials, 15.9% of depressed subjects discontinued treatment with citalopram due to adverse events (CPS, 2000).

Fluoxetine: Fluoxetine’s primary metabolite norfluoxetine has been found to be somewhat more potent than the parent drug at inhibiting the 5-HT uptake pump (CPS, 2000). The standard starting dose of fluoxetine is 20mg/day, with expected plasma fluoxetine levels of 255 ± 107 nmol/L (CPS, 2000). Fluoxetine is a safe drug. The most commonly reported side effects of fluoxetine include headache, nervousness, insomnia, somnolence, nausea and diarrhea. These side effects are generally transient and tend to disappear after 1 to 2 weeks. In premarketing clinical trials, 15% of depressed patients discontinued treatment due to side effects of fluoxetine (CPS, 2000).
Safety of Blood Sampling

A total of 12 blood samples (10 mL each) were taken per study session (over a period of 3 days). The first 10 samples were taken from a catheter inserted into the forearm cephalic vein by the study coordinator or a nurse, both experienced in the insertion of I.V. catheters. Insertion of the catheter involved little pain and allowed for collection of the majority of blood samples with only one puncture. Furthermore, the catheter remained inserted for a clinically short duration (8 hours). Remaining samples (on the second and third days of the study sessions) were collected by venipuncture by the study coordinator. Additional samples were taken on Days 1 and 7 of SSRI administration, and 7 days after discontinuation of medications, to monitor medication compliance and to determine SSRI levels. The study coordinator was trained in catheter insertion and venipuncture in accordance with standard training protocol at Sunnybrook and Women’s College Health Sciences Centre.

3.10 Data Analysis

All statistical analyses were done using SPSS 10.0 for Windows. The standard pharmacokinetic parameters of alprazolam (AUC 0-8 hrs, AUC 0-48 hrs, AUC 0-\(\infty\), \(C_{\text{max}}\), \(t_{\text{max}}\), \(t_{1/2}\)) were calculated using non-compartmental methods from the plasma concentration curve (Gibaldi M and Perrier D, 1975). Within-subjects changes were analyzed using paired t-tests (before and after SSRI administration), and independent samples t-tests were done to determine the differences between fluoxetine and citalopram groups. Correlation analyses were used to determine if SSRI concentration correlated with increase or decrease in alprazolam
concentration post-SSRI. Sedation was measured by the change from baseline in subject rated assessments on the visual-analogue scales. These scores are summarized by the computer as a Sedation Summary value. Psychomotor performance was measured by changes from baseline values in two tests: the DSST and the MTT. In the DSST, the number of correctly “translated” characters served as a measure of performance. In the MTT, performance was measured by the computer-generated mean variance (in cm) from the centre of the road and the proportion of time spent over the road. Baseline-corrected area under the curve for each of the pharmacodynamic measurements were calculated (AUC 0-3, 0-8), as well as maximal change from baseline, and time of maximal change from baseline. Pharmacodynamic analyses were carried out using analyses of variance (ANOVA) for repeated measures factoring to determine the influence of alprazolam and SSRI, both independently and together, on pharmacodynamic measures. Correlation analyses were carried out to detect possible correlation between change in alprazolam kinetics and change in dynamics. The change in alprazolam dynamic AUC (0-8) values post-SSRI versus pre-SSRI was calculated in this manner: \[ \left( \text{AUC (0-8) for alprazolam + SSRI} \right) - \left( \text{AUC (0-8) for alprazolam + placebo} \right) - \left( \text{AUC (0-8) for alprazolam alone} \right) - \left( \text{AUC (0-8) for placebo alone} \right) ] \]. This was done in order to control for placebo effect. Non-parametric (rank) correlation tests were carried out as well as parametric tests since the distribution the concentration AUC values may not have been evenly distributed due to the expected differences between citalopram and fluoxetine subjects with respect to change in kinetic values (e.g. most fluoxetine subjects were expected to experience a large increase in AUC, while citalopram subjects were expected to experience no AUC increase). Subsequently, regression
analyses were done to determine causal relationships. To determine whether the order of alprazolam and placebo administration during the first two study sessions and the final two sessions affected the pharmacodynamic results, an analysis of order effects was performed. For example, the sum (A+P) and difference (A-P) of the AUC (0-8) values, where A is the alprazolam response and P is the placebo response, from the DSST test for the first two sessions (alprazolam alone and placebo alone) were calculated. Independent t-tests were used to determine if there were differences between the group that received alprazolam first and the group that received placebo first. This was repeated for MTT and sedation tests for the two sessions before SSRI as well as the two sessions after SSRI administration. The addition (A+P) method detects whether there was a “period * treatment effect”; i.e. where the experimental conditions differ over two treatment periods and this period difference affects one group differently from the other; for example if the wash out period was not long enough this would affect only the group that got active treatment (alprazolam) first. The subtraction method (A-P) detects a consistent period effect.
4 Results

4.1 Subjects

Twenty one subjects completed the experiment (fig. 10). Sixty eight potential subjects were contacted through personal contact and advertisements posted at Sunnybrook Hospital and the University of Toronto after approval from the respective regulatory offices. Seventeen of these were ineligible, largely due to current use of exclusionary medications; oral contraceptives (n=10), antidepressants (n=2), finasteride (n=1), and marijuana (n=1). Three contacts were excluded due to medical conditions; one potential subject suffered from depression and was referred to an appropriate treatment facility, another potential subject suffered from Crohn's disease, which may have interfered with absorption of the study medications, and one other contact suffered from hypothyroidism controlled by levothyroxine, which was felt to be a potential confounding factor because preliminary studies have shown that iodothyronines may be involved in regulation of the expression of hepatic CYP3A4 in humans (Liddle, 1998).

Thirty one recruits completed the assessment; one potential subject was disqualified due to a positive urine screen for 3,4-Methylenedioxy-N-methamphetamine (MDMA; "ecstacy"), and the other 30 were randomized. Three subjects dropped out before the first session for personal reasons, and 27 subjects completed session 1. One subject developed a severe migraine headache during session 1, and dropped out
Figure 10. Subject recruitment (July, 1999-September, 2000)

- Contacted: 68
- Assessed: 31
- Not eligible: 17
- Not interested: 19
- Session 1: 27
  - Ineligible: 1
  - Drop out: 3
- Session 2: 22
  - Drop out: 1
- Session 3: 21
- Session 4: 21

Drop out: 5
at this point. Another four subjects dropped out after session 1 for personal reasons. One more subject dropped out after day two of fluoxetine treatment due to feelings of somnolence and anhedonia which she attributed to the medication. No other subjects dropped out due to side effects of any of the medications.

Eleven subjects randomized to fluoxetine completed the study, while 10 completed in the citalopram group. All urine drug screens were consistently negative for contraindicated medications, with the exception of three subjects. One subject in the fluoxetine group admitted to taking two Tylenol 2's (containing codeine) 24 hours prior to session 2, but only remembered half way through the session. Since the half life of codeine is very short (1.5 to 3.0 hours) (CPS, 2000), we felt it was acceptable to keep the data. This subject also tested positive for dextromethorphan and ephedrine after session 3, which she attributed to cold medications taken 2 days prior to the session, and her urine also was positive for ephedrine after session 4, though she denied taking any medications for over a week before this session. This subjects fluoxetine levels were not distinctly different from the other subjects so it was felt that the data could be used. As well, statistical analysis of the kinetic data was performed after excluding this subjects data, and all the kinetic differences remained statistically significant, so the data collected from this subject did not sway the results either way. Cannabinoids were detected in the urine of a second subject (citalopram group) after session 1. However, the reading for cannabinoids was 50.7μg, only slightly above the cutoff for a positive test (50μg), and the laboratory technician felt that since the urine sample was so dilute this reading may be unreliable. Subsequent screens were negative and the subject denied use of
marijuana within the previous 6 months. A third subject in the citalopram group tested positive for quinine after session 4, which was attributed to a lemonade and tonic drink two days prior to the session. This was not felt to be a factor that would interfere with the experiment.

Subject demographics in the two groups were very similar with respect to age, height, weight, gender and ethnicity (table 2.) Overall 52% of the subjects were women, and 48% were men (45% and 55% in the fluoxetine group, and 60% and 40% in the citalopram group respectively). Sixty seven percent of the subjects were Caucasian, and 33% were of Asian decent (64% and 36% in the fluoxetine group and 70% and 30% in the citalopram group, respectively). The average age of subjects was 25.9, the average height was 173.1 cm and the average weight was 71.25 kg.

Table 2. Subject Demographics

<table>
<thead>
<tr>
<th></th>
<th>Fluoxetine Group</th>
<th>Citalopram Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Caucasian</td>
<td>7 (64%)</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>Asian</td>
<td>4 (36%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Female</td>
<td>5 (45%)</td>
<td>6 (60%)</td>
</tr>
<tr>
<td>Male</td>
<td>6 (55%)</td>
<td>4 (40%)</td>
</tr>
<tr>
<td>Age</td>
<td>24.2 ±3.2 years</td>
<td>27.6 ± 5.8 years</td>
</tr>
<tr>
<td>Height</td>
<td>171.9 ± 9.8 cm</td>
<td>174.3 ± 6.5 cm</td>
</tr>
<tr>
<td>Weight</td>
<td>71.8 ± 16.7 kg</td>
<td>70.7 ± 8.7 kg</td>
</tr>
</tbody>
</table>
Most subjects reported symptoms of sedation after alprazolam administration; the most common were somnolence, spaciness, and dizziness. The incidence of reported adverse events associated with SSRI administration was slightly greater in the citalopram group (60%) as compared to the fluoxetine group (50%), although there was one subject who dropped out due to fluoxetine intolerance, while no one dropped out due to adverse effects associated with citalopram (tables 3 and 4). Six subjects randomized to citalopram (n=10) reported at least one adverse effect, the most common being somnolence (4 out of 10), nausea (3) and insomnia (2). One subject reported dry mouth, and another was seen by the study physician for a maculopapular rash that appeared on the torso and upper legs that was thought to be associated with citalopram since it appeared two days after initiation of the medication. The subject was warned to discontinue the medication if the rash spread or became worse in any way, and it disappeared a few days later. Six subjects randomized to fluoxetine (n=12 including one drop out) reported at least one adverse event after initiation of the medication. The most common reported side effects were nausea (25%), and somnolence (17%). One subject each reported each of the following side effects: insomnia, diarrhea, gastrointestinal pain, headache, tremor and anhedonia.
Table 3. Adverse events reported after initiation of SSRI medication (listed by subject number); includes one subject who dropped out after 2 days of fluoxetine

<table>
<thead>
<tr>
<th>Subject</th>
<th>Adverse Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>1002</td>
<td>none</td>
</tr>
<tr>
<td>1011</td>
<td>somnolence, nausea</td>
</tr>
<tr>
<td>1012</td>
<td>insomnia</td>
</tr>
<tr>
<td>1013</td>
<td>none</td>
</tr>
<tr>
<td>1014</td>
<td>none</td>
</tr>
<tr>
<td>1017</td>
<td>nausea, insomnia</td>
</tr>
<tr>
<td>1018</td>
<td>none</td>
</tr>
<tr>
<td>1021</td>
<td>somnolence, dry mouth, nausea</td>
</tr>
<tr>
<td>1024</td>
<td>somnolence</td>
</tr>
<tr>
<td>1027</td>
<td>rash, somnolence</td>
</tr>
<tr>
<td>1004</td>
<td>none</td>
</tr>
<tr>
<td>1006</td>
<td>somnolence, anhedonia (dropout)</td>
</tr>
<tr>
<td>1007</td>
<td>tremor</td>
</tr>
<tr>
<td>1009</td>
<td>none</td>
</tr>
<tr>
<td>1010</td>
<td>insomnia, nausea</td>
</tr>
<tr>
<td>1015</td>
<td>none</td>
</tr>
<tr>
<td>1016</td>
<td>GI pain</td>
</tr>
<tr>
<td>1019</td>
<td>none</td>
</tr>
<tr>
<td>1020</td>
<td>diarrhea</td>
</tr>
<tr>
<td>1022</td>
<td>none</td>
</tr>
<tr>
<td>1025</td>
<td>none</td>
</tr>
<tr>
<td>1026</td>
<td>headache, nausea, somnolence</td>
</tr>
</tbody>
</table>

Table 4. Percentage of subjects experiencing adverse event after initiation of SSRI medication (listed by adverse event); includes one subject who dropped out after 2 days of fluoxetine

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somnolence</td>
<td>4 (40%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Rash</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0</td>
</tr>
<tr>
<td>GI pain</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
</tr>
<tr>
<td>Tremor</td>
<td>0</td>
</tr>
<tr>
<td>Anhedonia</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somnolence</td>
<td>2 (17%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>Rash</td>
<td>0</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>GI pain</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>Headache</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>Tremor</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>Anhedonia</td>
<td>1 (8%)</td>
</tr>
</tbody>
</table>
Some subjects took the SSRI medication for longer than 21 days between the pre- and post- SSRI sessions. The average amount of time on medication between sessions two and three was 26.0 days in the fluoxetine group, and 23.8 days in the citalopram group. All but three subjects took the SSRI medication for less than 27 days between sessions two and three. Two subjects in the fluoxetine group took the medication for 34 days and 45 days between the sessions, and one subject in the citalopram group took the medication for 30 days between sessions. All subjects continued to take the SSRI until the end of session 4, which was between 7 and 16 days after session 3 for all subjects.

4.2 Pharmacokinetics

**Plasma levels of citalopram and fluoxetine**

Compliance was monitored by pill counting and SSRI plasma concentration monitoring. All pill counts done at the end of the study showed compliance. All subjects reported taking the medication at appropriate times each day, with the exception of one subject (in the citalopram group) who took the medication at erratic times for the first two weeks, but had taken the medication roughly 24 hours before sessions three and four, so we found this to be acceptable. Citalopram and its metabolites were present at similar levels over sessions three and four, indicating that steady state was reached. The average 24-hour trough steady state concentrations of citalopram, desmethylcitalopram and didemethylcitalopram were $81 \pm 31 \text{ nmol/L}$, $34 \pm 5.1 \text{ nmol/L}$, and $11 \pm 5 \text{ nmol/L}$, as measured over sessions
three and four (table 5). Desmethylcitalopram (DCIT) concentration was approximately 42% of the parent compound at steady state, while Didesmethylcitalopram (DDCIT) concentration was about 14%.

Table 5. Serum levels of citalopram throughout the study (n=10)

<table>
<thead>
<tr>
<th></th>
<th>CIT (nmol/L)</th>
<th>DCIT (nmol/L) (% of CIT)</th>
<th>DDCIT (nmol/L) (% of CIT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>26.8 ± 6.1</td>
<td>8.2 ± 1.5 (30%)</td>
<td>0.7 ± 1.5 (3%)</td>
</tr>
<tr>
<td>Day 7</td>
<td>73.9 ± 29.3</td>
<td>32.6 ± 5.13 (44%)</td>
<td>11.3 ± 4.4 (15%)</td>
</tr>
<tr>
<td>Session 3</td>
<td>80.1 ± 29.2</td>
<td>33.9 ± 5.1 (42%)</td>
<td>11.4 ± 5.2 (14%)</td>
</tr>
<tr>
<td>Session 4</td>
<td>82.2 ± 34.0</td>
<td>34.8 ± 5.45 (42%)</td>
<td>11.4 ± 5.2 (14%)</td>
</tr>
<tr>
<td>Day 7</td>
<td>7.8 ± 4.8</td>
<td>6.4 ± 2.5 (82%)</td>
<td>4.0 ± 3.0 (51%)</td>
</tr>
</tbody>
</table>

Trough concentrations of fluoxetine taken at sessions three and four were very similar (table 6), indicating that fluoxetine was at steady state over the post-SSRI sessions. The average 24-hour trough steady state concentration of fluoxetine was 232.8 ± 154.2 nmol/L, as measured at sessions three and four. Norfluoxetine was present at approximately 177% of the parent compound at steady state (414.2 ± 160.0 nmol/L), and this percentage increased dramatically 7 days after discontinuation of fluoxetine (463%).
Table 6. Serum levels of fluoxetine throughout the study (n=11)

<table>
<thead>
<tr>
<th></th>
<th>FLUOX (nmol/L)</th>
<th>NORFLUOX (nmol/L) (% of FLUOX)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>38.3 ± 69.4</td>
<td>50.5 ± 67.9 (132%)</td>
</tr>
<tr>
<td>Day 7</td>
<td>111.6 ± 63.6</td>
<td>163.9 ± 104.2 (146%)</td>
</tr>
<tr>
<td>Session 3</td>
<td>223.9 ± 157.5</td>
<td>397.4 ± 165.9 (177%)</td>
</tr>
<tr>
<td>Session 4</td>
<td>241.7 ± 158.1</td>
<td>431.0 ± 159.4 (178%)</td>
</tr>
<tr>
<td>Day 7 discontinuation</td>
<td>79.4 ± 76.7</td>
<td>367.8 ± 224.7 (463%)</td>
</tr>
</tbody>
</table>

Plasma levels of alprazolam alone

Over all subjects (n=21), the maximum concentration (C_{max}) of alprazolam alone was 84.6 ± 19.8 nmol/L, the time of maximum concentration (T_{max}) was 0.9 ± 0.6 hours, the elimination half-life (t_{1/2}) was 13.6 hours ± 3.3, and the plasma concentration area under the curve from zero to infinity (AUC (0-∞)) was 1090.8 ± 325.1 nmol/L. Independent samples t-tests confirm that there were no significant differences in any alprazolam pharmacokinetic parameters between the citalopram and fluoxetine groups before SSRI treatment.

Effect of fluoxetine on alprazolam pharmacokinetics

In the fluoxetine group (n=11), the pre-SSRI AUC (0-∞) of alprazolam was 1152.4 ± 386.2 nmol/L, the T_{max} was 1.0 ± 0.6 hour, the C_{max} was 87.6 ± 17.8 nmol/L, and the t_{1/2} was 14.2 ± 4.0 hours. There was a significant increase in the post-fluoxetine alprazolam AUC, as compared to pre-fluoxetine, for all time intervals: 0-3 hrs (p<0.05); 0-8hrs (p<0.005); 0-48hrs (p<0.001); and 0-∞ (p<0.001) (table 7; fig. 11, 12, 12a, 16, 18a). The AUC (0-∞) increased by 31.8% post-fluoxetine. As well, there was a significant increase in alprazolam t_{1/2} from 14.2 ± 4.0 hours pre-fluoxetine to
16.2 ± 3.4 hours post-fluoxetine (p<0.05). There was no significant increase in $C_{\text{max}}$ and no change in $T_{\text{max}}$. Higher steady state concentrations of fluoxetine did not correlate with greater increases in alprazolam AUC (data not shown).

Table 7. Alprazolam pharmacokinetic parameters before and after fluoxetine treatment (Mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Alprazolam (n=11)</th>
<th>Alprazolam+Fluoxetine (n=11)</th>
<th>Percent change</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (nmol/L)</td>
<td>87.6 ± 17.8</td>
<td>95.3 ± 28.7</td>
<td>8.8</td>
<td>.330</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (hrs)</td>
<td>1.0 ± 0.6</td>
<td>1.0 ± 0.5</td>
<td>0</td>
<td>.724</td>
</tr>
<tr>
<td>$t_{1/2}$ β (hrs)</td>
<td>14.2 ± 4.0</td>
<td>16.2 ± 3.4</td>
<td>1.4</td>
<td>.036</td>
</tr>
<tr>
<td>AUC$_0$-$\infty$ (nmol/L•h)</td>
<td>153.7 ± 21.9</td>
<td>177.8 ± 35.1</td>
<td>24.1</td>
<td>.020</td>
</tr>
<tr>
<td>AUC$_0$-$\infty$ (nmol/L•h)</td>
<td>360.1 ± 64.8</td>
<td>414.7 ± 85.4</td>
<td>15.2</td>
<td>.004</td>
</tr>
<tr>
<td>AUC$_0$-$\infty$ (nmol/L•h)</td>
<td>1005.4 ± 300.3</td>
<td>1319.5 ± 331.3</td>
<td>31.2</td>
<td>.001</td>
</tr>
<tr>
<td>AUC$_0$-$\infty$ (nmol/L•h)</td>
<td>1152.4 ± 386.2</td>
<td>1518.8 ± 431.8</td>
<td>31.8</td>
<td>.001</td>
</tr>
</tbody>
</table>

Effect of citalopram on alprazolam pharmacokinetics

The pre-citalopram AUC (0-$\infty$) of alprazolam was 1022.9 ± 243.7 nmol/L, the $T_{\text{max}}$ was 0.9 ± 0.8 hours, the $C_{\text{max}}$ was 81.4 ± 22.2 nmol/L, and the elimination $t_{1/2}$ was 13.0 ± 2.4 hours. After administration of citalopram, the $T_{\text{max}}$ of alprazolam increased significantly from 0.9 ± 0.8 hours to 1.4 ± 0.8 hours (p<0.05). There were no other significant changes in alprazolam pharmacokinetics post-citalopram as compared to pre-citalopram (table 8; fig. 11a, 12, 12a, 17, 18a). There was no correlation between citalopram steady state concentration and change in alprazolam AUC.
Table 8. Alprazolam pharmacokinetic parameters before and after citalopram treatment (Mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Alprazolam (n=10)</th>
<th>Alprazolam+Citalopram (n=10)</th>
<th>Percent change</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (nmol/L)</td>
<td>81.4 ± 22.2</td>
<td>74.4 ± 25.0</td>
<td>-8.5</td>
<td>.421</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (hrs)</td>
<td>0.9 ± 0.8</td>
<td>1.4 ± 0.8</td>
<td>55.5</td>
<td>.032</td>
</tr>
<tr>
<td>$T_{1/2}$ (hrs)</td>
<td>13.0 ± 2.4</td>
<td>12.0 ± 2.3</td>
<td>-7.7</td>
<td>.232</td>
</tr>
<tr>
<td>$\text{AUC}_{0-3}$ (nmol/L•h)</td>
<td>156.75 ± 32.2</td>
<td>137.31 ± 45.0</td>
<td>-12.4</td>
<td>.107</td>
</tr>
<tr>
<td>$\text{AUC}_{0-8}$ (nmol/L•h)</td>
<td>351.2 ± 52.5</td>
<td>345.0 ± 87.7</td>
<td>-1.7</td>
<td>.741</td>
</tr>
<tr>
<td>$\text{AUC}_{0-48}$ (nmol/L•h)</td>
<td>924.6 ± 213.8</td>
<td>970.8 ± 290.3</td>
<td>-5.0</td>
<td>.741</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty}$ (nmol/L•h)</td>
<td>1022.9 ± 243.7</td>
<td>1040.0 ± 336.7</td>
<td>1.7</td>
<td>.826</td>
</tr>
</tbody>
</table>
Fig. 11. Alprazolam kinetics before and after fluoxetine

Fig. 11a. Alprazolam kinetics before and after citalopram
Fig. 12. Change in Kinetic Parameters pre-SSRI versus post-SSRI: Citalopram vs. Fluoxetine

Fig. 12a. Change in Kinetic parameters pre-SSRI versus post-SSRI: Citalopram vs. Fluoxetine
Fig. 16. Alprazolam concentration AUC (0-48) before and after fluoxetine (plotted by subject)

Fig. 17. Alprazolam concentration AUC 0-48 before and after citalopram (plotted by subject)
4.3 Pharmacodynamics

**Pharmacodynamic effect of alprazolam alone**
Repeated measures ANOVA detected significant impairment in psychomotor function and significantly increased subjective sedation associated with alprazolam administration, as compared to placebo, regardless of SSRI administration or type of SSRI (table 9; fig. 13, 14 and 15). There was a significant decrease in the number of correctly translated characters on the DSST (p≤0.002), and significantly less time spent over the road on the MTT test (p≤0.002), as measured by AUC (0-8 and 0-3) and maximum change from baseline scores. As well, subjects experienced greater sedation as measured by the sedation summary scale AUC (0-8, 0-3) and maximum change from baseline on the sedation summary scale (p≤0.004).

**Pharmacodynamic effect of citalopram and fluoxetine alone**
Repeated measures ANOVA detected no significant differences in subjective or objective sedation and psychomotor impairment attributable to either SSRI alone, as measured by AUC (0-8) (table 9; figs. 13, 14, 15, 18b). However, there was a significant increase in performance on the DSST associated with fluoxetine as measured by AUC (0-3) (p=0.046) and maximum change from baseline (p=0.023), and a significant improvement in MTT performance associated with citalopram as measured by AUC (0-3) (p=0.031).
Pharmacodynamic effect of fluoxetine and citalopram on coadministered alprazolam

Repeated measures ANOVA detected no significant differences in alprazolam-associated subjective or objective sedation after either SSRI, as compared to before SSRI treatment (table 9; fig. 13, 14, 15, 18b). The only exception was that citalopram subjects experienced greater alprazolam-associated impairment on the MTT post-citalopram, as compared to pre-citalopram, as measured by AUC (0-8) (p=.020) and (0-3) (p=.016) but not maximum change from baseline scores. It appears from the graphs plotting Sedation, DSST and MTT performance over time after drug administration that the T\text{max} was shifted to the right after citalopram administration, however this was not a significant finding (table 9, Fig. 13, 14, 15). The variability in T\text{max} values was extremely high (Tables 11a, 11b). Non-parametric correlation analysis showed that subjects who experienced the greatest increase in alprazolam concentration AUC (0-\infty) post-SSRI also experienced the greatest increase in sedation AUC (0-8), as measured by the Sedation Summary Scale (p=.025, Spearman’s rho; p=.050, Kendall’s tau), as well as a trend towards increased maximum change from baseline scores on this measure (p=.057, Spearman’s rho; p=.091, Kendall’s tau)(table 10). Both parametric and non-parametric analyses also found a correlation between change in alprazolam concentration AUC (0-48) and change in maximum change from baseline score on the MTT (p=0.043, Pearson test; p=0.034, Kendall’s tau). No other correlations were detected. Regression analysis using the pre-SSRI to post-SSRI change in alprazolam concentration AUC (0-8, 0-48, 0-\infty) as independent variables and the changes on the dynamic measurements as dependent variables, found no significant causal relationships (data not shown).
Fig. 13. Sedation Summary

- placebo (n=21)
- alprazolam (n=21)
- citalopram (n=10)
- fluoxetine (n=11)
- citalopram + alprazolam (n=10)
- fluoxetine + alprazolam (n=11)

change from baseline score

0 2 4 6

time after alprazolam/placebo administration
Fig. 14. DSST

- Placebo (n=21)
- Alprazolam (n=21)
- Citalopram (n=10)
- Fluoxetine (n=11)
- Citalopram + Alprazolam (n=10)
- Fluoxetine + Alprazolam (n=11)

Change from baseline score vs. time after alprazolam/placebo administration.
Fig. 15. MTT

- placebo (n=21)
- alprazolam (n=21)
- citalopram (n=10)
- fluoxetine (n=11)
- citalopram + alprazolam (n=10)
- fluoxetine + alprazolam (n=11)
Table 9. Summary of significance testing for pharmacodynamic results, using repeated measures ANOVA (p-values)

<table>
<thead>
<tr>
<th></th>
<th>Area under the curve 0-3</th>
<th></th>
<th>Area under the curve 0-8</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluoxetine Group</td>
<td>Citalopram Group</td>
<td>Fluoxetine Group</td>
<td>Citalopram Group</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alpraz</td>
<td>Fluox</td>
<td>both</td>
<td>Alpraz</td>
<td>Cit</td>
</tr>
<tr>
<td>1. sed'n Sedation Summary</td>
<td>0.003</td>
<td>0.348</td>
<td>0.555</td>
<td>0.001</td>
<td>0.055</td>
</tr>
<tr>
<td>2. dsst correct</td>
<td>0.002</td>
<td>0.046</td>
<td>0.849</td>
<td>0.000</td>
<td>0.131</td>
</tr>
<tr>
<td>3. mtt mean time over road</td>
<td>0.001</td>
<td>0.151</td>
<td>0.194</td>
<td>0.000</td>
<td>0.031</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Maximum change from baseline</th>
<th></th>
<th>Tmax</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluoxetine Group</td>
<td>Citalopram Group</td>
<td>Fluoxetine Group</td>
<td>Citalopram Group</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alpraz</td>
<td>Fluox</td>
<td>Both</td>
<td>Alpraz</td>
<td>Cit</td>
</tr>
<tr>
<td>4. sed'n Sedation Summary</td>
<td>0.000</td>
<td>0.268</td>
<td>0.433</td>
<td>0.000</td>
<td>0.028</td>
</tr>
<tr>
<td>5. dsst correct</td>
<td>0.001</td>
<td>0.029</td>
<td>0.517</td>
<td>0.000</td>
<td>0.250</td>
</tr>
<tr>
<td>6. mtt mean time over road</td>
<td>0.000</td>
<td>0.220</td>
<td>0.184</td>
<td>0.000</td>
<td>0.104</td>
</tr>
</tbody>
</table>

alpraz = effect of alprazolam alone on pharmacodynamic measure
Fluox/Cit= effect of SSRI alone on pharmacodynamic measure
both = effect of both SSRI and alprazolam on pharmacodynamic measure
Table 10. Correlation tables (p-values & correlation coefficients) using Pearson correlation and Spearman’s non-parametric correlation analyses. Significant correlations (p<0.050) are highlighted in dark shade while trends towards significance (p<0.100) are highlighted in light shade.

<table>
<thead>
<tr>
<th></th>
<th>Change in conc. AUC (0-8)</th>
<th>Pearson Correlation</th>
<th>Change in conc. AUC (0-48)</th>
<th>Pearson Correlation</th>
<th>Change in conc. AUC (0-∞)</th>
<th>Pearson Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SED: change* in score</td>
<td>.194</td>
<td>.295</td>
<td>.197</td>
<td>.294</td>
<td>.101</td>
<td>.368</td>
</tr>
<tr>
<td>SED: change in max. change from baseline score</td>
<td>.344</td>
<td>.217</td>
<td>.230</td>
<td>.274</td>
<td>.126</td>
<td>.344</td>
</tr>
<tr>
<td>DSST: change in score</td>
<td>.351</td>
<td>.214</td>
<td>.363</td>
<td>.209</td>
<td>.735</td>
<td>.079</td>
</tr>
<tr>
<td>DSST: change in max. change from baseline score</td>
<td>.954</td>
<td>-.013</td>
<td>.546</td>
<td>.139</td>
<td>.634</td>
<td>.110</td>
</tr>
<tr>
<td>MTT: change in score</td>
<td>.483</td>
<td>.162</td>
<td>.997</td>
<td>.001</td>
<td>.916</td>
<td>-.025</td>
</tr>
<tr>
<td>MTT: change in max. change from baseline score</td>
<td>.133</td>
<td>.338</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3 Non-parametric tests

<table>
<thead>
<tr>
<th></th>
<th>Change in conc. AUC (0-8)</th>
<th>Spearman's Rho Correlation (n=21)</th>
<th>Change in conc. AUC (0-48)</th>
<th>Spearman's Rho Correlation</th>
<th>Change in conc. AUC (0-∞)</th>
<th>Spearman's Rho Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>SED: change* in score</td>
<td>.092</td>
<td>.378</td>
<td>.071</td>
<td>.401</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SED: change in max. change from baseline score</td>
<td>.242</td>
<td>.267</td>
<td>.120</td>
<td>.350</td>
<td>.057</td>
<td>.422</td>
</tr>
<tr>
<td>DSST: change in score</td>
<td>.527</td>
<td>.146</td>
<td>.366</td>
<td>.208</td>
<td>.754</td>
<td>.073</td>
</tr>
<tr>
<td>DSST: change in max. change from baseline score</td>
<td>.964</td>
<td>-.010</td>
<td>.862</td>
<td>.040</td>
<td>.889</td>
<td>.032</td>
</tr>
<tr>
<td>MTT: change in score</td>
<td>.733</td>
<td>.079</td>
<td>.758</td>
<td>-.071</td>
<td>.724</td>
<td>-.082</td>
</tr>
<tr>
<td>MTT: change in max. change from baseline score</td>
<td>.387</td>
<td>.199</td>
<td>.057</td>
<td>.422</td>
<td>.258</td>
<td>.259</td>
</tr>
</tbody>
</table>

*Change represents the difference between dynamic AUC (0-8) results from alprazolam alone session versus alprazolam + SSRI session. Note that the change in alprazolam dynamic AUC (0-8) values post-SSRI versus pre-SSRI was calculated in this manner: [(AUC (0-8) for alprazolam alone) – (AUC (0-8) for placebo alone)] – [(AUC (0-8) for alprazolam + SSRI) – (AUC (0-8) for alprazolam + placebo)].
Fig 18a. KINETICS: Change in AUC (0-infinity) of alprazolam concentration (pre-SSRI versus post-SSRI)

Fig 18b. DYNAMICS: Change in AUC (0-8) Sedation Summary Scale (pre-SSRI versus post-SSRI)

Black bars = fluoxetine subjects; White bars = citalopram subjects
### 4.4 Variability

#### Table 11a. Variability in kinetic changes pre- and post-SSRI

<table>
<thead>
<tr>
<th></th>
<th>Fluoxetine Group</th>
<th>Citalopram Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in AUC (0-8)</td>
<td>54.6 ± 48.3**</td>
<td>-6.2 ± 54.8</td>
</tr>
<tr>
<td>(nmol/L*h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in AUC (0-48)</td>
<td>314.1 ± 208.4**</td>
<td>46.1 ± 218.6</td>
</tr>
<tr>
<td>(nmol/L*h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in AUC (0-∞)</td>
<td>366.3 ± 244.5**</td>
<td>17.0 ± 226.7</td>
</tr>
<tr>
<td>(nmol/L*h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in ( t_{1/2} ) (h)</td>
<td>2.0 ± 2.4*</td>
<td>-1.0 ± 1.7</td>
</tr>
<tr>
<td>Change in ( C_{max} ) (nmol/L)</td>
<td>7.7 ± 24.8</td>
<td>-7.0 ± 24.6</td>
</tr>
<tr>
<td>Change in ( T_{max} ) (h)</td>
<td>-0.04 ± 0.5</td>
<td>0.5 ± 0.7*</td>
</tr>
</tbody>
</table>

Significance on paired t-test *p<0.05 **p<0.001

#### Table 11b. Variability in dynamic changes pre- and post- SSRI (placebo taken into account as described under Data Analysis heading in Methods section)

<table>
<thead>
<tr>
<th></th>
<th>Fluoxetine Group</th>
<th>Citalopram Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in Sedation Summary scores AUC (0-8)</td>
<td>73.7 ± 210.5</td>
<td>-55.2 ± 175.6</td>
</tr>
<tr>
<td>Change in DSST scores AUC (0-8)</td>
<td>-2.6 ± 50.3</td>
<td>5.0 ± 43.5</td>
</tr>
<tr>
<td>Change in MTT scores AUC (0-8)</td>
<td>-7.8 ± 54.4</td>
<td>27.0 ± 28.8*</td>
</tr>
<tr>
<td>Change in Sedation Summary scores ( T_{max} )</td>
<td>0.7 ± 1.93</td>
<td>0.85 ± 1.6</td>
</tr>
<tr>
<td>Change in DSST scores ( T_{max} )</td>
<td>-0.68 ± 4.84</td>
<td>0.7 ± 2.2</td>
</tr>
<tr>
<td>Change in MTT scores ( T_{max} )</td>
<td>-0.6 ± 5.0</td>
<td>1.2 ± 1.8</td>
</tr>
</tbody>
</table>

Significance on paired t-test *p<0.05
4.5 Order Effects

An analysis of order effect was performed to determine whether the order in which subjects received alprazolam and placebo both before and after SSRI administration affected the results of pharmacodynamic tests. For the two sessions before SSRI administration, there was no significant difference between the alprazolam-first and alprazolam-second groups with respect to performance on the manual tracking test or responses on the sedation measures. Analyses of order using the subtraction method (A-P) showed that subjects who received placebo first (n=11) in the two pre-SSRI sessions experienced greater psychomotor retardation on the DSST test than the patients who received alprazolam first (n=10). This may indicate a consistent difference in response to alprazolam that depended on the order of treatment. This was replicated when comparing the groups among the subjects who received fluoxetine (n=4) placebo first; n=7 alprazolam first, but not among the subjects who received citalopram (n=7 placebo first; n=3 alprazolam first), suggesting that the overall effect was driven by the four subjects who received placebo first in the fluoxetine group. This order effect was also not replicated when using the addition (A+P) method, nor did the effect occur in the post-SSRI sessions. Therefore these results should be interpreted with caution. Since an order effect was not found in any of the other tests for the two pre-SSRI sessions, and no tests at all during the post-SSRI sessions, order effect was not deemed to compromise our results.
5 Discussion

This study showed that fluoxetine (20mg/day) significantly impaired the metabolism of a single oral dose of the in vivo CYP3A4 probe alprazolam (1mg), leading to prolongation of the elimination $t_{1/2}$ and increased AUC over all time periods (0-3h, 0-8h, 0-48h and 0-$\infty$), whereas citalopram (20mg/day) did not. Fluoxetine caused an increase in the AUC (0-$\infty$) of alprazolam in all subjects, whereas citalopram caused little or no increase in the AUC (0-$\infty$) of alprazolam, or even a decrease in AUC (0-$\infty$), in all subjects except one (fig. 16 and 17). The change in alprazolam pharmacokinetics after fluoxetine was not, however, reflected by any significant changes in alprazolam pharmacodynamics.

5.1 Pharmacokinetics

The plasma levels of fluoxetine and citalopram in this study were consistent with previous reports. There was no significant difference between citalopram levels between sessions three and four, and the steady state trough concentrations were $81 \pm 31$ nmol/L and $34 \pm 5$ nmol/L for citalopram and desmethylcitalopram respectively (table 5). Other studies have found similar steady state trough levels for citalopram of around 69 nmol/L and 40 nmol/L for citalopram and desmethylcitalopram respectively after dosing of 20mg once daily in healthy volunteers (n=12) (Laine et al., 1997). Bezchlibnyk-Butler reports expected steady state trough concentration of citalopram at $130 \pm 70$ nmol/L after 20mg/day.
Variability in citalopram plasma concentration is around seven-fold (Fredricson, 1982), which may account for the somewhat lower observed plasma concentrations in our study. At steady-state, as well as after single doses, the ratio of metabolites in relation to parent compound have been found to be 30 to 50% for DCT, and 5 to 10% for DDCT (Bezchlibnyk-Butler, Aleksic, and Kennedy, 2000). This is consistent with our findings: 34% for DCT and 11% for DDCT.

There was no significant difference between fluoxetine levels between sessions three and four. The steady-state trough serum concentration of fluoxetine and norfluoxetine after 20mg/day fluoxetine were 232.8 ± 154 nmol/L and 414.2 ± 160 nmol/L respectively. After 30 days of dosing at 20mg/day, other studies have reported mean plasma concentrations of fluoxetine 255.86 ± 106.88 nmol/L and norfluoxetine 417.80 ± 136.02 nmol/L, very similar to our findings (CPS, 2000).

Independent samples t-tests confirm that there were no significant differences in any alprazolam pharmacokinetic parameters between the citalopram and fluoxetine groups before SSRI treatment. The alprazolam Cmax in our group of subjects (84.6 ± 19.8 nmol/L) was significantly higher than found in previous studies: Hassan et al. found a Cmax for oral alprazolam (1mg) of 39.7 ± 18.8 nmol/L (n=10) (Hassan, Sproule, Naranjo, and Herrmann, 2000), Greenblatt and Preskorn found a Cmax of 44.7 ± 3.5 nmol/L (oral alprazolam 1mg; n=12) (Greenblatt, Preskorn, Cotreau, Horst, and Harmatz, 1992) and Greenblatt and Wright found a Cmax of 47.6 nmol/L.
oral alprazolam 1mg; n=7) (Greenblatt et al., 1998). This may have been somewhat attributable to higher bioavailability of alprazolam in our study since subjects took the medication on an empty stomach and were not allowed any food until one hour after the dose was given, at which point the drug had been fully absorbed as indicated by a $T_{\text{max}}$ of 0.9 hours. In both the Greenblatt and Preskorn study and the Greenblatt and Wright study, subjects ate breakfast before the alprazolam was given, and therefore the drug may not have been fully bioavailable. However, this can not account entirely for the differences. Other possible factors that may have influenced this large difference in $C_{\text{max}}$ are method of encapsulation of alprazolam tablets, for blinding, and interindividual variation. The pre-SSRI alprazolam AUC (0-\infty) in our study (1090.8 \pm 325) was similar to that found by Greenblatt and Preskorn (913.3 \pm 90.7 nmol/L*h; n=12), and Greenblatt and Wright (767.6 nmol/L*h; n=7) but significantly higher than found by Hassan (571.0 \pm 176.8 nmol/L*h; n=10). Similarly, our $T_{\text{max}}$ (0.9 hours \pm 0.6) was comparable to those in the Greenblatt and Preskorn study and the Greenblatt and Wright study (1.4 hours \pm 0.2 and 1.4 h \pm 0.3 respectively) but much lower than that of Hassan’s (2.5 \pm 1.1 hours). These differences may be explained by the large interindivdual variation in the pharmacokinetics of alprazolam, and the relatively small sample size employed by Hassan. The $t_{1/2}$ of alprazolam in this study (13.6 hours \pm 3.3) was similar to those found by Hassan (11.7 \pm 2.5 hours), Greenblatt and Preskorn (17.4 \pm 1 hours) and Greenblatt and Wright (15.2 \pm 2.1 hours).
In the fluoxetine group, every subject experienced an increase in alprazolam AUC (0-48, 0-∞) after treatment with fluoxetine (fig 16 and 17). The observed increase in many of the pharmacokinetic parameters of alprazolam after a minimum of three weeks of coadministration with fluoxetine 20mg/day is consistent with the results from other studies that show the capacity of fluoxetine and norfluoxetine to impair the metabolism of other medications that are metabolized by CYP3A4, including alprazolam (Greenblatt, Preskorn, Cotreau, Horst, and Harmatz, 1992; Lasher, Fleishaker, Steenwyk, and Antal, 1991). Alprazolam metabolite formation is inhibited in vitro by ketoconazole, a potent CYP3A4 inhibitor, and anti-rat CYP3A1 antibodies (von Moltke, Greenblatt, Cotreau-Bibbo, Harmatz, and Shader, 1994b; von Moltke et al., 1993). In vivo, Greenblatt (1992) administered 1mg of alprazolam before and after 3 to 7 days of treatment with fluoxetine 40 mg/day, and found a 26% increase in the AUC (0-∞), and a 17% increase in the t1/2 of alprazolam (n=12) (Greenblatt, Preskorn, Cotreau, Horst, and Harmatz, 1992). We found a similar 32% increase in AUC (0-inf) of alprazolam and a 14% lengthening of the t1/2. Likewise, there was no significant change in Cmax or Tmax in the Greenblatt study, although a trend towards an increase in Cmax (Greenblatt, Preskorn, Cotreau, Horst, and Harmatz, 1992). We also found a non-significant increase in Cmax (from 87.6 nmol/L to 95.3 nmol/L), and no change in Tmax. This is the first study to show inhibition of alprazolam metabolism by fluoxetine at the lower therapeutically relevant dose of 20mg/day. Cmax might be expected to increase after inhibition of alprazolam metabolism by fluoxetine, however since the oral bioavailability of alprazolam is already very high (over 90%) due to minimal gastrointestinal or hepatic pre-systemic biotransformation, there is little room for detectable increases in Cmax of alprazolam after a single oral dose.
Erythromycin, a potent inhibitor of CYP3A4, has been shown to cause a 147% increase in the AUC (0-∞) of alprazolam (0.8mg), and prolong the half-life of alprazolam by 150% without any apparent change in C_m (Yasui et al., 1996). Likewise, ketoconazole, also a potent CYP3A4 inhibitor, has been shown to increase the AUC (0-∞) of alprazolam (1mg) by 300% and increase the t_{1/2} of alprazolam from 15.2 hours to 59 hours, without a significant change in C_{max} or T_{max} (Greenblatt, Wright, von Moltke, Harmatz, Ehrenberg, Harrel, Corbett, Counihan, Tobias, and Shader, 1998).

Citalopram 20mg/day did not affect the AUC, t_{1/2} or C_{max} of alprazolam, which is consistent with the in vitro data that suggests citalopram inhibits CYP3A4 to a lesser degree than fluoxetine and the other SSRIs. We found that the T_{max} of alprazolam was lengthened by half an hour (p<0.05), which may simply reflect the great deal of variation in T_{max} within our sample. Alternately, it may reflect a delay in absorption caused by citalopram without any metabolic effects. Since there was no significant change in the C_{max} of alprazolam post-citalopram, the prolongation of alprazolam T_{max} is probably due to an effect on the absorption of alprazolam. It is possible that citalopram may have a yet undiscovered effect on p-glycoproteins in the gut, or some other effect on the gut wall. The mean prolongation in alprazolam T_{max} of half an hour that we found would not be expected to have a significant effect on the clinical effect of alprazolam, however two subjects showed 2.0 and 1.0 hour delays, respectively, in the T_{max} of alprazolam post-citalopram as compared to the pre-citalopram session, which is quite significant and may reflect biochemical conditions unique to these subjects that are worth further study (fig. 19 and 20). It is also worth
noting at this point that there was a non-significant trend towards decreased alprazolam AUC at the very beginning of the kinetic curve (0-3 hours) during the citalopram + alprazolam session, as compared to the alprazolam only session (p=.107), which supports the delaying action of citalopram on alprazolam absorption.

Fig. 19. Subject 1017: Alprazolam kinetics pre- and post-citalopram (0-10 hours)

![Graph showing alprazolam kinetics pre- and post-citalopram (0-10 hours)]

Fig. 20. Subject 1027: Alprazolam kinetics pre- and post-citalopram (0-10 hours)

![Graph showing alprazolam kinetics pre- and post-citalopram (0-10 hours)]
During the course of this study, another paper was published supporting our results. Nolting et al. compared acute-dose pharmacokinetics of triazolam 0.25mg before and after 4 weeks of citalopram administration (20mg/day in week 1; 40mg/day for 3 weeks) and found no change in plasma triazolam AUC before and after citalopram, indicating little inhibition of the CYP3A4 enzyme by citalopram (Nolting and Abramowitz, 2000).

Based on the results of the study, which employed a therapeutically relevant dose of citalopram 20mg/day, we can not rule out the possibility that citalopram may inhibit the metabolism of alprazolam or other CYP3A4 substrates at higher therapeutic concentrations (between 40 to 80mg/day). A recent case study reported that a schizophrenic man experienced sedation, new-onset fatigue, confusion, enuresis and hypersalivation secondary to supratherapeutic levels of clozapine after augmentation with 40mg/day of citalopram (Borba and Henderson, 2000). Symptoms disappeared and clozapine levels dropped significantly after reduction of citalopram to 20mg/day, suggestive of possible CYP1A2 or 3A4 blockade at the higher dose of citalopram. However, Nolting found no effect of citalopram 40mg/day on CYP3A4-mediated triazolam metabolism (Nolting and Abramowitz, 2000). There are no other case reports which suggest higher doses of citalopram may affect the metabolism of CYP3A4 substrates.

It is interesting to note that there was a non-significant decline in alprazolam $C_{\text{max}}$ post-citalopram (from 81.4 nmol/L to 74.4 nmol/L). This may not be remarkable in the context of this experiment alone, but it is worthwhile to note that Hassan (2000)
found a similar trend in alprazolam kinetics after two weeks of sertraline treatment at three daily doses; 50mg (n=6), 100mg (n=4), and 150mg (n=6). The pre-sertraline $C_{\text{max}}$ of alprazolam was 39.7 nmol/L, while the post-sertraline $C_{\text{max}}$ was 27.7 nmol/L, 24.5 nmol/L and 34.2 nmol/L for the three doses respectively, and the post-sertraline change in $C_{\text{max}}$ at the lowest (50mg/day) dose was found to be significant ($p=0.05$) (Hassan, Sproule, Naranjo, and Herrmann, 2000). We also used low doses of citalopram (20mg/day). This may indicate that low doses of citalopram and sertraline, or indeed the SSRIs as a whole, may have some effect on the absorption or elimination of alprazolam that is currently unknown and not robust enough to have been described before. We found a significant delay in the $T_{\text{max}}$ of alprazolam after citalopram which may well affect the $C_{\text{max}}$ of alprazolam, although Hassan did not find a delay in alprazolam $T_{\text{max}}$ post-sertraline. These phenomena may or may not be related. This unknown effect may involve interactions of SSRIs with heretofore unknown p-glycoproteins in the gut or kidney, or another undescribed effect on the absorption or elimination of alprazolam.

In general, citalopram shows a linear dose-plasma concentration relationship, whereas fluoxetine does not (Preskorn, 1997). Both medications show a great deal of interindividual variability in plasma concentrations (Fredricson, 1982). The relationship between plasma concentration of citalopram or fluoxetine and inhibition of CYP enzymes in vivo is unknown. In this study, there was no relationship between higher concentrations of citalopram or fluoxetine and greater inhibition of alprazolam metabolism, according to correlation analyses. The only subject in the citalopram group who experienced a significant increase in alprazolam AUC ($0-\infty$) after
treatment with citalopram (fig. 16 and 17) in fact had the lowest citalopram levels at steady state. However, the $C_{\text{max}}$ of alprazolam alone for this subject was 72.8 nmol/L, which increased by 31% to 95.4 nmol/L after citalopram administration, so one explanation is that alprazolam was not fully absorbed during the pre-SSRI session, which would explain why the $C_{\text{max}}$ increased so significantly during the post-SSRI session and would also account for the increased AUC ($0-\infty$) post-SSRI.

5.2 Pharmacodynamics

**Pharmacodynamic effects of citalopram and fluoxetine alone**

Repeated measures ANOVA on the AUC (0-8) measurements detected no significant effect of either SSRI alone on the psychomotor or sedation scores. We found that the most common side effect of both citalopram and fluoxetine in this study was daytime somnolence, and although this was generally a transient effect, it was important to confirm that the SSRIs did not effect a pharmacodynamic change on their own, since this would have confounded the interpretation of our dynamic results.

Repeated measures ANOVA on the AUC (0-3) and maximum change from baseline scores did detect some significant differences between placebo and SSRI (table 9). However, these differences were not consistent and may therefore be due to a type I error, or else, in the case of the DSST and MTT, may be attributable to a learning effect. For example, ANOVA analysis found a significant improvement on the DSST for fluoxetine alone, as compared to placebo alone, as measured by AUC (0-3) and
maximum change from baseline. This may be due to a learning effect since the placebo alone session took place before the fluoxetine alone session (also see figure 14, page 68). This learning effect on the DSST is not evident in the AUC (0-8) measurements, nor is it evident in the citalopram group, and the effect may therefore be unimportant. There may also be a learning effect on the MTT, as evidenced by a statistically significant improvement on the MTT with citalopram as compared to placebo, but only as measured by AUC (0-3), not by AUC (0-8) or maximum change from baseline. Again, there did not appear to be a statistical difference in MTT scores between fluoxetine and placebo (also see figure 15, page 69). A learning effect may be important in the context of our dynamic results if scores on the MTT and DSST tests during the alprazolam alone sessions (which came before the alprazolam + SSRI sessions) appeared to be more impaired because of a lack of practice on the tests. However, there does not appear to be robust evidence for a significant learning effect. Subjects were required to practice each test for up to an hour, until they achieved comparable results three times in a row, before starting the first session.

Pharmacodynamic interaction effect of alprazolam + fluoxetine

The interaction effect of fluoxetine on alprazolam pharmacokinetics was not reflected by any significant change in the psychomotor function or subjective rated sedation scores (table 9). The dynamic assessment measures were sensitive enough to detect the significant effect associated with alprazolam administration as compared to placebo, but may not have been sensitive enough to detect the more subtle changes incurred by the 32% increase in AUC (0-∞) of alprazolam. Because the
C_{max} did not change, we would not have expected an increase in the maximum change from baseline sedation and psychomotor scores, but would have expected an increase in the AUC (0-8). Nonetheless, a similar study using erythromycin as a CYP3A4 inhibitor showed a pronounced increase in alprazolam AUC without detecting changes in dynamic measures (table 12). Yasui (1998) reported a nearly 150% increase in the AUC (0-\infty) of alprazolam after coadministered multiple-dose erythromycin, a potent CYP3A4 inhibitor, but found no evidence of altered psychomotor function parameters as measured by the DSST and sedation visual analogue scales, ascribed to a possible lack of test sensitivity or to the lack of change in the C_{max} of alprazolam (Yasui, Otani, Kaneko, Ohkubo, Osanai, Sugawara, Chiba, and Ishizaki, 1996).

It is important to address possible reasons why we did not detect significantly increased dynamic effects of alprazolam after fluoxetine administration. There appears to be no definite linear relationship between dose or plasma levels of alprazolam and response to alprazolam, although most studies in this area have focused on anxiolytic response, not sedation or psychomotor response, and since measurements of “response” are mostly subjective or third person, it is a very difficult phenomenon to study. In vitro and in vivo studies have indicated that the benzodiazepine receptor is not saturable at therapeutic plasma levels of alprazolam, therefore this is not a potential reason for our failure to detect a pharmacodynamic change Fujita. Since the C_{max} of alprazolam was unchanged after fluoxetine administration, this may account for the lack of change seen in the dynamic response to alprazolam plus fluoxetine, since from a subject’s point of view, post-
maximum dynamic response to alprazolam may be more difficult to gauge than maximum alprazolam response. Conversely, there have been many studies that are similar to ours (using different CYP3A4 inhibitors) that have found a dynamic change post-inhibitor without a significant change in alprazolam $C_{\text{max}}$ (table 12). Alternately, it is possible that the pre-SSRI plasma levels of alprazolam were high enough to have caused a “ceiling effect” such that further subjective and psychomotor changes were not detectable post-SSRI, suggesting that we may have needed to use lower doses of alprazolam. As well, the 32% increase in alprazolam AUC ($0-\infty$) may not have been robust enough for us to detect an effect. Most of the studies that have detected pharmacodynamic effects of a CYP3A4 inhibitor on alprazolam have reported lower initial alprazolam AUC and $C_{\text{max}}$ values, but have also detected far more robust changes in alprazolam AUC (table 12). Therefore, either of these factors could explain why we did not detect a pharmacodynamic change. Our pre- and post-SSRI alprazolam $C_{\text{max}}$ values were over twice those found in all of the other studies, meaning that we could expect much greater sedation on alprazolam days. Indeed, on alprazolam session days, most of our subjects appeared to be very sedated and slept throughout the entire session day, waking up only to perform the tests. Often subjects were not able to stay awake during the tests, and had to be awoken repeatedly. This may have made it difficult for subjects to gauge slight changes in their levels of sedation, and this is likely a significant factor in our inability to detect dynamic changes. Using doses of alprazolam even lower than 0.8mg should be investigated. It is notable, however, that Yasui et al. (1996) found a robust increase in alprazolam AUC (150%) after erythromycin coadministration, without a pharmacodynamic change (Yasui, Otani, Kaneko, Ohkubo, Osanai, Sugawara,
Chiba, and Ishizaki, 1996). The pre-treatment plasma alprazolam $C_{\text{max}}$ in this study was also much lower than ours, indicating that using a lower dose of alprazolam may not have allowed us to detect sedative and psychomotor effects of increased alprazolam with any added sensitivity. The minimal increase in alprazolam AUC(0-$\infty$) that we found after fluoxetine (32%) was probably also a significant factor in why we did not detect the expected dynamic changes. We used therapeutically low doses of fluoxetine (20mg/day) whereas most other interaction studies have used fluoxetine doses of 40 to 60mg/day (Greenblatt, Preskorn, Cotreau, Horst, and Harmatz, 1992a; Lasher, Fleishaker, Steenwyk, and Antal, 1991). It is conceivable that had we used a higher dose of fluoxetine, we would have detected greater kinetic changes and then possibly a dynamic effect. However, this can not be confirmed since little is known about the inhibitor dose to CYP3A4 inhibition relationship. Lastly, our inability to detect a pharmacodynamic effect of fluoxetine may be related to sample size. Initial sample size calculations were done with pharmacokinetic changes in mind, so that a sample size of 20 was calculated in order to detect a significant change in alprazolam kinetics, rather than dynamics. Had sample size been calculated with the goal of detecting a change in pharmacodynamic response, it is conceivable that the sample size would have been larger and there would have been more power to detect a dynamic change due to alprazolam inhibition by fluoxetine.
<table>
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<tbody>
<tr>
<td>Regimen</td>
<td>200mg bid for 4 days</td>
<td>4 doses of 200mg over three days</td>
<td>200mg od for 6 days</td>
<td>400mg tid for 10 days</td>
<td>20mg/day for 21 days</td>
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<td>1 mg</td>
<td>0.8 mg</td>
<td>0.8 mg</td>
<td>1 mg</td>
</tr>
<tr>
<td>N</td>
<td>7</td>
<td>8</td>
<td>10 males</td>
<td>12 males</td>
<td>11</td>
</tr>
<tr>
<td>AUC (0-\infty) before inhibitor (nmol/L*hr)</td>
<td>767.6</td>
<td>787.0</td>
<td>816.1</td>
<td>743.0</td>
<td>1152.4</td>
</tr>
<tr>
<td>AUC (0-\infty) after inhibitor (nmol/L*hr)</td>
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<td>1949.7</td>
<td>2173.1</td>
<td>1827.0</td>
<td>1518.8</td>
</tr>
<tr>
<td>Percent change in AUC (0-\infty) (Sig.)</td>
<td>300% (p&lt;0.001)</td>
<td>146% (p&lt;0.001)</td>
<td>166.3% (p&lt;0.001)</td>
<td>147.2% (p&lt;0.001)</td>
<td>32% (p=0.001)</td>
</tr>
<tr>
<td>$C_{\text{max}}$ before inhibitor (nmol/L)</td>
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<td>48.9</td>
<td>41.8</td>
<td>38.0</td>
<td>87.6</td>
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<td>$C_{\text{max}}$ after inhibitor (nmol/L)</td>
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<td>50.8</td>
<td>53.7</td>
<td>45.9</td>
<td>95.3</td>
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<tr>
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<td>28.5% (NS)</td>
<td>20.8% (NS)</td>
<td>8.8% (NS)</td>
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<td>YES</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
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</tr>
</tbody>
</table>

NS = Not a statistically significant difference

Pharmacodynamic interaction effect of alprazolam + citalopram

The only significant pharmacodynamic effect we found was in the citalopram group, where subjects experienced significantly more alprazolam-associated impairment on the MTT post-citalopram as compared to pre-citalopram, as measured by AUC over 0-3 hours and 0-8 hours (table 9). This effect was not replicated on either the DSST or in the sedation scores. As well, this result does not correspond with the pharmacokinetic results, which showed no effect of citalopram on alprazolam kinetics, except for a delay in $T_{\text{max}}$. We can offer no explanation for this finding except to suggest the possibility of a type I error, even though the effect was fairly robust.

Kinetically, citalopram caused a significant delay in the $T_{\text{max}}$ of alprazolam. It is interesting to note in the graphs plotting MTT and DSST performance, and sedation ratings, versus time after drug administration from 0 to 8 hours (figs. 13, 14 and 15), that there appears to be a delay in the time of maximum alprazolam effect after citalopram treatment on all three measures. These delays did not reach significance, probably due to the very large degree of variability in the time of maximum impairment (Tables 11a, 11b). Nevertheless, this corresponds well with the pharmacokinetic effect of citalopram.

Correlation and Regression analysis

Non-parametric correlations may be more appropriate than parametric when trying to correlate changes in kinetic and dynamic values. This is because the distribution of values would not be expected to be normal due to the expected differences
between citalopram and fluoxetine subjects with respect to change in kinetic values, since fluoxetine subjects experienced a change in alprazolam dynamics while citalopram subjects did not, however both were nonetheless carried out for the sake of comparison. Non-parametric correlation analysis (table 10) showed that subjects who experienced the greatest increase in alprazolam concentration AUC (0-∞) post-SSRI also experienced the greatest increase in sedation post-SSRI, as measured by the sedation summary scale. This correlation was not evident using parametric analysis, nor did the correlation hold for concentration AUC (0-8) or (0-48). Both parametric and non-parametric tests also revealed a correlation between change in alprazolam concentration AUC (0-48) and greater maximum change from baseline scores on the MTT post-SSRI. However there was no correlation between MTT scores and concentration AUC (0-8) or (0-∞). No significant correlation was noted for the DSST. These results may suggest that a greater change in alprazolam concentration post-SSRI, versus pre-SSRI, is correlated with greater sedation and psychomotor impairment post-SSRI. Since fluoxetine subjects showed a far more robust increase in alprazolam concentration AUC post-SSRI, this would provide evidence for a corresponding increase in dynamic effect in the fluoxetine group. However, regression analysis showed no relationship between changes in any dynamic measurement and changes in alprazolam concentration AUC. As well, the correlation analyses were by no means robust, and since no dynamic changes were detected using ANOVA, the significance of these results is questionable. At best, they allude to the possibility of a trend towards increased dynamic effect of alprazolam post-fluoxetine.
5.3 Clinical Implications of the Study

We observed that fluoxetine increased the AUC (0-3, 0-8, 0-48, 0-\infty) and $t_{1/2}$ of orally administered alprazolam (1mg) indicating that fluoxetine inhibits the hepatic metabolism of alprazolam, which is consistent with previous in vitro and other in vivo evidence. The metabolite of fluoxetine, norfluoxetine, probably contributes greatly to the metabolic inhibitory effect of fluoxetine, since it’s $K_i$ for CYP3A4 is greater than fluoxetine’s (table 1). Considering the extremely long half life of norfluoxetine, this has a significant clinical impact for patients discontinuing the medication. We found that levels of norfluoxetine were not significantly decreased one week after discontinuation of fluoxetine (table 6). Patients and physicians should be aware of the potential inhibitory effect of norfluoxetine for up to six weeks after discontinuation of fluoxetine. A study by Greenblatt found that the norfluoxetine metabolite was able to increase the plasma concentration of alprazolam 18 days after discontinuation of fluoxetine 40mg/day (Greenblatt, Preskorn, Cotreau, Horst, and Harmatz, 1992). We did not find a pharmacodynamic consequence of the increase in alprazolam AUC after multiple dose fluoxetine, indicating that the interaction is not clinically significant. Although we used only single dose alprazolam, the results are applicable to multiple-dose regimens, where the pharmacokinetic changes are likely to be amplified. We would expect fluoxetine to continue to augment the serum concentration of alprazolam after multiple doses. Indeed, multiple doses of fluoxetine (60mg/day od) coadministered with multiple-dose alprazolam (1mg qid) over four days resulted in increased alprazolam plasma concentrations as compared to
alprazolam (1 mg qid) alone, and this increase was greater after 48 hours of multiple
dose coadministration, than after 24 hours (Lasher, Fleishaker, Steenwyk, and Antal,
1991). Although we did not find an increase in the C\text{max} of single dose alprazolam
after fluoxetine treatment as compared to before fluoxetine, the C\text{max} of alprazolam
would be expected to be amplified after multiple-doses since the t\text{1/2} of alprazolam is
extended by fluoxetine. This is also confirmed by Lasher (1991). It should be noted
that alprazolam has a very wide margin of safety and therefore the potential for
serious harm is small, however potentially serious events may occur such as falls
(especially in the elderly) and car accidents.

Citalopram at 20 mg/day, in contrast to fluoxetine, does not appear to inhibit
CYP3A4 in vivo or lead to an accumulation of alprazolam as measured by AUC (0-
\infty), C\text{max} and t\text{1/2}. Although we can not rule out the possibility of interactions at higher
doses, it is unlikely that citalopram, at therapeutic doses, will interfere with CYP3A4
activity to a degree that is clinically significant. Nolting (2000) found that higher
therapeutic doses of citalopram (40 mg/day) did not affect the metabolism of the
CYP3A4 substrate triazolam (Nolting and Abramowitz, 2000). Since citalopram does
not appear to affect the elimination t\text{1/2} or the C\text{max} of alprazolam, it is unlikely that
alprazolam accumulation would occur after multiple doses. One interesting finding
was that citalopram significantly delays the T\text{max} of alprazolam, and furthermore, this
delay appears to be reflected in a delayed dynamic response to alprazolam (non-
significant). The clinical significance of a delay in alprazolam absorption may be
significant in patients where an immediate effect is desired.
5.4 Mechanism of CYP3A4-mediated interactions: findings that should be considered

The observed alterations in the kinetics of alprazolam after coadministration with multiple-dose fluoxetine may be due to the effects of fluoxetine on (i) absorption of alprazolam; (ii) metabolism of alprazolam; (iii) distribution of alprazolam or (iv) elimination of alprazolam. Since the C\text{max} and T\text{max} of alprazolam were unchanged after fluoxetine treatment, it is unlikely that fluoxetine affected the absorption of alprazolam. There is no evidence that fluoxetine affects the distribution or elimination of alprazolam, therefore the kinetic changes of alprazolam were almost certainly due to metabolic changes effected by fluoxetine. Previous in vitro and in vivo studies have shown that CYP3A4 is the major enzyme involved in alprazolam metabolism (von Moltke, Greenblatt, Harmatz, and Shader, 1993; von Moltke, Greenblatt, Grassi, Granda, Venkatakrishnan, Duan, Fogelman, Harmatz, and Shader, 1999; Yasui, Otani, Kaneko, Ohkubo, Osanai, Sugawara, Chiba, and Ishizaki, 1996) and alprazolam hydroxylation is strongly inhibited by potent and relatively specific CYP3A4 inhibitors in vitro and in vivo (von Moltke, Greenblatt, Cotreau-Bibbo, Harmatz, and Shader, 1994b) (table 12).

Research has demonstrated that some interactions with CYP3A4 inhibitors may also involve inhibition of P-glycoprotein; many drugs that are metabolized by human CYP3A4 are also transported by P-glycoprotein. P-glycoprotein is an integral cell membrane protein that serves as an ATP-dependant efflux pump (Silverman,
P-glycoprotein is expressed under physiological conditions in normal tissue in the apical domain of enterocytes, bile epithelial cells and in the renal proximal tubules. It was first studied in conjunction with antineoplastic drug resistance, where cancer cells were found to be actively exporting the drugs out of the cell via this protein. P-glycoprotein causes diminished absorption of some drugs from the gut, increased secretion with bile, and increased renal excretion. P-glycoprotein is also expressed in the luminal part of the endothelial cells of the brain capillaries, and this can diminish the CNS penetration of substrates. Drugs that induce the expression of p-glycoprotein (e.g. rifampin) enhance this effect whereas drugs that inhibit P-glycoprotein cause elevated plasma concentration through increased bioavailability and lowered secretion into bile and urine (e.g. digoxin) (Fromm, 2000; Westphal et al., 2000). It is unknown at this time whether alprazolam pharmacokinetics can be altered by inhibition or induction of P-glycoprotein, however studies indicate that expression of CYP3A4 and metabolism of midazolam and triazolam, both CYP3A4-metabolized benzodiazepines, are unaffected by inhibition of P-glycoprotein in mice (Perloff et al., 1999). Takano (1998) found that P-glycoprotein was not involved in the intestinal transport of midazolam across the epithelium, but midazolam may be able to interact with P-glycoprotein and inhibit the transport of some drugs (Takano et al., 1998). There is also little knowledge about the effect of citalopram and fluoxetine on P-glycoprotein, however it appears that neither SSRI is a substrate of P-glycoprotein since their passage across the blood brain barrier is independent of P-glycoprotein expression or activity (Rochat et al., 1999; Uhr et al., 2000). Thus it is tempting to suggest that fluoxetine is unlikely to
inhibit P-glycoprotein significantly, nor does it appear at this stage that alprazolam pharmacokinetics would be affected by p-glycoprotein inhibition.

Another consideration is that CYP3A5 and CYP3A7 may be more prevalent in the human liver than previously thought. A recent report suggests that CYP3A5 is present in 10-25% of human livers, and CYP3A7 in as many as 50% (Tsunoda et al., 1999). It has not been established whether the presence or absence of CYP3A5 and/or CYP3A7 in the intestine or liver contributes to a significant difference in metabolic capacity.

Finally, recent research has suggested that, although CYP3A does not display a bimodal enzyme activity-distribution curve, there may be population differences in CYP3A activity. After administration of oral alprazolam, Lin found that Asian subjects had significantly higher C\textsubscript{max} values than Caucasians, suggesting differential pharmacokinetics (Lin, 1988). A recent paper by Wandel found a significant difference in systemic clearance of midazolam and hepatic CYP3A activity between African Americans and European Americans, and these differences correlated well with a CYP3A4*B1 polymorphism in the 5' promoter region, a polymorphism found far more frequently in African Americans (Wandel, 2000). Similarly, Krecic-Shepard found that black subjects have significantly lower nifedipine clearance rates as compared to white subjects (Krecic-Shepard, 2000). We did not find any obvious differences between Asian and Caucasian subjects in our study.
5.5 Alprazolam as a probe of CYP3A4 activity in vivo

Several in vitro probes for CYP3A4 have been established including erythromycin, midazolam, alprazolam, terfenadine, nifedipine, and cyclosporin, however often these probes fail to accurately correlate with each other (Kinirons et al., 1999; Houston and Kenworthy, 2000). CYP3A4 exhibits several atypical kinetic properties in vitro such as activation, autoactivation, partial inhibition, substrate inhibition and biphasic saturation curves that often make it difficult to apply classical Michaelis-Menten kinetics (Korzekwa et al., 1998). For example, low concentrations of midazolam act as an activator of terfenadine metabolism, but inhibit the reaction at higher concentrations (Wang et al., 2000). It has been suggested that these atypical kinetic properties arise from the binding of multiple substrates simultaneously in the active site of CYP3A4. Korzekwa (1998) provided evidence of the binding of both 7,8-benzoflavone and phenanthrene simultaneously in the active site of CYP3A4 (Korzekwa, Krishnamachary, Shou, Ogai, Parise, Rettie, Gonzalez, and Tracy, 1998). Shou (1999) employed diazepam, temazepam, and nordiazepam as substrates to propose a model of CYP3A4 with two distinct but co-operative substrate-binding sites (Shou et al., 1994). Spectral titration studies by Hosea et al. (2000) have provided evidence for a three-site CYP3A4 model (Hosea et al., 2000). This model hypothesizes that there is one binding site where one and possibly two testosterone molecules can bind together with an overlapping midazolam. The peptide morphiceptin competes for binding at this site. A second site binds α-
naphthoflavone, and a third site binds a second midazolam in a location distinct from the first binding site. The interactions between CYP3A4 and its substrates and inhibitors appear to be complex and inhibition may be of a competitive, noncompetitive, or uncompetitive nature, partial inhibition or mechanism-based inhibition. This may have implications with respect to the interpretation of $K_i$ values, which are calculated using the Michaelis-Menten model.

As a consequence of the atypical characteristics of CYP3A4, interactions observed with one probe may not be representative of the action of other probes. A recent study by Kenworthy (1999) showed a lack of complete correlation between any two of ten different in vitro probes for CYP3A4, although some groups of probes whose molecular structures were similar correlated closely within themselves (Kenworthy et al., 1999). For example, diazepam, midazolam, triazolam and dextromethorphan correlated better with each other than with other probes, and likewise the larger molecular weight probes erythromycin, cyclosporin and testosterone were linked to each other. Therefore the interactions observed with one probe may not be representative of those observed with another substrate probe. This may impact significantly on the extrapolation of drug interactions from in vitro to in vivo. It has been suggested therefore that multiple CYP3A4 probes, one representing each of the different groups, be used for in vitro assessment of CYP3A4-mediated drug interactions.

There also appears to be a lack of correlation between in vivo probes. For example when the erythromycin breath test (ERBT), performed by administering radiolabelled
erythromycin iv and subsequently measuring the rate of exhalation of radiolabelled carbon dioxide, was compared with both oral and iv midazolam as a probe of CYP3A4 activity, no statistically significant correlations were observed and the phenotypic trait measures associated with these in vivo probes did not provide the same information about the catalytic activity of the enzyme (Kinirons, O'Shea, Kim, Groopman, Thummel, Wood, and Wilkinson, 1999). Similarly, alfentanil clearance did not correlate with ERBT (Krivoruk et al., 1994). A study comparing ERBT with dextromethorphan/3-methoxymorphanan urinary rations after oral dextromethorphan administration, and oral and i.v. verapamil clearance found only correlations between iv and oral verapamil clearance, and weak correlations between dextromethorphan/3-methoxymorphanan urinary rations and (1) iv verapamil clearance, (2) oral verapamil clearance and (3) plasma AUC (norverapamil)/AUC (verapamil) after oral verapamil. No correlations with ERBT were found (Krecic-Shepard et al., 1999). Only studies comparing ERBT with cyclosporin clearance show weak correlation between probes (Watkins et al., 1990; Turgeon et al., 1992). Lack of correlation between putative CYP3A4 probes may be attributable to route of administration, extrahepatic metabolism, probe characteristics, and inter/intra-subject variability, or possibly to the complicated nature of substrate binding to CYP3A4, although this has not been studied in vivo.

All of this suggests that in fact substrate binding to CYP3A4 and the subsequent enzyme kinetics are far more complicated than originally thought, and this has implications for in vitro probing of this, and possibly other, enzymes. The consensus seems to be that in fact there is no one “universal” probe for CYP3A4, but that the
probe to be utilized should depend on the drug(s) to be tested, and in many cases multiple probes should be used to study different aspects of the interaction. There is very little evidence regarding the in vivo consequences of the atypical kinetics noted in vitro, or of the complex nature of substrate binding to CYP3A4. The consequence of this is that it is unknown whether the results of this study can be applied to other situations, for example can we predict from this study that citalopram will not affect the metabolism of terfenadine (a CYP3A4 substrate)? This is debatable, since other studies have shown incongruous results. For example, eight days of fluoxetine administration at 60mg/day was not found to inhibit the metabolism of triazolam (0.25mg) while fluoxetine at 40mg/day and 20mg/day inhibited the metabolism of alprazolam (1mg), both of which are CYP3A4 metabolized triazolobenzodiazepines, even though ketoconazole inhibited the metabolism of both benzodiazepines (Wright et al., 1992; Greenblatt, Preskorn, Cotreau, Horst, and Harmatz, 1992). In this study, we looked at two substrate-enzyme interactions in vivo and have gleaned information about this particular set of variables, but if a different set of variables are used, it is conceivable that different results may be found if the binding of putative probes and/or inhibitors are different from those used here.

The most common type of inhibition is due to reversible, competitive inhibition of a CYP enzyme by a drug or drug metabolite. This phenomenon usually begins with the first dose of the inhibiting drug, and onset and offset of inhibition correlate well with the half life of the drug (Dresser, Spence, and Bailey, 2000; Cupp and Tracy, 1998). Irreversible, 'suicide' or mechanism-based inhibition occurs when a substrate becomes covalently bound to the enzyme, for example mifepristone (RU-486) and
grapefruit juice were demonstrated to be mechanism-based inhibitors of CYP3A4 (He et al., 1999; Chan et al., 1998). Time course of inhibition after mechanism based inactivation depends on both drug half life and enzyme turnover.

Therefore, the simplest model for the mechanism of interaction would involve competitive inhibition of alprazolam binding to the CYP3A4 enzyme by fluoxetine and citalopram, easily described by the Michaelis Menten model in vitro. We would conclude from the results of this study that fluoxetine and its metabolites are stronger inhibitors of alprazolam metabolism probably because they have a higher affinities for the CYP3A4 enzyme than citalopram and its metabolites. However, a main assumption of the Michaelis Menten model is the premise that substrate-enzyme interactions occur at only one site per enzyme and that each site operates independently from the others. There is abundant evidence that this is not the case for CYP3A4, and that the interaction may therefore be more complex. This may have consequences for in vivo inhibition as well. It is possible that fluoxetine and citalopram interact with the active site of CYP3A4 simultaneously with alprazolam, and fluoxetine causes allosteric changes to the enzyme to inhibit alprazolam hydroxylation, whereas citalopram does not. It is conceivable that the nature of the interaction is dose-dependant, or represents only partial inhibition. The mechanism is probably more complex than the simple competitive model.
6 Conclusions

The metabolism of a single oral dose of alprazolam (1mg) was inhibited by three weeks of administration of fluoxetine (20mg/day), leading to a 32% increase in the AUC (0-∞) of alprazolam with an associated 14% prolongation in the elimination t_{1/2} and no change in C_{max} or T_{max}, but no alprazolam-associated pharmacodynamic changes were noted. Citalopram (20mg/day) did not affect the AUC, C_{max} or t_{1/2} of alprazolam, but did produce a half-hour delay in the T_{max} that appears to have been reflected in the pharmacodynamic profile of alprazolam post-citalopram (not significant). Our kinetic results were consistent with previous results, except that no previous studies have found delayed T_{max} of alprazolam associated with citalopram treatment.

Although we did not find any clinical significance to the interaction of fluoxetine and single-dose alprazolam, the fact that the elimination t_{1/2} of alprazolam was increased after fluoxetine indicates that there would likely be a clinically significant interaction after multiple-dose alprazolam, as shown by Lasher (1991) (Lasher, Fleishaker, Steenwyk, and Antal, 1991). We predict that there is no danger of alprazolam accumulation after multiple doses when administered with concurrent citalopram. Physicians can use this information when determining which SSRI to prescribe to depressed patients suffering from comorbid anxiety, a common problem in psychiatry, or other medical problems requiring the use of alprazolam. Citalopram may be a more appropriate choice than fluoxetine in patients taking multiple doses of alprazolam for chronic anxiety problems, although sertraline has also been
demonstrated to have little effect on alprazolam metabolism. Furthermore, since there are many medications that are metabolized by CYP3A4 it may be possible to extend this knowledge to predict that citalopram will not interact with other CYP-3A4 medications such as midazolam, although this is not clear since in vivo probes do not always correlate well.
7  Final Comments and Future Recommendations

There has been considerable energy spent trying to decipher the patterns of drug interactions mediated by CYP enzymes, including CYP3A4, which plays a role in the metabolism of more than 50% of medications. The ultimate goal of this research is to develop the ability to predict drug interactions from in vitro and in vivo data. However, the diversity in pharmacokinetic and pharmacodynamic properties of potentially interacting drugs is great, and with the recent discovery that CYP3A4 may have a very complex mechanism of action, it may be more difficult than expected to reach this goal.

We detected some novel findings during the course of this study. We found a delay in alprazolam absorption after citalopram treatment that is also associated with a possible delay in alprazolam-associated psychomotor impairment. This may be indicative of a novel interaction that needs confirmation and further study. As well, the possibility that citalopram and sertraline may have a heretofore unknown effect on alprazolam absorption or elimination, leading to the observed decrease in $C_{\text{max}}$ seen in this study and others, should be probed further.
Reference List


von Moltke LL, Greenblatt DJ, Grassi JM, Granda BW, Venkatakrishnan K, Duan SX, Fogelman SM, Harmatz JS, and Shader RI (1999) Citalopram and


Appendix 1.

**Evaluation of the Interaction between Alprazolam and the SSRIs Citalopram and Fluoxetine**

**INFORMATION SHEET**

**Background and Purpose**

Several medications, such as antidepressants, may decrease the ability of the body to eliminate other drugs when the two are taken together. This may potentially result in drug side effects or changes in the concentration of a drug in the body. The purpose of this study is to determine whether the administration of citalopram or fluoxetine, antidepressant medications, affects the concentration or behavioural effects of another medication, alprazolam, in normal volunteers.

**Procedures**

If you agree to participate in this study, you will be assessed for any medical or psychological conditions which may preclude your participation. As part of this assessment, you will be asked about medications which you have used in the past month as well as your smoking and drinking (alcohol and coffee) habits. We will take a thorough medical history, and perform a brief medical examination, including routine blood tests, to screen for some medical problems. If you are female, a pregnancy test is required (urine). Females who are pregnant or breastfeeding will be excluded from the study. Females who may become pregnant will be expected to use an acceptable form of birth control other than an oral contraceptive during the study.

You will also attend four 3-day study sessions in the Human Psychopharmacology Research Laboratory at the Sunnybrook & Women's College Health Sciences Centre, Sunnybrook site. For the first day of each study session, you must remain in the Research Laboratory from approximately 8:00 a.m. to 5:00 p.m.; for the following 2 days of each session, you must return to the Research Laboratory at approximately 9:00 a.m. only. The total duration of the study is approximately 47 days from the first interview to the end of the study. The study sessions will be conducted on approximately days 4 to 6, 9 to 11, 33 to 35 and days 38 to 40. For 12 hours before and during the study sessions, you must abstain from alcohol and caffeine-containing foods or drinks (chocolate, coffee, tea, caffeinated soft drinks such as cola).

The procedure for the first day of each of the study sessions are as follows: You will be asked to fast from 11:00 p.m. on the night before the study sessions until you arrive at the study sessions at 8:00 a.m. and get at least 8 hours of sleep during the night. At the beginning of the session, you will be requested to provide a urine sample for the screening of drugs and to have a breathalyzer (alcohol breath) test. In 2 of the sessions you will receive alprazolam (an anti-anxiety agent) orally at 9:00 a.m., and in the other 2 you will receive placebo (an inactive substance) orally at 9:00 a.m. The capsules will look identical and neither you nor the person testing you will be aware of which you are receiving. The dose of alprazolam is 1 mg. Also at 8:00 a.m., we will insert a catheter into a vein in your
forearm in order to collect 10 blood samples (10 ml/sample) at the following times after the 9:00 a.m. medication administration time: 0.0, 0.5, 1.0, 1.5, 2, 2.5, 3, 4, 6, and 8 hours. Therefore the catheter will remain inserted for approximately 8 hours each day. During the study sessions, we will monitor your vital signs (temperature, pulse and blood pressure) at times when blood is drawn. You will also be asked to rate your current level of sedation, perform two computerized psychomotor tests and report any side effects you may experience at the time of blood testing. These testing procedures should take approximately 10 minutes each time.

On the second and third days of the study sessions, you will be asked to return to the Human Psychopharmacology Research Laboratory at either the Sunnybrook & Women's College Health Sciences Centre, Sunnybrook site or Women's College site at approximately 9:00 a.m. At these times, a small amount of blood (10 ml) will be taken from a vein on your forearm.

You will also be asked to return to the Human Psychopharmacology Research Laboratory at Sunnybrook & Women's College Health Sciences Centre, Sunnybrook site, for three additional brief visits the day after you start the medication, seven days after you start the medication, and seven days after you stop taking the medication, in order to have a small amount of blood taken from a vein in your forearm (on approximately Days 13, 19, and 47 of the study).

You will begin taking one 20 mg capsule of citalopram or fluoxetine after the first two study sessions have been completed and you will be required to take this medication for 30 consecutive days. You will be asked to take this medication at 8:00 each morning. Neither you nor the person testing you will know which of these two drugs you are taking.

If you need to take any other medication during the study, you must inform the study investigators. However, you should not take certain antihistamines such as Seldane or Hismanal. You will be required to abstain from grapefruit and grapefruit juice during the 47 day study.

Risks and Discomforts

Fluoxetine is a medication which has been used clinically for years to treat patients with depression. Therapeutic doses range from 20 to 60 mg per day. The most commonly reported side effects are nausea, diarrhea or loose stools, male sexual dysfunction, insomnia, anorexia, somnolence, tremor, increased sweating, dry mouth, asthenia and dizziness. The incidence of these symptoms in adults taking fluoxetine is 1.6 to 10% higher than subjects receiving placebo.

Citalopram is also an antidepressant. The most commonly reported side effects are nausea/vomiting, dry mouth, sexual dysfunction, headaches, increased sweating, tremor, somnolence, insomnia, dizziness and asthenia. The incidence of these symptoms in adults taking citalopram in therapeutic doses of 20 to 40 mg per day is 5 to 20% higher than subjects receiving placebo.

Alprazolam is a medication which is used clinically to treat patients with anxiety or panic disorder. However, the single dose of 1 mg which you will be taking on 2 separate days in this study is clinically low compared with doses of up to 10 mg per day given to those patients for long periods of time. The incidence of side effects with a 1 mg, single dose is
very low (less than 1%). Clinical studies of anxiety disorder patients taking alprazolam indicate that the incidence of side effects such as dizziness, muscle weakness, hypotension and lightheadedness are only slightly higher than among subjects taking placebo (less than 5%). The incidence of drowsiness is increased by approximately 20% in subjects taking alprazolam, when compared with subjects taking placebo.

Based on the literature, we expect that coadministration of fluoxetine and alprazolam, and perhaps citalopram and alprazolam, will produce an increase in alprazolam concentrations. Increases reported, however, are relatively small, with plasma drug concentrations remaining well within the wide margin of safety.

The intravenous catheter inserted on the first day of the study sessions involves little pain, will be inserted by an experienced person and does not involve any significant health risk. Insertion of the catheter allows for the collection of the majority of the blood samples with only one puncture. The blood samples taken on the second and third days of the study sessions will also be taken by an experienced person, involve little and brief pain, and will not involve any significant health risk. After insertion of a catheter (day 1 of each session) or a needle for blood draws there is some slight chance of bruising or inflammation.

You will be asked to fast from 11:00 p.m. prior to each study session. On the first day, you will be provided with a light breakfast (apple juice and muffin) and a light lunch. There is a risk of migraine among participants prone to migraine headaches. Other participants may experience headache. If medically necessary, you will be taken to your residence by a taxi after the study sessions. During the study sessions, magazines will be available so that you may spend your time comfortably between testing procedures.

Benefits

You may or may not receive any direct benefit by choosing to participate in this study. The information obtained in this study will help to predict and avoid certain medication interactions, leading to improvements in the treatment of health problems with medications.

Compensation

In consideration of your time, you will receive $700.00 upon completion of the 7 week study. If you withdraw early you will receive compensation on a prorated basis according to the number of days completed. You will also be reimbursed for parking and travel expenses (not including taxis).

Participation

Your participation in this study is voluntary and you can withdraw from the study at any time and for any reason. If you decide to withdraw from the study, this will not in any way affect your future medical care or any benefits to which you may be entitled.

Confidentiality

Your identity and the information obtained in this study will be kept strictly confidential and secure, available only to the researchers in the study. The data will be identified by your initials and date of birth only, and not by your name. Published reports and presentations at scientific meetings will refer to grouped data and no individual will be identifiable.
Evaluation of the Interaction between Alprazolam and the SSRIs
Citalopram and Fluoxetine

Informed Consent

The purpose of this research, the procedures to be followed and the possible risks associated with the study have been fully explained to me. I have had the opportunity to ask questions and my questions have been answered satisfactorily. I understand that I will be free to withdraw from the study at any time and this will not affect the care or the benefits to which I am entitled. I voluntarily consent to participate in this study.

I have been given a copy of this consent form to take home with me and I understand that I may contact Dr. Claudio Naranjo at (416) 480-6761 to ask any further questions I may have concerning the study.

______________________________
Name of Subject (print)

______________________________  _________________
Signature of Subject           Date

______________________________  _________________
Signature of Investigator       Date

______________________________  _________________
Signature of Witness            Date
Appendix 2
Alprazolam and Citalopram/Fluoxetine Interaction Study
Randomization Code
July 4, 1999

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1. Randomized citalopram or fluoxetine assignment in blocks of 4.
2. Randomized alprazolam or placebo to be given first in pre-SSRI sessions (1 & 2), in blocks of 4.
3. Randomized whether order of alprazolam and placebo would be the same or different in post-SSRI sessions (3 & 4) compared to pre-SSRI sessions, in blocks of 4.

Beth Sproule
Alprazolam and Citalopram/Fluoxetine Interaction Study
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April 26, 2000 extension

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4. Randomized citalopram or fluoxetine assignment in blocks of 4.
5. Randomized alprazolam or placebo to be given first in pre-SSRI sessions (1 & 2), in blocks of 4.
6. Randomized whether order of alprazolam and placebo would be the same or different in post-SSRI sessions (3 & 4) compared to pre-SSRI sessions, in blocks of 4.

Beth Sproule
Appendix 3.

**Sedation Measure: 100mm Visual Analog Scales**

Items on this scale:
1. Anxious
2. Bloated
3. Sad
4. Fatigued
5. Thinking speeded up
6. Tense
7. Spacey
8. Seclusive
9. Elated
10. Hungry
11. Pleasant
12. Nervous
13. Excited
14. Easily Irritated
15. Contented

These items are weighted to give a Sedation Summary value

**DSST**

Participants are shown a series of random box patterns, each of which correlates to a number. These patterns are changed each time the test is administered. During the test, numbers are displayed on the computer screen in a random order and subjects must use a light pen to quickly fill in a blank box with the pattern that corresponds to the displayed number.