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DOSE-RESPONSE EVALUATION OF THE INTERACTION BETWEEN SERTRALINE AND ALPRAZOLAM *IN VIVO*

by

Paul Christopher Hassan

A thesis submitted in conformity with the requirements for the degree of Master of Science, Graduate Department of Pharmacology, University of Toronto

DOSE-RESPONSE EVALUATION OF THE INTERACTION BETWEEN SERTRALINE AND ALPRAZOLAM *IN VIVO* by Paul Christopher Hassan for the degree of Master of Science, Graduate Department of Pharmacology, University of Toronto, 1998.

Based on *in vitro* data showing inhibition of alprazolam metabolism by sertraline via CYP3A4, the potential for similar *in vivo* inhibition was assessed in humans. Ten healthy volunteers participated in two test sessions (placebo/alprazolam 1mg p.o.) prior to initiating sertraline treatment. Blood samples were obtained over a 32 hour period and pharmacodynamic measures (sedation, psychomotor performance, memory function) were performed over an 8 hour period. Following two weeks of daily sertraline self-adminisration (50,100,150mg/day), test sessions were repeated. Alprazolam concentrations (n=6,4,6 at 50,100,150mg/day sertraline, respectively) showed no significant changes based on C_{max}, T_{max}, $t_{1/2(\beta)}$, and $AUC_{0-\infty}$, with the exception of a reduced C_{max} in the 50mg group. Similarly, dynamic data showed no significant variations based on peak effect, T_{max}, AUC_{0-3} and AUC_{0-8}, with the exception of increased peak impairment in one measure of psychomotor performance. These findings suggest that sertraline (50-150 mg/day) does not alter single-dose kinetics or dynamics of alprazolam.

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SECTION #1: INTRODUCTION

1.1 PURPOSE AND OBJECTIVE OF THE STUDY

This experiment was conducted to determine if therapeutic doses of sertraline (50, 100 and 150 milligrams per day, oral dose) would impair the metabolism of alprazolam (1 milligram, single oral dose), leading to clinically significant alterations in alprazolam kinetics and adverse effects, and to assess whether these changes were dependent on sertraline dosage.

1.2 REVIEW OF THE LITERATURE

1.2.1 THE CYTOCHROME P450 ENZYMES

The cytochrome P450 (CYP) enzymes are a superfamily of heme containing monoxygenase enzymes involved primarily in oxidative metabolism and biotransformation. Originally named for their ability to absorb light at a wavelength of 450 nanometers (Garfinkel 1958, Klingenberg 1958, Preskorn 1995), the CYP enzymes have been found to play important roles in human cellular function and metabolism. To date, more than 30 human CYP isozymes have been identified and may be found in various locations throughout the body. Although the highest

concentrations of xenobiotic or microsomal CYP enzymes are found in the liver, they are also found in the brain, gastrointestinal tissue and the kidney (Krishna and Klotz 1994, Ereshefsky et al. 1996).

Knowledge of the diversity of CYP enzymes has evolved to the point where individual CYP families, subfamilies and specific genes have been identified. The first division in classification and understanding of the CYP enzymes is characterization of the enzyme as either mitochondrial or microsomal (Harvey and Preskorn 1996a, Gonzalez and Nebert 1990). Mitochondrial or "steroidogenic" CYP enzymes are the more ubiquitous enzyme species, and are found in even single celled organisms. These enzymes are responsible for the maintenance of cellular integrity through the production of endogenous steroids, bile salts, cholesterol and prostaglandins (Gonzalez 1992, Guengerich 1994, Nebert and Gonzalez 1987). The microsomal CYP enzymes are found in the endoplasmic reticulum of animal cells and are thought to have originally evolved from the mitochondrial CYP enzymes to allow the elimination of plant toxins. The role of these enzymes has evolved to include the detoxification of drugs and other foreign substances (Gonzalez 1992, Gonzalez and Nebert 1990, Nebert and Gonzalez 1987, Harvey and Preskorn 1996a).

Division of the CYP enzymes into mitochondrial and microsomal subtypes is however not sufficient to distinguish individual enzymes. CYPs have therefore been classified using the nomenclature of molecular biology to families, subfamilies and specific genes coding for their

production. Enzymes within a family have at least 37% amino acid sequence homology and display broad functional similarities (Nebert et al. 1987, Nelson et al. 1993). Families are denoted by an Arabic number; for example, CYP families 7, 11, 17, 19, 21 and 27 are mitochondrial enzymes while families 1, 2, 3 and 4 are microsomal enzymes. These families are further divided into subfamilies which display sequence homology of at least 55 to 77% (Nebert et al. 1987, Nelson et al. 1993) and are denoted by a capital letter. Important subfamilies involved in human drug metabolism include CYP1A, CYP2C, CYP2D and CYP3A. Finally, these subfamilies are subdivided to specific genes which are denoted by a second Arabic number. For example, the subfamily CYP1A includes the isozymes CYP1A1 and 1A2.

1.2.2 DRUG INTERACTIONS AND THE CYTOCHROME P450 ENZYMES

In human drug metabolism, there are several CYP isozymes of particular importance. These include CYP1A2, CYP2C19, CYP2D6, and CYP3A4 (Gonzalez 1992, Wrighton and Stevens 1992). In terms of their relative abundance in the liver, CYP1A2 constitutes approximately 13% of total CYP content while CYP2C19, CYP2D6, and CYP3A4 constitute 20%, 2%, and 30%, respectively (Shimada et al. 1994). However, the relative abundance of an enzyme does not necessarily correlate with its metabolic capacity since enzymes possess different affinities for substrates. Therefore

some enzymes which are found in relatively low quantities may be responsible for the metabolism of large numbers of substrates (Bertz and Granneman 1997). For example, although CYP2D6 comprises a small proportion of total hepatic CYP content, it is responsible, at least in part, for the metabolism of many psychotropic medications including antipsychotics (haloperidol, perphenazine, risperidone), selective serotonin reuptake inhibitors (SSRIs) (fluoxetine, paroxetine, citalopram), tricyclic antidepressants (TCAs) (nortriptyline, amitriptyline, desipramine), and other antidepressants (venlafaxine, nefazodone) (Cholerton et al. 1992, Shen 1995, DeVane 1994a).

Additionally, the activity of some enzymes exhibit bimodal or polymodal distributions in the general population that are consistent with polymorphisms of the gene encoding their production (Daly et al. 1993). Of particular importance is the fact that some individuals exhibit significantly reduced enzymatic activity with respect to certain CYP enzymes. For example, in the Caucasian population, 5-10% of individuals are considered to be "poor metabolizers" (PMs) of CYP2D6 substrates and 3-5% are PMs of CYP2C19 substrates (Daly et al. 1993, Bertz and Granneman 1997).

The importance of CYP enzymes in psychopharmacology became widely recognized when it was discovered that SSRIs possessed an ability to inhibit the activity of one or more of these enzymes, potentially leading to clinically significant drug interactions. CYP2D6-mediated metabolism was the first to be explored and it was discovered that both paroxetine and

fluoxetine had the ability to significantly inhibit activity of this enzyme *in vivo* (Brosen et al. 1993, Brosen and Skjelbo 1991, Nemeroff et al. 1996). The inhibition could essentially convert individuals with otherwise normal enzymatic activity (extensive metabolizers or EMs) to a phenotype resembling a PM. Thus, at their usual doses, coadministration of CYP2D6 substrates with either paroxetine or fluoxetine could result in accumulation of the substrate, assuming that no significant alternative metabolic pathways existed. The clinical consequences of substrate accumulation would resemble those associated with elevated concentrations and toxicity of the substrate. For example, Ozdemir et al. (1997) found that both mean peak perphenazine concentrations and mean AUC_{0-8} increased approximately 6-7 times when subjects were pretreated with 20 mg paroxetine per day for 10 days. The increased concentrations resulted in significantly greater levels of sedation, extrapyramidal symptoms, and impairment of psychomotor performance and memory function.

1.2.3 THE ROLE OF CYP3A4 IN METABOLIC DRUG INTERACTIONS

CYP3A4 is another enzyme of considerable therapeutic importance. Psychotropic medications which are metabolized, at least in part, by CYP3A4 include antidepressants (imipramine, sertraline, nefazodone), anticonvulsants (carbamazepine, ethosuximide), and many benzodiazepines (alprazolam, triazolam, midazolam) (von Moltke 1995a,

Bertz and Granneman 1997, Ereshefsky et al. 1996, Nemeroff et al. 1996, Preskorn 1996a). The activity of CYP3A4 shows wide interindividual variation, but unlike CYP2D6, there is no evidence of a genetic polymorphism in the population (Daly et al. 1993, Ketter et al. 1995). Sources of variation have not been completely identified, but are thought to include factors such as age (Greenblatt et al. 1991a, Durnas et al. 1990, Wilkinson 1996), the presence of disease (Farrell 1987) or liver damage (von Moltke et al. 1995a), and environmental factors such as dietary exposure to inhibitors (e.g. grapefruit juice) or inducers (e.g. certain flavonoids) of enzymatic activity (Anderson 1988, Bailey et al. 1994, Guengerich et al. 1994). Medications can also either induce (e.g. rifampicin, carbamazepine) or inhibit (e.g. ketoconazole, erythromycin, certain SSRIs) the activity of CYP3A4 (Harvey and Preskorn 1996b, Ketter et al. 1995, von Moltke et al. 1995a), potentially leading to metabolic drug interactions if CYP3A4 substrates are coadministered. For example, in order to exert anti-histaminic effects, terfenadine must be metabolized to fexofenadine through CYP3A4. Normally, this process occurs during first-pass metabolism of the drug and the parent compound does not enter the systemic circulation. However, ketoconazole has the ability to inhibit CYP3A4 activity and therefore block the metabolism of terfenadine (von Moltke 1994a, Wilkinson 1996). Thus, in patients receiving concomitant therapy, large amounts of terfenadine enter the systemic circulation. Since terfenadine can cause lethal prolongation of the Q-T

interval, the consequences of this metabolic drug interaction may be severe (Zimmerman et al. 1992, Honig et al. 1993, Wilkinson 1996).

These types of interactions can be amplified if the coadministered medication undergoes extensive gut wall metabolism (through CYP3A4) prior to entering the systemic circulation. For example, CYP3A4 inhibition causes greater increases in cyclosporin plasma concentrations than can be accounted for by changes in first-pass hepatic metabolism alone (Hebert et al. 1992), thereby implying that gut wall metabolism is also inhibited. Conversely, some CYP3A4 substrates such as alprazolam and diazepam are unaffected by gut wall metabolism and therefore metabolic drug interactions involving these medications are mediated primarily through hepatic metabolism (Divoll et al. 1983, Smith et al. 1984, Lin et al. 1988).

In studying metabolic drug interactions, it is usually best to directly examine the effects of inhibitors or inducers on enzymatic activity. This is accomplished through the use of phenotyping procedures that estimate the activity of a particular enzyme *in vivo* through analysis of the metabolism of a probe drug. For CYP3A4, various tests have been proposed that involve either oral or intravenous administration of a probe drug followed by determination of the rate of formation of CYP3A4-specific metabolites and/or the rate of elimination of CYP3A4-specific parent compounds. Once these rates are determined, it is usually possible to form an index of CYP3A4 activity.

Two well-studied probe drugs for CYP3A4 are erythromycin and midazolam (Watkins 1994). The erythromycin test (Erythromycin Breath Test or EBT) is performed by administering radiolabeled erythromycin intravenously and subsequently measuring the rate of exhalation of radiolabeled carbon dioxide. The test is considered to be the most validated measure of hepatic CYP3A4 activity (Watkins 1994) but is unable to account for CYP3A4 activity in the gut wall and therefore fails to produce an estimate of total CYP3A4 activity (Lown et al. 1994). This problem is of particular importance if the information obtained from the test is being used to estimate the required dose of an orally administered CYP3A4 substrate that is affected by gut wall metabolism (e.g. cyclosporin). Similarly, the midazolam test, which also involves intravenous administration of drug, fails to account for gut wall metabolism (Kivisto and Kroemer 1997). Additionally, the midazolam test requires repeated blood sampling over a period of hours following administration of the drug in order to estimate CYP3A4 activity. As a result of such concerns, neither of these tests has been adopted as a standard for the measurement of CYP3A4 activity, and other drugs that are orally administered (such as dextromethorphan) are being investigated since they may be able to account for gut wall metabolism.

1.2.4 THE PREDICTION OF METABOLIC DRUG INTERACTIONS

The prediction of metabolic drug interactions has become increasingly important, particularly in the case of new medications that may rapidly gain widespread use. Ideally, the potential for such interactions would be assessed before the drug is approved for use in humans, and as a result, *in vitro* methods have been devised for this purpose. Metabolic pathways for new medications are now commonly studied through *in vitro* incubations with individually expressed human CYP enzymes (Wrighton et al. 1993). Once the enzymes involved in metabolism are identified, the potential for metabolic drug interactions with known inhibitors or inducers of enzymatic activity can be assessed.

The utilization of *in vitro* methods for prediction of *in vivo* response is of scientific and economic importance since it allows for an understanding of possible metabolic interactions without the expense, complications and potential hazards of *in vivo* studies (von Moltke et al. 1998a). Furthermore, it allows for the use of human tissue or cell lines rather than animal models for assessment of human tissue response. This can be particularly important in studies involving CYP enzymes since some enzymes are species-specific and others can differ in quantity, structure and function between species (von Moltke et al. 1998a).

Despite the potential advantages, there are several difficulties in the extrapolation of *in vitro* CYP metabolism to the *in vivo* situation

(Schmider et al. 1996). In particular, with respect to the *in vitro* results themselves, the applicability of the results is highly dependent on the *in vitro* model. For example, although human liver microsomes may appear to be the best *in vitro* model for assessment of drug interactions mediated by the CYP system, such experimental systems do not allow for transcriptional induction or inhibition of the enzyme and fail to take into account the presence of CYP enzymes in other body tissues (Schmider et al. 1996). In addition, *in vitro* experimental reactions may be influenced by factors such as temperature, growth medium, pH, electrolyte balance, protein content and phospholipid concentrations (Bertz and Granneman 1997). All of these factors can differ from conditions that are encountered *in vivo*.

Even if the conditions of *in vitro* experimentation are appropriate, extrapolation to the *in vivo* situation requires the consideration of many additional factors. In attempting to predict an *in vivo* metabolic drug interaction from *in vitro* data, most approaches compare expected *in vivo* hepatic concentrations of inhibitor to the *in vitro* inhibition constant (K_i) for a particular enzyme and substrate (von Moltke et al. 1998a). If the expected *in vivo* hepatic concentration of inhibitor greatly exceeds the K_i value, significant inhibition of substrate metabolism can occur and thus the potential for a metabolic drug interaction is high (Bertz and Granneman 1997). However, *in vivo* hepatic concentrations are impossible to determine on an individual basis since samples of liver

tissue are needed for analysis. Thus post-mortem studies are used to calculate liver to plasma concentration ratios which are subsequently used to estimate hepatic concentrations based on individual plasma concentrations (von Moltke et al. 1998a).

The use of this approach requires several assumptions. Firstly, the approach is only appropriate in situations where the mechanism of inhibition is noncompetitive or, in the case of competitive inhibition, where substrate concentrations are sufficiently low to allow the enzyme to function at less than 50% of maximum capacity (von Moltke et al. 1998a). Furthermore, the approach is only valid when *in vivo* clearance of the substrate is dependent on CYP activity, and when inhibition is reversible and concentration dependent. Finally, validity is critically dependent on the assumption that hepatic inhibitor concentrations can be accurately predicted and that hepatic concentrations are reflective of the concentrations to which the enzyme itself is exposed (Bertz and Granneman 1997).

Even with complex modeling of the *in vivo* situation, many questions remain unanswered. For example, if inhibition or induction is expected to occur *in vivo*, will the degree of inhibition be sufficient to cause clinically relevant consequences? Will metabolites of the substrate and inhibitor alter the potential for a clinically significant metabolic interaction? These and other factors will be considered in more detail in the discussion of the results of this study (Section 4).

1.2.5 MAJOR DEPRESSION

Major depression is one of the most debilitating psychiatric illnesses. Its symptoms include loss of energy and interest, feelings of guilt, decreased sleep, difficulty concentrating, loss of appetite, and suicidal ideation (APA 1994). In fact, major depression has been identified as the most common factor leading to both attempted and completed suicide. It has also been identified as an independent risk factor for mortality, increasing the likelihood of death by 59% in the first year after diagnosis (Rovner 1993, Shaffer 1988, Wells et al. 1989, Blazer et al. 1986, Barraclough et al. 1974). Major depression may occur either as a single episode or as a recurrent condition. Single episodes are estimated to occur in 30 to 50% of depressed individuals, with the remainder of patients experiencing repeated or recurrent episodes (Hirschfield and Cross 1982).

Epidemiological studies indicate that 15% of the North American population will suffer from a clinically significant major depressive episode at some point in their lives (Ancill and Holliday 1990, Blazer 1989, Regier et al. 1988). More recent estimates from the National Comorbidity Survey determined the lifetime prevalence of a major depressive episode among the noninstitutionalized United States population aged 15 to 54 years to be 17.1%, with significantly more women (21.3%) than men (12.7%) reporting an episode of depression (Kessler et al. 1994).

Despite its high prevalence, depression is consistently underdiagnosed and undertreated, leaving close to 50% of potential patients without adequate treatment (Hirschfeld et al. 1997). As a result, major depression is estimated to cause an annual loss of 172 million work days due to disability, absenteeism and reduced productivity (Tollefson 1993, Mintz et al. 1992, Wells et al. 1989) and an economic loss of approximately $36.5 billion in direct and indirect costs (Finkelstein et al. 1996) in the United States alone.

1.2.6 THE PHARMACOLOGICAL MANAGEMENT OF MAJOR DEPRESSION

Pharmacological treatments for major depression have been available for over 40 years and it has long been known that drug therapy can greatly improve the prognosis for a depressed patient. In fact, between 65 and 75% of depressed patients show improvement when pharmacological interventions are used, as compared to 30 to 40% of patients who improve with placebo (Baldessarini 1985). Response rates may increase when the medications are prescribed in appropriate doses, given adequate time to exert antidepressant efficacy, and monitored properly (Preskorn 1996b). The major classes of antidepressants include: the TCAs, such as nortriptyline, amitryptyline and imipramine; the SSRIs, such as fluoxetine, paroxetine and sertraline; the monoamine oxidase inhibitors (MAOIs), such as phenelzine and tranylcypromine; and

other agents such as trazodone and bupropion. Some newer agents such as venlafaxine and nefazodone, which do not fit into this classification scheme, are also currently available. Historically, the TCAs have been the drugs of choice and the standard of efficacy by which all other antidepressants are measured. However, the SSRIs have demonstrated similar efficacy to the TCAs (Kasper et al. 1992, Laird and Benefield 1995) with the advantage of a more benign side effect profile. In particular, the SSRIs lack the anticholinergic, antihistaminic and cardiovascular side effects that can be experienced with TCAs (Leonard 1993). More importantly, the SSRIs exhibit a high degree of safety in overdose, unlike the TCAs which can cause lethal prolongation of cardiac conduction with as little as 5 times the average daily dose (Preskorn and Irwin 1982). Both classes of drugs require approximately 4 to 6 weeks before the onset of antidepressant efficacy, although some symptoms such as loss of appetite and insomnia may remit earlier in treatment (Preskorn 1996b). Other agents, including the newest antidepressants and the MAOIs, have not demonstrated greater efficacy than either the TCAs or SSRIs in the treatment of major depression (Laird and Benefield 1995).

Given the wide array of available medications, the choice of a first-line antidepressant is complex one. Generally, the best indicator of potential success with an antidepressant is a past history of response to a particular agent by the patient or a close family member (Kaplan et al.

1994a). However, this information is usually not available to the clinician and therefore the choice of medication is based upon the perceived efficacy and the potential adverse effects of the drug. Thus SSRIs are frequently the drugs of choice when initiating treatment with an antidepressant (DeVane 1994b, Preskorn 1993, Montgomery 1994).

Among the five SSRIs that are currently marketed (fluoxetine, fluvoxamine, paroxetine, sertraline, citalopram), all have shown similar efficacy and side-effect profiles in the treatment of major depression (Kasper et al. 1992, Preskorn et al. 1995). There are, however, differences in the kinetics of the drugs and in their potential for drug-drug interactions. For example, all of the SSRIs have an elimination half-life of approximately one day with the exception of fluoxetine and its clinically active metabolite norfluoxetine which have half-lives of up to 4 and 15 days, respectively (DeVane 1994a). Sertraline and citalopram show increases in plasma concentrations that are proportional to increases in dose while fluoxetine, paroxetine and fluvoxamine may show greater than expected increases (Preskorn 1996a). In the area of drug interactions, each of the SSRIs differ in their respective abilities to inhibit isozymes of the cytochrome P450 system and therefore differ in their potential for interactions with substrates of these isozymes (Ereshefsky et al. 1996). For example, fluvoxamine can significantly inhibit CYP1A2, CYP2C19, and CYP3A4 while having a minimal effect on CYP2D6. Paroxetine can inhibit CYP2D6 without any substantial effect on other isozymes.

Fluoxetine can significantly inhibit CYP2D6, CYP3A4, and CYP2C19 without affecting CYP1A2. Sertraline and citalopram seem to have a mild to moderate inhibitory effect on CYP2D6 with little or unknown effects on other isozymes. Thus, the choice of an SSRI in the treatment of depression necessitates the consideration of many patient-specific factors and no individual SSRI is better than another in all situations.

1.2.7 SERTRALINE IN THE TREATMENT OF MAJOR DEPRESSION

Based on numbers of prescriptions, some SSRIs are more widely used than others. Sertraline has become the second most frequently prescribed SSRI in both the United States and Canada (American Druggist 1996, IMS Canada 1997), second only to the original SSRI, fluoxetine. As noted, this popularity may be due to a variety of factors. In comparison to other SSRIs, the advantageous properties of sertraline include an elimination half-life of approximately one day, a linear pharmacokinetic profile over its clinical dosing range, and kinetic properties which do not significantly differ in elderly and younger patients (DeVane 1994a, Preskorn 1993, Murdoch and McTavish 1992). Furthermore, the minimum effective dose of 50 milligrams (mg) per day is sufficient to cause remission of depressive symptoms in 58-76% of responders (Thompson et al. 1993, Bennie et al. 1995). The remaining 24-42% of responders may require higher doses of 100 to 200 mg per day to obtain

similar efficacy (Brown and Harrison 1995, Thompson et al. 1993, Bennie et al. 1995).

Sertraline is metabolized by the CYP3A4 enzyme to desmethylsertraline, and no other metabolites or metabolic pathways have been identified (Caccia 1998). Both compounds have the ability to inhibit reuptake of serotonin but the potency of desmethylsertraline is 10-25 times lower than the parent compound (Bolden-Watson and Richelson 1993, Cusack et al. 1994). Following oral dosing, peak sertraline plasma concentrations are achieved in 5-8 hours with steady-state concentrations achieved in approximately 5 days for sertraline and 14 days for desmethylsertraline (Auster 1993, Brown and Harrison 1995). Average steady-state sertraline plasma concentrations of 18, 45 and 85 nanograms per millilitre (ng/mL) are reached at doses of 50, 100 and 200 mg per day, respectively, while desmethylsertraline concentrations range 1.5-2.1 times higher (Ronfeld et al. 1997). However, there is wide interindividual variation in steady-state concentrations and, as with other SSRIs, there does not appear to be any correlation between plasma concentrations and antidepressant efficacy (Greist et al. 1995a).

1.2.8 ANXIETY DISORDERS

Anxiety disorders are often chronic illnesses with frequent recurrences and exacerbation of symptoms. Anxiety may be described as

an emotional response, with both physical and psychological components. The physical symptoms of anxiety include, but are not confined to: motor tension, restlessness, tachycardia, and nausea (APA 1994). The psychological manifestations include: worry, fear, difficulty concentrating and irritability (APA 1994). These symptoms may represent a normal physical and emotional response to situations which are perceived as stressful or threatening but, in some individuals, the anxiety which is experienced is excessive in either duration or intensity. Fears may become irrational or inappropriate to the point where they interfere with the activities of daily living or normal functioning. It is at this level that the anxiety response may be indicative of a psychological disorder (APA 1994).

Anxiety disorders are among the most frequently occurring mental illnesses. The National Institute of Mental Health, Epidemiologic Catchment Area Study estimated that the lifetime prevalence of anxiety disorders in the United States was 14.6% (Regier et al. 1990, Regier et al. 1993). More recently, the National Comorbidity Survey estimated the lifetime prevalence of any anxiety disorder to be 24.9% among the noninstitutionalized United States population aged 15 to 54 years (Kessler et al. 1994). Independent of the overall prevalence, the most commonly reported type of anxiety disorder is phobia, estimated to occur in up to 12.5% of individuals (Regier et al. 1990, Zajecka 1997), followed

by GAD and panic disorder at 10% and 3.5%, respectively (Shear and Schulberg 1995, Kessler et al. 1994).

Untreated anxiety disorders affect not only the individual but may also have social and economic consequences. For example, a Swedish study found that the risk of death by suicide was 5.7 times greater among patients who had been hospitalized with an anxiety disorder than among the general population (Allgulander 1994). Families, friends and co-workers also suffer when the illness begins to affect social function and the ability to work. Recent studies have found that up to 25% of GAD patients are unable to work and are receiving disability payments as a result of their illness (Simon et al. 1995a, Simon et al. 1995b). The same study documented marital problems in 10 to 20% of these patients. On a larger scale, the cost to society of untreated anxiety disorders is significant since patients with anxiety disorders are known to be heavy users of emergency rooms and other health care facilities (Katon et al. 1992, Klerman et al. 1991, Simon et al. 1995a, Salvador-Carulla et al. 1995). In total, the estimated annual cost of anxiety disorders in the United States alone is reported to be $46.6 billion, including both direct and indirect costs (Dupont et al. 1996).

1.2.9 THE PHARMACOLOGICAL MANAGEMENT OF ANXIETY DISORDERS

The pharmacological treatment of anxiety disorders can often be difficult due to their lifelong nature and to the high incidence of comorbid mental disorders (Rickels and Schwiezer 1990, Hoehn-Saric and McLeod 1985). Formerly, treatment of anxiety disorders was dominated by the barbiturates (phenobarbital, secobarbital, pentobarbital) and meprobamate - agents which are no longer widely used due to their lethality in overdose as well as excessive sedation, numerous drug interactions and high abuse potential (Hoehn-Saric and McLeod 1985). Other agents which may be used include buspirone and β-blockers such as propranolol (Grimsley 1995). By far the most widely prescribed agents in current treatment approaches are the benzodiazepines (e.g. alprazolam, chlordiazepoxide, clorazepate, diazepam, lorazepam, oxazepam, prazepam) (Kaplan et al. 1994b).

Although potency varies widely among the available benzodiazepines, when considered at equivalent doses, all possess similar anxiolytic and sedative-hypnotic properties. In fact, the clinical efficacy of individual benzodiazepines in the treatment of generalized anxiety disorder does not differ (Shader and Greenblatt 1993). Therefore, the most significant factor in clinical selection of a benzodiazepine is based on the pharmacokinetic and pharmacodynamic differences between various agents as well as on the desired clinical approach (Greenblatt and Shader 1987, Hayes and Kirkwood 1993).

Benzodiazepines may be subdivided according to their onset and duration of action, and according to their metabolism to active or inactive metabolites (Hayes and Kirkwood 1993, Grimsley 1995). If the clinical approach is the treatment of anxiety symptoms on an as needed basis then the use of a fast acting agent may be the appropriate therapeutic choice. Alternatively, if treatment is continuous or chronic, the use of a longer acting agent can allow for once daily dosing.

Classification of benzodiazepines to fast, intermediate or slow acting is based on onset of action following oral dosing and is determined primarily by the lipophilicity of the drug. Diazepam and clorazepate are fast acting agents with high lipophilicity, rapid penetration into the central nervous system (CNS) and onset of anxiolytic effect within 30 to 60 minutes (Greenblatt and Shader 1987, Hayes and Kirkwood 1993). Although this rapid onset is desirable from the perspective of rapid relief of anxiety, it may also produce rapid sedation, loss of control or may lead to feelings of euphoria (Greenblatt et al. 1983b), potentially increasing the abuse potential of these agents (Busto and Sellers 1986, Busto et al. 1990). Intermediate acting agents, such as lorazepam, alprazolam and chlordiazepoxide usually require 1 to 2 hours before anxiolytic effects are produced. They are relatively less lipophilic with slower penetration into the CNS (Arendt et al. 1983, Greenblatt et al. 1983c). Slower acting agents such as oxazepam are not recommended for the acute management of anxiety (Hayes and Kirkwood 1993) although sublingual

administration may expedite their onset of action in cases where such formulations are available.

Benzodiazepines are also classified according to their duration of action to long, intermediate and short acting agents. Fast acting agents generally have a shorter duration of action than would be predicted by single-dose studies due to their rapid redistribution into adipose tissue (Greenblatt et al. 1983b). Intermediate and slow acting agents do not redistribute as easily and therefore generally possess a longer duration of action (Greenblatt and Shader 1987). However, duration of action is in part dictated by metabolism, with those agents being metabolized to active metabolites exhibiting a duration of action that is longer than expected. Diazepam, chlordiazepoxide, clorazepate and prazepam are all metabolized through N-demethylation to desmethyldiazepam, an active metabolite with an elimination half life of 36 to 200 hours (Dommisse and Hayes 1987). Desmethyldiazepam is further oxidized to oxazepam, then conjugated and excreted. Agents undergoing this metabolic activation are very long acting, with half lives greater than 100 hours and an essentially continuous antianxiety effect at steady state concentrations (Grimsley 1995). However, steady state levels may not be reached for several weeks, which may complicate the process of dose titration or changes in dose. In addition, particularly if mechanisms of oxidation and elimination are impaired, extensive accumulation may occur leading to excessive drowsiness or sedation (Greenblatt 1983c).

These problems may be avoided through the use of intermediate to short acting agents such as alprazolam, lorazepam or oxazepam. The elimination half lives of these agents range from five to 20 hours and steady state concentrations may be attained rapidly with minimal accumulation following repeated dosing (Hayes and Kirkwood 1993). Of these agents, only alprazolam is metabolized through oxidation while lorazepam and oxazepam are conjugated to inactive metabolites and eliminated (Sproule et al. 1997b).

1.2.10 ALPRAZOLAM IN THE TREATMENT OF ANXIETY DISORDERS

Among the benzodiazepines, alprazolam has consistently been one of the most frequently prescribed in Canada (IMS Canada 1997) and the most widely prescribed in the United States since 1988 (Shader and Greenblatt 1993, American Druggist 1996). Alprazolam is indicated in the management of both generalized anxiety disorder and panic disorder, with recommended doses ranging from 0.25 to 3 mg per day for generalized anxiety and from 1 to 10 mg per day (average dose: 5-6 mg/day) for panic disorder (Upjohn 1995). Following a 1 mg oral dose, peak plasma concentrations of 12 to 22 ng/ml (average: 17 ng/ml) are achieved within 0.7 to 1.8 hours (average: 1.3 hours), with an elimination half-life of 9 to 16 hours (average: 11 hours) (Greenblatt and Wright 1993). Because of its relatively short half-life alprazolam is less likely, as

compared to the longer acting benzodiazepines, to accumulate in the CNS and cause excess sedation when multiple doses are administered (Shader and Greenblatt 1993). However, in order to maintain an anxiolytic effect, alprazolam must be administered two to three times per day.

Metabolites of alprazolam (4-hydroxyalprazolam and α-hydroxyalprazolam) are formed by oxidative metabolism almost exclusively through CYP3A4 (von Moltke et al. 1993, Venkatakrishnan et al. 1998). Both metabolites possess some anxiolytic activity, as indicated by GABA receptor affinity studies showing approximately 77% and 14% affinity as compared to intact alprazolam (Sethy and Harris 1982). However, both metabolites are considered clinically unimportant (Shader and Greenblatt 1993) due to their increased water solubility (Banks et al. 1992) and low concentrations that average less than 15% of intact alprazolam (Ciraulo et al. 1990). The production of 4-hydroxyalprazolam is favored over α-hydroxyalprazolam as indicated by plasma levels that are approximately 4 times greater at steady-state concentrations in humans (Ciraulo et al. 1990). Two other CYP isozymes, CYP2C9 and CYP2C19, have been implicated in the *in vitro* formation of α-hydroxyalprazolam (Venkatakrishnan et al. 1998) but their contribution is thought to be minor compared to the contribution of CYP3A4.

From clinical experience, therapeutic ranges for alprazolam have been defined. Steady state plasma concentrations of alprazolam have

been found to be related to both efficacy and toxicity (Lesser et al. 1992, Shader and Greenblatt 1993) with concentrations in the range of 20 to 40 ng/mL being sufficient to achieve remission from generalized anxiety (Greenblatt et al. 1993b). Concentrations above 48 ng/mL seem to be required for a reduction in the number of panic attacks while higher concentrations appear to be required for remission of phobias (Lesser et al. 1992). However, concentrations above 60 ng/mL were found to increase the frequency of unwanted side effects such as memory impairment, fatigue, and sedation (Lesser et al. 1992, Greenblatt et al. 1993b).

1.2.11 COMORBIDITY OF DEPRESSION AND ANXIETY DISORDERS

Several studies have confirmed that anxiety disorders and major depression are frequently comorbid conditions (Kessler et al. 1994, Wittchen et al. 1992, Tollefson 1993, Merikangas and Angst 1995, Andrade et al. 1994, Fawcett and Kravitz 1983). For example, Wittchen et al. (1991) found that 44% of a cohort of former psychiatric inpatients who fulfilled diagnostic criteria for anxiety or depression could be diagnosed with both disorders. Fawcett and Kravitz (1983) found that among 200 patients diagnosed with major depression, 62% experienced psychic anxiety, 42% experienced somatic anxiety, and 29% suffered from panic attacks. Other studies have documented comorbid depression

and anxiety disorders in up to 60% of psychiatric and primary care patients (Brown et al. 1996, Gorman and Coplan 1996, Merikangas and Angst 1995).

The impact of comorbidity on both the patient and community is severe. Comorbidity has been found to increase both severity and duration of either illness alone (Andrade et al. 1994, Grunhaus et al. 1994, Angst and Vollrath 1991). It has also been associated with poorer response to treatment, poorer outcome, and an increased risk of suicide (Bronisch and Wittchen 1994, Cox et al. 1994, Lepine et al. 1994, King et al. 1995). For example, in a group of patients with comorbid panic disorder and major depression, King et al. (1995) found that the risk of suicide increased dramatically in the comorbid condition with 26.9% of patients attempting suicide, as opposed to 16.8% of depressed patients without panic disorder. All of these factors combine to produce a 30 to 60% increase in utilization of health care services by these patients (Judd 1994). Furthermore, comorbid patients were found to require more medication and psychotherapy, as well as welfare and disability benefits (Nutt 1997) than patients with either disorder alone.

1.3 RATIONALE FOR HYPOTHESIS AND OBJECTIVE OF STUDY: THE POTENTIAL INTERACTION BETWEEN SERTRALINE AND ALPRAZOLAM

Clinically, due to the high rate of comorbidity between major depression and anxiety disorders, many patients receive combination antidepressant-anxiolytic pharmacotherapy. In fact, Mellinger et al. (1984) found that 18% of long-term anxiolytic users (at least 1 year) reported use of an antidepressant within the past 12 months. Thus, given that sertraline and alprazolam are among the most widely prescribed antidepressants and anxiolytics, respectively, their potential for concomitant administration in patients with comorbid anxiety and depression is high.

In any combination therapy, the potential for drug-drug interactions exists. Of particular importance to the potential interaction between sertraline and alprazolam is the fact that alprazolam metabolism is catalyzed almost exclusively by the cytochrome P450 enzyme CYP3A4 (von Moltke et al. 1993, Venkatakrishnan et al. 1998). As noted, metabolism through CYP3A4 may be inhibited by a variety of compounds and *in vitro* evidence from human, monkey, rat, and mouse liver microsomal preparations has shown that both sertraline and its metabolite possess the ability to inhibit alprazolam metabolism (sertraline K_i = 23.8 μM, desmethylsertraline K_i = 20.4 μM) (Figure 1) (von Moltke et al. 1994b, von Moltke et al. 1995a, von Moltke et al. 1995b, Preskorn 1996b). Thus, in

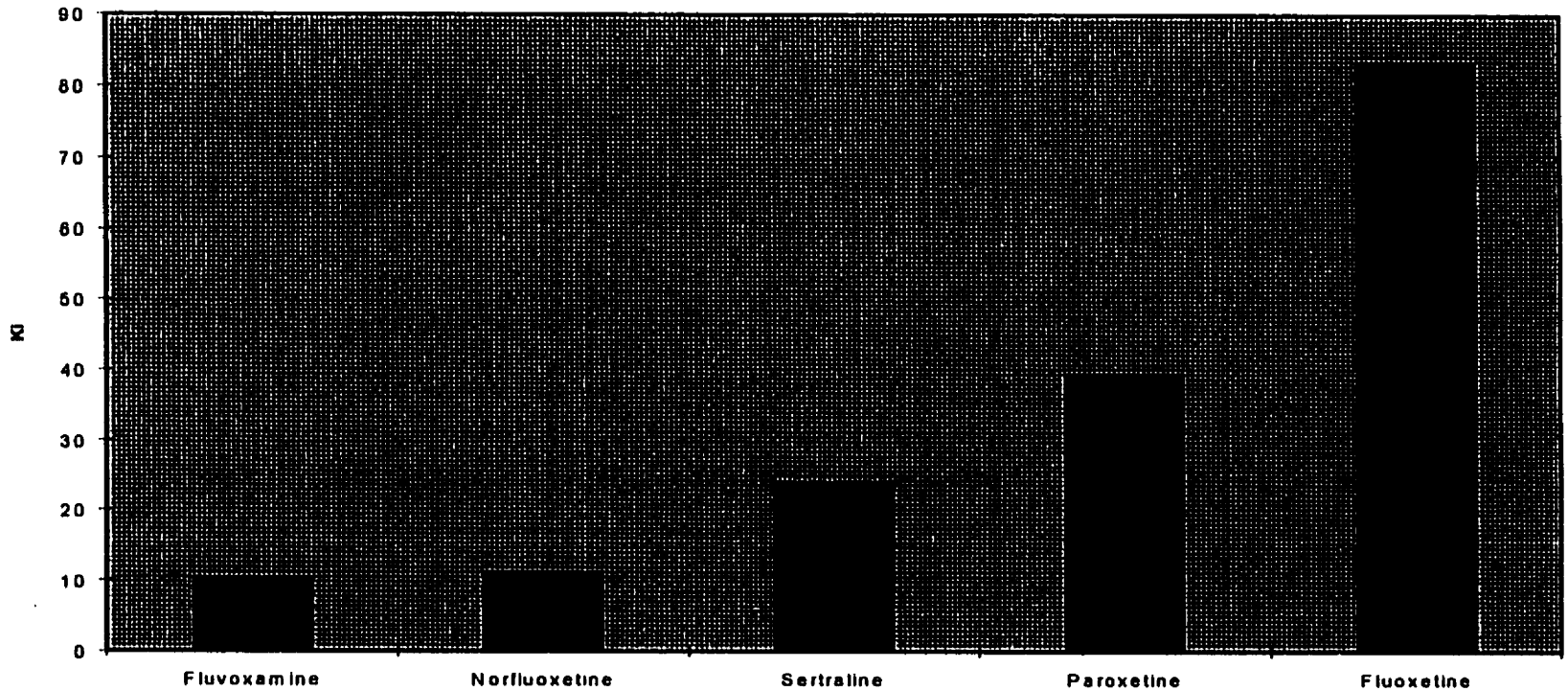


FIGURE 1: K_i values (μmol/L) of selective serotonin reuptake inhibitors for CYP3A4 activity using alprazolam as probe. K_i is the in vitro competitive inhibition constant where lower values indicate higher inhibitory potency.

(Ref: von Moltke LL, et. al. Br J Clin Pharmacol 1994;38:23-31, von Moltke LL, et. al. J Clin Psychopharmacol 1995;15:125-31)

humans, the potential for a drug interaction upon coadministration of alprazolam and sertraline exists.

Interaction studies between alprazolam and other SSRIs have documented significant increases in plasma alprazolam concentrations when coadministered with fluvoxamine (Fleishaker and Hulst 1994) and fluoxetine (Lasher et al. 1991, Greenblatt et al. 1992). In the study with fluvoxamine (Fleishaker and Hulst 1994), under a multiple dosing regimen of 1 mg alprazolam four times daily and 100 mg fluvoxamine once daily, mean peak alprazolam concentrations and AUC_{0-24} increased by approximately 100%, elimination half life increased by approximately 70%, and oral clearance decreased by 50% on Day 10 of the study. These changes caused clinically significant reductions in psychomotor performance (as measured by a digit-symbol substitution test and a continuous performance test) and clinically significant impairment of memory function (as measured by a digit span test) compared to alprazolam alone. Similarly, with fluoxetine (Lasher et al. 1991), under a multiple dosing regimen of 1 mg alprazolam four times daily and 60 mg fluoxetine once daily, mean peak alprazolam concentrations increased by approximately 30% while the elimination rate decreased by 21%. Psychomotor performance, as assessed by a digit-symbol substitution test, was significantly more impaired at 1 and 4 hours as well as all time points after 50 hours on the drug combination compared to alprazolam alone.

Although both of these studies were able to demonstrate a significant metabolic drug interaction with clinically significant consequences, fluvoxamine and fluoxetine are more potent *in vitro* inhibitors of alprazolam metabolism than either sertraline or desmethylsertraline (fluoxetine K_i = 83.3 μM, norfluoxetine K_i = 11.1 μM, fluvoxamine K_i = 10.2 μM) (Figure 1) (von Moltke et al. 1994b, von Moltke et al. 1995a, von Moltke et al. 1995b, Preskorn 1996b), and therefore it was not possible to predict the *in vivo* potential of sertraline to inhibit alprazolam metabolism based on these studies.

However, based on *in vitro* modeling techniques, some predictions could be made. Average steady-state sertraline and desmethylsertraline plasma concentrations following chronic dosing with 100 mg sertraline per day were reported to be approximately 117 nM and 234 nM, respectively (Ronfeld et al. 1997). Liver to plasma concentration ratios of 35.7 for sertraline and 22.4 for desmethylsertraline had also been reported (Levine et al. 1994). In combination, these data predict that concentrations of sertraline and desmethylsertraline in the liver should be approximately one quarter of their respective K_i values for inhibition of alprazolam metabolism. Thus *in vitro* modeling predicts less than 50% inhibition of alprazolam metabolism by sertraline and desmethylsertraline *in vivo*. However, the combined effect of the two inhibitors could not be established and thus the potential for a clinically significant metabolic interaction could not be fully assessed by *in vitro* methods.

SECTION #2: METHODS AND MATERIALS

2.1 STUDY DESIGN

The study was conducted in 10 healthy Caucasian volunteers, following a randomized, double-blind, placebo-controlled design. The study was approved by the Research Ethics Board of Sunnybrook Health Science Centre (a fully affiliated teaching hospital of the University of Toronto) and all subjects gave written consent to participate after procedures and possible side-effects were explained. Subjects were tested in the Human Psychopharmacology Laboratory which provided a controlled environment and minimized external influences.

Subjects between the ages of 18 and 55 were recruited by advertisement at local universities and teaching hospitals. All subjects were required to give a detailed medical history including the recent (past month) use of prescription, non-prescription, and herbal medicines. Subjects were also required to give a blood sample for clinical biochemistry (electrolytes, liver function, kidney function) and hematological (CBC) examinations, and a urine sample to screen for drug use (benzodiazepines, CNS depressants, cannabinoids, opiates, antidepressants, CNS stimulants, antipsychotics, antihistamines, decongestants, and antitussives) and pregnancy. Pregnant or breastfeeding mothers were excluded from the study and females of child-bearing potential were required to use an effective form of birth control other than oral contraceptives due to

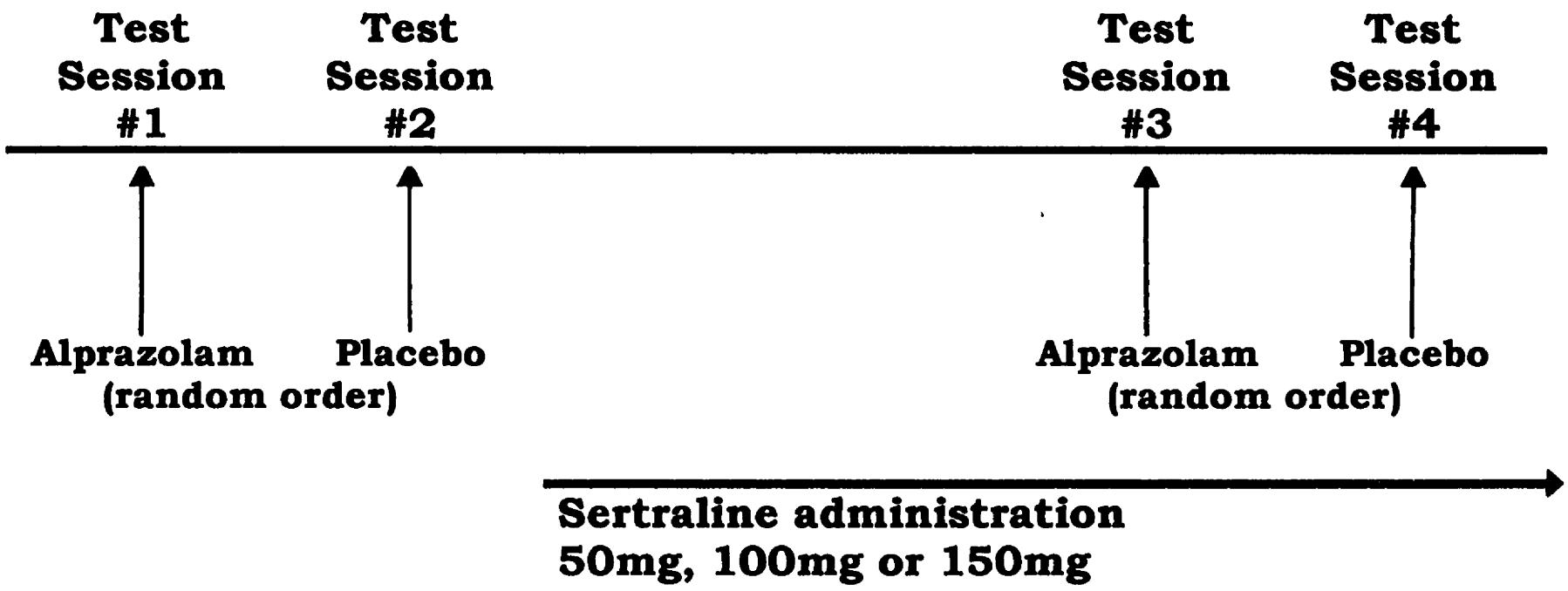
concerns about the interference of oral contraceptives with the metabolism and bioavailability of alprazolam (Kirkwood et al. 1991, Scavone et al. 1988, Stoehr et al. 1984).

Subjects were excluded from the study for a variety of other reasons. Elderly subjects (greater than 55 years) were excluded due to evidence of impaired alprazolam metabolism as compared to young controls (Greenblatt and Wright 1993, Greenblatt et al. 1983a). Subjects who used CYP3A4 substrates (e.g. clozapine) (Eiermann et al. 1997), inhibitors (e.g. erythromycin) (Yasui et al. 1996), or inducers (e.g. carbamazepine) (Kerr et al. 1994) were excluded since alprazolam metabolism could be altered by any of these medications. Cigarette smokers were excluded due to increased metabolic clearance of alprazolam (Smith et al. 1983). Subjects suffering from renal or hepatic disease as well as obese subjects in excess of 30% of ideal body weight were excluded since alprazolam clearance may be reduced (Ochs et al. 1986, Juhl et al. 1984, Abernethy et al. 1984) and sensitivity to the sedative effects of alprazolam may be heightened (Schmith et al. 1992). Users of illicit drugs were excluded since concurrent drug use could interfere with pharmacodynamic assessments of alprazolam (e.g. morphine could cause excessive sedation when combined with alprazolam). Finally, subjects with abnormal biochemical or hematological tests, as judged by the study physician, were excluded due

to concerns for their safety and the possibility of undiagnosed medical conditions.

In accordance with the exclusion criteria, subjects were not permitted to consume grapefruit juice at any time during the study, and were not permitted to consume alcohol or caffeine for at least 12 hours before attending a test session. Breathalyzer tests and urine drug screens were performed before each test session to help ensure compliance with the study protocol. During test sessions 1 and 2, subjects received alprazolam (1 mg orally) or placebo in a random order (Figure 2). Each subject was then assigned to receive one of three clinically relevant doses of sertraline: either 50, 100, or 150 mg per day, taken with dinner. Adherence to sertraline administration was assessed by plasma concentrations, pill counts, and a daily medication log. To achieve steady-state sertraline and desmethylsertraline concentrations, subjects administered sertraline for a minimum of 14 days at the assigned dose and, while continuing sertraline treatment, subjects attended test sessions 3 and 4 where they again received alprazolam or placebo in a random order. After completing these test sessions, five subjects elected to participate in two additional test sessions following 14 days of sertraline administration at a different dosage. One subject participated at three doses of sertraline. Therefore, we have data on 6 subjects at 50 mg sertraline, 4 subjects at 100 mg sertraline and 6 subjects at 150 mg sertraline.

FIGURE 2: Study Design



2.2 TEST SESSION PROCEDURES

At each test session, blood samples were taken at baseline, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 24, and 32 hours after receiving alprazolam or placebo. Over the first 8 hours of each session, immediately following each blood sample, subjects were asked to perform a series of tests to assess adverse effects of alprazolam (sedation, psychomotor impairment, and memory impairment). Selection of the testing procedures was based on a variety of factors including their use in similar studies involving benzodiazepines, their specificity in measuring the desired adverse effect, our past experience with the tests, and their availability.

Sedation was assessed using questions from the Tufts University Benzodiazepine Scale (TUBS). The TUBS is composed of a series of 10 centimeter visual analog scales (VAS) that are presented to the subject both before (baseline) and after receiving alprazolam or placebo. Although results from any individual VAS can be reported, the scale assesses overall sedation by averaging the responses on key questions pertaining to sedation (e.g. tiredness, sleepiness, "spaced out"). The resulting sedation summary score was used in this study since it allowed subjects to report their feelings on a number of specific components of sedation rather than leaving interpretation of the term "sedation" to the subject. Furthermore, the TUBS has been used extensively by other investigators in the pharmacodynamic assessment of sedative effects of

benzodiazepines (Shader et al. 1986, Greenblatt et al. 1988, Greenblatt et al. 1994).

Psychomotor impairment was assessed using a computerized manual tracking test and a digit symbol substitution test (DSST), presented before (baseline) and after administration of alprazolam or placebo. The manual tracking test requires subjects to use a sensitive joystick apparatus to keep a computerized plane centered over a road that randomly scrolls across the screen. Each assessment consists of three 20 second trials and the mean percent time spent over the road is used as the final measure of psychomotor function. The DSST requires subjects to translate a series of single digits (0-9) into symbols using a legend which the subject is able to see at all times. The legend utilizes the same set of 10 symbols in all tests, but their pairing with digits is randomized for each test. The subject is given 90 seconds in which to make as many substitutions as possible, and the number of correct substitutions is used as a measure of psychomotor function. Both the manual tracking test (Shaw et al. 1989, Sullivan et al. 1989, Naranjo et al. 1984) and the DSST (Ozdemir et al. 1997, Greenblatt et al. 1991b, Shaw et al. 1989) have been used by other investigators in the assessment of psychomotor function.

Memory impairment (immediate and delayed recall) was assessed using an auditory memory test based on the Rey Auditory Verbal Learning Test (Lezak 1995). Subjects were presented with an audio

listing of 15 common words after which they were asked to recall as many of the words as possible, in any order, within a one minute time frame. This process was repeated twice with the same list of words being presented in different (randomized) orders. The maximum number of correctly recalled words over the three trials was used as our measure of immediate recall. Following a period of approximately 10 minutes during which other pharmacodynamic testing was completed, subjects were asked to recall as many words as possible from the list, in any order, within a one minute time frame. The number of correctly recalled words was used as our measure of delayed recall. New word lists were used for each administration of the test and words were not repeated. Similar testing procedures have been used to assess immediate and delayed recall in many other studies involving benzodiazepines (Shader et al. 1986, Greenblatt et al. 1988, Greenblatt et al. 1994) and this particular procedure has been used by one other investigator (Ozdemir et al. 1997)

To reduce learning effects (the potential of subjects to improve their scores as they gain more experience with the tests) subjects were trained and required to obtain acceptable and consistent results in all tests before being allowed to continue in the study. In the DSST, subjects needed to correctly substitute at least 30 symbols in 90 seconds while in the manual tracking test subjects were required to have a mean score of at least 80% time over the road. In the immediate and delayed recall tests, subjects needed to correctly recall at least 10 of the 15 words.

2.3 CYP3A4 PHENOTYPING

Phenotyping of subjects for CYP3A4 activity was performed before the first test session (as a baseline measure of enzyme activity) and following 14 days of sertraline administration at an assigned dose. The procedure was based on a method by Jones et al. (1996) which used oral dextromethorphan (DM) as a probe drug. Subjects were asked to empty their bladders, swallow a 30 mg oral dose of DM, and then to collect all of their urine for a period of 72 hours. An aliquot of urine could then be analyzed for concentrations of DM and its CYP3A4 metabolite, 3-methoxymorphinan (3-MM). The ratio of DM to 3-MM was to be used as an index of CYP3A4 activity.

The method was selected for use in this study because it posed advantages over tests such as the Erythromycin Breath Test and the midazolam test. In particular, it allowed for oral administration of the probe drug which did not require the presence of the investigator or medical personnel. Furthermore, it did not expose the subject to radioactivity (as in the EBT) or to repeated blood sampling (as in the midazolam test). It was however a newly developed procedure that had not been validated by other investigators.

2.4 MEASUREMENT OF ALPRAZOLAM AND SERTRALINE PLASMA CONCENTRATIONS

Blood samples were centrifuged at 3400 rpm for 10 minutes, after which plasma was separated and frozen at -20°C until analysis. All samples were assayed for total plasma alprazolam concentrations. Due to the lack of clinical significance and low concentrations of 4-hydroxy-alprazolam and alpha-hydroxyalprazolam metabolites following a single 1 mg oral dose, assays were not performed to determine their concentrations (Greenblatt et al. 1993a). As a measure of adherence to the study protocol, the final sample from each test session was assayed for sertraline and desmethylsertraline plasma concentrations. All assays were performed at the Psychopharmacology Analytical Laboratory, Queen Street Mental Health Division of the Addiction and Mental Health Services Corporation, Toronto, Canada.

Alprazolam plasma concentrations were assayed by gas chromatography - mass spectrometry (GCMS) (Greenblatt 1990, Greenblatt et al. 1983d). A Fisions VG TRIO-1000 GCMS with a quadruple system and AS800 autosampler was used. The column was a 7.5 m DB-5 J & W and the carrier gas was helium at a flow-rate of 4 ml/min. Source temperature was set at 250°C, and splitless injector temperature at 240°C. Initial oven temperature rose at 20°C/min from 140°C to 265°C. Base peaks for alprazolam and adinazolam (internal standard) were 273 and 308 nm respectively, and quantitation was

performed by calculating the alprazolam to adinazolam ratio. Alprazolam samples were processed by solid phase extraction using Waters LC-18 cartridges. Briefly, adinazolam was added to the solid phase columns followed by 0.5 ml unknown plasma or known standards (0, 2.5, 5.0, 7.5, 10, 15, 20, 40, 60 nM) or plasma quality controls (blank plasma spiked with 5, 10, 20, 50 nM alprazolam). Samples were pumped through the columns and washed with 1 ml wash solution (50 mM H3PO4, 5% MeOH, pH 9) then 1 ml rinse solution (40% MeOH, 15 mM phosphate, pH 9). Samples were then eluted with 1 ml elution solution (37.5 mM phosphate, 80% MeOH, pH 2) and collected into 3 ml reacti-vials. All eluents were basified with 400 µl of 4M NH_4OH and extracted into 200 µl butyl acetate before injection into the GCMS. The minimum level of detection was 1.0 nM. For concentrations of 3 to 50 nM, the coefficient of variation was 4.8%. For concentrations below 2.5 nM, the coefficient of variation was 27%.

Sertraline and desmethylsertraline plasma concentrations were assayed by reversed-phase liquid chromatography on a Hewlett-Packard HP1050 machine with an autosampler set to an injection volume of 100 µl. The flow rate was 1.5 ml/min at 40°C and the detection wavelength was set at 215 nm. Separation was performed on a reverse phase 25 cm X 4.6 mm CSC ODS2 C_{18} 5 µm column. The mobile phase consisted of 34.65 µM dimethyloctylamine in a mixture of 50 mM phosphate buffer (pH 2) and acetonitrile mixed 65:35 by volume. Extraction was performed

using solid phase LC-8 tubes, which were washed with two 1 ml aliquots of methanol, two 1 ml aliquots of elution solution (37.5 mM H_3PO4 and 70% MeOH), and one 1 ml aliquot of wash solution (50 mM sodium phosphate adjusted to pH 9 with 50 mM H_3PO4, 200 mM NaCl and 5% MeOH by volume). Briefly, 200 µL of doxepin (1200 nM, internal standard) were added to each tube, followed by 0.5 ml unknown plasma. Tubes were washed with two 1 ml aliquots of wash solution, rinsed with 1 ml rinse solution (15 mM phosphate pH 8.5 and 70% MeOH by volume), and eluted with 0.5 ml elution solution under vacuum for injection into the HPLC. Standards were prepared by addition of 200 µl internal standard to known amounts of sertraline and desmethylsertraline standard solutions made to 1000 nM in plasma (final concentrations ranged from 10 nM to 500 nM). All subject and control sample concentrations were determined using the peak height ratio of sertraline/doxepin and desmethylsertraline/doxepin. The limit of detection was 2 nM for both sertraline and desmethylsertraline. The coefficients of variation for sertraline and desmethylsertraline were 11.8% and 5.1% respectively for concentrations below 25 nM, and 4.1% and 3.4% for concentrations above 50 nM.

2.5 DATA ANALYSIS

Alprazolam kinetic parameters - peak concentration (C_{max}), time to peak concentration (t_{max}), elimination half-life ($t_{1/2(\beta)}$), and area under the concentration-time curve from baseline to infinity ($AUC_{0-\infty}$) - were calculated using non-compartmental methods. For each subject, regression analysis of the terminal portion of the log concentration-time graph, over a period of at least 21 hours, was used to determine the elimination constant (K_e) (Gibaldi and Perrier 1975). Pharmacodynamic parameters (peak effect, time to peak effect, AUC_{0-3} hours and AUC_{0-8} hours) were calculated for each test. AUC for pharmacodynamic testing was defined as a measure of the overall effect of treatment(s) during a specified time period, independent of fluctuations occurring between time points. Calculations of AUC_{0-3} hours were replaced by AUC_{0-4} hours in immediate and delayed recall analyses due to the lack of a time point at 3 hours. Peak effect was calculated as the maximum percent increase or decrease from baseline. AUC was calculated using the trapezoidal method (Gibaldi and Perrier 1975).

All statistical comparisons within dosage groups were made using paired tests (t-tests or Wilcoxon signed-rank test) and ANOVA, where possible (SPSS 1993). Kinetic data were compared for alprazolam C_{max}, T_{max}, $t_{1/2(\beta)}$, and $AUC_{0-\infty}$ before and after sertraline treatment for each dosage group. Pharmacodynamic data were similarly compared for peak effect, time to peak effect, AUC_{0-3} or AUC_{0-4} hours, and AUC_{0-8} hours

before and after sertraline treatment for each dosage group. The effect of alprazolam alone was compared to the effect of placebo alone (n = 10) to determine if our tests were sensitive to the effects of alprazolam alone. The effect of sertraline&placebo was compared to the effect of placebo alone (n = 16) to determine if sertraline produced any significant changes in test results in the absence of alprazolam. The interaction between sertraline and alprazolam was assessed by comparing the effect of the drug combination (alprazolam&sertraline - placebo alone) to the sum of the effect of alprazolam alone (alprazolam alone - placebo alone) and sertraline alone (sertraline&placebo - placebo alone). If a significant interaction was detected, the effect was quantified using linear regression to determine if the interaction depended on the dose of sertraline. The effect was also quantified by ANOVA to examine how the interaction depended on sertraline dosage in the absence of a linear relationship.

All pharmacodynamic data were assessed for order of treatment effects by two-way ANOVA using sertraline dosage as an independent variable to compare subjects who received alprazolam first to those who received placebo first for each pair of test sessions (i.e. test sessions 1 and 2 formed the first pair, test sessions 3 and 4 formed the second pair). If an order effect was detected, individual dosage groups were assessed by two-sample t-tests (Armitage and Berry 1994). Two methods were used to derive the scores used assess order of treatment effects: subtraction of scores and addition of scores. Subtraction of scores

calculated the effect of alprazolam alone (alprazolam alone - placebo alone) for test sessions before sertraline pretreatment and calculated the effect of alprazolam in the presence of sertraline (alprazolam&sertraline - placebo&sertraline). Addition of scores calculated the total response to alprazolam and placebo before sertraline pretreatment (alprazolam alone + placebo alone) and after sertraline pretreatment (alprazolam&sertraline + placebo&sertraline). Calculated scores were then categorized into two groups according to the order in which alprazolam was administered (alprazolam-first and placebo-first) and compared by two-sample t-tests.

SECTION #3: RESULTS

3.1 SUBJECT CHARACTERISTICS

The mean age of the ten subjects was 26 years (range: 20 to 43 years); eight were female and two were male (Table 1). All subjects were deemed to be compliant with the study protocol. Breathalyzer tests (conducted on all subjects) and urine drug screens (conducted on 17% of collected samples) concurred with subject reports that no drugs known to interact with CYP3A4 were consumed during the study period and that no drugs known to cause central nervous system effects were consumed before or during the test sessions. Sertraline plasma concentrations varied between 22 and 384 ng/ml (desmethylsertraline range: 23-357 ng/ml), consistent with reported ranges during chronic dosing (Greist et al. 1995b). Nine subjects reported adverse effects from sertraline including nausea, sleep disturbances, decreased appetite, sexual dysfunction, sweating, and headache. These effects were generally transient and no subject discontinued treatment. There were no adverse events associated with the test sessions.

3.2 DETECTION OF THE EFFECT OF ALPRAZOLAM ALONE

On each pharmacodynamic test (sedation, DSST, manual tracking, immediate recall and delayed recall) a significant alprazolam effect was

TABLE 1: Subject Characteristics

Subject Number	Gender	Doses of Sertraline Received (mg/day)	Age (years)	Weight (kg)	Concomitant Medications
1	Male	150	23	66	none
2	Male	50, 100, 150	43	105	levothyroxine 0.1mg (p.o., once daily)
3	Female	50	26	55	none
4	Female	50	33	48	salbutamol (100 μg/dose, inhaler)*, cromolyn sodium (2 mg qid, inhaler)
5	Female	50	25	64	none
6	Female	50, 150	24	64	none
7	Female	50	20	69	none
8	Female	100, 150	23	66	none
9	Female	100, 150	22	52	none
10	Female	100, 150	22	66	fluticasone propionate (100 mg, bid, inhaler)

*prn (not used on study days)

detected compared to placebo alone for peak effects, AUC_{0-3} or AUC_{0-4}, and AUC_{0-8} (n=10) (Table 2). Time to peak effect was not significantly different from placebo in any test.

3.3 DETECTION OF THE EFFECT OF SERTRALINE ALONE

Pharmacodynamic testing performed after receiving sertraline&placebo showed no significant differences from placebo alone in measures of peak effect, time to peak effect, AUC_{0-3} or AUC_{0-4}, and AUC_{0-8} (n=16) (Table 2).

3.4 INFLUENCE OF SERTRALINE ON ALPRAZOLAM KINETICS

Alprazolam kinetic parameters (C_{max}, T_{max}, $t_{1/2(\beta)}$, $AUC_{0-\infty}$) did not change in the presence of sertraline (Table 3) with the exception of a decreased C_{max} in the 50 mg sertraline group. Although alprazolam C_{max} was lower in all three sertraline dosage groups (Figure 3) compared to alprazolam alone, only the 50 mg group reached statistical significance ($p = 0.05$).

TABLE 2: Summary of Significance Testing for Pharmacodynamic Results (p-values)

	Analysis of Drug Effects & Interaction Effects					*Analysis of Order Effects*									
	Identify Effect/Interaction			Quantification		First 2 days		Sertaline days (Note:100mg group not tested)							
	alp vs. pla n=10	*sert vs. pla n=16*	*Interaction effect n=16*	*Interaction (by sertraline dosage group) n=16*		*t-tests n=10 alp first=4 pla first=6*		*t-tests n=16 alp first=7 pla first=2*		*t-tests 50mg n=6 alp first=4 pla first=2*		*t-tests 150mg n=6 alp first=3 pla first=3*		*anova (with sert dosage as a factor)*	
Measure	t-test	t-test	Wilcoxon	LR	anova	diff scores	add scores	diff scores	add scores	diff scores	add scores	diff scores	add scores	diff scores	add scores
Sedation															
- Peak Effect	<.001	0.70	0.20	0.40	0.40	0.40	0.50	0.10	0.10	0.02	0.03	0.30	0.80	0.90	0.10
- Time to Peak	0.30	0.20	0.70	0.03	0.08	0.40	0.90	0.40	0.60	0.60	0.20	0.70	0.50	0.02	0.20
- AUC (0-3 hrs)	<.009	0.50	0.80	0.20	0.30	0.40	0.40	0.20	0.50	0.30	0.40	0.80	0.80	0.50	0.60
- AUC (0-8 hrs)	0.05	0.50	0.40	0.30	0.30	0.70	0.30	0.50	0.60	0.01	0.10	0.70	0.60	0.80	0.30
Tracking															
- Peak Effect	0.00	0.60	0.03	0.06	0.06	0.20	0.10	0.80	0.60	0.60	0.50	0.20	0.40	0.07	0.10
- Time to Peak	0.40	0.50	0.30	0.90	0.90	0.30	0.40	0.10	0.60	0.70	0.60	0.30	0.70	0.50	0.60
- AUC (0-3 hrs)	0.01	0.40	0.20	0.20	0.20	0.08	0.50	0.60	0.30	0.80	0.90	0.50	0.80	0.20	0.50
- AUC (0-8 hrs)	0.02	0.90	0.10	0.10	0.20	0.30	0.60	0.90	0.30	0.80	0.50	0.06	0.50	0.10	0.60
DSST															
- Peak Effect	<.001	0.90	0.30	0.07	0.06	0.20	0.40	0.50	0.40	0.40	0.20	0.40	0.70	0.07	0.20
- Time to Peak	0.60	0.40	0.80	0.30	0.20	0.30	0.70	0.09	0.40	0.80	0.70	0.40	0.60	0.01	0.70
- AUC (0-3 hrs)	<.001	0.20	0.80	0.10	0.09	0.20	0.70	0.30	0.30	0.50	0.10	0.70	0.80	0.09	0.40
- AUC (0-8 hrs)	<.001	0.20	0.30	0.06	0.10	0.50	0.40	0.30	0.30	1.00	0.10	1.00	0.50	0.01	0.20
Imm. Recall															
- Peak Effect	0.00	0.30	0.30	0.60	0.10	0.05	0.60	1.00	0.40	0.50	0.10	0.00	0.30	0.00	0.03
- Time to Peak	0.60	0.20	0.30	0.80	0.60	0.20	0.30	1.00	0.30	0.70	0.70	0.40	0.80	0.06	0.50
- AUC (0-3 hrs)	<.001	0.30	0.30	0.90	0.30	0.80	0.70	0.90	0.80	0.70	0.30	0.02	1.00	0.00	0.70
- AUC (0-8 hrs)	<.001	0.60	0.20	0.20	0.10	0.30	0.90	0.70	1.00	0.40	0.40	0.01	1.00	0.00	0.20
Del. Recall															
- Peak Effect	<.001	0.20	0.20	0.90	0.90	0.07	0.04	1.00	1.00	0.70	0.20	0.20	0.40	0.50	0.60
- Time to Peak	0.60	0.30	0.60	0.08	0.20	0.20	0.30	0.40	0.80	0.80	0.60	0.20	0.20	0.30	0.60
- AUC (0-3 hrs)	<.001	0.72	0.09	0.70	0.09	0.60	0.20	0.30	0.40	0.40	0.30	0.10	0.40	0.10	0.08
- AUC (0-8 hrs)	<.001	0.40	0.10	0.60	0.80	0.10	0.50	0.30	0.80	0.50	0.30	0.08	1.00	0.06	0.10

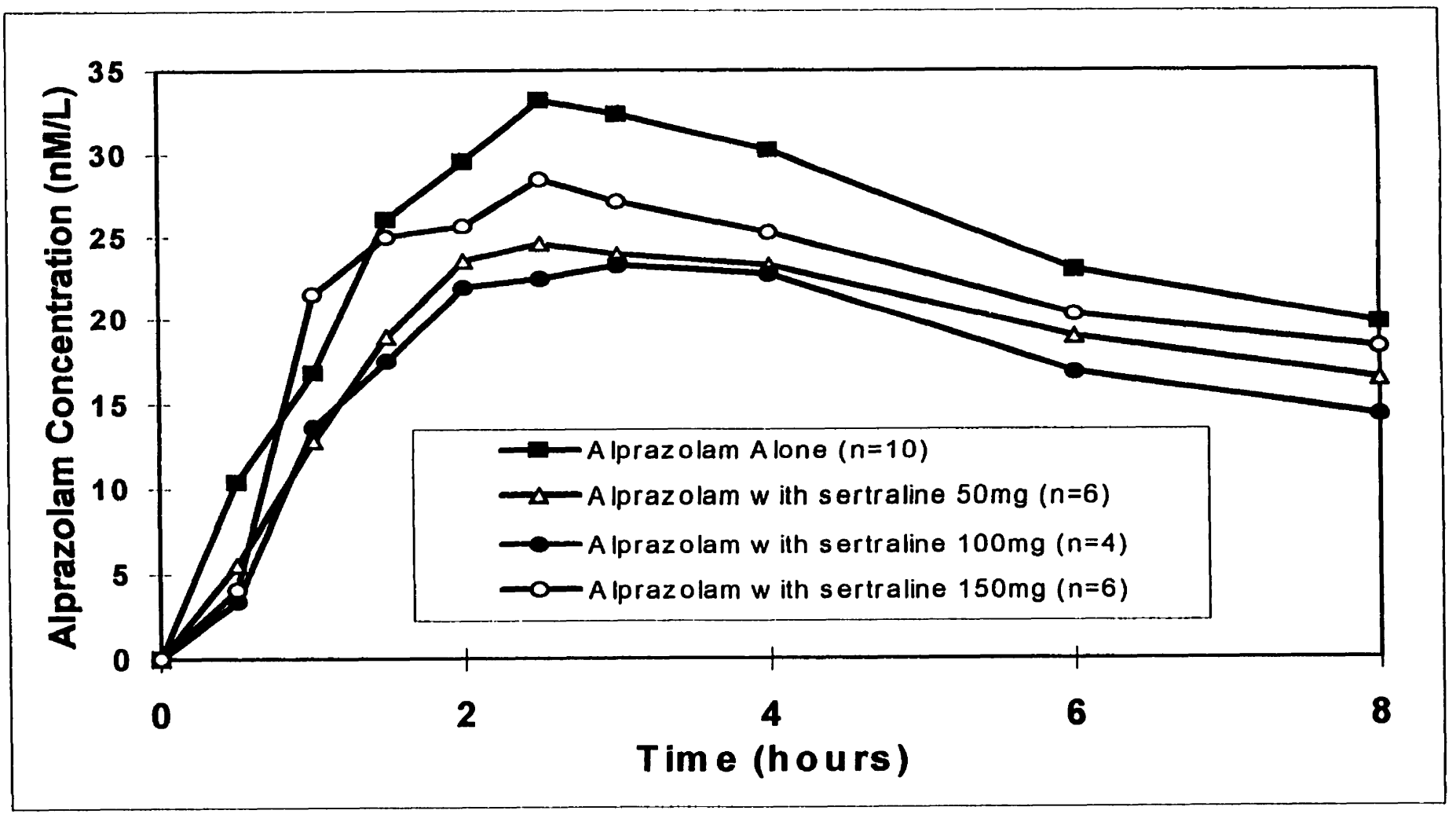
Abbreviations: alp=alprazolam, sert=sertraline, pla=placebo, LR=linear regression, diff=difference, add=addition, AUC=area under curve. DSST=Digit Symbol Substitution Test, Imm=immediate, Del=delayed. Highlighting indicates p values less than 0.05

TABLE 3: Alprazolam Pharmacokinetic Parameters (Mean ± SD)

	Alprazolam n=10	Alprazolam/ Sertraline 50mg n=6	Alprazolam/ Sertraline 100mg n=4	Alprazolam/ Sertraline 150mg n=6
C_{max}	39.7 nM/L (± 18.8)	27.7 nM/L* (±7.4)	24.5 nM/L (± 3.4)	34.2 nM/L (± 11.7)
T_{max}	2.5 hours (± 1.1)	2.5 hours (± 0.9)	2.6 hours (± 1.3)	2.0 hours (± 0.8)
$t_{1/2}$	11.7 hours (± 2.5)	12.2 hours (± 3.3)	12.4 hours (± 5.6)	14.2 hours (± 7.1)
$AUC_{0-\infty}$	571.0 (± 176.8)	458.8 (± 85.9)	422.7 (± 113.2)	566.3 (± 185.6)

*Wilcoxon matched-pairs significance compared to alprazolam alone, p=0.05

FIGURE 3: Mean Alprazolam Concentrations Over Time



3.5 INFLUENCE OF SERTRALINE ON ADVERSE EFFECTS OF ALPRAZOLAM

No interactions between sertraline and alprazolam were detected in sedation, DSST, immediate and delayed recall scores for any parameter (peak effect, time to peak effect, AUC_{0-3} or AUC_{0-4}, and AUC_{0-8}) at any dose of sertraline. In the manual tracking test, an interaction effect was detected in peak performance ($p=0.03$) such that the peak decrease in performance on the drug combination was greater than would be expected by adding the effects of sertraline and alprazolam alone. This interaction was not significantly related to sertraline dosage, although there was a trend toward an increased interaction at higher doses of sertraline (multiple regression, $p=0.06$). No interaction effects were observed in any other manual tracking test parameters. Figures 4A to 4D illustrate the mean peak effect, time to peak effect, AUC_{0-3} hours, and AUC_{0-8} hours for the sedation scale in each of the four test sessions, divided into sertraline dosage groups. Figures 5, 6, 7 and 8 illustrate the same parameters for the DSST, manual tracking, immediate recall, and delayed recall tests, respectively. Mean values for all pharmacodynamic parameters are summarized in Table 4.

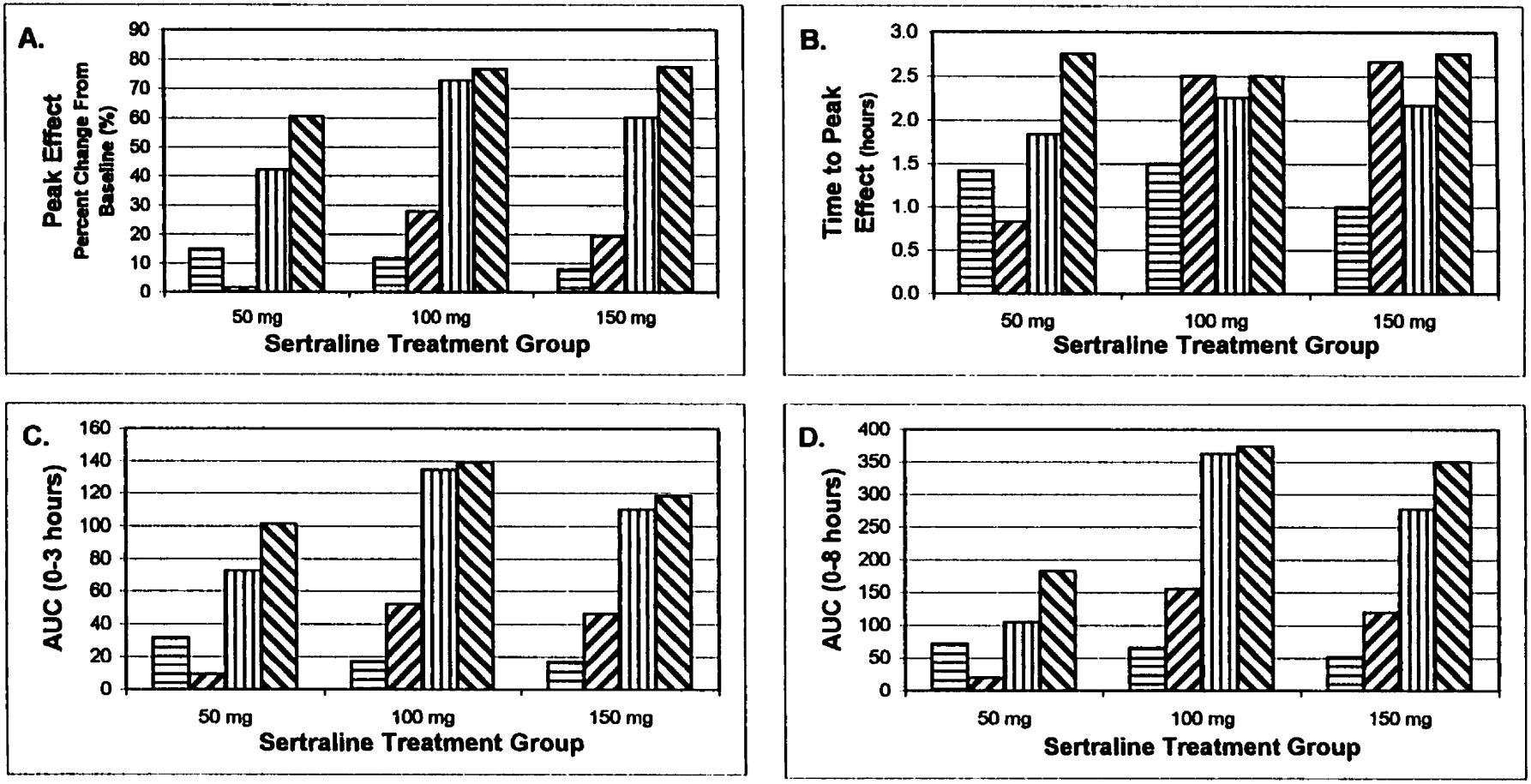


FIGURE 4: ***Summary of the Sedation Test***

Mean peak percent increase from baseline (A), time to peak effect (B), AUC 0-3 (C), AUC 0-8 (D) for Sedation Summary Scores, divided according to sertraline dosage groups. Legend: = placebo alone, = placebo + sertraline, = alprazolam alone, = alprazolam + sertaline. AUC = area under curve.

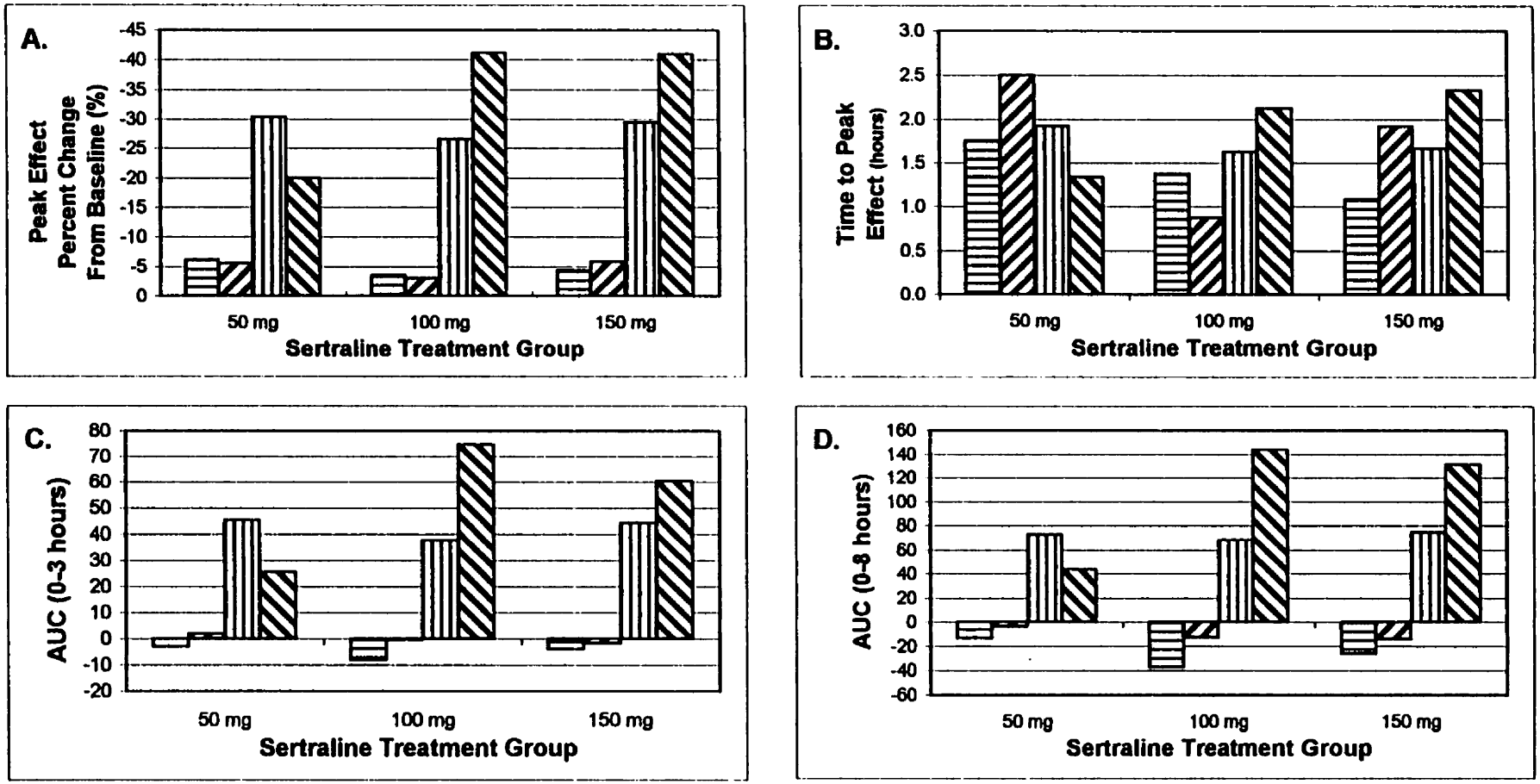


FIGURE 5: ***Summary of the Digit Symbol Substitution Test (DSST)***

Mean peak percent increase from baseline (A), time to peak effect (B), AUC 0-3 (C), AUC 0-8 (D) for Sedation Summary Scores, divided according to sertraline dosage groups. Legend: = placebo alone, = placebo + sertraline, = alprazolam alone, = alprazolam + sertaline. AUC = area under curve.

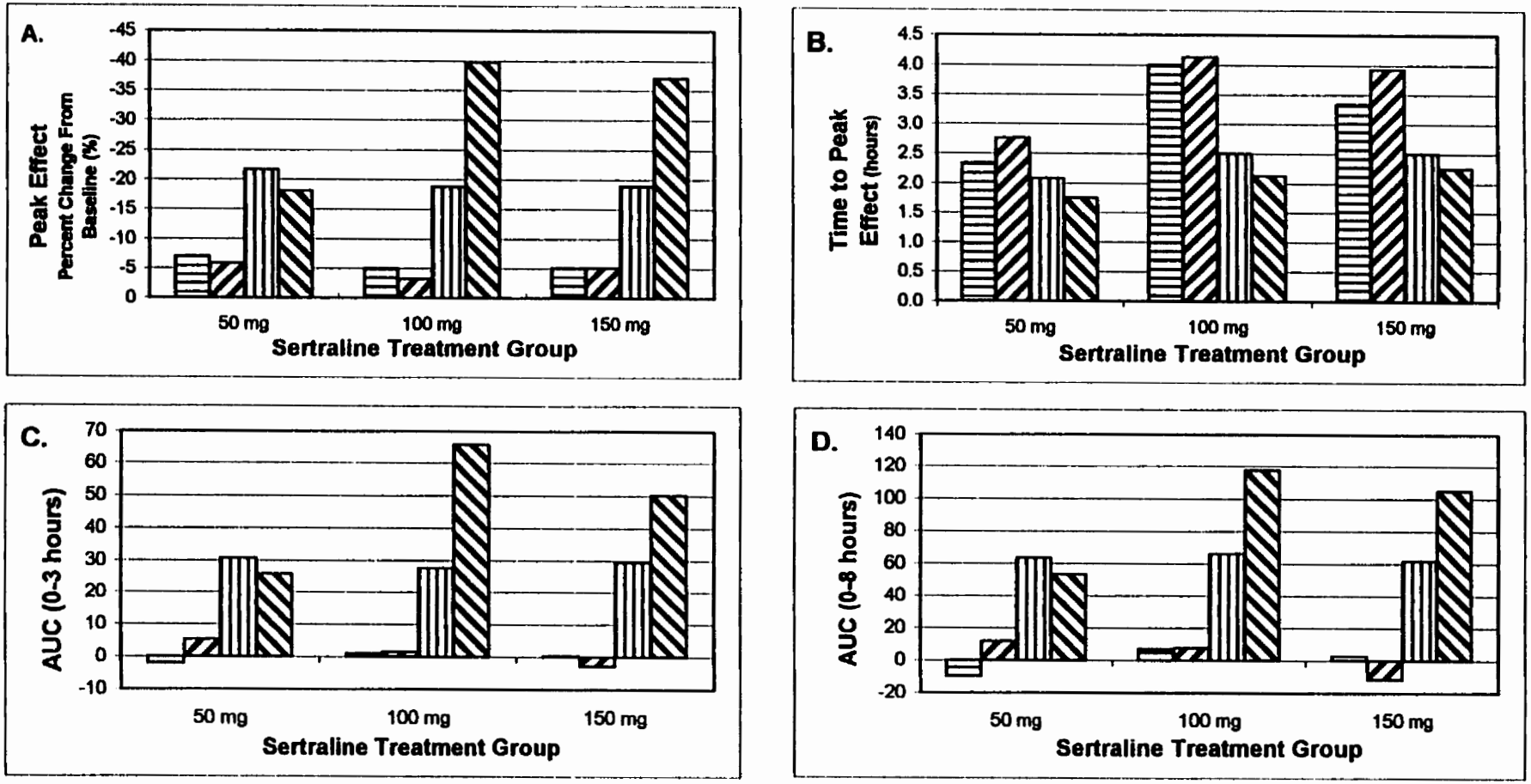


FIGURE 6: ***Summary of the Manual Tracking Test***

Mean peak percent increase from baseline (A), time to peak effect (B), AUC 0-3 (C), AUC 0-8 (D) for Sedation Summary Scores, divided according to sertraline dosage groups. Legend: = placebo alone, = placebo + sertraline, = alprazolam alone, = alprazolam + sertaline. AUC = area under curve.

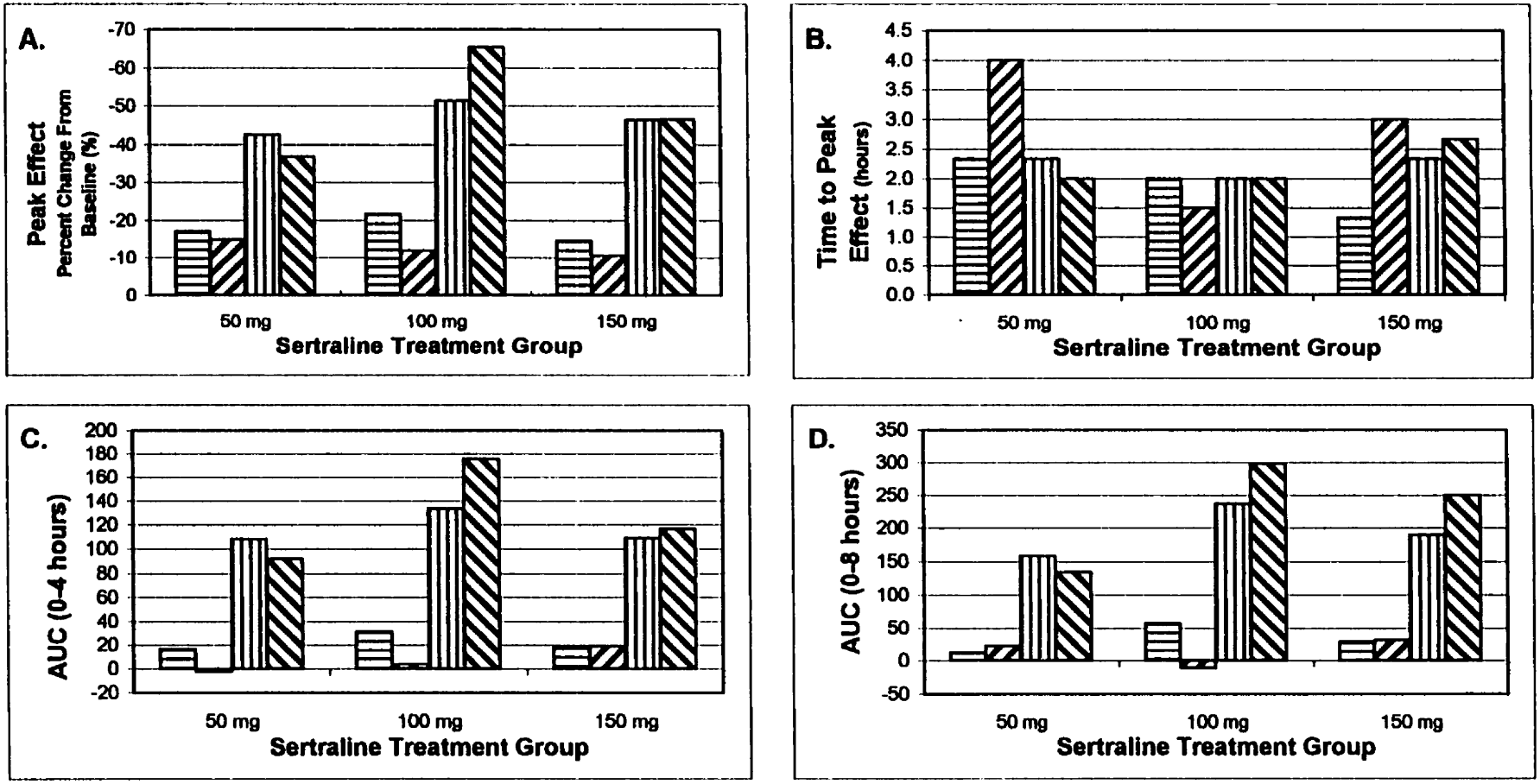


FIGURE 7: *Summary of the Immediate Recall Test*

Mean peak percent increase from baseline (A), time to peak effect (B), AUC 0-4 (C), AUC 0-8 (D) for Sedation Summary Scores, divided according to sertraline dosage groups. Legend: = placebo alone, = placebo + sertraline, = alprazolam alone, = alprazolam + sertaline. AUC = area under curve.

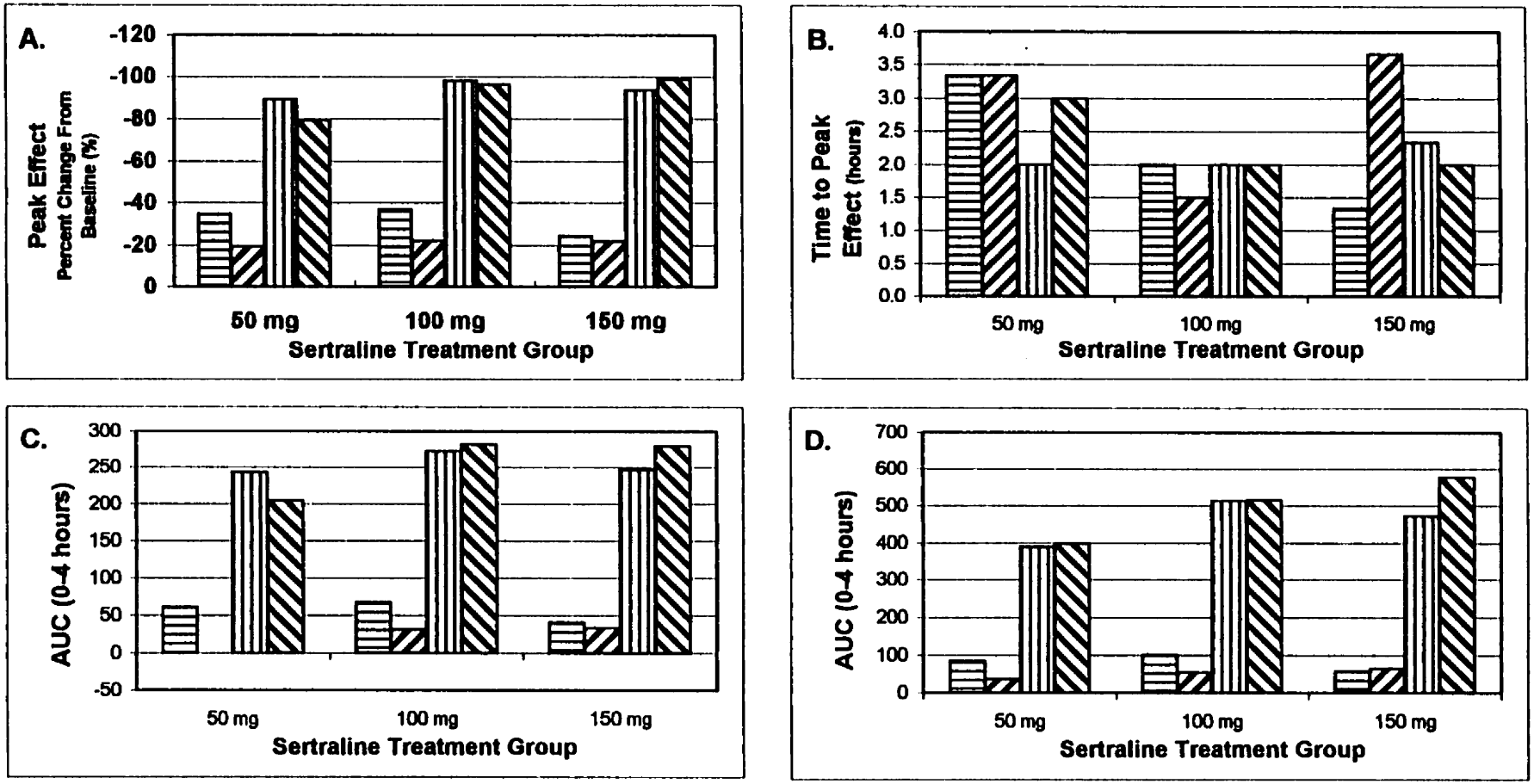


FIGURE 8: ***Summary of the Delayed Recall Test***

Mean peak percent increase from baseline (A), time to peak effect (B), AUC 0-4 (C), AUC 0-8 (D) for Sedation Summary Scores, divided according to sertraline dosage groups. Legend: = placebo alone, = placebo + sertraline, = alprazolam alone, = alprazolam + sertaline. AUC = area under curve.

TABLE 4: Mean values for pharmacodynamic parameters grouped by dosage of sertraline

	ALPRAZOLAM			PLACEBO			ALPRAZOLAM & SERTRALINE			PLACEBO & SERTRALINE		
	50 mg group	100 mg group	150 mg group	50 mg group	100 mg group	150 mg group	50 mg group	100 mg group	150 mg group	50 mg group	100 mg group	150 mg group
SEDATION												
- Peak Effect*	42.2	72.8	60.2	14.8	11.8	7.8	60.5	76.5	77.3	1.5	27.8	19.2
- Time to Peak Effect**	1.8	2.3	2.2	1.4	1.5	1.0	2.8	2.5	2.8	0.8	2.5	2.7
- AUC (0-3 hours)	72.6	134.1	110.0	31.6	17.0	16.9	101.4	138.9	118.0	9.3	52.1	46.0
- AUC (0-8 hours)	104.4	362.6	276.6	71.1	65.6	51.7	182.0	374.1	350.0	20.5	154.5	119.2
DSST												
- Peak Effect*	-30.4	-26.6	-29.4	-6.2	-3.5	-4.3	-20.0	-41.2	-41.0	-5.6	-3.0	-5.8
- Time to Peak Effect**	1.9	1.6	1.7	1.8	1.4	1.1	1.3	2.1	2.3	2.5	0.9	1.9
- AUC (0-3 hours)	45.4	37.8	44.3	-3.0	-8.1	-4.1	25.3	74.7	60.4	1.8	-0.5	-1.8
- AUC (0-8 hours)	72.8	68.6	74.6	-13.2	-36.9	-25.9	43.7	143.9	131.1	-3.3	-12.5	-13.9
MANUAL TRACKING												
- Peak Effect*	-21.7	-18.8	-19.0	-6.9	-5.0	-5.1	-18.0	-39.7	-37.1	-5.8	-3.2	-5.1
- Time to Peak Effect**	2.1	2.5	2.5	2.3	4.0	3.3	1.8	2.1	2.3	2.8	4.1	3.9
- AUC (0-3 hours)	30.7	27.5	29.4	-2.0	1.0	0.4	25.7	65.7	50.1	5.3	1.5	-2.8
- AUC (0-8 hours)	63.3	66.1	61.7	-9.8	6.7	2.7	53.3	117.3	104.8	11.4	7.5	-11.4
IMMEDIATE RECALL												
- Peak Effect*	-42.6	-51.3	-46.4	-17.1	-21.8	-14.5	-36.8	-65.5	-46.5	-14.9	-11.9	-10.6
- Time to Peak Effect**	2.3	2.0	2.3	2.3	2.0	1.3	2.0	2.0	2.7	4.0	1.5	3.0
- AUC (0-4 hours)	108.2	133.5	108.9	16.0	30.9	17.8	92.1	175.5	116.6	-2.6	3.7	19.2
- AUC (0-8 hours)	158.3	235.8	189.0	12.4	57.0	28.2	134.3	298.5	249.7	23.2	-10.8	31.2
DELAYED RECALL												
- Peak Effect*	-89.4	-97.9	-93.8	-34.8	-36.7	-24.5	-79.0	-96.2	-98.7	-19.3	-22.0	-21.8
- Time to Peak Effect**	2.0	2.0	2.3	3.3	2.0	1.3	3.0	2.0	2.0	3.3	1.5	3.7
- AUC (0-4 hours)	242.7	272.7	246.8	61.2	67.4	40.4	204.2	281.5	279.3	-0.4	31.1	33.2
- AUC (0-8 hours)	388.8	512.1	471.3	84.1	102.4	59.2	398.4	514.6	576.6	36.4	54.4	65.2

* The peak effect measure is given as mean percent change from baseline; ** Time to peak effect represents time, in hours, required to reach the peak effect; The AUC measures represent area under the curve from 0 - 3 hours, 0-4 hours or 0-8 hours

AUC = Area under curve; DSST = Digit Symbol Substitution Test

3.6 INFLUENCE OF TREATMENT ORDER ON ADVERSE EFFECTS OF ALPRAZOLAM

To assess order of treatment effects, results from each parameter of all tests were analyzed by ANOVA. No order effects were detected in measures of sedation, DSST, manual tracking, or delayed recall (Table 2). For immediate recall scores, a dose-related order effect was detected and t-tests determined that the effect was evident only in the 150 mg sertraline group for the two visits which took place in the presence of sertraline. Peak impairment ($p = 0.01$), AUC_{0-4} ($p = 0.02$) and AUC_{0-8} ($p = 0.01$) were significantly affected by the order in which subjects received alprazolam or placebo. T-tests also detected an order effect in peak sedation scores ($p = 0.02$) and AUC_{0-8} ($p = 0.01$) for the two days in the presence of sertraline in 50 mg group, but as previously stated, this effect was not evident in the overall analysis by ANOVA and was therefore not given further consideration. It should be noted that subjects from the 100 mg group could not be separately assessed for order effects since all subjects were randomized to receive placebo first. However, these subjects were used in the overall analysis by ANOVA. Analysis of the first two test sessions (in the absence of sertraline) detected a borderline significant order effect in peak scores for delayed recall ($p = 0.04$) by one method of analysis (addition of scores) but this

effect was not confirmed by the other method (difference of scores) and was therefore deemed inconsistent and was not considered further.

3.7 INFLUENCE OF SERTRALINE ON CYP3A4 ACTIVITY

An analysis of the urine samples collected in the CYP3A4 phenotyping procedures was not performed.

SECTION #4: DISCUSSION, CONCLUSIONS, RECOMMENDATIONS

4.1 GENERAL DISCUSSION

Our results indicate that sertraline does not significantly alter alprazolam kinetics and adverse effects when the two drugs are coadministered. This conclusion was based on the use of clinically relevant doses of sertraline (50 to 150 mg per day) and alprazolam (1 mg). Although only a single dose of alprazolam was administered during the study, the results are directly applicable to the use of alprazolam on an "as needed" basis and can be extrapolated to conditions of multiple-dosing where any change in single-dose alprazolam kinetics and dynamics would likely be amplified. Since few differences were observed in alprazolam kinetics and dynamics, the likelihood of an interaction between the two drugs under a multiple-dosing regimen is small.

Support for the absence of an interaction is derived from the kinetic data. Parameters of alprazolam T_{max}, $t_{1/2(\beta)}$ and $AUC_{0-\infty}$ were consistent with observations from other investigators following a single 1 mg oral dose (Greenblatt et al. 1993b) and were not significantly altered by the presence of sertraline. Alprazolam C_{max}, however, was significantly lowered by the combination of alprazolam&sertraline in the 50 mg treatment group compared to alprazolam alone. The reduction was also evident in other dosage groups but failed to reach statistical significance.

Although the reasons for these reductions remain unexplained, a reduced C_{max} in the absence of changes in other kinetic parameters may imply that sertraline alters the absorption and/or distribution of alprazolam without affecting its metabolism. The clinical significance of such a change would likely be minimal.

Nonetheless, our findings are contrary to the hypothesis that sertraline may cause inhibition of CYP3A4-mediated alprazolam metabolism *in vivo*. The most definitive method for establishing a lack of CYP3A4 inhibition would involve analysis of enzymatic activity directly, through the use of phenotyping procedures. In this study, the method described in Section 2.3 (CYP3A4 Phenotyping) would have allowed a within-subject comparison of CYP3A4 activity before and after sertraline administration to determine if inhibition of the enzyme was occurring, independent of the effects on alprazolam kinetics and adverse effects. However, analysis of the phenotyping samples collected in this study was not performed for several reasons.

Following further study of dextromethorphan as a probe for CYP3A4 activity, evidence was presented that disputed its validity and reliability. In particular, use of the urinary ratio of DM to 3-MM concentrations as an index of CYP3A4 activity was called into question when *in vitro* studies discovered that 3-MM was not produced exclusively by CYP3A4, but could also be produced by CYP2C19 and CYP2C9 (von Moltke et al. 1998b). In addition, studies failed show a correlation between CYP3A4 activity

measured by the DM probe and activity measured by other validated methods such as the midazolam test (Gorski et al. 1998) and the cortisol test (Lindley et al. 1998). As a result, the value of analyzing our phenotyping samples came into question and was delayed indefinitely. Statistical analysis of the alprazolam data then revealed that sertraline did not significantly alter the kinetics or adverse effects of alprazolam and it was therefore determined that an analysis of the effect of sertraline on CYP3A4 activity was not needed to provide additional support for the lack of a clinically significant interaction. Thus, although no definitive conclusions can be made concerning CYP3A4 activity, it seems likely that sertraline did not inhibit CYP3A4 and therefore it also seems likely that no significant metabolic interactions would exist between sertraline and other CYP3A4 substrates such as clozapine, triazolam, and midazolam.

Our findings also demonstrated that the combination of sertraline and alprazolam did not potentiate the sedation, psychomotor impairment or delayed recall impairment produced by alprazolam alone. One exception was observed in the manual tracking test where the peak percent decrease from baseline was significantly larger ($p = 0.03$) on the drug combination versus alprazolam alone. The magnitude of this effect was not related to the dose of sertraline but showed a trend toward increased impairment at higher doses of sertraline (multiple regression, $p = 0.06$). However, other parameters on the manual tracking test (T_{max}, AUC_{0-8}) were not significantly different on the drug combination versus

alprazolam alone. Furthermore, in our other measure of psychomotor performance, a digit symbol substitution test, no significant changes were observed in any parameters. This result is not surprising since the DSST is sensitive to changes in attention and concentration whereas the manual tracking test, while also requiring attention and concentration, is strongly influenced by changes in fine motor coordination. Thus the results of the two tests imply that peak fine motor coordination is significantly reduced on the drug combination compared alprazolam alone while concentration and attention remain unaffected.

To ensure the validity of our results, all parameters from each pharmacodynamic test were assessed to determine whether the order in which subjects received alprazolam or placebo affected the results of the test (i.e. an order of treatment effect). Analysis of the order effect detected in the immediate recall test showed that subjects who received alprazolam first (n = 3) experienced a greater degree of impairment during their alprazolam&sertraline test session than those who received placebo first (n = 3), for the two test sessions in the presence of sertraline. This result is consistent with a learning effect since subjects who received placebo first were able to gain more experience with the test before receiving alprazolam and therefore could be expected to have better performance. The results of the immediate recall test should therefore be interpreted with caution. However, in light of the fact that

consistent order effects were not detected in any other tests, the overall results of the study were not compromised.

Another potential area of concern in this study is the variability of the pharmacodynamic data. Due to the small sample sizes in each sertraline dosage group (n = 6,4,6), large deviations from mean values in the responses of one or two subjects could cause the comparison of mean values to not reach statistical significance. Such large variations were created in subjects who responded similarly to both placebo and alprazolam. For example, graphs of individual subject data for the delayed recall test show that subject #204 responded to placebo (in the absence of sertraline) in a manner that was quite similar to the alprazolam response (in the absence of sertraline) (Figure 9). Similarly, for the sedation test, subject #210 responded to both placebo and alprazolam in the presence of sertraline (Figure 10). Furthermore, placebo responses were generally consistent through all of the pharmacodynamic tests for a given time point (i.e. a placebo response in sedation scores was generally evident in DSST, manual tracking and recall scores as well). Although there is no method of eliminating such responses, increases in sample sizes would likely reduce the impact of the responses on significance testing.

A further limitation of the study that relates to the small sample size involves the use of subjects in more than one treatment group. Due to difficulties in the recruitment of subjects and to financial concerns, the

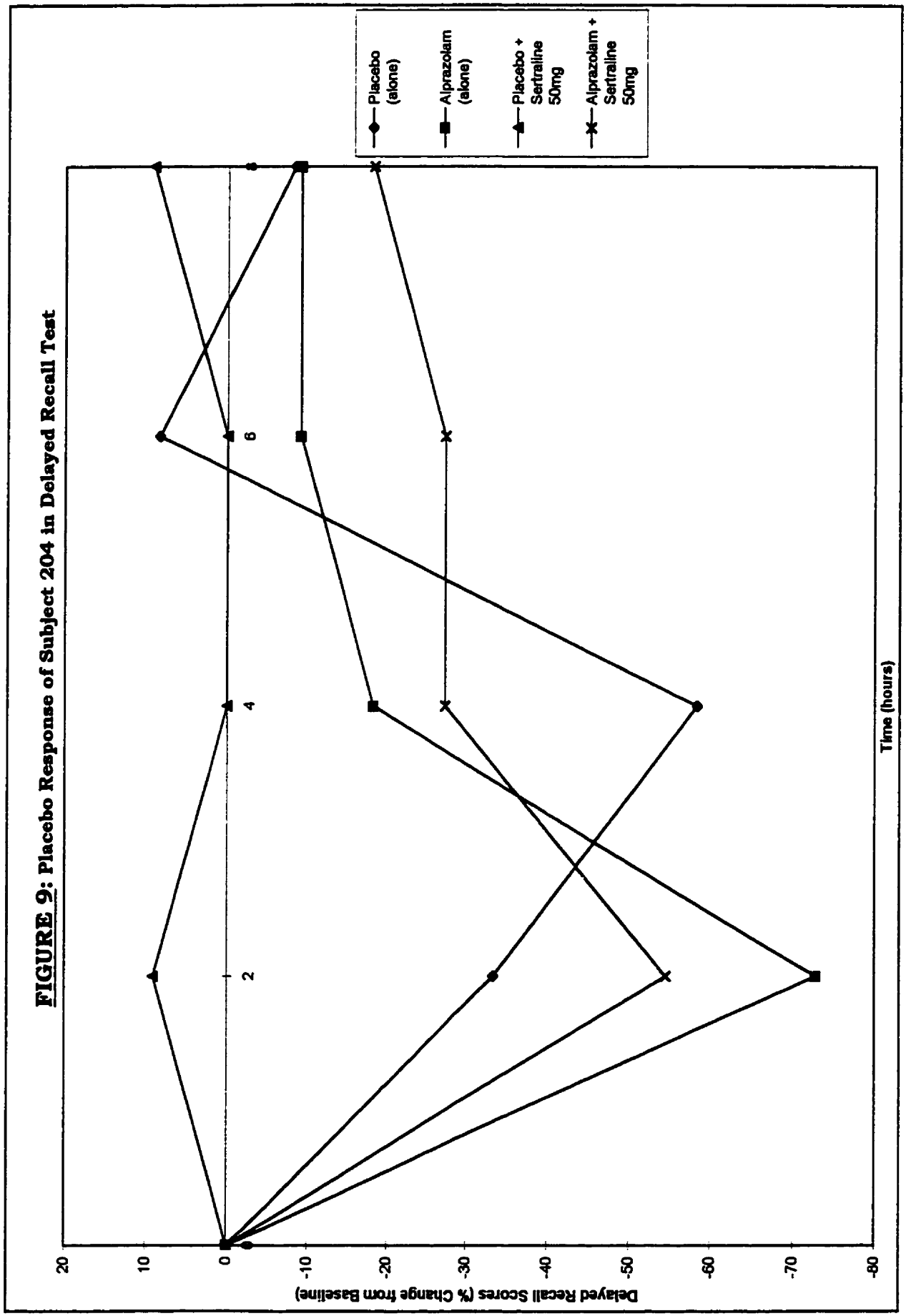
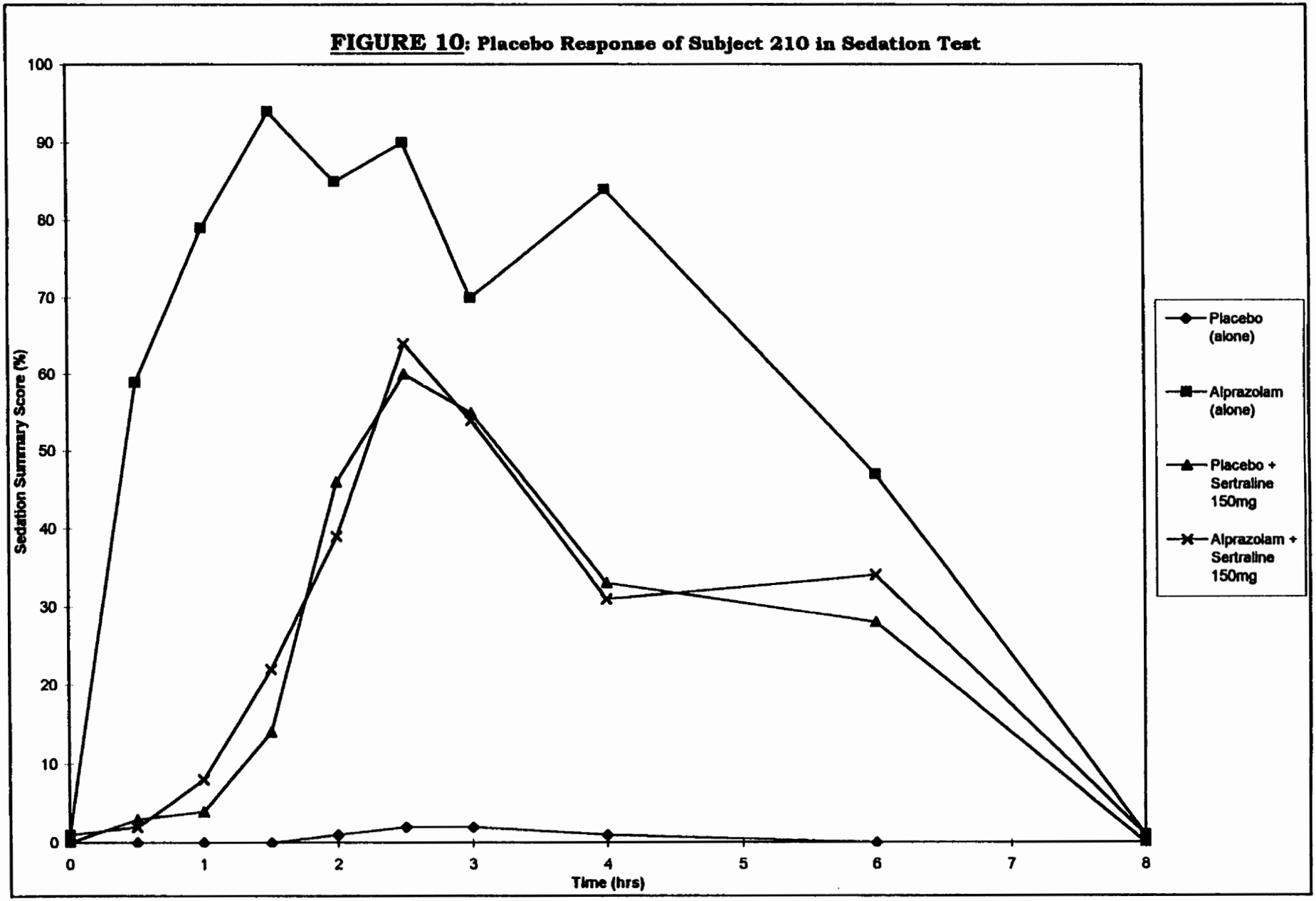


FIGURE 9: Placebo Response of Subject 204 in Delayed Recall Test

FIGURE 10: Placebo Response of Subject 210 in Sedation Test



decision was made to allow subjects to enroll in multiple treatment groups. As detailed in Section 2.1 (Study Design), five subjects participated in one treatment group, four subjects participated in two groups, and one subject participated in all three groups. This decision complicated the analysis of the data since treatment groups were no longer independent of each other, nor were they all dependent on each other. Thus a repeated measures analysis of variance, usually performed in studies of this nature, was not appropriate for the design of the study. Furthermore, in some cases, the assumption of independent groups needed to be made in order to complete the analysis. In retrospect, the choice of independent or dependent groups should have been fixed and should not have been altered, despite the difficulties in subject recruitment.

Having acknowledged the limitations of the study, it is important to consider possible reasons for the conflict between our *in vivo* findings and the *in vitro* data on inhibition of alprazolam metabolism by sertraline. Such a conflict could be caused by a variety of factors since there are many considerations in assessing the potential for a clinically relevant drug interaction from *in vitro* data. Sproule et al. (1997a) have detailed these considerations in terms of pharmacokinetic, pharmacodynamic, and patient related factors.

Pharmacokinetic factors emerge as the most likely reason for the observed lack of a clinically significant interaction. They include factors

such as: the relative *in vitro* potency of the inhibitor or inducer compared to known *in vivo* inhibitors or inducers; the predicted concentration of the inhibitor at the site of biotransformation compared to the concentration required to inhibit the enzyme *in vitro*; the saturability of the enzyme at clinically relevant concentrations of substrate and inhibitor *in vivo*; and the possibility of alternate metabolic pathways that were not explored *in vitro*. As they relate to this study, the relative *in vitro* potency of sertraline in the inhibition of alprazolam metabolism was known to be lower than that of fluoxetine and fluvoxamine, both of which had documented *in vivo* interactions with alprazolam. However the *in vivo* inhibitory potency of sertraline could not be clearly defined from these studies since they utilized more potent inhibitors of alprazolam metabolism. Furthermore, the combined inhibitory effect of sertraline and its metabolite had not been explored *in vitro*, again limiting extrapolation to the *in vivo* condition. Similarly, although the *in vitro* inhibition constant (K_i) for sertraline was known, concentrations of sertraline and desmethylsertraline in the liver (or more precisely at the site of CYP3A4 biotransformation) could only be estimated by plasma concentrations and liver to plasma concentration ratios. Saturation of CYP3A4 was considered highly unlikely since plasma alprazolam concentrations were known to be well below the K_m values for alprazolam metabolite formation (von Moltke et al. 1994b, Greenblatt and Wright 1993). Finally, the possibility of significant alternate metabolic pathways

for alprazolam was considered unlikely given the extensive *in vitro* evidence implicating CYP3A4 as the major pathway for biotransformation (von Moltke et al. 1993). Thus the most likely reason for the observed lack of an *in vivo* interaction is that sertraline and desmethylsertraline concentrations in the liver were not sufficient to cause inhibition of CYP3A4.

Pharmacodynamic factors could have also contributed to the lack of an observed interaction. Such factors include: the change in substrate concentration required to produce undesired effects; and the impact of changes in concentrations of active metabolites. However both of these factors were well-established for the medications involved in this study. For example, it was known that alprazolam plasma concentrations above 60 ng/mL were associated with adverse effects. Thus, if alprazolam metabolism had been sufficiently impaired, concentrations above 60 ng/mL and associated adverse effects would have become more severe (if peak concentrations increased) and/or would have been sustained for longer periods of time (if AUC was increased). Metabolites of alprazolam, while active, were known to contribute negligibly to these effects due to their low concentrations *in vivo*. Furthermore, if sertraline was able to inhibit alprazolam metabolism, concentrations of these metabolites would fall, thereby decreasing their involvement in the interaction. Thus it is unlikely that pharmacodynamic factors contributed to the lack of an interaction in this study.

Patient related factors could also have influenced the potential for an interaction. These factors include: the inherent enzyme activity of the patient as it relates to the potential impact of an inhibitor or inducer; and the susceptibility of the patient to the effects of a potential drug interaction. For example, the impact of sertraline on CYP3A4 could have been influenced by the baseline activity of subjects in the study. In particular, it is possible that sertraline inhibits CYP3A4 activity more potently in subjects with high baseline activity than those with low baseline activity. This phenomenon, known as clearance normalization, has been demonstrated with other CYP3A4 inhibitors such as ketoconazole and itraconazole (Olkkola et al. 1994). Thus if a large percentage of subjects in the study had low baseline activity, the inhibitory effects of sertraline could have been masked. Although possible, this seems to be an unlikely explanation for the observations given the random nature of subject recruitment and the continuous distribution of CYP3A4 activity in the population.

Finally, in considering the results of this study, it is important to acknowledge the results of similar studies. An earlier study by Rapeport et al. (1996) demonstrated that sertraline (200 mg per day) had no effect on the kinetics and dynamics of carbamazepine (200 mg b.i.d.). However carbamazepine is not a model substrate for metabolism through CYP3A4 since the drug induces its own metabolism (Bernus et al. 1994) and since its metabolism may be partially dependent on the activity of enzymes

other than CYP3A4 (Levy et al. 1988, Kerr et al. 1994). Thus the results obtained with carbamazepine were not directly applicable to other CYP3A4 substrates. However, a paper published by Preskorn et al. (Preskorn et al. 1997) during the course of this study demonstrated that 50 mg of sertraline per day did not alter the kinetics of alprazolam (1 mg p.o. single dose). Our study confirms this finding and further demonstrates that alprazolam kinetic parameters are not altered at higher doses of sertraline (100 and 150 mg per day) and that changes in adverse effects associated with alprazolam (sedation, psychomotor impairment, memory impairment) are not evident at any dose of sertraline.

4.2 CONCLUSIONS

In conclusion, the coadministration of sertraline and alprazolam does not increase the risk of adverse events associated with alprazolam toxicity. This finding is of particular importance to clinicians who prescribe alprazolam in combination with an SSRI since the documented interactions between alprazolam and fluvoxamine or fluoxetine can now be avoided. Our findings are also of importance to elderly populations where drug interactions that cause unexpected sedation and psychomotor impairment may lead to falls and serious resulting complications. Furthermore, our findings suggest that sertraline does not

produce significant inhibition of CYP3A4-mediated drug metabolism over its clinically relevant dosing range. Thus, unlike other SSRIs which can cause significant inhibition of CYP1A2 (Spina et al. 1993), 2C19 (Lemberger et al. 1988, Perucca et al. 1994), 2D6 (Ozdemir et al. 1997, Brosen et al. 1993) and/or 3A4 (Fleishaker and Hulst 1994, Lasher et al. 1991), sertraline causes either mild or clinically insignificant inhibition of these enzymes (Sproule et al. 1997a, Ozdemir et al. 1998, Preskorn et al. 1994). Our findings therefore support the use of sertraline in patients who are receiving multiple medications in order to reduce the risk of drug interactions.

4.3 RECOMMENDATIONS

Future studies may wish to focus on the effects of sertraline on the CYP3A4 enzyme itself, perhaps investigating the paradoxical finding of a reduction in peak alprazolam concentrations in the presence of sertraline. A suitable probe for total CYP3A4 activity will be needed to account for potential changes in gut wall metabolism, and perhaps an investigation of alprazolam as such a probe would prove worthwhile given the apparent specificity of alprazolam for CYP3A4-mediated metabolism.

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