

Effect of Sertraline on the Pharmacokinetics and Protein Binding of Diazepam in Healthy Volunteers

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Summary

A double-blind randomised placebo-controlled study was conducted in healthy male volunteers to determine the effects of sertraline on the pharmacokinetics of diazepam and its primary metabolite, *N*-demethyl diazepam. The effect of sertraline on the plasma protein binding of diazepam was also studied. Sertraline 50 mg/day titrated over a 10-day period to 200 mg/day or placebo was administered for 32 days. A single intravenous dose of diazepam 10mg was given before the start, and after 21 days of sertraline or placebo treatment. The pharmacokinetic analyses were based on data from 20 individuals.

The systemic clearance of diazepam decreased by 32% (-0.100 ml/min/kg) in the sertraline group compared with a 19% decrease (-0.054 ml/min/kg) in the placebo group ($p = 0.0266$). However, this small difference (13%) between the 2 groups was not considered meaningful.

Other than a prolonged time to maximum plasma concentration for *N*-demethyl diazepam, no other pharmacokinetic parameters were significantly altered by sertraline. The plasma protein binding of diazepam was unchanged by concomitant administration of sertraline. These results suggest that sertraline at the maximum recommended dosage under steady-state conditions, and demethylsertraline, the principal metabolite of sertraline, are unlikely to exert significant inhibitory effects on the CYP2C19 and CYP3A3/4 hepatic isoenzymes responsible for the metabolism of diazepam. Therefore, it would be expected that sertraline would, similarly, have a minimal effect on the pharmacokinetic profile of other drugs metabolised by these hepatic isoenzymes.

Diazepam and other benzodiazepines are frequently administered to patients with psychiatric disorders, including those receiving long term antidepressant therapy; therefore, the potential for interactions between benzodiazepines and antidepressants is an important clinical concern. Diazepam is metabolised primarily by *N*-demethylation to its active metabolite *N*-demethyl diazepam. Biotransformation of diazepam to tem-

azepam (3-hydroxydiazepam) is mediated entirely by the hepatic enzyme cytochrome P450 (CYP) 3A3/4; transformation of diazepam to *N*-demethyl diazepam is mediated by CYP2C19 at low plasma concentrations but can also be mediated by CYP3A3/4 at higher concentrations.^[1-6] *N*-Demethyl diazepam is subsequently hydroxylated to oxazepam, which is then conjugated with glucuronic acid and eliminated via the kidneys.

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Thus, diazepam can be used as a model substrate to test the effects of drugs on the functional integrity of the CYP3A3/4 and CYP2C19 isoenzymes.

The present study was conducted to examine the effect of coadministration of sertraline, a selective serotonin reuptake inhibitor (SSRI) antidepressant, on the pharmacokinetics of diazepam and its active metabolite *N*-demethyldiazepam and the plasma protein binding of diazepam in healthy male volunteers. Most importantly, this study provides a rigorous approach to determining whether sertraline, at its maximum recommended dose, affects diazepam clearance. By extrapolation, this method allows for evaluation of the effects of sertraline on the CYP3A3/4 and CYP2C19 isoenzymes, which are responsible for diazepam metabolism.

1. Materials and Methods

1.1 Study Design

This double-blind placebo-controlled randomised parallel-group study was conducted in healthy adult male volunteers aged between 18 and 35 years. Smokers and individuals with any condition that might affect drug absorption were excluded from participation. Participants were also excluded if they had evidence or a history of allergic, haematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic or neurological disease; known drug or alcohol dependence; or drug allergies. All drugs other than the study medications were withdrawn at least 2 weeks before the start of the study. No concomitant drug therapy or donation of blood or blood components was permitted for its duration. Written informed consent was obtained from all study participants, and institutional review board approval was obtained.

1.2 Drug Administration Protocol

All volunteers received a 2-minute intravenous infusion of diazepam 10mg on day 1. Individuals were randomly assigned to receive sertraline or placebo orally once daily for 32 days (days 15 to 46), beginning 14 days after the first diazepam

infusion. The initial sertraline dosage of 50 mg/day was titrated up to the maximum recommended dosage of 200 mg/day (4 times the typical effective dose) in 50mg increments at 3-day intervals (50, 100, 150 and 200mg). The 200 mg/day dosage was then maintained for the remaining 22 days of the study. All individuals received a second infusion of diazepam on study day 35 (1 hour after receiving the study medication).

1.3 Laboratory Evaluations

Blood was collected before each diazepam infusion for assessment of plasma protein binding. Blood samples were obtained prior to and immediately after the infusion, and at 0.08, 0.25, 0.5, 1, 2, 3, 4, 8, 12, 24, 48, 72, 96, 144, 192, 240 and 288 hours postinfusion. Samples were analysed for diazepam and *N*-demethyldiazepam concentrations. On days 15, 35, 36 and 41, plasma samples were collected just before participants received the study medication and the concentration of sertraline was measured.

1.4 Analysis of Samples

The plasma concentrations of diazepam and *N*-demethyldiazepam were determined by gas chromatography-electron capture, with lower and upper quantification limits of 5 and 400 µg/L, respectively.^[7] Plasma sertraline concentrations were also measured by gas chromatography-electron capture, with upper and lower quantification limits of 10 and 100 µg/L, respectively.^[8] Diazepam plasma protein binding was determined by equilibrium dialysis; an equal volume of sodium phosphate buffer (pH 7.4) at ambient temperature (22°C) for 18 hours was used.

1.5 Pharmacokinetic Parameters

The following pharmacokinetic parameters were determined for diazepam:

- Terminal elimination rate constant (k_{el}), estimated by least-squares regression analysis of plasma concentration-time data obtained during the terminal log-linear elimination phase.

- Mean terminal elimination half-life ($t_{1/2\beta}$), calculated as $0.693/\text{mean } k_{el}$.
- Total area under the plasma concentration-time curve (AUC_{∞}), calculated as AUC_t [to the last time (t) with a measurable plasma concentration (C)] plus $AUC_{t-\infty}$ (from time 't' to infinity). AUC_t was estimated using linear trapezoidal approximation. $AUC_{t-\infty}$ was estimated as $C_{t,est}/k_{el}$, where $C_{t,est}$ represents the estimated concentration at time 't' based on the regression analysis described above for k_{el} .
- Systemic clearance (CL), estimated as the ratio of dose to AUC_{∞} .
- Apparent volume of distribution at steady-state (V_{ss}), estimated as $(\text{dose}/AUC_{\infty}) \cdot (AUMC/AUC_{\infty})$, where AUMC, which represents the area under the C • time versus time curve extrapolated to infinity (first moment), was estimated in the same manner as AUC_{∞} . Its effect on mean residence time was ignored because of the brevity of the infusion.
- Plasma protein binding, determined by calculating the percentage of unbound diazepam in plasma.

The following pharmacokinetic parameters were determined for *N*-demethyldiazepam:

- Total area under the plasma concentration-time curve from zero to 288 hours (AUC_{288h}), estimated by linear trapezoidal approximation.
- Maximum observed plasma concentration (C_{max}) and time to C_{max} (t_{max}).

1.6 Statistical Analysis

Two sample t-tests were used to compare the sertraline plus diazepam and placebo plus diazepam treatment groups in terms of the differences in pharmacokinetic parameters between day 1 and day 35. The 95% confidence limits on the difference between mean change scores were also computed for all comparisons.

2. Results

24 individuals (20 volunteers plus 4 designated 'extras') were enrolled to ensure that 20 individuals would complete the study. If any of the 20

Table I. Baseline demographic data for the male study participants (n = 20)

	Study group	
	sertraline	placebo
No. of study participants	10	10
Mean age (y)	26.2	28.2
Age range (y)	18-35	21-35
Mean bodyweight (kg)	75	76.8
Bodyweight range (kg)	65.0-89.1	62.3-88.6
Caucasian	6	8
African-American	1	0
Other	3	2

volunteers discontinued the study, data from the same number of the 'extra' 4 would be substituted. In fact, 2 volunteers did discontinue the study, and samples from 2 of the 4 'extra' individuals were used in their place. Thus, the final data presentation includes data from 20 individuals who completed the study.

Individuals assigned to the sertraline and placebo groups were similar with respect to age and bodyweight (table I). Mean age was 26.2 years in the sertraline group versus 28.2 years in the placebo group. Mean bodyweight was 75.0kg in the sertraline group versus 76.8kg in the placebo group.

Table II lists baseline (day 1) and final (day 35) values for pharmacokinetic parameters of diazepam and *N*-demethyldiazepam and plasma protein binding of diazepam. Mean plasma concentrations of diazepam and *N*-demethyldiazepam before and after 21 to 32 days of treatment with sertraline or placebo are shown in figures 1 and 2, respectively.

The V_{ss} , k_{el} , and plasma protein binding of diazepam were not significantly altered by daily sertraline administration (table II).

The CL of diazepam in sertraline-treated individuals decreased by 32% from a mean value of 0.313 to 0.213 ml/min/kg on day 35, while diazepam CL in the placebo group decreased by 19% from a mean baseline value of 0.285 to 0.231 ml/min/kg on day 35. This small (13%) difference between the 2 groups was statistically significant ($p = 0.0266$). The mean CL values on day 35,

Table II. Mean pharmacokinetic parameters (\pm SD) for diazepam and *N*-demethyldiazepam and diazepam protein binding

Parameter	Sertraline		Placebo	
	baseline (day 1)	final (day 35)	baseline (day 1)	final (day 35)
Diazepam				
CL (ml/min/kg)	0.313 \pm 0.079	0.213 \pm 0.041	0.285 \pm 0.073	0.231 \pm 0.061*
V_{ss} (L/kg)	0.94 \pm 0.20	0.76 \pm 0.17	1.17 \pm 0.19	0.92 \pm 0.16
k_{el} (h^{-1})	0.0180 \pm 0.0065	0.0161 \pm 0.0056	0.0126 \pm 0.0035	0.0141 \pm 0.0050
$t_{1/2\beta}$ (h) ^a	38.5	43.1	55.0	49.2
Unbound drug in plasma (%)	1.4 \pm 0.4	1.1 \pm 0.3	1.6 \pm 0.7	1.3 \pm 0.6
<i>N</i>-Demethyldiazepam				
AUC _{288h} (μ g/L \cdot h)	6446 \pm 1150	7939 \pm 1680	6104 \pm 1504	7041 \pm 1707
C_{max} (μ g/L)	42 \pm 9	48 \pm 15	32 \pm 8	39 \pm 9
t_{max} (h)	60 \pm 32	74 \pm 49	89 \pm 34	72 \pm 20*

a Harmonic mean values are presented.

Abbreviations and symbol: AUC_{288h} = area under the concentration-time curve between 0 and 288 hours; CL = systemic clearance from the plasma; C_{max} = maximum plasma concentration; k_{el} = terminal elimination rate constant; t_{max} = time taken to reach C_{max} ; $t_{1/2\beta}$ = terminal elimination half-life; V_{ss} = volume of distribution at steady-state; * $p < 0.05$, between sertraline and placebo.

0.213 ml/min/kg in the sertraline group and 0.231 ml/min/kg in the placebo group, differed by only 8%.

There was no statistically significant difference between the C_{max} and AUC_{288h} values for *N*-demethyldiazepam before and after administration of sertraline. The t_{max} of *N*-demethyldiazepam was significantly prolonged by sertraline treatment

relative to placebo. However, interpretation of these changes was complicated by a substantially larger t_{max} at baseline in the placebo group (89 hours) compared with that in the sertraline group (60 hours). Although the t_{max} values for the metabolite at day 35 were almost identical in the placebo and sertraline groups (72 and 74 hours, re-

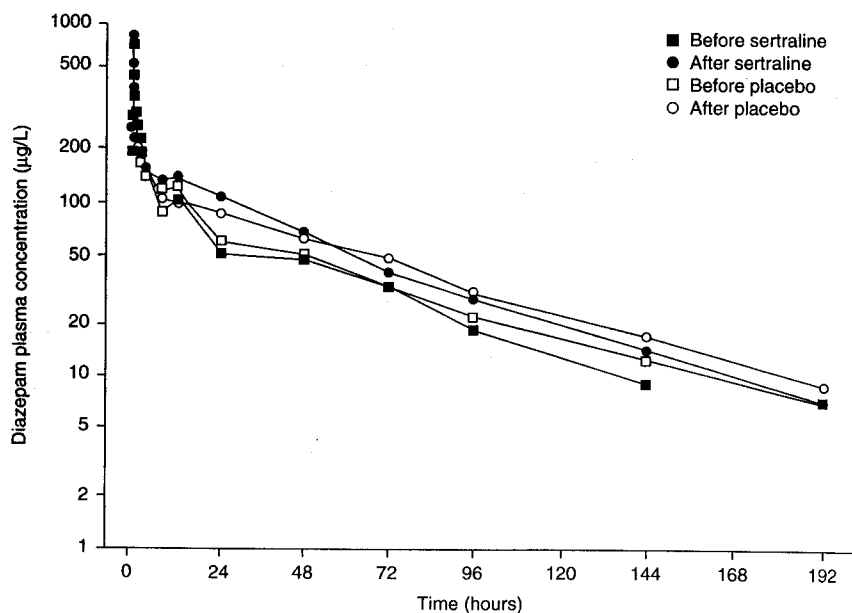


Fig. 1. Mean plasma concentrations of diazepam before and during daily administration of sertraline or placebo; plasma concentrations were determined beginning at baseline and on day 21 of treatment with sertraline or placebo. The sertraline dosage was titrated from 50 to 200 mg/day over the first 10 days of treatment and maintained at 200 mg/day for the remaining 22 days.

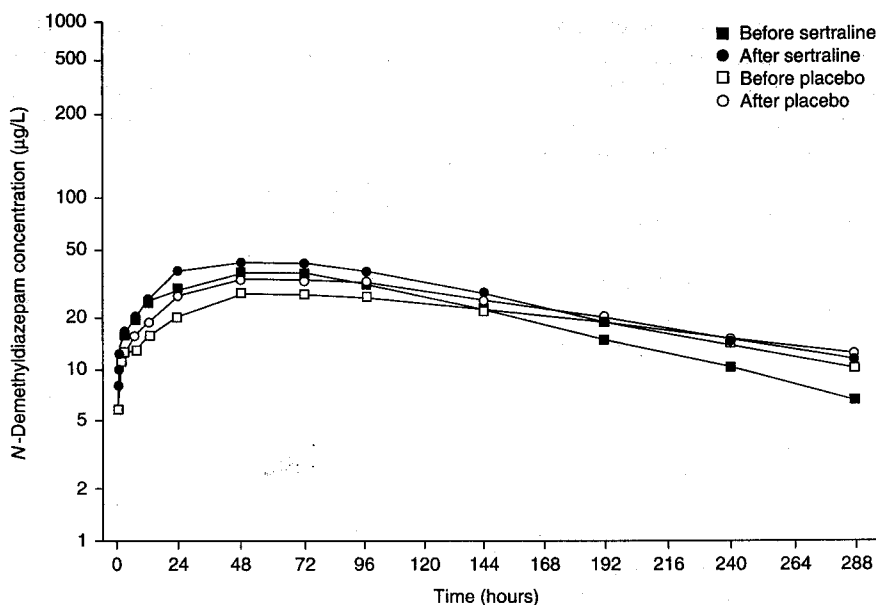


Fig. 2. Mean plasma concentrations of *N*-demethyl diazepam before and during daily administration of sertraline or placebo; plasma concentrations were determined beginning at baseline and on day 21 of treatment with sertraline or placebo. The sertraline dosage was titrated from 50 to 200 mg/day over the first 10 days of treatment and maintained at 200 mg/day for the remaining 22 days.

spectively), these values represented a 23% increase in t_{max} with concomitant sertraline administration but a 19% decrease with placebo administration. This difference was statistically significant ($p = 0.0227$). Sertraline plasma concentrations ranged from 22 to 104 µg/L.

3. Discussion

The objectives of this study were to examine the effect of sertraline, administered at the maximum recommended daily dose, on the pharmacokinetics of diazepam and *N*-demethyl diazepam, a metabolite that accounts for approximately 50% of diazepam clearance.^[9] Displacement of diazepam and other highly protein-bound drugs by sertraline could increase the free fraction of these drugs, possibly magnifying their pharmacological effects. Because SSRIs are highly protein bound, the possibility of such an interaction exists with all drugs

of this class, although few studies have examined the potential of SSRIs other than sertraline with regard to protein binding displacement.

The findings of this study indicate that sertraline, when given at the maximum recommended dose, does not alter the plasma protein binding of diazepam. These results are consistent with those of the sertraline interaction studies with tolbutamide^[10] and warfarin,^[11] which indicated no biologically significant displacement of these highly protein-bound drugs by sertraline. The fact that diazepam, tolbutamide and warfarin are structurally distinct suggests that these findings are likely to extend to a wide variety of other highly protein-bound drugs that bind at the same protein-binding site as these drugs.

The antidepressant drug fluoxetine, an SSRI, is a known inhibitor of CYP3A3/4^[12,13] and some CYP2C isoenzymes.^[14] The inhibitory effect of fluoxetine on these enzymes is believed to account

for the alterations in the pharmacokinetic profile of diazepam reported when fluoxetine and diazepam were administered concomitantly.^[15,16] In one non-comparative well-controlled study, clearance of orally administered diazepam decreased by approximately 40% from baseline when it was given to healthy volunteers treated with fluoxetine 60mg for 8 days.^[16] Sertraline at 200mg for 22 days produced a modest 13% net decrease in the clearance of diazepam relative to placebo. This effect is considerably less than the decrease produced by fluoxetine at its usually effective dose. In addition, it was likely that steady-state plasma concentrations were not achieved in the fluoxetine study^[16] and, therefore, maximum enzyme inhibition may not have been obtained.^[17]

The fact that sertraline 200 mg/day produced only a slight decrease in diazepam CL relative to placebo probably reflects minimal inhibition of the CYP2C hepatic enzyme responsible for the metabolism of diazepam and *N*-demethyldiazepam. The degree of enzyme inhibition is concentration-dependent;^[18] therefore, because sertraline exhibits linear pharmacokinetics throughout its therapeutic dosage range, it would be expected that the usual therapeutic dosage of sertraline 50 mg/day would have a lesser effect on the pharmacokinetic profile of diazepam and other substrates for CYP2C19 and CYP3A3/4.

Perucca et al.^[19] found that coadministration of fluvoxamine and diazepam was associated with increased mean C_{max} values of diazepam and statistically significant reduction in apparent oral clearance and prolongation of diazepam half-life. The effects of paroxetine on diazepam have not been studied; although there is evidence that paroxetine is a more potent inhibitor of the CYP2D6 enzyme than fluoxetine,^[20,21] the potential effects of paroxetine on the CYP2C enzymes remain unknown.

4. Conclusions

The effects of sertraline on diazepam pharmacokinetics demonstrated in this study suggest that sertraline has no significant inhibitory effect on the

CYP2C or CYP3A3/4 isoenzymes. Therefore, only a minimal pharmacokinetic interaction would be expected between sertraline and other drugs metabolised by these same enzyme systems.

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