### Title

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# Determination of Minimal Steady-State Plasma Level of Diazepam Causing Seizure Threshold Elevation in Rats

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## Summary

**Objective—**Diazepam, administered by the intravenous, oral or rectal routes, is widely used for the management of acute seizures. Dosage forms for delivery of diazepam by other routes of administration, including intranasal, intramuscular and transbuccal, are under investigation. In predicting what dosage amounts are necessary to terminate seizures, the minimal exposure required to confer seizure protection must be known. Here we administered diazepam by continuous intravenous infusion to obtain near-steady-state levels, which allowed an assessment of the minimal levels that elevate seizure threshold.

**Methods—**The thresholds for various behavioral seizure signs (myoclonic jerk, clonus and tonus) were determined with the timed intravenous pentylenetetrazol seizure threshold test in rats. Diazepam was administered to freely-moving animals by continuous intravenous infusion via an indwelling jugular vein cannula. Blood samples for assay of plasma levels of diazepam and metabolites were recovered via an indwelling cannula in the contralateral jugular vein.

**Results—**The pharmacokinetic parameters of diazepam following a single 80-μg/kg intravenous bolus injection were determined using a non-compartmental pharmacokinetic approach. The derived parameters $V_d$, CL, $t_{½\alpha}$ (distribution half-life) and $t_{½\beta}$ (terminal half-life) for diazepam were, respectively, 608 ml, 22.1 ml/min, 13.7 min and 76.8 min, respectively. Various doses of diazepam were continuously infused without or with an initial loading dose. At the end of the infusions, the thresholds for various behavioral seizure signs were determined. The minimal plasma diazepam concentration associated with threshold elevations was estimated as approximately 70 ng/ml. The active metabolites nordiazepam, oxazepam, and temazepam achieved levels that are expected to make only minor contributions to the threshold elevations.

**Significance—**Diazepam elevates seizure threshold at steady-state plasma concentrations lower than previously recognized. The minimally effective plasma concentration provides a reference

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**Disclosure**

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. The authors declare no competing financial interests.

that may be considered when estimating the diazepam exposure required for acute seizure treatment.

**Keywords**

diazepam; continuous infusion; seizure threshold; time intravenous pentylenetetrazol seizure test; pharmacokinetics

## Introduction

Diazepam is a long acting benzodiazepine that is often prescribed for its anxiolytic, sedative-hypnotic and muscle relaxant properties.[1] In addition, diazepam is an effective antiseizure agent that is used in the acute treatment of seizure exacerbations, seizure clusters (acute repetitive seizures) and status epilepticus.[2,3,4] Benzodiazepines as a class act as positive allosteric modulators of $GABA_A$ receptors, which are γ-aminobutyric (GABA)-gated chloride channels that are the principal mediators of fast synaptic inhibition in the central nervous system.[2,5,6,7] Diazepam is administered by the oral, intravenous, intramuscular and rectal routes.[3] In the United States, the only product approved for the out-of-hospital acute treatment of seizures is the diazepam rectal gel formulation dispensed in a proprietary rectal delivery system. The rectal gel is indicated for intermittent use to control bouts of increased seizure activity. Alternative, more convenient or more rapidly acting formulations intended for other routes of administration could replace the rectal formulation in managing acute and repetitive seizure attacks, provided they deliver adequate levels of diazepam required for antiseizure activity.[8] Intranasal and buccal formulations have been investigated.[9,10]

Although diazepam has been used clinically for more than 50 years and its pharmacokinetic properties have been studied extensively in preclinical and clinical studies,[11] little information exists on the minimal blood concentration required for its antiseizure effect. In experimental studies in animal models, diazepam is typically administered by bolus and the levels fall rapidly during the time of antiseizure testing. The rate of fall in blood and brain concentrations is particularly rapid following a bolus in a naïve animal due to redistribution. Therefore, it has not been possible to obtain reliable estimates of the blood concentrations required for antiseizure activity. In one such study in unanesthetized rats, Marcucci et al.[12] observed that concentrations above 150 ng/ml were associated with antiseizure activity. However, in unanesthetized cats with a penicillin focus, Celesia et al.[13] estimated that diazepam concentrations above 1500–2000 ng/ml are required to inhibit PTZ-evoked and spontaneous electrographic seizures.

Human studies have also resulted in a range of estimates. Based on levels achieved after bolus intravenous administration, it has been proposed that serum levels above 400 ng/ml are required for anticonvulsant effects.[11,14] Some investigators have used electrographic measures to assess antiseizure activity. Photoconvulsive responses and spontaneous epileptiform discharges were suppressed in one study with concentrations, respectively, in the range of 100–500 ng/ml and 500–1400 ng/ml.[15] In a separate study, interictal spike activity was reduced with serum levels between 80–410 ng/ml (mean, 210 ng/ml),[16] whereas in another study in children, serum concentrations greater than 250 ng/ml were associated

with a decrease in paroxysmal activity in the EEG.[17] A direct correspondence between effects on electrographic measures and prevention of clinical seizures has not been established. Estimates of the threshold plasma concentrations of diazepam required to terminate or prevent behavioral seizures are available in only a limited number of instances. In a 15 year-old girl with focal epilepsy who received a bolus intravenous diazepam infusion, plasma levels greater than 130 ng/ml were associated with seizure freedom.[18] In another report, a child in whom seizures had been arrested by diazepam had recurrence of convulsions when diazepam plasma levels dropped to 130 ng/ml.[19] However, in one adult with severe refractory seizures occurring in clusters, seizures recurred after rectal administration of diazepam when serum concentrations would have been above 170 ng/ml.[16]

Given the uncertainties in attempts to estimate seizure threshold values following bolus dosing or when patients are experiencing the unpredictable occurrence of spontaneous seizures, we sought to establish in rats the minimum steady-state concentration of diazepam in plasma that elevates the seizure threshold by using an approach where redistribution is complete and blood levels are maintained at a near constant concentration. Both the left and right jugular veins of the rats were permanently catheterized to allow continuous infusion of diazepam into one jugular and withdrawal of well-mixed blood for plasma level determinations from the other jugular. In an initial series of experiments, we characterized the pharmacokinetic parameters of diazepam and its metabolites following a bolus dose using a non-compartmental analysis approach. The derived parameter estimates allowed us to plan the dosing regimens to be applied in the remainder of the study. We used two dosing paradigms to achieve near steady-state diazepam plasma concentrations: (*a*) continuous intravenous infusion of diazepam for 4 half-lives, which is expected to bring the diazepam plasma concentration to 94% of the steady-state level, and (*b*) a loading dose bolus followed by continuous intravenous infusion for 1 half-life, which allowed a near steady-state level of diazepam to be achieved more rapidly. In both dosing paradigms, exposure to diazepam was sufficiently prolonged so that redistribution was complete and blood levels were not changing rapidly during the time of seizure threshold measurement. The timed intravenous pentylenetetrazol (PTZ) seizure threshold test was used to quantify the extent of elevation of the seizure threshold. This test is a sensitive measure of the antiseizure activity of drugs that act as positive modulators of $GABA_A$ receptors, such as benzodiazepines including diazepam.[20] In the protocol applied in our laboratory, the seizure threshold values are determined for three different behavioral seizure signs, myoclonic jerks, clonus, and tonus, that usually occur sequentially as the total dose of PTZ infused increases.[21]

## Materials and Methods

### Animals

Male Sprague-Dawley rats (225–400 g) obtained from Charles River Laboratories were kept in a vivarium under controlled environmental conditions with an artificial 12-h light/dark cycle. Animals were allowed to acclimatize in the vivarium at least 3 days before beginning of any experimental procedure. Experiments were performed during the light phase of the light/dark cycle after a 30 min period of acclimation to the experimental laboratory. The animal facilities were fully accredited by the Association for Assessment and Accreditation

of Laboratory Animal Care. All studies were performed under protocols approved by the Institutional Animal Care and Use Committee of the University of California, Davis in strict compliance with the Guide for the Care and Use of Laboratory Animals of the National Research Council (National Academy Press, Washington, D.C.). The principles outlined in the Basel declaration (http://www.basel-declaration.org) including the 3R concept were considered when planning the experiments.

### Diazepam and vehicle solutions

A commercially available formulation of diazepam (5 mg/ml diazepam injection, USP solution; Hospira Inc., Lake Forest, IL, USA) was used in the present study. Diazepam is not water-soluble and is formulated in an aqueous vehicle containing 40% propylene glycol, 10% alcohol, 5% sodium benzoate and benzoic acid, and 1.5% benzyl alcohol. To dilute the commercial diazepam formulation, we used a solution of the same composition as the vehicle in the commercial preparation. This vehicle was administered in some experiments as control. The pH of the diazepam or vehicle solution was maintained between 6.2–6.5 as in the commercial formulation.

### Catherization of the jugular vein

Animals were anesthetized using the combination of ketamine (50–100 mg/kg, i.p.) and dexmedetomidine (0.5 mg/kg, i.p.). The ventral portion of the neck was shaved and an incision was made starting at the base of the neck and extending to chest at the clavicle. The external right and left jugular veins were isolated and catherized using polyurethane tubing (0.025 in × 0.0140 in; Instech Laboratories Inc., Plymouth Meeting, PA, USA). The patency of the cannula was confirmed by injecting 200–250 μL of sterile saline followed by a slow withdrawal of the blood. After the patency of the cannula was confirmed, it was securely tied to the vein with caudal and rostral sutures; both were positioned over the polyurethane overlap in order to avoid occlusion of the tubing. The cannulae were exteriorized at the back of the neck. The catheters were locked with heparin (500 U/ml)-dextrose catheter lock solution (SAI Infusion Technologies, Lake Villa, Illinois, USA). The exteriorized end of the catheters was sealed with a stainless steel plug. The anesthesia was reversed using atipamezole hydrochloride (1 mg/kg, i.p.; Antisedan®, Orion Corporation, Espoo, Finland). The animals were allowed to recover from the surgery for at least 7 days before testing. During these 7 days, the cannulae were flushed occasionally with the heparin solution using a blunt needle (23 G) and 1 ml syringe in order to prevent blocking by clots. The instilled solution was withdrawn, aspirated fluid/blood was discarded, the cannula was flushed with saline to clear it of blood, and refilled with fresh heparin-dextrose lock solution.

### Timed intravenous PTZ seizure threshold test

To evaluate the effect of diazepam on seizure threshold, the $GABA_A$ receptor antagonist PTZ was infused via a jugular vein catheter and the doses required to elicit various seizure signs were determined. PTZ (20 mg/ml) was infused at a constant rate of 0.25 ml/min using a Becton Dickinson 5 ml syringe mounted on an infusion pump (Model ‘11’ plus syringe pump; Harvard Apparatus, Holliston, MA). The thresholds to the following endpoints were determined: (*i*) the first myoclonic jerk; (*ii*) the onset of generalized clonus with loss-of-righting reflex; and (*iii*) the onset of tonic phase. The infusion was stopped at the onset of

tonic phase. Latencies were measured from the start of the PTZ infusion to the onset of each behavior. The PTZ threshold (minimum) dose (in mg/kg) to achieve each endpoint was determined according to the following formula: (infusion duration [s] × infusion rate [ml/min] × convulsant drug concentration [mg/ml] × 1000)/(60 [s] × weight of rat [g]).

### Blood collection and assay

Blood was collected by reterobital sinus puncture in K2-EDTA tubes. The whole blood was centrifuged at 3,000 RPM for 10 min at 4 °C and the clear plasma supernatant was collected and stored at −80 °C until analyzed. Plasma diazepam and metabolite levels were determined using a validated liquid chromatographic and tandem mass spectrometric assay (LC–MS/MS; Covance Laboratories, Durham, NC). In the case of diazepam and nordiazepam, the assay had a lower limit of quantitation of 1 ng/ml and was validated for concentrations up to 500 ng/ml with standards. In the case of oxazepam and temazepam, the assay was validated for concentrations in the range of 0.1–50 ng/ml.

### Pharmacokinetic and statistical analysis

Pharmacokinetic analysis was conducted with Kinetica® software (ThermoFisher Scientific).

Results are expressed as mean ± SEM; the significance of the difference in the responses of treatment groups with respect to control is based on one-way ANOVA followed by specific *post hoc* comparisons using Tukey's test. Differences were considered statistically significant when the probability of type I error was less than 0.05.

## Results

### Determination of pharmacokinetic parameters of diazepam and metabolites after intravenous bolus administration of diazepam in rats

Diazepam (80 μg/kg) was administered by intravenous bolus injection over 5 s via the lateral tail-vein in 8 rats. For each rat, blood was collected at intervals from 5 to 240 min following the bolus and the concentrations of diazepam, nordiazepam, temazepam, and oxazepam were determined in the plasma. As shown in Figure 1, the mean diazepam plasma concentration fell monotonically over time during the 240 min period following injection. At the last time-point sampled (240 min), diazepam could be detected in only 3 of the 8 animals; the levels in the remainder were below the limit of detection. The metabolite levels overall were much lower than the levels of diazepam; levels of the des-methyl congener nordiazepam (nordazepam), considered to be the principal metabolite of diazepam, were modestly greater than those of the 3-hydroxy metabolites oxazepam and temazepam. These latter two metabolites showed a delayed rise. Nordiazepam was detected in 3, 8, 6, and 2 of 8 animals at 5, 10, 30 and 60 min, respectively, and in none of the animals at the later time points. The levels of temazepam in rat plasma, although low, could be detected in 8 of 8 animals at the 5 min to 60 min time points, and in 4, 2 and 0 of 8 animals at 90, 120 and 240 min, respectively. Like diazepam, oxazepam was detectable in all animals at all time points except at the 240 min time point when levels were detectable in only 3 of 8 animals.

Noncompartmental analysis (Kinetica software; ThermoFisher®) was used to calculate the following pharmacokinetic parameters from plasma diazepam levels in each of the 8 rats: maximum observed plasma concentration ($C_{max}$), area under the curve for time zero to last sampling time ($AUC_{0–240}$), initial (disposition) and elimination half-lives ($t_{½\alpha}$ and $t_{½\beta}$), volume of distribution ($V_d$), clearance (CL), and elimination rate constant $k_{el}$. These values are provided in Table 1.

## Effect of continuous intravenous infusion of diazepam on diazepam and metabolite levels and seizure sign thresholds

Asuming first order kinetics, the infusion rate $R$ to achieve a steady-state concentration $C_{ss}$ is given by $R = C_{ss} \times CL$. To achieve near $C_{ss}$ of 89, 200, 400 ng/mg with CL= 22.14 ml/min implies $R \sim 2$, 4.5 and 9 μg/min. (The maximum target concentration is approximately the peak diazepam concentration achieved by diazepam rectal gel as specified in the FDA-approved label.) Continuous intravenous infusion of diazepam at these rates via the right jugular vein (flow rate in all cases was 1 ml/h) for a total of 308 min resulted in mean ± S.E.M. plasma levels of 55.0 ± 9.1, 130.1 ± 15.5 and 360 ± 55 ng/ml, respectively (Fig. 2). Regression of mean plasma level achieved versus infusion rate revealed a linear relationship with slope of 0.044 min/ml and intercept −48 μg/min ($r^2$=0.99). The levels of the 3 diazepam metabolites also generally rose in a corresponding fashion but the absolute concentrations were <10-fold those of the parent, consistent with a prior study which found diazepam to undergo only limited metabolism in the rat.[12]

The intravenous PTZ infusion test was conducted at the end of the 308 min infusion for vehicle and each of the 3 infusion rates. We have found the vehicle elevated the threshold for all 3 seizure signs, with greater effect on clonus and tonus than on myoclonic jerks. Vehicle was demonstrated to elevate the threshold for tonus (see caption to Figure 3), which is likely due to the various alcohols that are included to solubilize diazepam. To account for this effect, we assessed changes in threshold with respect to vehicle treatment. Statistically significant elevations in all 3 seizure endpoints were observed with doses of 4.5 and 9 μg/min but not 2 μg/min. Thus, seizure threshold elevation was obtained with an uncorrected mean concentration of 130 ng/ml (achieved by continuous infusion of 4.5 μg/min) but not 55 ng/ml (achieved by continuous infusion of 2.0 μg/min). The PTZ infusion was administered via the jugular vein cannula used for diazepam infusion so that the diazepam infusion was terminated at the time the PTZ infusion was begun. The diazepam plasma concentration is expected to drop slightly during the time that the PTZ infusion test was conducted. We therefore corrected the threshold values using a correction factor $e^{-t \ln(2)/t_{½\beta}}$, where $t$ is the time of onset of clonus after initiation of PTZ infusion. Correcting for the drop-off in concentration during the on average 10.4 min and 5.7 min between onset of PTZ infusion (and termination of diazepam infusion) and seizure sign (clonus) responses for the 4.5 μg/min and 2 μg/min PTZ infusion, respectively, the estimated concentration of diazepam that is associated with elevation in the seizure sign threshold is approximately 118 ng/ml whereas an estimated concentration of 50 ng/ml is not associated with elevation in any seizure sign.

### Effect of loading dose and continuous intravenous infusion of diazepam on diazepam and metabolite levels and seizure sign thresholds

The continous intravenous infusion paradigm requires a relatively long period of infusion, which may lead to tolerance, a well-known liability of benzodiazepines.[22] To reduce the duration of exposure to diazepam prior to seizure testing, we administered a bolus dose of diazepam to rapidly bring the diazepam level to the desired steady-state value followed by continuous infusion to maintain the target level. The maintenance doses (MD) were chosen to target $C_{ss}$ values spanning the range demonstrated in the previously described continuous infusion experiments to be at a level below that associated with activity to a level that is strongly active. The latter was arbitrarily set at 3.5 μg/min, which was expected based on the experiment of Figure 2 to achieve a strongly active plasma level. Additional continuous infusion rates were set at approximately 40% (1.4 μg/min) and 60% (2.0 μg/min) of this value. The loading doses were chosen to overshoot the target based on the formula LD (μg) = $C_{ss} \times V_d$ (608.4 ml), where LD is the loading dose. Thus, to achieve peak targets concentrations of 60 ng/ml, 90 ng/ml and 150 ng/ml, loading doses of 36.5 μg, 54.8 μg and 91.4 μg were administered.

Using the dosing schemes LD 91.4 μg MD 3.5 μg/min; LD 54.8 μg MD 2.0 μg/min; and LD 36.5 μg MD 1.4 μg/min, mean plasma levels of 78.3 ± 12.2 ng/ml, 35.0 ± 6.2 ng/ml and 16.3 ± 2.1 ng/ml were obtained at the end of the 77 min infusion (Fig. 4A). The mean levels of the 3 metabolites were in all cases >10-fold less than that of diazepam (Fig. 4B–D).

The intravenous PTZ infusion test was conducted at the end of the 77 min infusion for rats receiving vehicle or diazepam administered with each of the 3 dosing schemes (Fig. 5). Statistically significant elevations in all 3 seizure endpoints were observed only with the high dose scheme (LD 91.4 MD 3.5 μg/min). The intermediate dose scheme (LD 54.8 μg MD 2.0 μg/min) was associated with a statistically significant increase in thresholds for myclonic jerks and tonus but not clonus. The low dose scheme (LD 36.5 μg MD 1.4 μg/min) failed to elevate the threshold for any of the seizure endpoints. The uncorrected mean plasma concentrations at the end of the 77 min infusions for the high, intermediate and low dose schemes were 78.3, 35.0 and 16.3 ng/ml, respectively. Correcting for the drop-off in concentration during the on average 5.9 min, 3.9 min and 2.7 min between onset of PTZ infusion and seizure sign responses in the high, intermediate and low diazepam dosing scheme groups, the plasma concentration of diazepam that is associated with a clear elevation in the seizure threshold is approximately 74 ng/ml (high dosing scheme) whereas an estimated concentration of 34 ng/ml (intermediate dosing scheme) is equivocal and an estimated concentration of 16 (low dosing scheme) was not associated with a threshold alteration for any seizure sign.

## Discussion

This study for the first time has provided an estimate of the minimum plasma level of diazepam under near-steady-state conditions that elevates the seizure threshold. To assay seizure threshold, we used the timed intravenous PTZ infusion test, a seizure model that is highly sensitive to diazepam and readily allows quantification of diazepam effects on seizure threshold.[22] To achieve the near-steady-state condition, diazepam was administered by

continuous intravenous infusion at different doses without and with an initial loading dose. Diazepam was infused through a permanently implanted jugular vein cannula and blood for determination of diazepam and metabolites was withdrawn from a permanently implanted catheter in the contralateral jugular vein. In the initial set of experiments, a highly significant elevation in seizure threshold occurred at an estimated plasma concentration of 118 ng/ml whereas there was no threshold elevation with an estimated plasma concentration of 50 ng/ml. In a second set of experiments, an estimated concentration of 34 ng/ml was associated with threshold elevation in some but not all endpoints whereas a concentration of 74 ng/ml caused unequivocal threshold elevation in all endpoints. It is noteworthy that the threshold concentration values are slightly lower in the second set of experiments, in which exposure to diazepam was shorter (77 min versus 308 min), suggesting that a minimal amount of tolerance may have occurred during the more prolonged exposure in the initial set of experiments. Focusing on the second set of experiments as it provides a finer estimate of the minimal effective concentration, we conclude that the plasma concentration associated with elevation in threshold of all 3 endpoints is within the range of about 70 ng/ml, but effects on threshold may occur at concentrations as low as 30 ng/ml. These values are modestly below the level of 150 ng/ml previously believed to be associated with antiseizure activity in rats and are also modestly below the levels proposed to be associated with suppression of electrographic seizure activity and arrest of seizures in humans (see Introduction). However, the literature values are based on a limited data set and subject to substantial uncertainty. While our results indicate that antiseizure activity occurs at lower steady-state plasma concentrations than previously recognized, the human clinical relevance of our findings is uncertain. We further note that the potency of diazepam varies in different seizure models and that the potency against PTZ-induced seizures is particularly robust.[2] With these caveats, our results suggest that plasma levels of diazepam in the range of 70 ng/ml and possibly even lower could have clinical activity.

Diazepam is well recognized to undergo demethylation and oxidative metabolism to active metabolites. The principal metabolite is nordiazepam, which is roughly 4-fold less potent than diazepam as a modulator of $GABA_A$ receptors and also as an antiseizure agent in rats.[23,24] However, levels of nordiazepam in our experiments were only a fraction of that of diazepam even after sufficient time had elapsed to nearly achieve steady-state. This observation is consistent with prior studies showing little accumulation of nordiazepam in cultured rat hepatocytes and in rats in vivo.[12,25,26] Overall, nordiazepam is expected to have contributed negligibly to the antiseizure effects observed in the present study. Nordiazepam is metabolized to oxazepam, which is also active as an anticonvulsant but is also substantially less potent than diazepam.[27,28] Diazepam is additionally metabolized to temazepam (N-methyloxazepam), which is also metabolized to oxazepam. However, levels of temazepam and oxazepam were even lower than that of nordiazepam and would therefore have even less influence on the outcome. There is greater production of the active metabolites in humans.[17,19] Therefore, the threshold diazepam concentration determined here in rats may overestimate to some extent the level required to confer seizure protection in humans, particularly with dosing schemes that allow the accumulation of substantial levels of active metabolites.

The pharmacokinetic parameters obtained in our study with bolus dosing are quantitatively similar to values reported in the literature. Following acute administration of diazepam, blood levels of the parent are well described by a two-compartment model with a rapid distribution phase (α) followed by a longer elimination phase (β).[29] Our half-life values for $t_{½\alpha}$ and $t_{½\beta}$ of, respectively, 0.23 h and 1.3 h are in good agreement with the values previously reported by Klotz et al.[29] of 0.29 h and 1.1 h. In another study, Löscher and Schwark[30] obtained a $t_{½\beta}$ value of 1.4 h. Klotz and colleagues[29] obtained a plasma clearance of 24.5 ml/min, which is similar to the clearance obtained in the present study of 22.14 ml/min.

Currently, in the United States, an intravenous formulation of diazepam is approved for the treatment of status epilepticus and severe recurrent convulsive seizures.[4,31] A rectal gel formulation (Diastat®) is approved for the outpatient treatment of bouts of increased seizure activity (seizure clusters).[32] The rectal formulation of diazepam is limited by poor patient and caregiver acceptance. Rectal formulations also often exhibit erratic and unpredictable absorption. Alternative routes of diazepam administration, including intranasal, buccal or intramuscular, may be preferred.[3,33,34] Nasal spray formulations that administer diazepam doses of 4 to 20 mg have been reported to result in mean diazepam blood levels of approximately 100 ng/ml to 400 ng/ml.[33,35] Our study confirms that these exposures are associated with relevant pharmacodynamic activity and indeed, modestly lower exposures may be sufficient to confer seizure protection, which may avoid adverse effects including oversedation, hypotension and respiratory depression that can occur with high diazepam doses.

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**Key Points**

- Various dosage forms of diazepam are under development to treat acute seizures but target blood levels are poorly defined
- Estimates of levels conferring seizure protection in animals and humans have been based on studies in which levels are rapidly changing
- To obtain a more reliable estimate, continuous infusion in rats was used to maintain the concentration at near steady-state levels
- Seizure threshold was assessed using the timed intravenous pentylenetetrazol seizure test
- Plasma concentrations of diazepam greater than about 70 ng/ml were found to elevate the seizure threshold

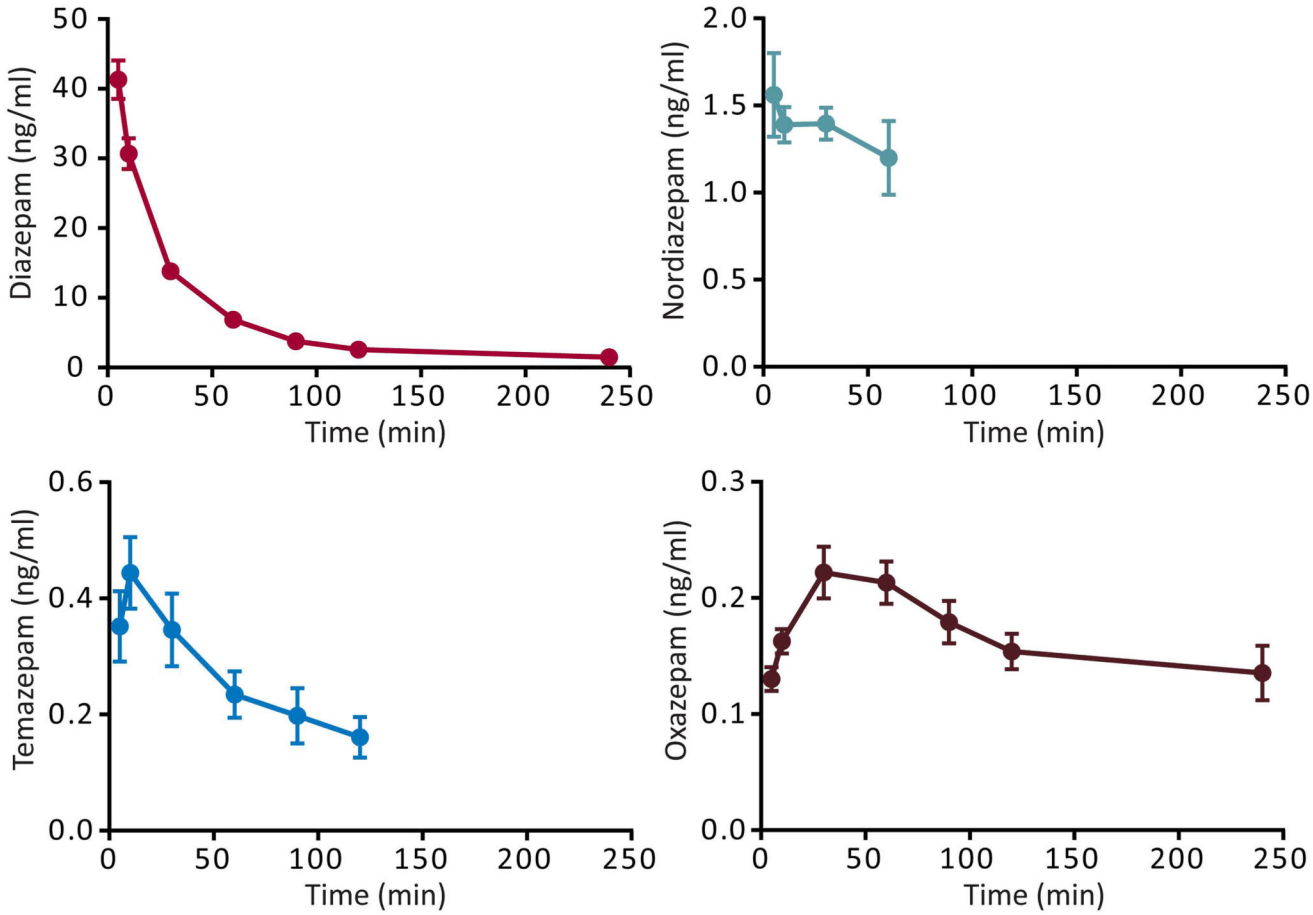


**Figure 1.**

Plasma levels of diazepam and its metabolites nordiazepam, temazepam and oxazepam after an acute i.v. bolus injection of diazepam (80 μg/kg). Blood samples were collected 5, 10, 30, 60, 90, 120 and 240 min after the diazepam bolus. Data point represent mean ± S.E.M. of measurements in 8 animals. The levels of nordiazepam and temazepam were below the limit of detection in all animals at 90 and 240 min, respectively, and therefore no data values are shown at or after these time points.

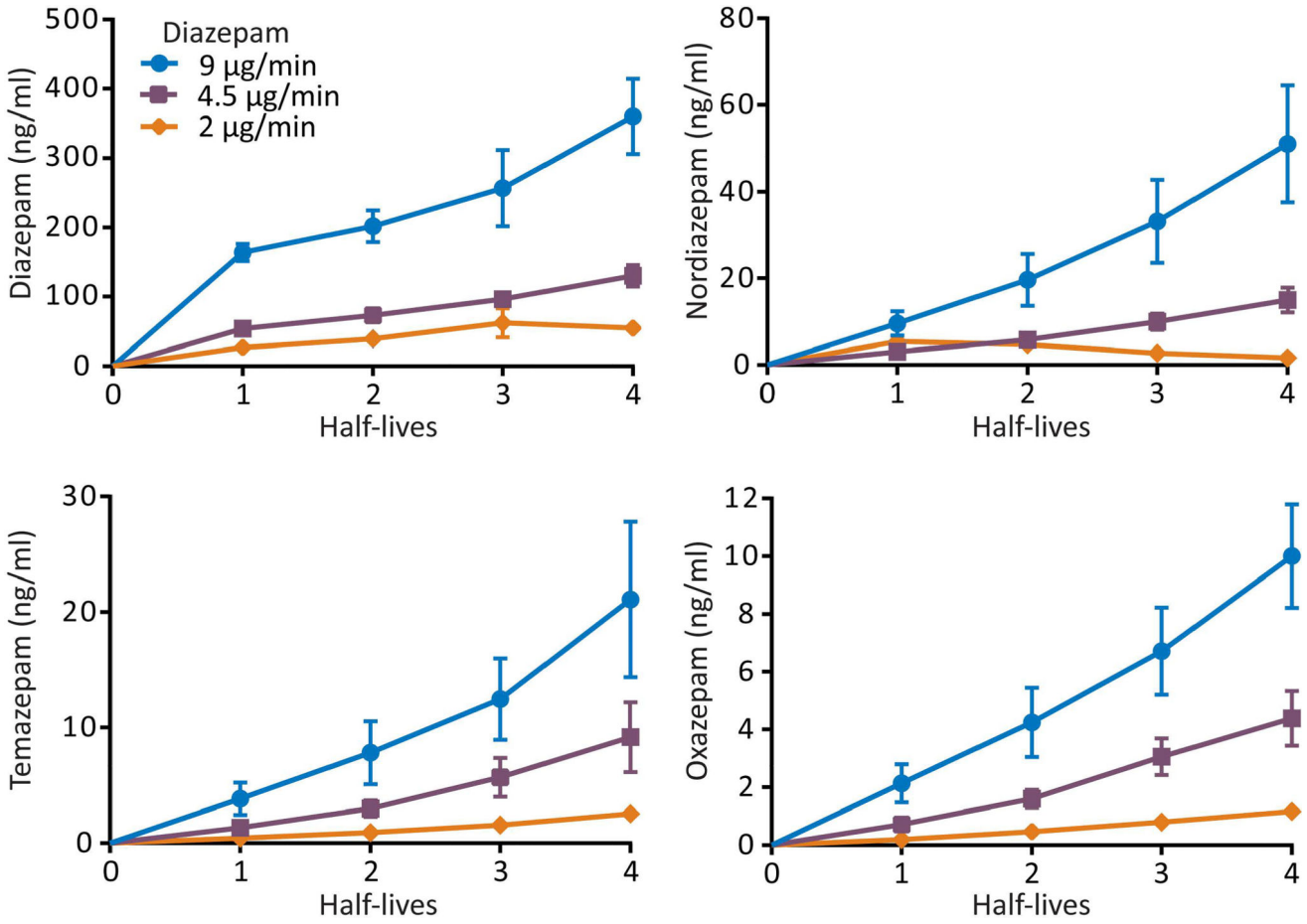


**Figure 2.**

Plasma levels of diazepam and its metabolites nordiazepam, temazepam, and oxazepam at various time points during continuous i.v. administration of diazepam at the indicated rates. Blood samples were collected at intervals equal to multiples of the terminal elimination half-life ($t_{½\beta}$) value (77, 154, 231 and 308 min). Data point represent mean ± S.E.M. of measurements in 6 animals. Diazepam area under the curve for time zero to last sampling time ($AUC_{0–308}$) increased linearly with dose [slope, 5,818 ± 471 (ng/ml • min) $(mg/kg)^{-1}$].

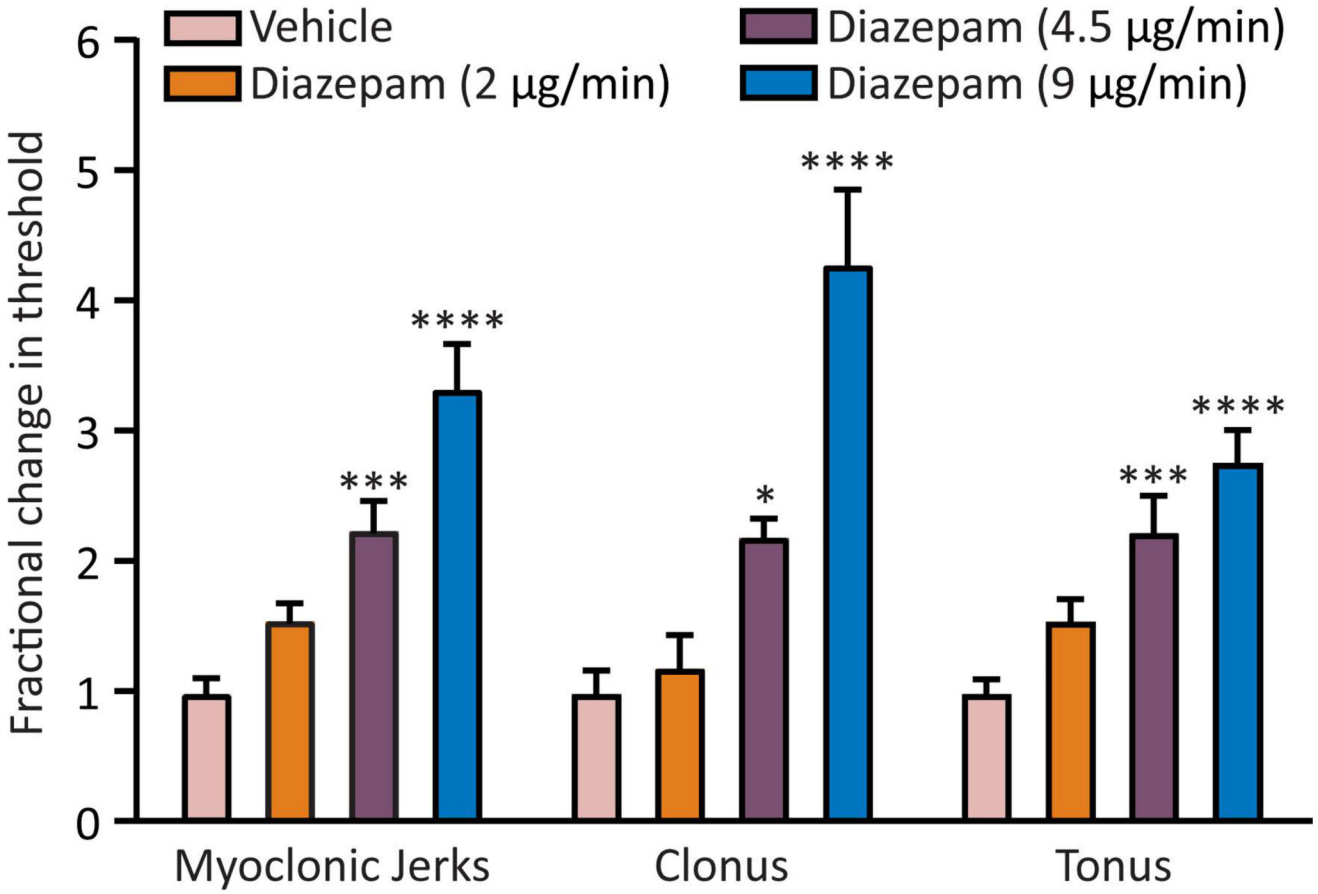


**Figure 3.**

Effect of continuous intravenous infusion of diazepam (2, 4.5 and 9 µg/min for 4 half-lives or 308 min) on the threhold for myoclonic jerks, generalized clonus, and tonus in the timed intravenous PTZ test in rats. PTZ threshold was measured at the end of continuous diazepam infusion. The values indicate mean ± S.E.M. of measurements in 6 to 8 rats normalized with respect to the thresholds in the vehicle-treated groups, which for myoclonic jerks, clonus, and tonus were, respectively, 26.5 ± 2.6, 68.7 ± 10.9 and 111.7 ± 9.5 mg/kg. To assess the effect of the vehicle, threshold values were determined following infusion of a 24% cyclodextrin solution for 5 h in 3 rats, which resulted in threshold values of 24.1 ± 6.2, 29.1 ± 7.2 and 63.7 ± 22.7 mg/kg. *$p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$ compared with the vehicle control group (ANOVA followed by Tukey's Multiple Comparison test).

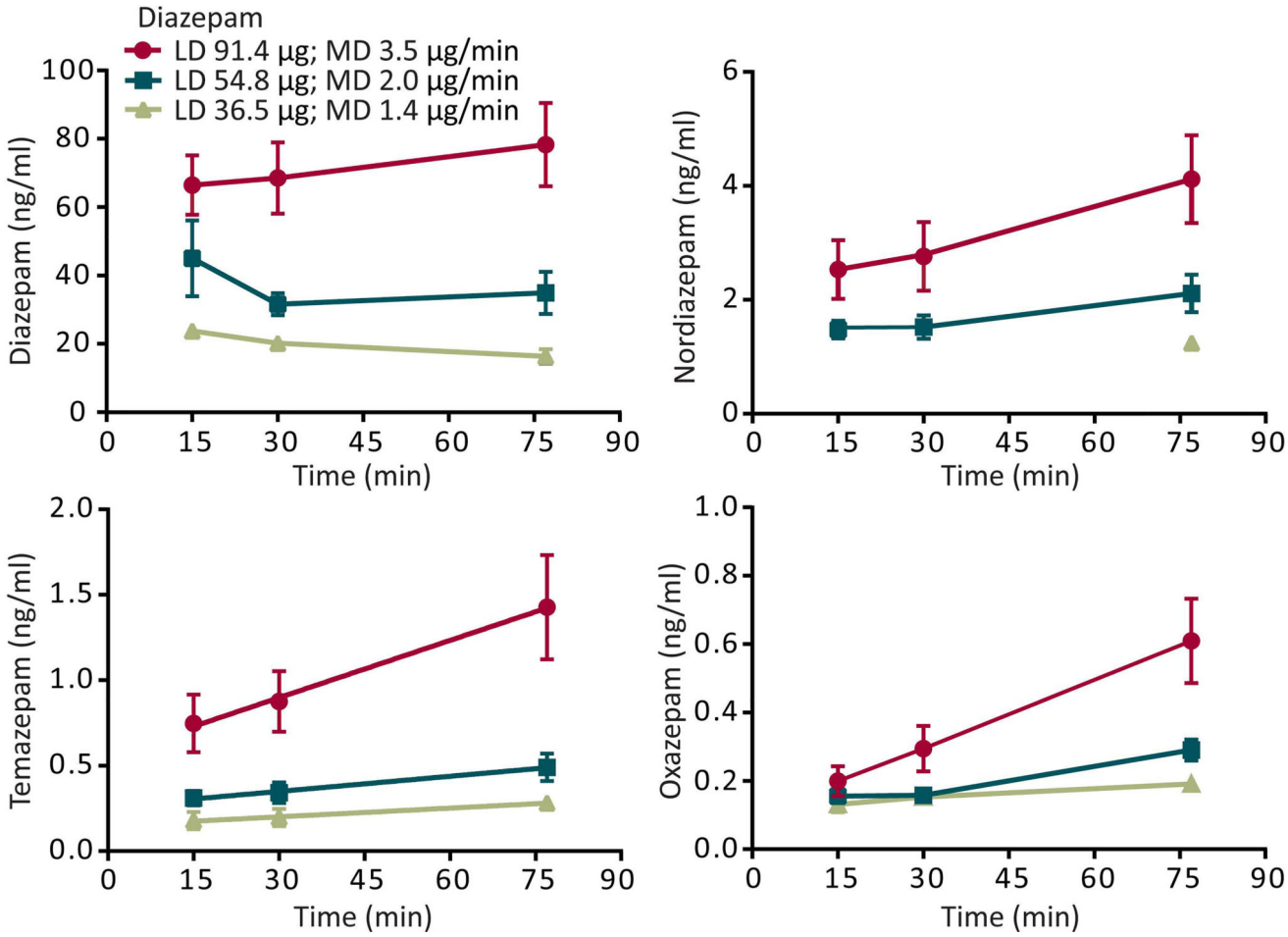


**Figure 4.**

Plasma levels of diazepam and its metabolites nordiazepam, temazepam, and oxazepam at various time points after an intravenous loading dose and during continuous intravenous administration of diazepam (maintenance dose). The legend shows the dosing schemes. Blood samples were collected at 15, 30 and 77 min after the loading dose. The first 2 blood collections were during the maintenance phase and the last blood collection was at the end of the maintenance phase, which equals the mean $t_{½\beta}$ value (Table 1). Data point represent mean ± S.E.M. of measurements in 6 animals. LD, loading dose; MD, maintenance dose. The levels of nordiazepam at 15 and 30 min were below the assay limit of detection. Diazepam area under the curve for first to last sampling time ($AUC_{15–77}$) increased linearly with dose [slope, 1,164 ± 101 (ng/ml • min) $(MD\ mg/kg)^{-1}$].

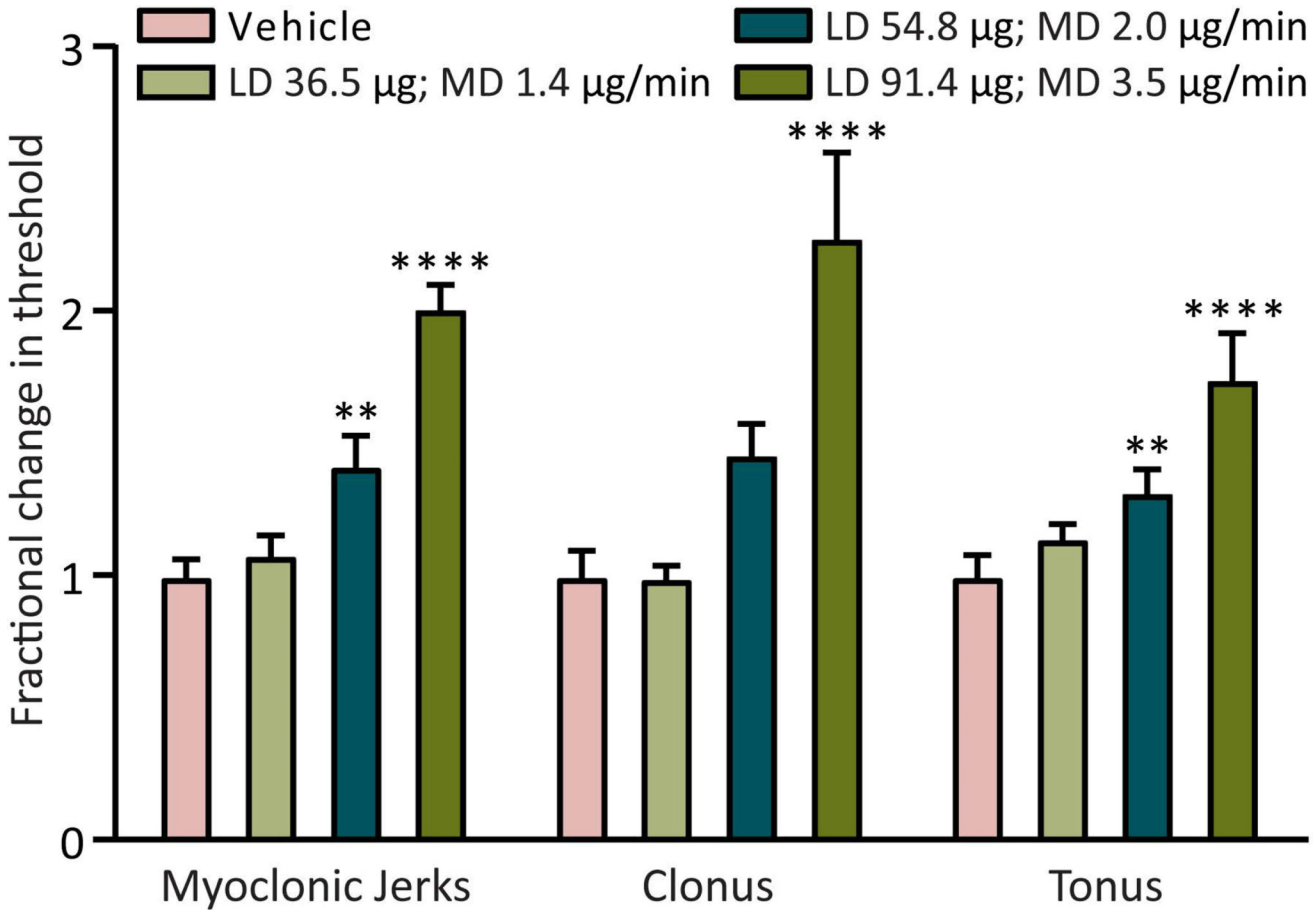


**Figure 5.**

Effect of continuous intravenous infusion of diazepam at different loading and maintenance doses on myoclonic jerk, generalized clonus, and tonic extension in response to intravenous PTZ infusion in rats. PTZ threshold was measured at the end of continuous diazepam infusion. Bars indicate mean ± S.E.M. of values from 6 to 8 rats normalized with respect to the thresholds in the vehicle-treated groups (V), which (in milligrams per kilogram) for myoclonic jerk, clonus, and tonic extension, respectively, were 26.49 ± 1.65, 36.88 ± 3.48, 92.82 ± 6.97. ** $p < 0.001$; **** $p < 0.0001$ compared with the vehicle control group (ANOVA followed by Tukey's test).

**Table 1**

Individual animal noncompartmental parent pharmacokinetic parameters after a bolus intravenous injection of diazepam (80 μg/kg).

| Rat Number | Body Weight (g) | $C_1$ (ng/ml) | $AUC_{0–240}$ (min•ng/ml) | $t_{½\alpha}$ (min) | $t_{½\beta}$ (min) | $V_d$ (ml) | $V_d$ (ml/kg) | CL (ml/min) |
|---|---|---|---|---|---|---|---|---|
| 1 | 366 | 45.2 | 1520 | 14.4 | 85.2 | 497.6 | 1359.6 | 19.27 |
| 2 | 352 | 28.9 | 1000 | 19.6 | NC | 922.5 | 2620.7 | 28.16 |
| 3 | 376 | 49.5 | 1363 | 7.5 | 43.3 | 493.8 | 1313.4 | 22.08 |
| 4 | 360 | 39.0 | 1292 | 17.4 | NC | 656.4 | 1823.3 | 22.29 |
| 5 | 360 | 45.1 | 1245 | 5.8 | 39.1 | 517.8 | 1438.4 | 23.13 |
| 6 | 355 | 51.2 | 2039 | 7.7 | 102.6 | 308.6 | 869.2 | 13.93 |
| 7 | 353 | 38.4 | 1564 | 16.9 | 115.2 | 575.8 | 1631.2 | 18.05 |
| 8 | 360 | 33.3 | 954 | 20.3 | 75.2 | 894.9 | 2485.8 | 30.17 |
| **MEAN ± SEM** | **360 ± 3** | **41.3 ± 2.8** | **1372 ± 122** | **13.7 ± 2.1** | **76.8 ± 12.6** | **608.4 ± 74.1** | **1692.7 ± 211.7** | **22.14 ± 1.86** |

$AUC_{0–240}$, area under the curve from time of bolus injection to the time of last sample (240 min); $C_1$, plasma concentration at first measurement after bolus injection (5 min); $t_{½\alpha}$, initial or disposition half-life; $t_{½\beta}$, elimination half-life; $V_d$, volume of distribution; CL, clearance, NC, not calculated.