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Pharmacokinetics of dexmedetomidine, MK-467, and their combination following intravenous administration in male cats

Short title: PK of IV dexmedetomidine and MK-467 in cats

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Abstract

This study characterized the pharmacokinetics of dexmedetomidine, MK-467, and their combination following intravenous bolus administration to cats. Seven 6 to 7 year old male neutered cats, weighting 5.1 ± 0.7 kg were used in a randomized, cross-over design. Dexmedetomidine [12.5 (D12.5) and 25 (D25) µg/kg), MK-467 [300 µg/kg (M300)] or dexmedetomidine (25 µg/kg) and MK-467 [75, 150, 300 or 600 µg/kg - only the plasma concentrations in the 600 µg/kg group (D25M600) were analyzed] were administered intravenously, and blood was collected until 8 hours thereafter. Plasma drug concentrations were analyzed using liquid chromatography/mass spectrometry. A two-compartment model best fitted the data. Median (range) volume of the central compartment (mL/kg), volume of distribution at steady-state (mL/kg), clearance (mL/min/kg) and terminal half-life (min) were 342 (131-660), 829 (496-1243), 14.6 (9.6-22.7) and 48 (40-69) for D12.5; 296 (179-982), 1111 (908-2175), 18.2 (12.4-22.9), and 52 (40-76) for D25; 653 (392-927), 1595 (1094-1887), 22.7 (18.5-36.4), and 48 (35-60) for dexmedetomidine in D25M600; 117 (112-163), 491 (379-604), 3.0 (2.0-4.5), and 122 (99-139) for M300; and 147 (112-173), 462 (403-714), 2.8 (2.1-4.8), and 118 (97-172) for MK-467 in D25M600. MK-467 moderately but statistically significantly affected the disposition of dexmedetomidine, whereas dexmedetomidine minimally affected the disposition of MK-467.

Keywords: Cats, pharmacokinetics, dexmedetomidine, MK-467, intravenous

Introduction

Dexmedetomidine, the active isomer in the racemic medetomidine, is an alpha-2 adrenoceptor agonist, and is widely used to produce sedation and analgesia, or as premedication prior to general anesthesia, in a variety of species, including cats (Granholm et al., 2006; McSweeney et al., 2012). Like other drugs in this class, medetomidine and dexmedetomidine cause vasoconstriction, bradycardia and decreased cardiac output (Lamont et al., 2001; Selmi et al., 2003; Pypendop et al., 2011). These effects may be detrimental, particularly in older or sick cats, and may limit the clinical use of dexmedetomidine in these patients.

MK-467, previously known as L-659,066 is an alpha-2 adrenoceptor antagonist; however, contrary to other alpha-2 antagonists, it does not appear to cross the blood-brain barrier (Clineschmidt et al., 1988). Because the desirable effects from alpha-2 agonists (sedation, analgesia) are mediated at the level of the central nervous system, but the cardiovascular effects (vasoconstriction, bradycardia) are, at least in part, mediated peripherally (i.e. outside of the central nervous system), combining medetomidine or dexmedetomidine with MK-467 has been proposed to decrease the vasoconstriction and bradycardia, while minimally affecting the sedative (and presumably analgesic) effect. Such benefits have been demonstrated in dogs, sheep and horses (Pagel et al., 1998; Enouri et al., 2008; Honkavaara et al., 2008; Raekallio et al., 2010; Honkavaara et al., 2011; Restitutti et al., 2011; Rolfe et al., 2012; Vainionpaa et al., 2013).

Dexmedetomidine likely affects its own disposition by producing bradycardia and vasoconstriction (Dutta et al., 2000; Pypendop et al., 2013). These effects would be expected to decrease the volume of distribution and clearance, resulting in longer

terminal half-life and increased exposure. If MK-467 prevents or decreases the magnitude of dexmedetomidine-induced cardiovascular depression, it would affect the disposition of dexmedetomidine, as has been reported in dogs (Honkavaara et al., 2012).

The aim of this study was to characterize the pharmacokinetics of dexmedetomidine, MK-467, and their combination, following IV[1] administration to cats. We hypothesized that MK-467 would significantly alter the pharmacokinetics of dexmedetomidine (i.e. would increase clearance/decrease exposure), while dexmedetomidine would not significantly alter the disposition of MK-467.

Materials and methods

The results reported here were obtained as part of a larger study, aiming at defining the optimal dose of MK-467 preventing dexmedetomidine-induced bradycardia without affecting dexmedetomidine-induced sedation (Honkavaara et al., 2016). The methods and results for heart rate measurement and sedation are presented in detail elsewhere.

Animals

Seven healthy 6- to 7-year old neutered male cats were used in the study (mean ± SD body weight 5.1 ± 0.7 kg, body condition score 5/9). The study was approved by the Institutional Animal Care and Use Committee at the University of California, Davis. All cats were acclimatized to laboratory conditions and handling prior to commencing the study.

Instrumentation

Prior to the study, all cats were anesthetized for implantation of a subcutaneous telemetric ECG and blood pressure transmitter and vascular access port; the catheters of the

1 Intravenous

transmitter and port were placed in a carotid artery. However, due to technical problems with the vascular access ports early in the study, it was decided not to use them for blood collection.

At least 12 hours prior to the study, cats were anesthetized with isoflurane in oxygen for placement of a jugular and medial saphenous venous catheter. The former was used for blood sample collection, and the latter for drug administration. Catheter insertion sides (left / right) were alternated during the course of the investigation.

Treatments

Each cat received a total of seven treatments: dexmedetomidine at two doses (12.5 and 25 µg kg^{-1}; D12.5 and D25, respectively), MK-467 (Vetcare Ltd, Mäntsälä, Finland) alone (300 µg kg^{-1}; M300), and dexmedetomidine (25 µg/kg) combined with MK-467 (75, 150, 300 and 600 µg/kg; D25M75, D25M150, D25M300 and D25M600, respectively). The order of treatments was randomized according to a computer-generated randomization list (www.randomizer.org) and there were at least two weeks between successive treatments. MK-467, in powder form, was dissolved in sterile 0.9% saline to a concentration of 2 mg/mL and aspirated into a syringe through a 0.2 µm filter (Fisherbrand, Fischer Scientific, PA, USA). Dexmedetomidine was diluted with 0.9% saline to a concentration of 100 µg/mL. Combination treatments were mixed in the same syringe, and all drugs were diluted to a final volume of 3 mL with 0.9% saline. Treatments were prepared less than an hour prior to administration as an IV bolus.

Blood sampling

Blood samples (2 mL) were obtained from the jugular catheter prior to drug administration, and 1, 2, 4, 8, 15, 30, 60, 120, 240, and 480 minutes following drug

administration. Blood was transferred into tubes containing EDTA and immediately placed on ice. Blood was centrifuged within 30 minutes of collection, the plasma separated and frozen at -20˚C until analyzed for dexmedetomidine and/or MK-467 concentrations.

Drug analysis

Dexmedetomidine and MK-467 concentrations were determined in protein-precipitated plasma samples using liquid chromatography/mass spectrometry, according to previously reported methods (Escobar et al., 2012; Honkavaara et al., 2012). The limit of quantitation was 0.1 ng/mL for both dexmedetomidine and MK-467. For dexmedetomidine, accuracy (% nominal concentration) was verified at 0.3, 5 and 30 ng/mL and ranged from 92 to 111%. Intra-assay and inter-assay precision, verified at the same concentrations, ranged from 2 to 13% and from 6 to 16%, respectively. Based on the pharmacodynamic results (data presented elsewhere), the D25M600 was considered the combination group of interest, and plasma dexmedetomidine and MK-467 concentrations were determined for that combination only (in addition to the D12.5, D25 and M300 groups).

Pharmacokinetic analysis

All pharmacokinetic analyses were performed using Phoenix WinNonlin 6.2 (Certara, Princeton, NJ). Nonlinear least squares regression was performed on the plasma dexmedetomidine concentration-time data. Data were weighted by the reciprocal of the observed plasma concentrations squared (D12.5, dexmedetomidine in D25M600) or the reciprocal of the predicted concentrations squared (D25, M300, MK-467 in D25M600) and fitted to 2-, and 3-compartment models with bolus input into, and elimination from

the central compartment. The appropriate model was selected by observation of the residuals plot and by use of Akaike's information criterion. Parameters estimated by the model were A, B, α and β in the equation $C_t = A \cdot e^{-at} + B \cdot e^{-bt}$, where C_t is the plasma drug concentration at time t. Other pharmacokinetic parameters were calculated by use of standard pharmacokinetic equations.

Protein binding

Protein binding of dexmedetomidine and MK-467 was determined using equilibrium dialysis. Briefly, MK-467 and dexmedetomidine were added to cat plasma to reach concentrations of 0.1, 1, and 10 µg/mL for MK-467, with or without 20 ng/mL dexmedetomidine, and 5, 20, and 100 ng/mL for dexmedetomidine; protein binding of dexmedetomidine 20 ng/mL was also determined with 0.1, 1, or 10 µg/mL MK-467. Samples were incubated for 4 hours with continuous shaking at 37˚C in an equilibrium dialysis device using phosphate buffered saline as a receiver side solution. After incubation, the plasma phase was diluted with an equal volume of phosphate buffered saline, and the phosphate buffered saline phase was diluted with an equal volume of blank plasma. All samples were protein-precipitated using acetonitrile. After centrifugation the supernatants were analyzed in triplicate for dexmedetomidine and/or MK-467 concentrations using liquid chromatography/mass spectrometry. The unbound drug fraction (%) was calculated as 100 x peak area in phosphate buffered saline phase/peak area in plasma phase.

Statistical analysis

Dexmedetomidine pharmacokinetic parameters were compared between the D25 and D25M600 groups using the Wilcoxon signed rank test for paired data. One-tailed tests

were used, to match our stated hypothesis that MK-467 increased the clearance of/decreased the exposure to dexmedetomidine. MK-467 pharmacokinetic parameters were compared between the M300 and D25M600 groups, following correction for the difference in dose where appropriate, using the two-tailed Wilcoxon signed rank test for paired data. In addition, plasma dexmedetomidine concentration at time 0 (C_0) and area under the time-dexmedetomidine concentration curve (AUC) were tested for bioequivalence between D12.5 and D25M600, and between D25 and D25M600. Similarly, MK-467 C_0 and AUC, indexed to the dose, were tested for bioequivalence in the M300 and D25M600 groups. Bioequivalence was defined according to the WHO Guidelines on Evaluation of Similar Biotherapeutic Products[2] and the EMA Guideline on the Investigation of Bioequivalence[3], in which the 90% confidence interval (CI) for the ratio of the test (D25M600) and reference (D12.5, D25 or M300, respectively) products should fall within the 80-125% range. Significance was set at $P < 0.05$. Data is presented as median (range) except were specified otherwise.

Results

Due to a treatment error, data was available for 6 cats in the D25 group, and 7 cats in the other groups. A 2-compartment model with bolus input into, and elimination from the central compartment best fitted the time-plasma concentration data for dexmedetomidine (Figure 1) and MK-467 (Figure 2) in all treatment groups. Pharmacokinetic parameters for dexmedetomidine in D12.5, D25 and D25M600 are presented in Table 1.

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2 http://www.who.int/biologicals/areas/biological_therapeutics/BIOTHERAPEUTICS_FOR_WEB_22APRIL2010.pdf, accessed on 7/30/2015.

3 http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/01/WC500070039.pdf, accessed on 7/30/2015

Pharmacokinetic parameters for MK-467 in M300 and D25M600 are presented in Table 2. The disposition of dexmedetomidine was significantly affected by the co-administration of MK-467, as shown by significant differences in A ($p = 0.0313$), AUC ($p = 0.0469$), area under the first moment curve ($p = 0.0488$), clearance ($p = 0.0469$), and C_0 ($p = 0.0283$). For MK-467, K10 was significantly larger in M300 compared to D25M600 ($p = 0.0234$); no other significant difference was found. Dexmedetomidine 25 µg/kg combined with MK-467 600 µg/kg was not bioequivalent to either dexmedetomidine 12.5 µg/kg or 25 µg/kg [ratio of AUC (90% CI) 113% (79-162%) and 74% (56-98%) for D25M600/D12.5 and D25M600/D25, respectively; ratio of C_0 98% (57-166%) and 55% (31-97%) D25M600/D12.5 and D25M600/D25, respectively]. MK-467 600 µg/kg combined with dexmedetomidine 25 µg/kg fulfilled the criteria for bioequivalence with MK-300 µg/kg based on AUC/dose [ratio (90% CI) 103% (97-109%)] but not on C_0 [ratio (90% CI) 87% (78-97%)].

Results for protein binding of dexmedetomidine and MK-467 are presented in Table 3.

Discussion

In this study, the disposition of dexmedetomidine was influenced by the co-administration of MK-467. This is presumably related to the antagonism of dexmedetomidine-induced bradycardia and vasoconstriction as has been reported in other species, influencing the distribution and metabolism of dexmedetomidine (Enouri et al., 2008; Honkavaara et al., 2008; Raekallio et al., 2010; Honkavaara et al., 2011; Rolfe et al., 2012; Vainionpaa et al., 2013). The changes in the pharmacokinetics of dexmedetomidine observed with co-administration of MK-467 are in agreement with a previous study in dogs, in which co-administration of MK-467 halved the area under the

time-plasma dexmedetomidine concentration curve, and doubled the volume of distribution of dexmedetomidine (Honkavaara et al., 2012). However, the changes seen in this study were more modest in comparison; median dexmedetomidine AUC was approximately 20% smaller when MK-467 was co-administered than when it was not, and median volume of distribution was approximately 44% larger when MK-467 was co-administered, although the difference was not statistically significant. Several factors may have contributed to these differences between dogs and cats. First, the ratio of doses for MK-467 and dexmedetomidine was 24:1 in this study, and 25-75:1 in the dog study. It is possible that the lower MK-467:dexmedetomidine dose ratio used here resulted in less complete antagonism of dexmedetomidine-induced peripheral cardiovascular effects. Second, the previous study compared the pharmacokinetics of dexmedetomidine, with or without co-administration of MK-467, for 90 minutes following administration, compared to 8 hours in this study. At the dose used, the cardiovascular effects of dexmedetomidine are expected to last only for approximately 2-3 hours, therefore the magnitude of the difference would be expected to decrease as drug concentration data is obtained over a longer time. Third, the dose of dexmedetomidine in this study (25 µg/kg) was 2.5 time larger than the dose in the previous study, possibly resulting in larger decreases in heart rate and increases in systemic vascular resistance, which, when coupled with a lower MK-467:dexmedetomidine dose ratio may have resulted in less complete peripheral antagonism of these changes. Finally, the differences may be simply related to the fact that 2 different species were studied.

Dexmedetomidine administration appeared to have minimal influence on the disposition of MK-467 in cats. The reason for the significant difference in K_{10} is unclear, but may be

related to the fact that MK-467 incompletely antagonized dexmedetomidine-induced cardiovascular effects, and that these cardiovascular effects influence the rate at which MK-467 was eliminated. Alternatively, the higher dose of MK-467 in the combination group may be responsible for the difference. In any case, the magnitude of the difference was small, and while clearance was also slightly larger for MK-467 alone, the difference did not reach statistical significance. Interestingly, MK-467 combined with dexmedetomidine did fulfill the criteria for bioequivalence with MK-467 alone (following correction for the difference in dose) based on exposure (i.e. AUC) but not based on C_0. Again, the reason for this lack of bioequivalence is unclear; if related to partial antagonism of early dexmedetomidine-induced cardiovascular effects, one would expect that dose-corrected C_0 would be larger for MK-467 combined with dexmedetomidine than for MK-467 alone, as the lower cardiac output due to lower heart rate and larger increase in systemic vascular resistance following dexmedetomidine would be expected to cause a decrease in the volume of the central compartment. However, in this study, dose-corrected C_0 was larger for MK-467 alone. MK-467 may have dose-dependent pharmacokinetics in cats, as to the author's knowledge, this is the first study in this species, and only one dose of MK-467 was tested; however, dose-dependent pharmacokinetics typically result in differences in AUC. Finally, the reason for the lack of acceptance of bioequivalence in C_0 may be related to lack of statistical power: the lower 90% CI fell just outside of the 80-125% range, and this is likely related to the small study sample, as a larger sample would likely result in a smaller CI around the mean, and would likely fall within the 80-125% range. It should be noted that 7 subjects would be considered too low based on regulatory agency recommendations to test

bioequivalence; therefore, rejection of bioequivalence should be interpreted with caution. A larger number of subjects would tend to narrow the confidence interval, and it is possible that parameters for which the ratio is close to 100%, bioequivalence would be demonstrated in a larger study. However, it is unlikely that a larger study would change the findings for parameters for which bioequivalence was confirmed.

Plasma protein binding was high for dexmedetomidine and moderate for MK-467. In addition, while no statistical analysis was conducted on protein binding data, due to triplicate determination in a single sample at each concentration, plasma protein binding for either drug appeared to be independent of the presence of the other drug. Within the range of concentrations studied, plasma protein binding appeared concentration-independent for dexmedetomidine, whereas it was lower at the lowest MK-467 concentration examined. The reasons for this finding are unclear, but it is suspected to be related to the assay, as a consistent carryover was observed in the chromatographic system during MK-467 analyses, and this may have influenced the observed binding at the lowest MK-467 concentration. Overall, total drug concentrations (as measured in this study) are expected to be a good predictor of the free, active drug concentrations.

The dose of dexmedetomidine used in this study (25 µg/kg) was selected to produce profound sedation for approximately 2 hours, based on the results of a previous study (Pypendop & Ilkiw, 2014b). The additional dexmedetomidine group (D12.5) was selected based on the dog study, in which exposure to dexmedetomidine was approximately halved by the addition of MK-467, in an attempt to include a dexmedetomidine alone group with similar exposure to dexmedetomidine as in the group including MK-467 (Honkavaara et al., 2012). However, as illustrated by the lack of bioequivalence between

the D25M600 and either the D12.5 or D25 groups, the exposure to dexmedetomidine when combined with MK-467 at the dose used was not similar to that following 12.5 µg/kg dexmedetomidine. Nevertheless, the ratios for AUC and C_0 suggest that, overall, the disposition of 25 µg/kg dexmedetomidine combined with 600 µg/kg MK-467 was more similar to the disposition of 12.5 µg/kg than that of 25 µg/kg dexmedetomidine alone. The dose of MK-467 (600 µg/kg) in the combination was selected based on the pharmacodynamic part of the study, as it was the dose achieving the largest reduction in dexmedetomidine-induced bradycardia (Honkavaara et al., 2016). The dose of MK-467 alone (300 µg/kg) was selected because it was an intermediate dose in the planned combination groups.

The pharmacokinetics of dexmedetomidine in cats following bolus administration of 5, 20, and 50 µg/kg have been previously reported (Pypendop & Ilkiw, 2014a). A 3-compartment model best fitted the time-concentration data following 20 and 50 µg/kg, whereas a 2-compartment model best fitted the data in this study. Overall, the disposition of dexmedetomidine following 20 µg/kg was fairly similar to that in this study. When compared to the 25 µg/kg group from the present investigation, median AUC and clearance were 11% larger and 30% lower in the previous study, respectively. Terminal half-life was 52 min in this study and 56 min in the previous study. Volume of distribution was approximately half in the previous study compared to this study. The methods in the previous study were similar to those in this study; however 5 female spayed cats were used, compared to 7 male cats in this study. It is possible that differences in body composition between the 2 study groups account for some of the differences observed.

This study has several limitations. The study sample was small, resulting in limited statistical power; this was worsened for the D25 group due to the treatment error. Due to the small sample, it was decided that non-parametric statistics were preferable, again decreasing the likelihood of reaching statistical significance. Only one dose of dexmedetomidine and MK-467 were used in combination, and the dose of MK-467 alone was different from the dose used in the combination. It is possible that the pharmacokinetic interaction between dexmedetomidine and MK-467 would be different at different doses or different dose ratios. Finally, only male neutered cats were used; to the authors' knowledge, there is no data available on sex differences in the disposition of either dexmedetomidine or MK-467. It is therefore possible that the findings would be different in female cats.

In conclusion, the pharmacokinetics of dexmedetomidine and MK-467, alone and in combination, following intravenous bolus administration in male cats were described. At the doses used, co-administration of MK-467 moderately influenced the disposition of dexmedetomidine, whereas co-administration of dexmedetomidine minimally influenced the disposition of MK-467.

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Table 1: Median (range) pharmacokinetic parameters for dexmedetomidine, following intravenous bolus administration of 12.5 µg/kg (D12.5), 25 µg/kg (D25), or 25 µg/kg dexmedetomidine combined with 600 µg/kg MK-467 (D25M600) in 7 cats. Due to a treatment error, data is available for 6 cats only in the D25 group.

Parameter	D12.5	D25	D25M600
A (ng/mL)	25 (12-78.7)	68.3 (17.1-115.8)	25.1 (14.8-42.4)*
B (ng/mL)	10.1 (2-19)	16.5 (8.4-23.7)	12.3 (9-21.5)
α (/min)	0.3 (0.03-1.49)	0.28 (0.1-1.08)	0.35 (0.11-1.01)
β (/min)	0.014 (0.01-0.017)	0.013 (0.009-0.017)	0.014 (0.012-0.02)
$t_{1/2\alpha}$ (min)	2.3 (0.5-23.1)	3.6 (0.6-6.7)	2 (0.7-6.4)
$t_{1/2\beta}$ (min)	48 (40-69)	52 (40-76)	48 (35-60)
V_1 (mL/kg)	342 (131-660)	296 (179-982)	653 (392-927)
V_2 (mL/kg)	588 (169-696)	744 (579-1193)	904 (496-1147)
V_{ss} (mL/kg)	829 (496-1243)	1111 (908-2175)	1595 (1094-1887)
CL (mL/min/kg)	14.6 (9.6-22.7)	18.2 (12.4-22.9)	22.7 (18.5-36.4)*
CL_D (mL/min/kg)	40.4 (2-153.9)	60.9 (20.4-163.4)	118.7 (37.6-247.5)
K_{10} (/min)	0.043 (0.025-0.083)	0.05 (0.023-0.106)	0.045 (0.025-0.052)
K_{10} $T_{1/2}$ (min)	16.3 (8.4-27.8)	14.9 (6.5-29.7)	15.3 (13.4-28.3)
K_{12} (/min)	0.142 (0.003-1.164)	0.176 (0.049-0.803)	0.182 (0.041-0.629)
K_{21} (/min)	0.086 (0.012-0.262)	0.07 (0.035-0.198)	0.128 (0.038-0.351)
C_0 (ng/mL)	36.5 (18.9-95.7)	86.2 (25.5-139.5)	38.3 (27-63.8)*
AUC (ng.min/mL)	858 (552-1304)	1382 (1090-2023)	1099 (686-1349)*
AUMC (ng.min^2/mL)	61113 (26662-75576)	89045 (61595-150187)	77430 (34348-91517)*
MRT (min)	58 (48-73)	63 (53-95)	64 (46-81)

A, B: coefficients, α, β: exponents in the equations $C_t = A \times e^{-\alpha t} + B \times e^{-\beta t}$, where C_t is drug concentration at time t; $t_{1/2\alpha}$: distribution half-life; $t_{1/2\beta}$: elimination half-life; V_1: apparent volume of the central compartment; V_2: apparent volume of the peripheral compartment; V_{SS}: apparent volume of distribution at steady-state; Cl: clearance; Cl_D: distribution clearance; K_{10}, K_{12}, K_{21}: rate constants; C_0: concentration at time 0; AUC: area under the plasma concentration curve; AUMC: area under the first moment curve; MRT: mean residence time. *Significantly ($P<0.05$) different from D25 (only the D25 and D25M600 groups were compared).

401Table 2: Median (range) pharmacokinetic parameters for MK-467, following intravenous 402bolus administration of 300 µg/kg (M300), or 600 µg/kg MK-467 combined with 25 403µg/kg dexmedetomidine (D25M600) in 7 cats.

Parameter	M300	D25M600
A (ng/mL)	1933.9 (1379.6-2242.9)	2973.7 (2438-3974)
B (ng/mL)	562.5 (431.9-730.3)	1194.9 (731.7-1402.8)
α (/min)	0.29 (0.22-0.5)	0.37 (0.23-0.53)
β (/min)	0.006 (0.005-0.007)	0.006 (0.004-0.007)
$t_{1/2\alpha}$ (min)	2.4 (1.4-3.2)	1.9 (1.3-3.1)
$t_{1/2\beta}$ (min)	122 (99-139)	118 (97-172)
V_1 (mL/kg)	117 (112-163)	147 (112-173)
V_2 (mL/kg)	356 (263-492)	298 (287-561)
V_{ss} (mL/kg)	491 (379-604)	462 (403-714)
CL (mL/min/kg)	3 (2-4.5)	2.8 (2.1-4.8)
CL_D (mL/min/kg)	23.9 (20.1-43)	33.6 (20.5-43.7)
K_{10} (/min)	0.023 (0.017-0.04)	0.02 (0.015-0.031)*
K_{10} $T_{1/2}$ (min)	29.6 (17.1-41)	34.2 (22.4-45.6)
K_{12} (/min)	0.199 (0.141-0.379)	0.268 (0.139-0.367)
K_{21} (/min)	0.072 (0.059-0.115)	0.075 (0.062-0.147)
C_0 (ng/mL)	2558.2 (1842.9-2680)	4069.8 (3464.6-5345)
AUC (ng.min/mL)	100665 (66124-152054)	214892 (126217-281694)
AUMC (ng.min^2/mL)	16577189 (8802645-29246856)	33971353 (18959367-53741134)
MRT (min)	165 (133-192)	160 (130-236)

404*Significantly ($P<0.05$) different from M300 (dose-dependent parameters were indexed 405to the dose for comparison). See table 1 for remainder of key.

406Table 3: Mean±SD protein-binding of dexmedetomidine, with and without MK-467, and

407MK-467, with and without dexmedetomidine, in cat plasma. Mean and SD were

408calculated from triplicate measurements.

Drug and concentration	Fraction bound in plasma (%)
Dexmedetomidine 5 ng/mL	91.2±1.9
Dexmedetomidine 20 ng/mL	92.5±0.2
Dexmedetomidine 100 ng/mL	91.4±0.8
Dexmedetomidine 20 ng/mL with MK-467 0.1 µg/mL	92.2±0.4
Dexmedetomidine 20 ng/mL with MK-467 1 µg/mL	92.3±0.6
Dexmedetomidine 20 ng/mL with MK-467 10 µg/mL	92.8±0.4
MK-467 0.1 µg/mL	53.5±5.9
MK-467 1 µg/mL	65.8±3.4
MK-467 10 µg/mL	64.4±3.8
MK-467 0.1 µg/mL with dexmedetomidine 20 ng/mL	56.1±2.6
MK-467 1 µg/mL with dexmedetomidine 20 ng/mL	65.9±5
MK-467 10 µg/mL with dexmedetomidine 20 ng/mL	64.1±8.1

409

410Figure legends

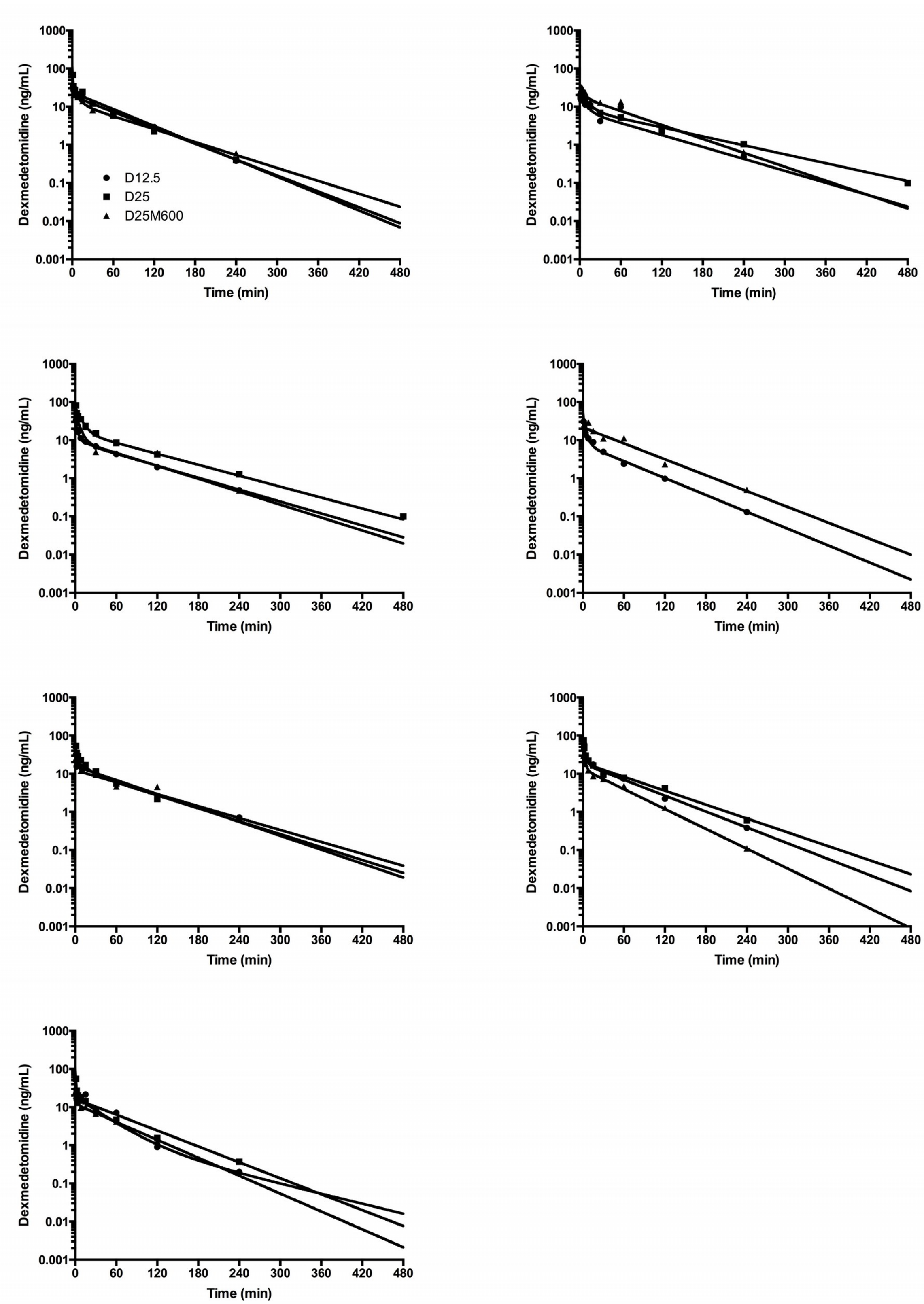
411

412Figure 1: Observed (markers) and predicted (lines) plasma dexmedetomidine

413concentrations following intravenous bolus administration of 12.5 µg/kg (D12.5), 25

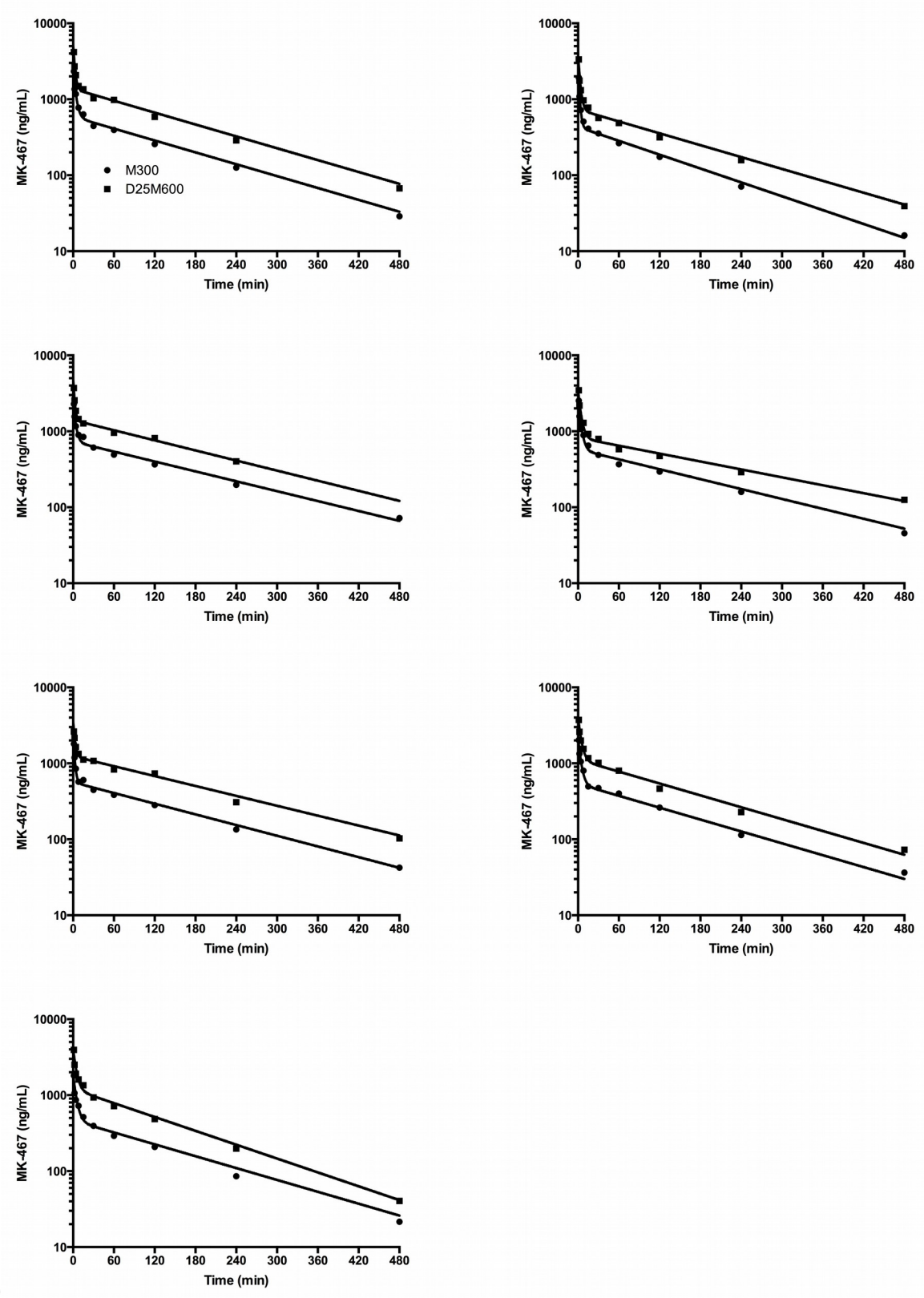
414µg/kg (D25), or 25 µg/kg dexmedetomidine combined with 600 µg/kg MK-467

415(D25M600) in 7 cats. Due to a treatment error, data is only available in 6 cats for D25.


Dexmedetomidine (ng/mL)
1000
100
10
1
0.1
0.01
0.001
Time (min)
0
60
120
180
240
300
360
420
480
D12.5
D25
D25M600

416

Figure 2: Observed (markers) and predicted (lines) plasma MK-467 concentrations following intravenous bolus administration of 300 µg/kg (M300), or 600 µg/kg MK-467 combined with 25 µg/kg dexmedetomidine (D25M600) in 7 cats.


MK-467 (ng/mL)
10000
1000
100
10
0
60
120
180
240
300
360
420
480
Time (min)
M300
D25M600

420