Influence of MK-467, a Peripherally Acting $\alpha_2$-Adrenoceptor Antagonist on the Disposition of Intravenous Dexmedetomidine in Dogs

Juhana Honkavaara, Flavia Restitutti, Marja Raekallio, Kati Salla, Erja Kuusela, Ville Ranta-Panula, Valtteri Rinne, Outi Vainio, and Mika Scheinin

Department of Equine and Small Animal Medicine, University of Helsinki, Helsinki, Finland (J.H., F.R., M.R., K.S., E.K., O.V.); Department of Pharmacology, Drug Development and Therapeutics, University of Turku, Turku, Finland (V.R.-P., V.R., M.S.); and Unit of Clinical Pharmacology, Turku University Hospital, Turku, Finland (V.R.-P., V.R., M.S.)

Received September 6, 2011; accepted November 23, 2011

ABSTRACT:
Growing evidence supports the use of (2R-trans)-N-[2-(1,3,4,7,12b-hexahydro-2′-oxo-spiro(2H-benzo[furo[2,3-a]quinolizine-2,4′-imidazo[3′,4′-y]ethyl])methanesulfonamide (MK-467), a peripherally acting $\alpha_2$-adrenoceptor antagonist, in conjunction with the sedative-anesthetic agent dexmedetomidine in animals to avoid hemodynamic compromise. We evaluated the possible effects of different doses of MK-467 on the plasma concentrations of dexmedetomidine in eight beagle dogs. Both drugs were administered intravenously. Each dog received five treatments: dexmedetomidine alone (10 $\mu$g/kg), MK-467 alone (250 $\mu$g/kg), MK-467 alone (500 $\mu$g/kg), and dexmedetomidine (10 $\mu$g/kg) combined with different doses of MK-467 (250, 500, and 750 $\mu$g/kg) in a randomized, crossover fashion. Selected pharmacokinetic parameters were calculated. The area under the time-concentration curve of dexmedetomidine was significantly greater after dexmedetomidine alone (by 101 ± 20%, mean ± 95% confidence interval) compared with that after dexmedetomidine and 250 $\mu$g/kg MK-467. Increasing the dose of the antagonist had no further effect on the exposure to dexmedetomidine. The apparent volume of distribution of dexmedetomidine was significantly smaller after dexmedetomidine alone compared with that after all treatments that included MK-467. Dexmedetomidine (10 $\mu$g/kg) did not significantly influence the plasma concentrations of MK-467 (250 $\mu$g/kg). The results suggest that the peripherally acting $\alpha_2$-adrenoceptor antagonist MK-467 markedly influenced the early disposition of dexmedetomidine without obvious effects on the later plasma concentrations of the drug.

Introduction

Dexmedetomidine [(+)-4-[1-(2,3-dimethylphenyl)ethyl]-1H-imidazo[2,3-b]quinolizine-2,4′-imidazolidin]-3′-yl]methanesulfonamide (MK-467) is a potent $\alpha_2$-adrenoceptor agonist that is widely used in veterinary and human medicine as a sedative and analgesic agent (Murrell and Hellebrekers, 2005; Tan and Ho, 2010). However, especially in dogs, dexmedetomidine has undesirable cardiovascular effects characterized by marked vasoconstriction and consequent reductions in heart rate, followed by decreases in the cardiac index and tissue oxygen delivery (Pypendop and Verstegen, 1998). This effect is mediated by $\alpha_2$-adrenoceptors located on vascular smooth muscle cells (Flacke et al., 1993; Bloom et al., 1992a; Link et al., 1996). In humans, the adverse cardiovascular effects are commonly not very pronounced, probably because of more conservative dosing regimens and a possibly lesser sensitivity of humans than dogs to the peripheral vasoconstrictive effects of dexmedetomidine. However, humans also show these typical $\alpha_2$-adrenoceptor agonist-mediated vasoactive effects, especially when higher than therapeutically recommended plasma concentrations are reached (Bloor et al., 1992b; Ebert et al., 2000; Snapir et al., 2006). Dexmedetomidine has also been shown to dose dependently reduce its own clearance in humans, a phenomenon mediated via reduced cardiac output (Dutta et al., 2000). Likewise, dexmedetomidine has been suggested to alter its own pharmacokinetics in dogs (Salonen et al., 1995; Kuusela et al., 2000). It also reduces the distribution of thiopental in humans (Bührer et al., 1994).

(2R-trans)-N-[2-(1,3,4,7,12b-hexahydro-2′-oxo-spiro(2H-benzo[furo[2,3-a]quinolizine-2,4′-imidazolidin]-3′-yl)ethyl]methanesulfonamide (MK-467; also known as L-659,066) was first introduced by Clineschmidt et al. (1988) as a peripherally acting $\alpha_2$-adrenoceptor antagonist that did not prevent dexmedetomidine-induced sedation in rats (Doze et al., 1989). It has since been shown to be capable of attenuating or preventing the initial vasoconstriction and the consequent hemodynamic disturbances induced by dexmedetomidine in dogs (Pagel et al., 1998; Enouri et al., 2008; Honkavaara et al., 2008, 2011) and sheep (Bryant et al., 1998; Raekallio et al., 2010) while preserving the centrally mediated desired effects (Honkavaara et al., 2008; Restitutti et al., 2011).

ABBREVIATIONS: MK-467, (2R-trans)-N-[2-(1,3,4,7,12b-hexahydro-2′-oxo-spiro(2H-benzo[furo[2,3-a]quinolizine-2,4′-imidazolidin]-3′-yl)ethyl]methanesulfonamide; AUC, area under the time-concentration curve.
However, neither the possible pharmacokinetic interactions between MK-467 and dexmedetomidine nor the plasma concentrations and pharmacokinetics of MK-467 have been reported in any species. Therefore, we decided to evaluate the plasma concentrations of intravenously administered dexmedetomidine in dogs when coadministered three different doses of MK-467. The focus was on the 1st h after drug administration, when dexmedetomidine is known to have its peak cardiovascular and sedative effects (Pyndop and Verstegen, 1998; Kuusela et al., 2000; Honkavaara et al., 2011; Restiutti et al., 2011). We hypothesized that MK-467 would reduce the exposure to dexmedetomidine because of an improved cardiac index compared with dexmedetomidine alone. Furthermore, plasma concentrations of MK-467 and the effects of dexmedetomidine on them were assessed.

Materials and Methods

Animals. The study was approved by the National Animal Experimentation Board of Finland. Eight healthy beagles (6 males and 2 females), aged 15.1 ± 2.3 months (mean ± S.D.) and weighing 14.9 ± 1.8 kg at study start, were used. The dogs were housed in groups and fed a commercial diet. Before the experiments, food was withheld for 12 h, but water was provided ad libitum. The dogs were considered healthy on the basis of clinical examination, complete blood counts, and routine serum chemistry results.

Instrumentation. Anesthesia was induced with sevoflurane via a mask, and the dogs were intubated. Five milligrams of lidocaine (Lidocain 20 mg/ml; Orion, Turku, Finland) were infiltrated over the jugular vein, and an 18-gauge double-lumen central venous catheter (Arrow International, Reading, PA) was inserted and sutured to the adjacent skin. A 22-gauge intravenous catheter (Optiva-2; Medex Medical Ltd., Lancashire, UK) was placed into the cephalic vein. A minimum of 60 min was allowed between extubation and drug administration to ensure full recovery. Hemodynamic data and assessments of the central nervous system effects were also recorded for separate analysis and reporting (Honkavaara et al., 2011; Restiutti et al., 2011).

Study Protocol. Each dog was treated five times using a randomized crossover design with washout periods of 14 days between the treatments. The treatments were as follows: 10 μg/kg dexmedetomidine (Dexdomitor 0.5 mg/ml; Orion Pharma, Turku, Finland) (D), 10 μg/kg dexmedetomidine + 250 μg/kg MK-467 (Merck Sharp and Dohme, Whitehouse Station, NJ) (DM25), 10 μg/kg dexmedetomidine + 500 μg/kg MK-467 (DM50), 10 μg/kg dexmedetomidine + 750 μg/kg MK-467 (DM75), or 250 μg/kg MK-467 alone (M25). MK-467 was supplied as a powder and was dissolved in sterile saline. Dexmedetomidine was diluted with saline to a concentration of 50 μg/ml. Immediately before use, calculated doses of both drugs were mixed in a single syringe and further diluted with saline to a standard volume of 10 ml. All treatments were administered intravenously via the cephalic vein during 30 s and flushed with 10 ml of saline. Venous blood samples were obtained via the central venous catheter at baseline and at 1, 3, 5, 10, 20, 30, 45, 60, and 90 min after drug administration. After treatment M25, samples were only collected until 60 min. The samples were then centrifuged, and plasma was stored at −20°C until analyzed.

Concentrations of dexmedetomidine in plasma were analyzed with liquid chromatography-mass spectrometry as described previously (Snapiar et al., 2006). In six of the dogs, MK-467 concentrations in plasma were analyzed with liquid chromatography-mass spectrometry after liquid-liquid extraction and with yohimbine as an internal standard. After reverse-phase separation (Gemini 5-μm C18 110A column, 150 × 2.0 mm; Phenomenex, Torrance, CA), quantitative detection was performed in multireaction monitoring mode with a triple quadrupole mass spectrometer (AB Sciex API 4000; Applied Bioscience, Foster City, CA). For MK-467 and yohimbine, the precursor ions were scanned at m/z of 419.0 and 355.0, respectively. The fragment ions monitored for MK-467 had m/z values of 127.0 and 200.0 and values for yohimbine were 212.0 and 144.0. The chromatograms were analyzed and processed using AB Sciex software (Analyst 4.1; Applied Biosystems). The lower limits of quantitation were 0.02 ng/ml for dexmedetomidine and 1.00 ng/ml for MK-467. Pharmacokinetic estimates for the area under the concentration-time curve (AUC0-90 or AUC0-60), total body clearance (Cl), volume of distribution (Vd), and rate constants and half-lives for the distribution and elimination phases (t1/2α and t1/2β) were calculated using standard pharmacokinetic software and both noncompartmental methods and a two-compartment intravenous bolus model with no lag time and first-order elimination (WinNonlin version 5.2; Pharsight, Mountain View, CA).

Statistical Analyses. Treatments were compared as follows for plasma concentrations of dexmedetomidine (n = 8): AUC0-90, Vd, Cl, and distribution and elimination t1/2 were compared between D, DM25, DM50, and DM75. For MK-467 (n = 5; one data set was excluded because of failed quality controls), the following comparisons were made: AUC0-90 and Vd between M25 and DM25 and AUC0-90 and Vd, Cl, and t1/2 between DM25, DM50, and DM75. To assess linearity between dose and exposure to MK-467, multiples of AUC0-90 were compared against DM75 (1.5 × DM50 and 3.0 × DM25, respectively). Post hoc t tests with the Bonferroni correction were used to compare the calculated parameters between treatments. The Shapiro-Wilk test was used to evaluate normality of the distributions of the calculated parameters. Values of P < 0.05 were considered statistically significant.

Results

All treatments were well tolerated. Concentration-time curves of dexmedetomidine in plasma after each treatment are presented in Fig. 1, and the calculated pharmacokinetic parameters of dexmedetomidine are summarized in Table 1. The AUC0-90 for dexmedetomidine, when it was dosed alone, was approximately twice as high as compared with treatments when MK-467 was coadministered. The calculated apparent clearance and apparent volume of distribution of dexmedetomidine were approximately doubled by the concomitant administration of the antagonist. In two-compartment modeling, no statistically significant differences were observed for either t1/2α or t1/2β estimates of dexmedetomidine between the treatments. However, on visual inspection of the data, it was evident that the distribution of dexmedetomidine from the central compartment appeared to be more rapid when the drug was given together with the antagonist. Modeling after dexmedetomidine alone provided rather poor fits with the use of a simple two-compartment model, which resulted in poor accuracy and wide scatter of the t1/2α estimates.

The observed plasma concentrations of MK-467 are presented in Fig. 2. The results from one dog had to be excluded because of failed quality controls in the analysis. There were no significant differences between M25 and DM25 in AUC0-90 or Vd. The calculated parameters for MK-467 are presented in Table 2. The dose-corrected AUCs were nearly equal for DM25 and DM50, but no further dose-proportional increase in exposure was evident for DM75.

Discussion

The peripherally acting α2-adrenergic receptor antagonist MK-467 had a very marked influence on the plasma concentrations of intravenously administered dexmedetomidine in dogs. Exposure to dexmedetomidine, as judged by AUC0-90 values, was approximately halved when dexmedetomidine was administered in combination with the antagonist. This was due to the early disposition of dexmedetomidine being markedly enhanced by MK-467 compared with that of dexmedetomidine alone, probably because of preserved cardiac function and tissue perfusion. A reduction in blood flow to peripheral vascular beds has been suggested to decrease the Vd of drugs that would otherwise be rapidly distributed to tissues (De Paeppe et al., 2002). A comparison of the hemodynamic effects of the treatments from the present study is the subject of another report and is thus not discussed in detail here. In brief, MK-467 dose dependently attenuated or prevented dexmedetomidine-induced increases in systemic vascular resistance and blood pressure and the consequent reductions in heart rate and cardiac index (Honkavaara et al., 2011). Increasing the dose of MK-467 to greater than 250 μg/kg had little effect on the plasma concentrations of dexmedetomidine because only minor differences were detected...
between the treatments involving different dose levels of the antagonist. This was probably because the lowest dose of MK-467 used was already sufficient to prevent most of the hemodynamic effects induced by the $\alpha_2$-adrenoceptor agonist (Honkavaara et al., 2011). The slight reduction in dexmedetomidine-induced clinical sedation after administration of increasing doses (up to 10 $\mu$g/kg) of dexmedetomidine (Lawrence et al., 1996). Although organ-specific perfusion measurements were not performed in this study, it could be expected that the rate-limiting step in the elimination of dexmedetomidine is more dependent on the metabolism of the parent compound than on liver blood flow. In previous studies investigating the metabolism of racemic medetomidine, the importance of biotransformation on the terminal clearance rate in dogs has been highlighted (Salonen et al., 1989). Furthermore, in an in vitro study, Kaivosaa et al. (2002) postulated that canine hepatocytes produce the glucuronide conjugate of dexmedetomidine at a much slower rate than do human hepatocytes. These findings might explain the similar rate of decline in later plasma concentrations among all treatments in the present study. However, the hemodynamic

There were no statistically significant differences in the calculated $t_{1/2}$ of dexmedetomidine between the treatments. This result might suggest that after the initial distribution phase, the drug’s rate of elimination was relatively independent of organ perfusion (e.g., hepatic perfusion that was probably reduced after dexmedetomidine alone). However, in dogs anesthetized with either chloralose-urethane or fentanyl-halothane, only a moderate reduction in blood flow through the hepatic artery was observed after administration of increasing doses (up to 10 $\mu$g/kg) of dexmedetomidine (Lawrence et al., 1996). Although organ-specific perfusion measurements were not performed in this study, it could be expected that the rate-limiting step in the elimination of dexmedetomidine is more dependent on the metabolism of the parent compound than on liver blood flow. In previous studies investigating the metabolism of racemic medetomidine, the importance of biotransformation on the terminal clearance rate in dogs has been highlighted (Salonen et al., 1989). Furthermore, in an in vitro study, Kaivosaa et al. (2002) postulated that canine hepatocytes produce the glucuronide conjugate of dexmedetomidine at a much slower rate than do human hepatocytes. These findings might explain the similar rate of decline in later plasma concentrations among all treatments in the present study. However, the hemodynamic

### TABLE 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AUC$_{0-90}$</th>
<th>$C_{1\text{,min}}$</th>
<th>$V_d$</th>
<th>$Cl$</th>
<th>$t_{1/2p}$</th>
<th>$t_{1/2a}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>421 ± 67*</td>
<td>15.8 ± 3.8</td>
<td>1.1 ± 0.2*</td>
<td>20.9 ± 3.4*</td>
<td>36.8 ± 4.7</td>
<td>6.9 ± 6.6</td>
</tr>
<tr>
<td>DM25</td>
<td>215 ± 24</td>
<td>12.6 ± 1.8</td>
<td>2.5 ± 0.4</td>
<td>38.9 ± 5.8</td>
<td>45.5 ± 7.5</td>
<td>4.2 ± 1.5</td>
</tr>
<tr>
<td>DM50</td>
<td>188 ± 26**</td>
<td>11.6 ± 1.8</td>
<td>3.0 ± 0.6</td>
<td>43.3 ± 9.9</td>
<td>52.7 ± 19.0</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td>DM75</td>
<td>189 ± 25</td>
<td>11.6 ± 2.4</td>
<td>2.8 ± 0.5</td>
<td>44.1 ± 8.7</td>
<td>46.4 ± 10.7</td>
<td>4.0 ± 3.9</td>
</tr>
</tbody>
</table>

* $P < 0.01$ significantly different from all other treatments.
** $P < 0.05$ significantly different from DM25. See text for treatment key.

*The values in the sixth column, $t_{1/2a}$, are derived from a two-compartment model. The first five parameters in the table were calculated with noncompartmental methods and should be treated as rough estimates. For a discussion of this, see the paragraph on the limitations of the study in the Discussion.*

FIG. 1. Mean plasma concentrations (error bars indicate 95% confidence intervals) of dexmedetomidine in eight dogs during the first 10 min after drug administration on a linear scale. Inset, log-linear plot for the whole 0 to 90 min sampling period. See text for treatment key.
differences between treatments in the present study cannot be ignored either. Hepatic clearance can be influenced not only by the capacity of hepatocytes to eliminate a drug from the bloodstream but also by drug delivery regulated by liver blood flow (Wilkinson, 1987). For example, in dogs, reductions in cardiac output and liver blood flow induced by propranolol markedly reduced the clearance of lidocaine without affecting its hepatic extraction ratio (Branch et al., 1973). Thus, after a reduction in liver perfusion, total hepatic clearance would remain unaffected only if the rate of elimination was independent of drug delivery to the hepatocytes via blood flow (Nies et al., 1974). Whereas Lawrence et al. (1996) found no significant effect by dexmedetomidine on arterial hepatic perfusion, their results are not necessarily applicable to conscious dogs because the general anesthetics used in their study could have caused significant alterations in organ blood flow (i.e., impairing the validity of baseline values and obscuring the possible cardiovascular effects of dexmedetomidine). Furthermore, organ perfusion measurements were made 15 min after administration of dexmedetomidine, thus excluding detection of the early cardiovascular disturbances induced by the agonist. In the present study, differences in both the systemic hemodynamic performance (i.e., distribution) and liver perfusion (i.e., clearance) might have then contemporaneously affected the plasma concentrations during the whole of the sampling period, especially because the differences in cardiac index between treatments remained obvious throughout (Honkavaara et al., 2011). In fact, Salonen et al. (1995) concluded that administration of atipamezole, a centrally and peripherally acting α2-adrenoceptor antagonist, increased the clearance of racemic medetomidine in dogs by restoring hepatic blood flow. However, cardiac output or hepatic perfusion was not measured in their study. Because MK-467 markedly increased the apparent Vd of dexmedetomidine, the terminal clearance and half-life estimates may have been affected by differences in drug disposition, because the true elimination phase may not have been sufficiently represented within the sampling period. However, our aim was to study the interactions of the drugs early after their administration when their cardiovascular effects reached their maximum and thus calculation of the pharmacokinetic parameters for the 1st hour would describe these effects better than extrapolating the plasma drug concentrations to infinity. Because the detailed relationship between the intrinsic hepatic clearance and liver blood flow on the total clearance of dexmedetomidine in dogs remains unknown, further in vivo studies are nevertheless necessary especially because only parent drug concentrations were measured in the present study.

The AUC0–60 of MK-467 was not significantly different between M25 and DM25, suggesting that 10 μg/kg simultaneously administered dexmedetomidine did not markedly affect the disposition of MK-467. Because MK-467 alone induced tachycardia and an increase in the cardiac index, compared with moderate reductions in both baseline and hepatic blood flow on the total clearance of dexmedetomidine in dogs remains unknown, further in vivo studies are nevertheless necessary especially because only parent drug concentrations were measured in the present study.

The AUC0–60 of MK-467 was not significantly different between M25 and DM25, suggesting that 10 μg/kg simultaneously administered dexmedetomidine did not markedly affect the disposition of MK-467. Because MK-467 alone induced tachycardia and an increase in the cardiac index, compared with moderate reductions in both baseline and hepatic blood flow on the total clearance of dexmedetomidine in dogs remains unknown, further in vivo studies are nevertheless necessary especially because only parent drug concentrations were measured in the present study.

The AUC0–60 of MK-467 was not significantly different between M25 and DM25, suggesting that 10 μg/kg simultaneously administered dexmedetomidine did not markedly affect the disposition of MK-467. Because MK-467 alone induced tachycardia and an increase in the cardiac index, compared with moderate reductions in both baseline and hepatic blood flow on the total clearance of dexmedetomidine in dogs remains unknown, further in vivo studies are nevertheless necessary especially because only parent drug concentrations were measured in the present study.
ALTERED DISPOSITION OF DEXMEDETOMIDINE BY MK-467 IN DOGS

the central compartment. Of interest, though, increasing the dose of MK-467 did not linearly increase its AUC_{0–90}, whereas it dose dependently opposed the hemodynamic changes induced by dexmedetomidine (Papel et al., 1998; Honkavaara et al., 2011). Although the AUC_{0–90} of MK-467 did increase linearly with DM50 compared with DM25, the exposure to MK-467 did not differ between DM50 and DM75. As with dexmedetomidine, the small differences in cardiovascular function between DM50 and DM75 might be attributable to the absence of an effect of dose on exposure because both distribution and clearance could have been superior with the higher dose of the antagonist. It is unfortunate that neither the principal route(s) of elimination nor metabolism of MK-467 was described in the published literature. Thus, more specific information on the disposition, biotransformation, and clearance of MK-467, with or without dexmedetomidine, would be required, especially if both compounds are to be concomitantly administered in clinical practice.

The present investigation is compromised by the very short sample collection time after the drug administrations. Therefore, the calculated pharmacokinetic parameters can only be considered as rough estimates. For instance, with dexmedetomidine, AUC_{0–90} covered only 52 to 94% of estimated AUC_{0–∞} (average, 81%). This makes the estimates of V_d and Cl inaccurate. However, the main conclusion of the study remains valid, because the conclusion of a significantly altered disposition of dexmedetomidine after concomitant administration of MK-467, compared with that after dexmedetomidine alone, is unaffected by the short sampling time. The present study was focused on the clinically most relevant 1st h after drug administration and was not primarily designed as a pharmacokinetic investigation.

In conclusion, MK-467 reduced the plasma concentrations of intravenously administered dexmedetomidine in dogs, most likely because of an improved cardiac index and tissue perfusion, which increased the disposition of the drug compared with that for dexmedetomidine alone. Dexmedetomidine did not affect the exposure to MK-467, but the latter showed nonlinear dose exposure at least in the presence of the agonist drug. Further studies are needed to fully characterize the mechanisms of the pharmacokinetic interactions of these two compounds, both in vitro and in vivo. It remains to be evaluated whether the dosage of dexmedetomidine needs to be adjusted in clinical practice if the drug is administered in combination with a peripherally acting α_2-adrenoceptor antagonist.

Acknowledgments

MK-467 was a generous gift from Merck, Sharp and Dohme.

Authorship Contributions

Participated in research design: Honkavaara, Restitutti, Raekallio, Kussela, Vainio, and Scheinin.

Conducted experiments: Honkavaara, Restitutti, Raekallio, Kussela, and Rinne.

Performed data analysis: Honkavaara, Salla, Ranta-Panula, and Scheinin.

Wrote or contributed to the writing of the manuscript: Honkavaara, Restitutti, Raekallio, Salla, Kussela, Rinne, Vainio, and Scheinin.

References


