Title page

Influence of MK-467, a peripherally acting \( \alpha_2 \)-adrenoceptor antagonist on the disposition of intravenous dexmedetomidine in dogs

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Running title page

Running title: Altered disposition of dexmedetomidine by MK-467 in dogs

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Introduction: 430

Discussion: 1454

List on non-standard abbreviations:

AUC = Area Under Time Concentration Curve, CI95% = 95% Confidence Interval, CI = Total Body Clearance, m/z = mass-to-charge ratio, $T_{1/2\alpha}$ = Distribution Half-time, $T_{1/2\beta}$ = Elimination Half-time, $V_d$ = Volume of Distribution,
Abstract

Growing evidence supports the use of MK-467, a peripherally acting $\alpha_2$-adrenoceptor antagonist, in conjunction with the sedative-anaesthetic 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 [D]), MK-467 alone (250 $\mu$g/kg [M25]), and dexmedetomidine (10 $\mu$g/kg) combined with different doses of MK-467 (250 $\mu$g/kg [DM25], 500 $\mu$g/kg [DM50] and 750 $\mu$g/kg [DM75]) in a randomized, cross-over fashion. Selected pharmacokinetic parameters were calculated. The area under the time-concentration curve (AUC$_{0-90}$) of dexmedetomidine was significantly greater after dexmedetomidine alone (by 101 ± 20 %, mean ± CI95%) when compared to DM25. 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 D when compared to 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 dexmedetomidine’s early disposition without obvious effects on the drug’s later plasma concentrations.
Introduction

Dexmedetomidine ((+)-4-[1-(2,3-dimethylphenyl)ethyl]-1H-imidazole) is a potent $\alpha_2$-adrenoceptor agonist that is widely used in veterinary and human medicine as a sedative and analgesic agent (Murrell & Hellebrekers, 2005; Tan & 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 & Verstegen, 1998). This effect is mediated by $\alpha_2$-adrenoceptors located on vascular smooth muscle cells (Bloor et al., 1992b; Flacke et al., 1990 & 1993; Link et al., 1996). In humans, the adverse cardiovascular effects are commonly not very pronounced, probably due to more conservative dosing regimens and a possibly smaller sensitivity of humans than dogs to the peripheral vasoconstrictive effects of dexmedetomidine. However, also humans show these typical $\alpha_2$-adrenoceptor agonist–mediated vasoactive effects, especially when higher than therapeutically recommended plasma concentrations are reached (Bloor et al., 1992a; 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). Similarly, 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 (Buhrer et al., 1994).

MK-467 (also known as L-659,066) (2R-trans)-N-(2-)1,3,4,7,12b-hexahydro-2'-oxo-spiro(2H-benzofuro,(2,3-a)quinolizine-2,4'-imidazolidin-3'-yl)ethyl methanesulphonamide) 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). 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 co-administered with three different doses of MK-467. The focus was on the first hour after drug administration, when dexmedetomidine is known to have its peak cardiovascular and sedative effects (Pypendop & Verstegen, 1998; Kuusela et al., 2000; Honkavaara et al., 2011; Restitutti et al., 2011). We hypothesized that MK-467 would reduce the exposure to dexmedetomidine because of an improved cardiac index when compared to 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, 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. Prior to the experiments, food was withheld for 12 hours but water was provided ad libitum. The dogs were considered healthy based on clinical examination, complete blood counts and routine serum chemistry.

Instrumentation. Anesthesia was induced with sevoflurane via a mask and the dogs were intubated. Five mg of lidocaine (Lidocain® 20 mg/ml, Orion, Turku, Finland) were infiltrated over the jugular vein and a 18 G double-lumen central venous catheter (CV-50016, Arrow® International, Reading, PA, USA) was inserted and sutured to the adjacent skin. A 22 G 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; Restitutti et al., 2011).

Study protocol. Each dog was treated five times using a randomized cross-over design with wash-out periods of 14 days between the treatments. The treatments were as follows: dexmedetomidine (Dexdomitor® 0.5 mg/ml, Orion Pharma, Turku, Finland) 10 µg/kg (D), dexmedetomidine 10 µg/kg + MK-467 (Merck, Sharp & Dohme, PA, USA) 250 µg/kg (DM25), dexmedetomidine 10 µg/kg + MK-467 500 µg/kg (DM50), dexmedetomidine 10 µg/kg + MK-467 750 µg/kg (DM75) or MK-467 alone at 250 µg/kg (M25). MK-467 was supplied as a powder and was dissolved
in sterile saline at a concentration of 1 mg/ml. Dexmedetomidine was diluted with saline to a concentration of 50 µg/ml. Immediately prior to 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 i.v. via the cephalic vein during 30 seconds 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 minutes after drug administration. After treatment M25, samples were only collected until 60 minutes. 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 previously described (Snapir 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 internal standard. After reversed-phase separation (Gemini 5 µm C₁₈ 110A column, 150 x 2.0 mm, Phenomenex), quantitative detection was performed in multi-reaction monitoring mode (MRM) with a triple quadrupole mass spectrometer (AB Sciex API 4000). 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 those for yohimbine were 212.0 and 144.0. The chromatograms were analyzed and processed using AB Sciex software (Analyst 4.1). 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 (AUC₀-₆₀ or AUC₀-₉₀), total body clearance (Cl), volume of distribution (V₅), 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 non-compartmental
methods and a two-compartment i.v. bolus model with no lag time and first-order elimination (WinNonlin version 5.2, PharSight Corporation, Mountain View, CA, USA).

Statistical analyses. Treatments were compared as follows for plasma concentrations of dexmedetomidine (n=8): AUC\textsubscript{0-90}, V\textsubscript{d}, Cl and distribution and elimination T\textsubscript{1/2} were compared between D, DM25, DM50 and DM75. For MK-467 (n=5; one dataset was excluded because of failed quality controls), the following comparisons were made: AUC\textsubscript{0-60} and V\textsubscript{d} between M and DM25 and AUC\textsubscript{0-90}, V\textsubscript{d}, Cl and T\textsubscript{1/2} between DM25, DM50 and DM75. To assess linearity between dose and exposure to MK-467, multiples of AUC\textsubscript{0-90} were compared against DM75 (1.5 x DM50 and 3.0 x DM25, respectively). Post hoc t-tests with Bonferroni correction were used to compare the calculated parameters between treatments. Shapiro-Wilks’ 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 Figure 1 and the calculated pharmacokinetic parameters of dexmedetomidine are summarized in Table 1. Dexmedetomidine AUC$_{0-90}$, when dosed alone, was approximately twice as high compared to treatments where MK-467 was co-administered. 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 $T_{1/2\alpha}$ or $T_{1/2\beta}$ estimates of dexmedetomidine between the treatments. Still, 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 employed simple two-compartment model, which resulted in poor accuracy and wide scatter of the $T_{1/2\alpha}$ estimates.

The observed plasma concentrations of MK-467 are presented in Figure 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 AUC$_{60}$ or $V_d$. 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-adrenoceptor 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 to 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 V₅₀ of drugs that would otherwise be rapidly distributed to tissues (Gonzalez et al., 2011). 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. Briefly, 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 above 250 µg/kg had little effect on the plasma concentrations of dexmedetomidine as only minor differences were detected between the treatments involving different dose levels of the antagonist. This was likely because the lowest employed dose of MK-467 was already sufficient to prevent most of the hemodynamic effects induced by the α₂-adrenoceptor agonist (Honkavaara et al., 2011). The slight reduction of dexmedetomidine-induced clinical sedation by MK-467 reported earlier in dogs (Honkavaara et al., 2008) and also seen in this study (comprehensive results are published separately [Restitutti et al., 2011]) were probably caused by this decrease in plasma dexmedetomidine concentrations. Large variation was observed between the dogs in early plasma concentrations and
in $T_{1/2\alpha}$ when dexmedetomidine was administered alone. We suggest that the marked reductions in cardiac output (CO was less than the estimated total blood volume of the dog) affected the early disposition of dexmedetomidine to such an extent that the assumption of a maximum concentration reached immediately after intravenous administration should be rejected (i.e. incomplete early distribution within the central compartment). For example, Grimsrud et al. (2009) reported a range of 1-6 minutes for the observed $T_{max}$ of detomidine after intravenous administration to horses which could be explained by cardiovascular effects (Nyman et al., 2009), comparable with the present study.

There were no statistically significant differences in the calculated $T_{1/2\beta}$ of dexmedetomidine between the treatments. This 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 µg/kg) of dexmedetomidine (Lawrence et al., 1996). While 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 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, Kaivosaari et al. (2002) postulated that canine hepatocytes produce the glucuronide conjugate of dexmedetomidine at a much slower rate than human hepatocytes. These findings
might explain the similar rate of decline in later plasma concentrations between all treatments in the present study. However, the hemodynamic 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). Consequently, 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). While Lawrence et al. (1996) found no significant effect by dexmedetomidine on arterial hepatic perfusion, their results are not necessarily applicable to conscious dogs as 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 minutes 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 as 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. As MK-467 markedly increased the apparent V_d of dexmedetomidine, the terminal clearance and half-life estimates may have been affected by differences in drug disposition, as 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 calculating the pharmacokinetic parameters for the first hour would describe these effects better than extrapolating the plasma drug concentrations to infinity. As 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 as only parent drug concentrations were measured in the present study.

The AUC_{0-60} of MK-467 was not significantly different between M25 and DM25, suggesting that 10 µg/kg of simultaneously administered dexmedetomidine did not markedly affect the disposition of MK-467. As MK-467 alone induced tachycardia and an increase in the cardiac index, compared to moderate reductions in both with DM25 (Honkavaara et al., 2011), the differences in hemodynamic behavior between the doses chosen for comparison were thus unlikely to noticeably influence the plasma concentrations of MK-467. This could be explained by the small volume of distribution of MK-467, also detected in this study, as despite changes in the cardiac index, the majority of the drug would nevertheless stay within the central compartment. Interestingly, though, increasing the dose of MK-467 did not linearly increase its AUC_{0-90} while it dose-dependently opposed the hemodynamic changes induced by dexmedetomidine (Pagel et al., 1998; Honkavaara et al., 2011). Although
the AUC$_{0-90}$ of MK-467 did increase linearly with DM50 when compared to 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 as both distribution and clearance could have been superior with the higher dose of the antagonist. Unfortunately, neither the principal route(s) of elimination nor metabolism of MK-467 have been 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 52-94 % of estimated AUC$_{0-\text{inf}}$ (average, 81 %). This makes the estimates of $V_d$ and Cl inaccurate. The main conclusion of the study, however, remains valid, as the conclusion of a significantly altered disposition of dexmedetomidine after concomitant administration of MK-467, compared to dexmedetomidine alone, is unaffected by the short sampling time. The present study was focused on the clinically most relevant first hour 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 when compared to dexmedetomidine alone. Dexmedetomidine did not affect the exposure to MK-467, but the latter showed non-linear 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 α₂-adrenoceptor antagonist.
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Authorship contributions:

Participated in research design: Honkavaara, Kuusela, Raekallio, Restitutti, Scheinin, Vainio

Conducted experiments: Honkavaara, Kuusela, Raekallio, Restitutti, Rinne

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

Wrote or contributed to the writing of the manuscript: Honkavaara, Kuusela, Raekallio, Restitutti, Rinne, Salla, Scheinin, Vainio
References


Footnotes:

Some of the results have been presented at the 10th World Congress of Veterinary Anaesthesia, 2009, Glasgow, Scotland.
Legends for the Figures:

Figure 1. Mean plasma concentrations (error bars indicate 95 % confidence intervals) of dexmedetomidine in eight dogs during the first ten minutes after drug administration on a linear scale. Insert: Log-linear plot for the whole 0-90 minute sampling period. See text for treatment key.

Figure 2. Mean plasma concentrations (error bars indicate 95 % confidence intervals) of MK-467 in five dogs. See text for treatment key.
Table 1. Calculated pharmacokinetic parameters of dexmedetomidine in eight dogs (mean ± 95% confidence interval).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AUC0-90 (ng/min/ml)</th>
<th>C1min (ng/ml)</th>
<th>Vd (l/kg)</th>
<th>Cl (ml/kg/min)</th>
<th>T1/2β (min)</th>
<th>T1/2α* (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>421±67(^a)</td>
<td>15.8±3.8</td>
<td>1.1±0.2(^a)</td>
<td>20.9±3.4(^a)</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(^b)</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>

\(^a\) p < 0.01 significantly different from all other treatments, \(^b\) p < 0.05 significantly different from DM25. See text for treatment key. * The values in the sixth column, T1/2α, are derived from a two-compartment model. The first five parameters in the Table were calculated with non-compartmental 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.
Table 2. Calculated pharmacokinetic parameters of MK-467 in five dogs (mean ± 95% confidence interval). 

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AUC&lt;sub&gt;0-90&lt;/sub&gt; (ng/min/ml)</th>
<th>C&lt;sub&gt;1min&lt;/sub&gt; (µg/ml)</th>
<th>V&lt;sub&gt;d&lt;/sub&gt; (l/kg)</th>
<th>Cl (ml/kg/min)</th>
<th>T&lt;sub&gt;1/2β&lt;/sub&gt; (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM25</td>
<td>31700±8400</td>
<td>1.81±0.47</td>
<td>0.49±0.15</td>
<td>6.5±2.6</td>
<td>53.8±8.3</td>
</tr>
<tr>
<td>DM50</td>
<td>67400±28700</td>
<td>2.89±0.72</td>
<td>0.42±0.1</td>
<td>6.2±2.1</td>
<td>52.4±13.1</td>
</tr>
<tr>
<td>DM75</td>
<td>68700±26400</td>
<td>2.74±0.55</td>
<td>0.62±0.23</td>
<td>8.8±3.3</td>
<td>50.6±7.2</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-60&lt;/sub&gt; (ng/min/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M25</td>
<td>26600±9100</td>
<td>1.44±0.26</td>
<td>0.41±0.13</td>
<td>7.8±3.4</td>
<td>39±7.6</td>
</tr>
</tbody>
</table>

See text for treatment key. Note: parameters for treatment M25 are calculated from 0 until 60 minutes and no statistical comparisons with the other treatments were performed.
Figure 1
Figure 2

Concentration (ng/ml) vs. Time (min)

- M25
- DM25
- DM50
- DM75