In vitro evaluation of differences in phase 1 metabolism of ketamine and other analgesics among humans, horses, and dogs

Livia Capponi, Dr med vet; Andrea Schmitz, Dr med vet; Wolfgang Thormann, Dr phil nat, Dr habil; Regula Theurillat; Meike Mevissen, Dr med vet, Dr habil

Objective—To investigate cytochrome P450 (CYP) enzymes involved in metabolism of racemic and S-ketamine in various species and to evaluate metabolic interactions of other analgesics with ketamine.

Sample Population—Human, equine, and canine liver microsomes.

Procedures—An analgesic was concurrently incubated with luminogenic substrates specific for CYP 3A4 or CYP 2C9 and liver microsomes. The luminescence signal was detected and compared with the signal for negative control samples. Ketamine and norketamine enantiomers were determined by use of capillary electrophoresis.

Results—A concentration-dependent decrease in luminescence signal was detected for ibuprofen and diclofenac in the assay for CYP 2C9 in human and equine liver microsomes but not in the assay for CYP 3A4 and methadone or xylazine in any of the species. Coincubation of methadone or xylazine with ketamine resulted in a decrease in norketamine formation in equine and canine liver microsomes but not in human liver microsomes. In all species, norketamine formation was not affected by ibuprofen, but diclofenac reduced norketamine formation in human liver microsomes. A higher rate of metabolism was detected for S-ketamine in equine liver microsomes, compared with the rate for the S-enantiomer in the racemic mixture when incubated with any of the analgesics investigated.

Conclusions and Clinical Relevance—Enzymes of the CYP 3A4 family and orthologs of CYP 2C9 were involved in ketamine metabolism in horses, dogs, and humans. Methadone and xylazine inhibited in vitro metabolism of ketamine. Therefore, higher concentrations and diminished clearance of ketamine may cause adverse effects when administered concurrently with other analgesics. (Am J Vet Res 2009;70:777–786)
S-ketamine is its greater affinity for N-methyl-D-aspartate receptors, compared with the affinity of R-ketamine.\textsuperscript{11} The S-enantiomer has been successfully used in experiments involving dogs\textsuperscript{9} and other laboratory animals. Recently, S-ketamine has been introduced into veterinary practice for use in cats. Clinically, the anesthetic and analgesic potency of the S-isomer is approximately 3 to 4 times that of the R-isomer.

In horses, ketamine should not be used as the sole induction agent because its use results in extensor rigidity, dog-sitting posture, extreme muscle spasms, purposeless movements, excited facial expressions, profuse sweating and salivation, and sometimes convulsions.\textsuperscript{13} Therefore, it is recommended that ketamine be administered in combination with xylazine and diazepam. The combination of racemic ketamine with the α\textsubscript{2}-adrenergic receptor agonist xylazine has been widely used in horses to induce and maintain anesthesia.\textsuperscript{14} A more rapid recovery was evident in ponies sedated with xylazine administered in conjunction with S-ketamine, compared with recovery after administration of xylazine and racemic ketamine.\textsuperscript{15} The more rapid elimination of S-ketamine, compared with elimination of the racemic mixture, could explain the more rapid recovery detected with S-ketamine. In contrast, pharmacokinetic variables of racemic ketamine or the single S-enantiomer in ponies anesthetized with isoflurane did not significantly differ.\textsuperscript{16} These are indications that concurrently administered drugs are of importance for the pharmacokinetics of ketamine.

In humans, in vitro studies\textsuperscript{17–19} have revealed that CYP 3A4, CYP 2C9, and CYP 2B6 are responsible for N-demethylation of racemic ketamine, with CYP 3A4 being the enzyme primarily involved in biotransformation. In vitro biotransformation experiments have revealed that ketamine is demethylated in the liver and lungs of horses.\textsuperscript{20} Preliminary data from our laboratory group have confirmed that the CYP 3A4 and CYP 2C9 orthologs are the metabolizing enzymes for racemic ketamine in equine liver microsomes. Taking into account the important role of ketamine in anesthesia and analgesia in many species, it has been our intent to evaluate metabolic interactions of ketamine with routinely coadministered drugs, such as methadone, xylazine, ibuprofen, and diclofenac. Methadone is used as an analgesic agent for transportation to our laboratory. Tissues were frozen and stored at −70°C until used for microsomal preparation, as described elsewhere.\textsuperscript{26} Total CYP protein was determined by use of a method reported elsewhere.\textsuperscript{27} The final concentration of CYP protein was 461 pmol/mg of protein. Human\textsuperscript{4} and canine\textsuperscript{5} liver microsomes were obtained from a commercial source and stored at −70°C. Baculovirus-insect-cell–expressed human CYP 3A4 plus P450 reductase plus cytochrome b\textsubscript{5} (referred to as the CYP 3A4 supersomes) and human CYP 1A2 plus P450 reductase plus cytochrome b\textsubscript{5} (referred to as the CYP 1A2 supersomes) were also obtained from a commercial source.\textsuperscript{8}

**Materials and Methods**

**Sample population**—Liver samples were collected from the cadavers of 3 healthy horses at a local slaughterhouse. Horses were crossbred horses or Franches-Montagnes of both sexes; horses were 13 to 30 years old. The horses had no history of drug treatment for at least 6 months preceding slaughter. Liver samples were collected within 30 minutes after the horses were stunned. Samples were immediately placed on dry ice for transportation to our laboratory. Tissues were frozen and stored at −70°C until used for microsomal preparation, as described elsewhere.\textsuperscript{26} Total CYP protein was determined by use of a method reported elsewhere.\textsuperscript{27} The final concentration of CYP protein was 461 pmol/mg of protein.

**Luminescence signal of liver microsomes incubated with substrate specific for CYP 3A4 and CYP 2C9**—A luminogenic CYP assay\textsuperscript{4} couples CYP enzyme activity to the light generation of firefly luciferase.\textsuperscript{28} Luciferase uses ATP and D-luciferin to generate light in the presence of ambient oxygen and magnesium. Luminogenic CYP substrates are derivatives of D-luciferin, but they are not active with luciferase.

In an initial step, human, canine, and equine liver microsomes were incubated with ketamine, methadone, xylazine, ibuprofen or diclofenac, and D-luciferin for 30 minutes, and the derivates were converted by the CYP enzyme to D-luciferin products when they reacted with the respective CYP. Luciferin-H is highly selective for CYP 2C9 in human microsomes, whereas luciferin-BE reacts with CYP 3A4, CYP 2C9, and CYP 4F12. However, the expression of CYP 3A7 and CYP 4F12 is minimal in the liver of humans.\textsuperscript{28} The luminescence signal was detected in a second step by the addition of luciferin detection reagent, which contained luciferase and ATP in a reaction mixture limited only for D-luciferin. Luciferin detection reagent simultaneously stopped the CYP en-
zymatic activity and initiated a luciferase reaction that generated an amount of light directly proportional to the luciferin product produced by the CYP. Concurrent incubation of the luminogenic substrate and the compound of interest (eg, ketamine) was expected to attenuate the luminescence signal, compared with the signal for a control substance, when the 2 substances interacted with the same CYP. Assays were conducted in accordance with the manufacturer’s instructions. Positive control samples were inhibitors for human CYP 3A4 (ketocazolene1) and CYP 2C9 (diclofenac2). Potassium phosphate buffer3 (100mM potassium dihydrogen phosphate mixed with 100mM dipotassium hydrogen phosphate [pH, 7.4]) was used to perform negative control reactions. Luminogenic assays were conducted in a white opaque polystyrene nontreated flat-bottom 96-well plate.4 A luciferin standard curve was prepared for each assay; concentrations ranged from 0.016 to 1µM beetle luciferin.5 The microsomal protein content in each well was 20 µg. Racemic ketamine1 was used at final concentrations of 1, 10, 50, 100, and 200µM; S-ketamine6 was used at concentrations of 0.5, 5, 25, 50, and 100µM; methadone5,6 xylazine hydrochloride,6 and ibuprofen6 were each used at final concentrations of 10, 50, 100, 200, and 300µM; and diclofenac was incubated at final concentrations of 1, 5, 10, 50, and 100µM. Ketonazolene (an inhibitor for human CYP 3A4) was used as a positive control compound at a final concentration of 0.2µM, and diclofenac (a selective inhibitor for CYP 2C9) was used at a final concentration of 20µM. Ketonazolene was dissolved in methanol to yield a final methanol concentration of 0.02% in the incubation mixture. The final concentration of the potassium phosphate buffer was 100mM. The luminescence signal was detected at 20°C by use of a luminometer and computer software.7 The NADPH required for CYP activity was supplied by use of an NADPH regeneration system.8 Incubations were performed in triplicate. Unspecific toxic effects of the inhibitors and ketamine against any enzymes were ruled out in preliminary experiments in which luciferin was incubated (37°C for 20 minutes) with the respective inhibitors and the commercial luciferin detection reagent containing the luciferase enzyme. In addition, incubation of racemic ketamine with human CYP 1A2 supersomes was used to test for unspecific quenching of luminogenesis by the substrate. Use of the respective substrates did not reveal a reaction.

Microsomal incubations with ketamine and other compounds—The incubation mixture (250 µL) comprised human, equine, or canine liver microsomes (0.5 mg/mL of microsomal protein), racemic ketamine at a concentration of 26µM or S-ketamine at a concentration of 13µM, potassium phosphate buffer (100mM [pH, 7.4]), and the NADPH regenerating system9 (1.3mM NADP+, 3.3mM glucose-6-phosphate, glucose-6-phosphate dehydrogenase [0.4 U/mL], 3.3mM MgCl2, and 5µM sodium citrate). The mean value for the Michaelis constant (ie, Km) for racemic ketamine was calculated to be approximately 65µM. Racemic ketamine or S-ketamine was incubated concurrently with xylazine or ibuprofen at concentrations ranging from 0 to 300µM, with methadone at concentrations ranging from 0 to 200µM, or with diclofenac at concentrations ranging from 0 to 100µM. The incubation mixture was vortexed and preincubated for 3 minutes at 37°C. Microsomal protein was added to achieve a final concentration of 0.5 mg/mL in a final volume of 250 µL, and the enzymatic reaction was started at 37°C. Samples were incubated for 8 minutes without shaking. An incubation period of 8 minutes was chosen on the basis of preliminary experiments conducted with equine and canine microsomes. Incubations were stopped by the addition of 500µL of 0.2M NaOH,6 and vials were placed on ice. Incubations were performed in duplicate.

Analytic procedure and assay specifications—Enantioselective analysis of ketamine and norketamine was performed by use of capillary electrophoresis10 with modifications described in another report10 and conditions used in the in vitro experiments of other investigators.11 The assay was based on liquid-liquid extraction at alkaline pH followed by capillary electrophoresis analysis of the reconstituted extract by use of a Tris-phosphate buffer (50mM [pH, 2.5]) containing 10 µg of sulfated β-cyclodextrin/mL as a chiral selector. The β-cyclodextrin was a mixture (35 mg from 1 lot12 and 15 mg from a second lot,13 which was dissolved in 5 mL of Tris-phosphate buffer). To avoid an overlap of peaks between R-ketamine and xylazine, only β-cyclodextrin from 1 lot was used (50 µg, which was dissolved in 5 mL of Tris-phosphate buffer that consisted of 0.303 g of Tris14 and 144 µL of orthophosphoric acid [85%]15 in 50 mL of distilled water with xylazine) as the chiral selector for measuring the incubations. Briefly, samples (200 µL) were mixed with 0.5 mL of 0.2M sodium hydroxide containing 30 µL of the internal standard (+)-pseudophedrine hydrochloride.16 Samples were evaporated, and remaining residues were dissolved in 30 µL of Tris-phosphate buffer (5mM), A capillary electrophoresis instrument17 with an on-column variable wavelength detector set to 195 nm and a 50-µm (internal diameter) fused-silica capillary18 with a total length of 45 cm (effective length, approx 34 cm) was used. Applied voltage was –20 kV, and temperature of the circulating cooling fluid in the capillary cartridge and around the sample trays was set at 20°C. Samples were injected with a vacuum of 6.895 kPa for 5 to 7 seconds; run time was 15 to 20 minutes. Quantitation was based on multilevel internal calibration by use of corrected peak areas.20 All chemicals were analytic grade.

Data analysis—All values were reported as mean and SD. Data were statistically analyzed by use of an ANOVA for repeated measures followed by the Bonferroni multiple comparison test; statistical analyses were conducted by use of commercially available software.21 Values of P < 0.05 were considered significant.

Results

Identification of CYP orthologs involved in metabolism of ketamine and analgesics in human, equine, and canine species—In the first part of our study, our intent was to identify CYP orthologs involved in the metabolism
of ketamine and analgesic compounds in human, canine, and equine liver microsomes. Luminogenic CYP assays were used to accomplish this objective.

Concurrent incubation of racemic ketamine, the substrate for CYP 3A4, and liver microsomes from all 3 species resulted in a significant (P = 0.01) concentration-dependent decrease in the luminescence signal in human and equine microsomes (Figure 1). No significant concentration-dependent results were obtained in canine liver microsomes. Ketoconazole, an inhibitor of CYP 3A4, caused an inhibition of the luminescence signal of approximately 30% in all 3 species. At the highest S-ketamine concentration (100 µM), inhibition of the luminescence signal was approximately 25% in all species investigated. At the lowest concentration investigated (0.5 µM), activity of the signal was approximately 85% for human and canine liver microsomes, whereas no effect was evident for equine liver microsomes. No difference was detected for the 3 species for data obtained with incubations consisting of 50 µM S-ketamine and 100 µM racemic ketamine.

A significant (P = 0.01) concentration-dependent reduction in the luminescence signal was obtained when the luminogenic substrate for CYP 3A4 was concurrently incubated with S-ketamine (Figure 1). This effect was evident with human, canine, and equine liver microsomes, and no marked difference was evident among the 3 species. At the highest S-ketamine concentration (100 µM), inhibition of the luminescence signal was approximately 25% in all species investigated. At the lowest concentration investigated (0.5 µM), activity of the signal was approximately 85% for human and canine liver microsomes, whereas no effect was evident for equine liver microsomes. No difference was detected for the 3 species for data obtained with incubations consisting of 50 µM S-ketamine and 100 µM racemic ketamine.

Human, equine, and canine liver microsomes were incubated with the substrate for CYP 3A4 and various concentrations of methadone or xylazine. No effect on luciferase was obtained for methadone in human, equine, or canine liver microsomes at any concentration investigated (data not shown). With respect to the limited specificity of the CYP 3A4 substrate provided by the supplier, methadone was incubated with human CYP 3A4 supersomes to provide evidence that this enzyme is involved in methadone biotransformation. A significant (P < 0.001) concentration-dependent inhibition of the luminescence signal was detected (Figure 2). Inhibition of the signal was approximately 25% at the lowest concentration used (10 µM). The decrease in activity was 45% at a concentration of 50 µM, and no further decrease in the luminescence signal was detected at the higher concentrations (100 to 300 µM).
Minor inhibition of the luminescence signal was only detected at the highest concentration (300 \mu M) when xylazine was incubated with human and equine liver microsomes and the substrate for CYP 3A4. With canine liver microsomes, no decrease in the luminescence signal was detected. A decrease of 23% and 13% in the luminescence signal was detected at the highest concentration investigated for human and equine liver microsomes, respectively (Table 1). Inhibition of the luminescence signal of the positive control compound (0.2 \mu M ketoconazole) was 51%, 35%, and 26% for human, equine, and canine liver microsomal preparations, respectively.

Incubation of human and equine liver microsomes with various concentrations of the NSAID ibuprofen and the substrate for CYP 2C9 resulted in a significant \((P < 0.001)\) concentration-dependent inhibition of the luminescence signal for both species (Figure 3). In equine liver microsomes, inhibition of the signal was more pronounced, compared with that for human liver microsomes; inhibition differed significantly between the 2 species at 3 concentrations of ibuprofen. Incubation of the NSAID diclofenac with the substrate specific for human CYP 2C9 resulted in a significant \((P < 0.001)\) concentration-dependent inhibition of the luminescence signal in human and equine liver microsomes (Table 1). The decrease in activity was approximately 65% at the highest concentration of diclofenac in human and equine liver microsomes, compared with results for the solvent control sample (which was assigned a value of 100%). Ibuprofen (200 \mu M) was used as a positive control compound, and inhibition of approximately 20% and 30% was evident in human and equine liver microsomes, respectively, compared with results for the solvent control sample.

Drug-drug interactions of analgesics with ketamine in human, equine, and canine liver microsomes—Increasing methadone concentrations resulted

### Table 1—Results for incubation of human, equine, and canine liver microsomes with xylazine, diclofenac, and CYP inhibitors.*

<table>
<thead>
<tr>
<th>CYP</th>
<th>Compound</th>
<th>Concentration (\mu M)</th>
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<th>Equine</th>
<th>Canine</th>
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<td>3A4</td>
<td>Ketoconazole</td>
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<td>49 ± 16.0</td>
<td>65 ± 11.4</td>
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<td></td>
<td>Xylazine</td>
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<td>100 ± 3.5</td>
<td>85 ± 14.6</td>
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<tr>
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<td>50</td>
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<td>97 ± 3.4</td>
<td>99 ± 6.3</td>
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<tr>
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<td>100</td>
<td>102 ± 4.5</td>
<td>94 ± 5.1</td>
<td>97 ± 5.6</td>
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<td>200</td>
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<td>91 ± 6.4</td>
<td>85 ± 6.7</td>
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<tr>
<td></td>
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<td>300</td>
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<td>87 ± 4.0</td>
<td>97 ± 8.7</td>
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<td>2C9</td>
<td>Ibuprofen</td>
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<td>86 ± 9.6</td>
<td>70 ± 9.6</td>
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<td>100 ± 10.1</td>
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<td>34 ± 5.2</td>
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Values reported are mean ± SD of triplicate incubations.

*Ketoconazole is a standard inhibitor for CYP 3A4, and ibuprofen is a standard inhibitor for CYP 2C9.

ND = Not determined.

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**Figure 2**—Mean ± SD results for generation of the luminescence signal for various concentrations of methadone incubated with baculovirus-insect-cell–expressed human CYP 3A4 plus P450 reductase plus cytochrome b5 (ie, human CYP 3A4 supersomes) and the luminogenic substrate specific for human CYP 3A4. Ketoconazole (0.2 \mu M) was used as a positive control compound. See Figure 1 for remainder of key.

**Figure 3**—Mean ± SD results for generation of the luminescence signal for various concentrations of ibuprofen incubated with human (A) and equine (B) liver microsomes and the luminogenic substrate specific for CYP 2C9. Diclofenac (20\mu M) was used as a positive control compound. The luminescence signal obtained is shown, compared with the signal for the solvent control sample. See Figure 1 for remainder of key.
in a decrease in metabolite formation (S- and R-norketamine) in equine and canine microsomal preparations; consequently, significantly \( (P < 0.001) \) higher amounts of ketamine were detected in the reaction mixture (Figure 4). In contrast, increasing concentrations of methadone decreased norketamine formation in human liver microsomes only at the 2 highest concentrations investigated, and results did not differ significantly. In human, equine, and canine liver microsomes, R-ketamine concentrations exceeded S-ketamine concentrations when ketamine was incubated alone or concurrently with methadone. Concentrations of S-norketamine were larger than concentrations of R-norketamine in all cases.

Coincubation of xylazine and ketamine resulted in a decrease in norketamine formation in equine liver microsomes; this was a significant \( (P = 0.01) \) concentration-dependent effect. Consequently, higher amounts of ketamine were detected with increasing xylazine concentrations (Figure 5). No change was detected for human liver microsomes (data not shown), and the effect on norketamine formation was small but significant for canine liver microsomes. Concentrations of R-ketamine \( > 10 \mu M \) in combination with high xylazine concentrations could not be accurately determined, which explained missing R-ketamine data. The amount of S-norketamine exceeded the amount of R-norketamine at all xylazine concentrations.

![Figure 4](image1.png)  
**Figure 4**—Mean ± SD concentrations of R-ketamine (circles and solid line), S-ketamine (squares and solid line), R-norketamine (circles and dashed line), and S-norketamine (squares and dashed line) when incubated with methadone and 26 µM racemic ketamine in human (A), equine (B), and canine (C) liver microsomes. The incubation period was 8 minutes. Data represent results for duplicate incubations. In panels B and C, there is a significant \( (P < 0.05) \) decrease in norketamine formation with increasing concentrations of methadone.

![Figure 5](image2.png)  
**Figure 5**—Mean ± SD concentrations of R-ketamine (circles and solid line), S-ketamine (squares and solid line), R-norketamine (circles and dashed line), and S-norketamine (squares and dashed line) when incubated with xylazine and 26 µM racemic ketamine in equine (A) and canine (B) liver microsomes. The incubation period was 8 minutes. Data represent results for duplicate incubations. For both species, there is a significant \( (P < 0.05) \) decrease in norketamine formation with increasing concentrations of xylazine.
concentrations investigated in equine and canine liver microsomes, whereas this effect was not evident in human liver microsomes (data not shown).

Norketamine formation was not affected by the concentration of ibuprofen in all 3 species investigated (data not shown). Diclofenac had no significant effect on norketamine formation at any concentration investigated when equine and canine liver microsomes were incubated with racemic ketamine. A small but significant decrease in norketamine formation with increasing diclofenac concentrations was detected for human liver microsomes. Higher R-ketamine concentrations were obtained, compared with S-ketamine concentrations, for equine and canine liver microsomes (Figure 6). Biotransformation rates were higher for S-ketamine than for R-ketamine at all concentrations of diclofenac in equine and canine liver microsomes but not in human liver microsomes (data not shown).

Concentrations of the norketamine enantiomers after incubation with human liver microsomes for 8 minutes were approximately 70% to 95% lower than the norketamine enantiomer concentrations of the equine and canine liver microsomes when incubated with ketamine. This difference was also detected when the incubation included ketamine with methadone, xylazine, and ibuprofen.

A higher rate of metabolism was found for S-ketamine in equine liver microsomes, compared with the metabolic rate for the S-enantiomer, in the racemic mixture when concurrently incubated at various concentrations of methadone, xylazine, ibuprofen, and diclofenac. However, there was a decrease in the biotransformation rate of S-ketamine, compared with the rate for racemic ketamine, in equine liver microsomes incubated with increasing concentrations of the various compounds (Figure 7).

**Discussion**

In the study reported here, racemic and S-ketamine competed for the substrate specific for human CYP 3A4 in human, equine, and canine liver microsomes, which supported the hypothesis that CYP 3A4 is involved in biotransformation in all 3 species. Other studies revealed that CYP 3A4, CYP 2C9, and CYP 2B6 were the enzymes responsible for N-demethylation of racemic ketamine, with CYP 3A4 being the major pathway. No effect on the luminescence signal was detected when methadone or xylazine was concurrently incubated with the substrate for CYP 3A4 in the 3 species investigated, even though methadone is metabolized via CYP 3A4 in humans and xylazine is metabolized via CYP 3A in rats. Methadone is metabolized by CYP 3A4, CYP 2B6, CYP 2C9, CYP 2D6, CYP 2C9, and CYP 2C19 in humans, whereas the major pathways are linked to CYP 3A4 and CYP 2B6. It can be concluded that the substrate provided by the manufacturer is not selective for CYP 3A4, and therefore, biotransformation by CYPs other than CYP 3A4 can be assumed. Even though there is competition between the substrate for CYP 3A4...
and methadone, the amount of luminescence may not be inhibited because the substrate can be metabolized by other CYPs to form luciferin. We must emphasize that it can only be suggested that orthology of human CYP 3A4 metabolize the same compounds in other species. Although CYP enzymes are classified into distinct subfamilies on the basis of amino acid sequence, a high degree of sequence identity does not necessarily indicate similar catalytic specificity. Substitution of a single amino acid can cause a change in substrate specificity. Therefore, investigations that use single CYPs are needed to identify a specific CYP involved in biotransformation. For this reason, we incubated human single CYP 3A4 supersomes with methadone and the substrate for CYP 3A4, and a concentration-dependent decrease in the resulting luminescence signal was obtained. These results support the hypothesis that CYP 3A4 is involved in metabolism of methadone, but CYP 3A4 was not detected in the luminogenic assay because the luminogenic substrate was not specific for CYP 3A4.

Xylazine is reported to be metabolized by CYP 3A in rats, but evidence for involvement of CYP 3A4 in xylazine metabolism is still lacking. In the study reported here, no effect on generation of the luminescence signal was obtained when the substrate for CYP 3A4 was incubated concurrently with xylazine in human, equine, and canine liver microsomes. Again, a lack of specificity of the substrate may have been the reason for the lack of effect.

Canine liver microsomes are able to metabolize substrates that are markers for human CYP 3A4 and CYP 2C9 activity. Analysis of results for the study reported here revealed an inhibition in luminescence signal when racemic ketamine was concurrently incubated with human and equine liver microsomes and a luminogenic substrate for human CYP 2C9. This result was evidence that CYP 2C9 is involved in biotransformation in these 2 species. Investigations on canine liver microsomes with the substrate for CYP 2C9 could not be performed because canine microsomes do not metabolize the substrate specific for human CYP 2C9. Additional investigations would be needed with canine liver microsomes and a substrate specific for canine CYP 2C21, which is the equivalent for human CYP 2C9 in dogs.

It has been reported that CYP 2C9 is involved in biotransformation of ibuprofen and diclofenac in humans, and results of our study are in agreement with these findings. Ibuprofen caused a concentration-dependent decrease in luminescence signal in human and equine liver microsomes. A concentration-dependent decrease in the resulting luminescence signal was obtained for diclofenac in human and equine liver microsomes, which indicated that CYP 2C9 is involved in diclofenac biotransformation in the liver of these 2 species.

Our intent in the second part of the study was to identify and characterize possible drug-drug interactions between ketamine and other analgesic drugs. Incubations of equine and canine liver microsomes with racemic ketamine and methadone or xylazine followed by stereoselective analysis of the enantiomers of ketamine and its most important metabolite norketamine revealed that increasing concentrations of methadone and xylazine resulted in a decrease in formation of R- and S-norketamine. Increasing methadone concentrations resulted in a decrease in metabolite formation (S- and R-norketamine) in equine and canine liver microsomes, whereas methadone had only a minor effect on norketamine formation in human liver microsomes at the concentrations investigated. A possible reason may have been a difference in affinity of the CYPs for these compounds. Furthermore, methadone reportedly is metabolized by CYP pathways that are not used for ketamine biotransformation. The norketamine concentrations were approximately 80% less when ketamine was concurrently incubated with methadone and human liver microsomes, compared with concentrations when incubated with equine and canine liver microsomes. It can be assumed that a longer period is necessary for human liver microsomes to metabolize ketamine into norketamine, compared with the interval for metabolism by equine and canine liver microsomes. The CYP content of equine and canine liver microsomes is approximately 460 and 330 pmol/mg of protein, respectively, whereas human liver microsomes have a metabolic rate of 240 to 250 pmol/mg of protein. This may explain the differences in the intensity of metabolism among the 3 species.

Concurrent incubation of xylazine and ketamine resulted in a decrease in norketamine formation in equine and canine liver microsomes. The major enzyme for metabolism of xylazine in rats is CYP 3A. In the study reported here, we determined that a higher xylazine concentration resulted in formation of a lower amount of norketamine; consequently, more parent compound remained in the incubation mixture. Xylazine inhibited the CYP-mediated metabolism of ketamine, which could lead to higher ketamine concentrations in the body and may cause adverse effects in vivo. Ketamine and xylazine are both metabolized by CYP 3A4 in humans and CYP 3A in rats. Because the major pathway for both compounds is probably the same, it can be assumed that the CYP 3A family is also involved in biotransformation of xylazine in horses and dogs. We confirmed that biotransformation of ketamine into norketamine was inhibited by xylazine for the equine and canine liver microsomes, and we presume that both are metabolized by the same CYP.

In our study, increasing concentrations of ibuprofen or diclofenac had no or only a small effect on norketamine formation in human, equine, and canine liver microsomes. It can be concluded that diclofenac and ibuprofen concentrations investigated in this study do not affect ketamine biotransformation under the experimental conditions used. Based on the assumption that CYP 2C9 is involved in the biotransformation of these compounds, ketamine could be transformed into norketamine by other pathways, including that of CYP 3A4.

Analysis of our data revealed that N-demethylation of ketamine is stereoselective. Stereoselective N-demethylation of ketamine has also been hypothesized in an in vivo study in horses and an in vitro study in human liver microsomes. A higher clearance of the
S-enantiomer in the absence of the R-enantiomer was reported for an in vivo study with human volunteers and surgical patients, compared with clearance for the S-enantiomer in the racemic mixture. Another study conducted by our laboratory group revealed stereoselective biotransformation of ketamine to norketamine in equine liver and lung microsomes, with slower elimination of S-ketamine in the presence of R-ketamine. In the study reported here, a higher rate of metabolism of S-ketamine in the absence of R-ketamine, compared with the metabolism rate for S-enantiomer in the racemic mixture, was detected at all concentrations of methadone, xylazine, ibuprofen, and diclofenac in equine liver microsomes. Ponies sedated with xylazine have a lower elimination half-life and mean residence time for S-ketamine administered as S-ketamine alone, compared with results for S-ketamine administered in a racemic mixture. This is in agreement with the findings of the study reported here. We hypothesize that this phenomenon is attributable to mutual inhibition of S- and R-ketamine via the same enzymatic pathways. In addition, the CYP enzymes involved may have a higher affinity for the S-enantiomer.

An enzyme-substrate competition between R- and S-ketamine can delay the N-demethylation of S-ketamine to S-norketamine, which would lead to slower elimination of this enantiomer when administered as part of a racemic mixture. Conversely, ponies anesthetized with isoflurane did not have significant differences for the pharmacokinetic variables estimated for the S-enantiomer of ketamine and norketamine after administration of a combination of R- and S-ketamine or S-ketamine alone.

It has been reported that S-ketamine has a more prolonged analgesic effect (lasting 4 times as long as racemic ketamine) and is approximately twice as potent as the racemic mixture for inhibiting a central summation of pain. This indicates advantages for administration of the S-enantiomer (instead of a racemic mixture) to horses. Ponies anesthetized with xylazine and S-ketamine achieve a standing position during recovery significantly faster than ponies anesthetized with xylazine and racemic ketamine. The authors of that study concluded that the more rapid elimination of S-ketamine and its active metabolite S-norketamine could explain the faster recovery detected for S-ketamine. Investigations on the biotransformation rate of S-ketamine in other species are needed.

References


