Inhibition of cytochrome P450 enzymes involved in ketamine metabolism by use of liver microsomes and specific cytochrome P450 enzymes from horses, dogs, and humans

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Objective—To identify and characterize cytochrome P450 enzymes (CYPs) responsible for the metabolism of racemic ketamine in 3 mammalian species in vitro by use of chemical inhibitors and antibodies.

Sample—Human, canine, and equine liver microsomes and human single CYP3A4 and CYP2C9 and their canine orthologs.

Procedures—Chemical inhibitors selective for human CYP enzymes and anti-CYP antibodies were incubated with racemic ketamine and liver microsomes or specific CYPs. Ketamine N-demethylation to norketamine was determined via enantioselective capillary electrophoresis.

Results—The general CYP inhibitor 1-aminobenzotriazole almost completely blocked ketamine metabolism in human and canine liver microsomes but not in equine microsomes. Chemical inhibition of norketamine formation was dependent on inhibitor concentration in most circumstances. For all 3 species, inhibitors of CYP3A4, CYP2A6, CYP2C19, CYP2B6, and CYP2C9 diminished N-demethylation of ketamine. Anti-CYP3A4, anti-CYP2C9, and anti-CYP2B6 antibodies also inhibited ketamine N-demethylation. Chemical inhibition was strongest with inhibitors of CYP2A6 and CYP2C19 in canine and equine microsomes and with the CYP3A4 inhibitor in human microsomes. No significant contribution of CYP2D6 to ketamine biotransformation was observed. Although the human CYP2C9 inhibitor blocked ketamine N-demethylation completely in the canine ortholog CYP2C21, a strong inhibition was also obtained by the chemical inhibitors of CYP2C19 and CYP2B6. Ketamine N-demethylation was stereoselective in single human CYP3A4 and canine CYP2C21 enzymes.

Conclusions and Clinical Relevance—Human-specific inhibitors of CYP2A6, CYP2C19, CYP3A4, CYP2B6, and CYP2C9 diminished ketamine N-demethylation in dogs and horses. To address drug-drug interactions in these animal species, investigations with single CYPs are needed. (Am J Vet Res 2011;72:1505–1513)
ketamine metabolism is performed by CYPs. Both ketamine enantiomers are N-demethylated into S- and R-norketamine by CYPs in the liver of humans, dogs, rats, and rabbits. Ketamine is metabolized enantioselectively to norketamine, dehydronorketamine, and 3-hydroxylated norketamine metabolites in humans, dogs, and horses. Norketamine is the major metabolite that remains pharmacologically active.

Evidence suggests that drug–drug interactions are associated with adverse effects or treatment failure. More than 2 million cases of adverse human drug reactions occur annually in the United States, resulting in 100,000 deaths. To predict possible drug–drug interactions, knowledge of the CYPs involved in drug biotransformation is essential. Earlier studies performed in our laboratory showed that methadone and xylazine inhibited the metabolism of ketamine in vitro. The antiancancer agent cyclophosphamide, which is a CYP2B6 substrate, increases the duration of ketamine anesthesia in mice. Medetomidine, an inhibitor of human CYP3A4 and CYP2C9, is often used for veterinary anesthesia and also inhibits ketamine N-demethylation in vitro.

Identification and characterization of the CYPs involved in ketamine metabolism will provide a basis to study drug–drug interactions in horses and dogs to prevent treatment failure or adverse effects resulting from alterations in drug metabolism. Impairment of drug metabolism due to enzyme inhibition has obvious clinical implications, including toxic effects as a result of increased bioavailability and decreased clearance. In humans, studies involving heterologously expressed human CYPs showed that mainly CYP3A4, CYP2B6, and CYP2C9 are responsible for the biotransformation of ketamine to its active metabolite, norketamine. Another study revealed that CYP3A4, CYP2C9, CYP2A6, CYP2D6, CYP2B6, and CYP2C19 are responsible for ketamine N-demethylation in humans. The single human CYP2B6 has high affinity for ketamine, but its capacity for ketamine metabolism in humans is low. On the other hand, CYP3A4 and CYP2C9 have low affinity but high capacity for ketamine. Furthermore, CYP3A4 is the major enzyme for ketamine metabolism in humans, and CYP2B6 and CYP2C9 contribute to a lesser extent to ketamine N-demethylation at therapeutic drug concentrations. In contrast, recently published data indicate that the highest demethylation activity is achieved with human single CYP2B6 followed by CYP3A4 and CYP2C19.

Little information is available on CYPs involved in ketamine metabolism in horses and dogs. Theoretically, adverse effects might occur when anesthetics and analgesic drugs given concomitantly with ketamine are metabolized via the same CYPs. Recombinant CYPs have been produced for various mammalian species, but single CYPs from dogs and horses are not commercially available, with the exception of purified heterologously expressed canine CYP3A12 and CYP2C21. Additional knowledge about equine CYP enzymes has been derived almost completely from functional studies conducted to evaluate the metabolism of known marker substrates for special human CYPs in horse microsomes. To identify the CYPs responsible for the metabolism of a drug, the US FDA Center for Drug Evaluation and Research proposed various methods, including inhibition studies with compounds that selectively inhibit a certain CYP in humans. Our group demonstrated that orthologous enzymes of human CYP3A4 and CYP2C9 are involved in ketamine metabolism in horses and dogs in vitro. In that study, screening of canine and equine microsomes in a high-throughput luminescence assay was performed.

The purpose of the study reported here was to assess the inhibitory potency of known human CYP inhibitors toward ketamine N-demethylation in horses and dogs. Inhibitors of interest included general CYP inhibitor ABT, ketoconazole (CYP3A4), sulfaphenazole (CYP2C9), 2-phenylecyclopenta-mine (tranylcypromine; CYP2A6), nootkatone (CYP2C19), clopidogrel (CYP2B6), and quinidine (CYP2D6) as well as anti-human CYP antibodies against CYP3A4, CYP2C9, and CYP2B6. Such information would help to avoid therapeutic failure and unexpected toxic effects in veterinary patients. Furthermore, by identifying the enzymes involved in metabolic clearance and their relative contributions, the degree of inhibition and induction of CYPs could be estimated, both of which might be a concern for drug–drug interactions in clinical settings.

Materials and Methods

Sample—Liver tissue samples were obtained from the carcasses of 7 previously healthy horses that had no history of drug treatment in the preceding months and had been humanely slaughtered by use of captive bolt for meat. The horses were Franches-Montagnes or crossbreeds of both sexes and ranged in age from 8 to 17 years. The samples were collected from various sites in each liver immediately after stunning and were placed on dry ice for transportation to the laboratory. Tissues were frozen and kept at −80°C until used for microsomal preparation.

Preparation of liver microsomes and single CYPs—Equine liver microsomes were prepared as described elsewhere. Briefly, pieces of liver tissue were collected less than half an hour after horses were stunned and were placed on ice immediately. Microsomes were prepared by grinding the frozen tissue, then homogenizing it in a homogenizer. The suspension was then pelleted at 9,000 X g for 15 minutes. The pellet was discarded, and the supernatant was pelleted at 19,000 X g for 15 minutes, followed by 100,000 X g for 60 minutes. Microsomes were used immediately or stored frozen at −80°C at a protein concentration of 2 mg/mL. Total CYP protein content was determined by spectrophotometric assessment. Aliquots containing a final concentration of 412 pmol of CYP/mg of total protein were frozen and stored at −80°C until used. Pooled canine and human liver microsomes were obtained from a commercial source. The canine pool was derived from the livers from 30 dogs and contained 550 pmol of CYP/mg of total protein. Human pooled microsomes originated from 120 donors and contained 250 pmol of CYP/mg of total protein. Microsomes were
In vitro metabolism experiments—A typical incubation mixture with a final volume of 250 µL consisted of racemic ketamine\textsuperscript{a} at a concentration of 25 µM, NADPH-regenerating system\textsuperscript{b} (1.25 mM NADP\textsuperscript{+}, 3.3 mM glucose-6-phosphate, glucose-6-phosphate dehydrogenase [0.4 U/mL], 3.3 mM MgCl\textsubscript{2}, and 3 mM sodium citrate), and potassium phosphate buffer (100 mM [pH, 7.4]). The preincubation step was performed at 37°C for 4 minutes. The enzymatic reaction was started at 37°C after adding microsomes to a final protein concentration of 0.5 mg/mL and single CYPs to a final concentration of 25 pmol/mL. A sample of 200 µL was withdrawn from the reaction mixture after 8 minutes of incubation for reactions with equine, canine, and human liver microsomes and after 10, 20, 30, and 60 minutes for incubations with human CYP3A4, canine CYP3A12, canine CYP2C21, and human CYP2C9, respectively. The 8-minute incubations were performed in the linear range of norketamine formation with respect to time and protein content. The samples were mixed immediately with 500 µL of ice-cold sodium hydroxide\textsuperscript{c} (0.2 M) to terminate enzymatic reactions and 50 µL of the internal standard solution containing (+)-pseudoephedrine hydrochloride\textsuperscript{d} (149 µM) or, to avoid interferences, 20 µL of a lamotrigine\textsuperscript{e} solution (120 µM) in experiments in which tranylcypromine was used as inhibitor. All samples were prepared in duplicate.

Inhibition of ketamine N-demethylation with chemical inhibitors—To assess the role of CYP enzymes involved in ketamine metabolism, the effect of the general CYP inhibitor ABT\textsuperscript{1} was investigated at 200 and 500 µM in pooled microsomes from the 3 species.\textsuperscript{26} Samples with single CYPs and liver microsomes were coincubated with 6 chemical compounds: the CYP3A4 inhibitor ketoconazole,\textsuperscript{f} the CYP2C9 inhibitor sulfaphenazole,\textsuperscript{g} the CYP2A6 inhibitor tranylcypromine,\textsuperscript{h} the CYP2C19 inhibitor nootkatone,\textsuperscript{i} the CYP2B6 inhibitor clopidogrel,\textsuperscript{j} and the CYP2D6 inhibitor quinidine.\textsuperscript{1} Inhibitor concentrations of 2 and 10 µM were selected for use on the basis of published data.\textsuperscript{28–36} All inhibitors except ABT were preincubated with ketamine (25 µM) and the NADPH-regeneration system in potassium phosphate buffer for 4 minutes at 37°C, followed by addition of the microsomes.

1-aminobenzotriazole was dissolved in water (stock solutions of 2 and 5 mM). Ketoconazole, sulfaphenazole, and clopidogrel were dissolved in methanol\textsuperscript{b} (high-performance liquid chromatography grade), quinidine and tranylcypromine in water, and nootkatone in dimethyl sulfoxide\textsuperscript{d} to achieve stock solutions with concentrations of 2 and 10 mM. Stock solutions were diluted with potassium phosphate buffer before use. To exclude a possible organic solvent effect on enzyme activity, control samples devoid of the inhibitor were evaluated in parallel. All inhibitors were incubated with equine microsomes without ketamine to exclude a possible interference of the inhibitors and their main metabolites with the analysis of S- and R-norketamine. 1-aminobenzotriazole was preincubated with microsomes from all species for 30 minutes, ketamine was added, and the reaction was performed as previously described.

Inhibition of ketamine N-demethylation with antibodies—Aliquots of liver microsomes from all species investigated as well as the human single CYP3A4 and its canine ortholog CYP3A12 were preincubated for 15 minutes with a CYP-specific antibody at room temperature (20°C). The antibody used for inhibition of human CYP2B6\textsuperscript{f} was monoclonal, whereas the antibodies for inhibition of CYP2C9\textsuperscript{f} and CYP3A4\textsuperscript{f} were polyclonal. Volumes of 10, 14.8, and 18.3 µL of anti-CYP2B6 and anti-CYP2C9 antibodies and 15, 27.7, and 33 µL of anti-CYP3A4 antibody were used for incubations with human, equine, and canine microsomes, respectively. The anti-CYP2B6 contained 10 mg of protein/mL; no information on protein content was provided for the other antibodies used. To obtain antibody saturation and a strong inhibition, the highest concentration provided by the manufacturer was used.\textsuperscript{37} Therefore, the polyclonal antibodies were tested in human microsomes with an ascending amount of antibody. The amount for canine and equine microsomes was subsequently estimated on the basis of the CYP content in pooled microsomes. The reaction was started by adding to the microsomes the preheated mixture, which contained the NADPH-regenerating system, ketamine, and potassium phosphate buffer. Additional steps were performed as described for the chemical inhibitors.

Analytic procedure—Enantioselective analysis of ketamine and norketamine was performed by use of capillary electrophoresis as described elsewhere.\textsuperscript{1,38–40} The assay was based on liquid-liquid extraction at an alkaline pH, reconstitution of the dried residue in 50 µL of 25 mM Tris-phosphate buffer (pH, 2.5), and capillary electrophoresis analysis of the reconstituted extract in a Tris-phosphate buffer (50 mM [pH, 2.5]) containing highly sulfated β-cyclodextrin\textsuperscript{f} (10 mg/mL) as chiral selector. Sulfated β-cyclodextrin was used as a mixture of 2 batches as described elsewhere.\textsuperscript{40} A capillary electrophoresis instrument\textsuperscript{f} with an on-column variable wavelength detector set to 195 nm and a 30-µm (internal diameter) fused-silica capillary\textsuperscript{f} 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 to 20°C. A pressure of 0.6895 kPa (0.1 psi) to induce a buffer flow toward the anode was applied during the entire run. Samples were injected with a pressure of 6.895 kPa for 6 seconds, and run time was 16 minutes. Quantitation based on multilevel internal calibration was performed by use of corrected peak areas in the range of 0.5 to 30 µM for S- and R-ketamine and 0.25 to 15 µM for S- and R-norketamine. All chemicals were of analytic grade.

Statistical analysis—For each norketamine enantiomer, the percentage of inhibition was calculated from the extent of ketamine N-demethylation, which was based on the change in the presence of the inhibi-
tor or inhibitory antibody, compared with the norket-amine formation in absence of an inhibitor. All values are reported as the mean of duplicate determinations. Commercially available statistical software was used to perform a repeated-measures ANOVA (with Bonferroni correction used for multiple comparisons) to compare ketamine N-demethylation at various inhibitor concentrations within each species and among all 3 species. Values of \( P < 0.05 \) were considered significant.

**Results**

Stereoselectivity of ketamine N-demethylation—Formation of S-norketamine was faster than that of R-norketamine. This was evident in the electropherograms (Figure 1). Ketamine metabolism was also stereoselective in incubations with canine CYP2C21 and human CYP3A4 (data not shown). No significant difference in the inhibition of ketamine N-demethylation was evident for S-ketamine versus R-ketamine in all species (human, equine, and canine liver microsomes) evaluated.

Effect of ABT on ketamine N-demethylation in liver microsomes—1-aminobenzotriazole blocked ketamine N-demethylation in a concentration-dependent manner in liver microsomes from all 3 species (Figure 2). In equine microsomes, addition of 200µM ABT resulted in an inhibition of 60%, and even at a concentration of 500µM, an incomplete inhibition was obtained (70%). At both concentrations (200 and 500µM), ABT resulted in a stronger inhibition in human (\( P = 0.007 \))

![Figure 1](image-url)
and canine (P = 0.005) liver microsomes than in equine liver microsomes. At a concentration of 500µM ABT, inhibition of ketamine N-demethylation in the equine microsomes was extensive but not complete.

**Effect of chemical inhibitors on ketamine N-demethylation in single CYPs**—Experiments with single CYP3A4, CYP3A12, CYP2C9, and CYP2C21 demonstrated that the N-demethylation rates of ketamine were higher for CYP3A4 and CYP3A12 than those observed for CYP2C9 and CYP2C21 (data not shown). Because of the unknown selectivity of the chemical inhibitors toward canine CYPs, their possible inhibitory potential toward other CYP isoforms was investigated through use of the commercially available canine single CYP3A12 and CYP2C21. The effects of the 6 specific human CYP inhibitors on ketamine N-demethylation were investigated and compared with the effects of their human orthologs, CYP3A4 and CYP2C9. Ketocnazole completely suppressed ketamine N-demethylation in human CYP3A4 and the canine ortholog CYP3A12 at the lower concentration of 2µM. Inhibitors that inhibit CYPs other than CYP3A4 (in humans) or CYP3A12 (in dogs) did not affect ketamine N-demethylation in canine microsomes at the lower concentration of 2µM. In contrast, sulfaphenazole and tranylcypromine caused a small inhibition of approximately 14% in CYP3A4 at a concentration of 2µM. Decreases in N-demethylation activities of 17% and 10% were obtained with 10µM sulfaphenazole in CYP3A4 (in human microsomes) and CYP3A12 (in canine microsomes), respectively. N-demethylation was diminished by approximately 30% and 25% at the higher concentration of clopidogrel in CYP3A4 and CYP3A12, respectively. Tranylcypromine diminished ketamine N-demethylation in CYP3A4 by 25%, but no inhibition was obtained in CYP3A12. Nootkatone and quinidine had no effect on ketamine biotransformation in both enzymes at the 2 concentrations investigated (data not shown).

Sulfaphenazole, the inhibitor of the human CYP2C9 and its canine ortholog CYP2C21, inhibited ketamine N-demethylation concentration dependently. The inhibition was complete in both species at 10µM, and 2µM sulfaphenazole caused decreases in ketamine metabolism of 90% and 40% with CYP2C9 and CYP2C21, respectively. None of the other chemical inhibitors had an effect on ketamine biotransformation of human CYP2C9, but they decreased ketamine N-demethylation in a concentration-dependent manner in canine CYP2C21, with 10 µM resulting in a more pronounced effect. In CYP2C21, the strongest inhibition of ketamine N-demethylation at a concentration of 2µM was obtained with clopidogrel (79%), followed by sulfaphenazole (40%) and nootkatone (33%; Figure 3). At the higher concentration, clopidogrel and nootkatone reduced ketamine metabolism by 90% and 70%, respectively. Quinidine, tranylcypromine, and ketoconazole inhibited ketamine biotransformation in CYP3C21 to a minor extent (15%).

**Effects of chemical inhibitors on ketamine N-demethylation in liver microsomes**—The extent of inhibition of ketamine N-demethylation in the 3 species of liver microsomes by the 6 chemical inhibitors was dependent on inhibitor concentration (Figure 4). Ketoconazole, a potent and selective CYP3A4 inhibitor, significantly reduced ketamine-N-demethylation in all 3 species, with the effect being most prominent in humans (2µM; 52%). Compared with the effects of ketoconazole in human microsomes, the effects in canine (P < 0.001) and equine (P < 0.001) microsomes were significantly smaller. Sulfaphenazole, an inhibitor of human CYP2C9, had a minor inhibitory effect in canine and human microsomes and almost no effect in equine microsomes at the lower concentration of sulfaphenazole used (2µM). A concentration of 10µM diminished the norketamine formation by approximately 14% in CYP3A4 at a concentration of 2µM. The inhibition was complete in both species at 10µM, and 2µM sulfaphenazole caused decreases in ketamine metabolism of 90% and 40% with CYP2C9 and CYP2C21, respectively. None of the other chemical inhibitors had an effect on ketamine biotransformation of human CYP2C9, but they decreased ketamine N-demethylation in a concentration-dependent manner in canine CYP2C21, with 10 µM resulting in a more pronounced effect. In CYP2C21, the strongest inhibition of ketamine N-demethylation at a concentration of 2µM was obtained with clopidogrel (79%), followed by sulfaphenazole (40%) and nootkatone (33%; Figure 3). At the higher concentration, clopidogrel and nootkatone reduced ketamine metabolism by 90% and 70%, respectively. Quinidine, tranylcypromine, and ketoconazole inhibited ketamine biotransformation in CYP3C21 to a minor extent (15%).

**Effects of chemical inhibitors on ketamine N-demethylation in liver microsomes**—The extent of inhibition of ketamine N-demethylation in the 3 species of liver microsomes by the 6 chemical inhibitors was dependent on inhibitor concentration (Figure 4). Ketoconazole, a potent and selective CYP3A4 inhibitor, significantly reduced ketamine-N-demethylation in all 3 species, with the effect being most prominent in humans (2µM; 52%). Compared with the effects of ketoconazole in human microsomes, the effects in canine (P < 0.001) and equine (P < 0.001) microsomes were significantly smaller. Sulfaphenazole, an inhibitor of human CYP2C9, had a minor inhibitory effect in canine and human microsomes and almost no effect in equine microsomes at the lower concentration of sulfaphenazole used (2µM). A concentration of 10µM diminished the norketamine formation by approxi-
mately 32% in human microsomes, 28% in canine, and 12% in equine.

Tranylcypromine, a selective inhibitor of human CYP2A6, decreased norketamine formation significantly in all species investigated. In equine microsomes, tranylcypromine (2 µM) caused the strongest inhibition (35%) of all inhibitors used. Strong differences between the higher and the lower concentration of tranylcypromine were found for human (P = 0.003) and canine (P = 0.001) microsomes but not for equine microsomes. Nootkatone (CYP2C19) inhibited the N-demethylation pathway at 2 µM in a species-dependent manner. Ketamine biotransformation was suppressed by 35% in human microsomes and approximately 25% in equine and canine microsomes. The 2 most potent inhibitors in canine microsomes were nootkatone and tranylcypromine. Clopidogrel, an inhibitory compound for human CYP2B6, blocked ketamine N-demethylation at 2 µM in microsomes from all 3 species with the effect being most pronounced in canine microsomes. Furthermore, quinidine, an inhibitor of human CYP2D6, reduced ketamine metabolism only to a small extent in humans and horses but not in dogs.

Most chemical inhibitors resulted in a significant concentration-dependent inhibition, with the effect more pronounced at a concentration of 10 µM than at the lower 2 µM concentration. An inhibitor concentration of 10 µM is selective for sulfaphenazole,

Discussion

Studies on possible drug-drug interactions are particularly important when it is common practice to administer certain drugs in combination with others, as is the situation with ketamine. In the present study, inhibition experiments were performed to evaluate CYPs involved in ketamine biotransformation in humans, dogs, and horses. Our results showed that most chemical CYP inhibitors were able to reduce ketamine biotransformation in liver microsomes from all 3 species, suggesting the involvement of several CYPs in ketamine N-demethylation. In liver microsomes from humans, CYP3A4, CYP2B6, and CYP2C9 were identified as mainly responsible for ketamine biotransformation in all 3 species, dogs, and horses. Our results showed that most chemical CYP inhibitors were able to reduce ketamine biotransformation in liver microsomes from all 3 species, suggesting the involvement of several CYPs in ketamine N-demethylation. In liver microsomes from humans, CYP3A4, CYP2B6, and CYP2C9 were identified as mainly responsible for ketamine biotransformation. The chemical inhibitors specific for human CYP2C19 and CYP2A6 diminished ketamine N-demethylation in all 3 types of microsomes. Furthermore, quinidine, an inhibitor of human CYP2D6, reduced ketamine metabolism only to a small extent in humans and horses but not in dogs.

Most chemical inhibitors resulted in a significant concentration-dependent inhibition, with the effect more pronounced at a concentration of 10 µM than at the lower 2 µM concentration. An inhibitor concentration of 10 µM is selective for sulfaphenazole,
whereas lower concentrations are selective for all other chemical inhibitors used in our study.26-30 In theory, the sum of the effects of all inhibitors should result in an inhibition of approximately 100% in liver microsomes. This was the situation for experiments with equine and canine microsomal preparations at selective inhibitor concentrations. At the higher concentration, the sum of inhibitor effects was > 100% (271%, 233%, and 196% for human, canine, and equine liver microsomes, respectively), suggesting an inhibitor can have an effect on > 1 CYP.

1-aminobenzotriazole is reportedly a general and unspecific inhibitor of CYPs in humans that is used to distinguish CYP-related metabolism from metabolism by other enzymes.26-27 Therefore, ABT was used to test for the involvement of CYP enzymes in the N-demethylation of ketamine.28 This compound almost completely blocked ketamine N-demethylation in human and canine microsomes at a concentration of 500µM, indicating that most or even all involved enzymes were blocked. These findings agree with other findings that ABT inhibits human CYP isoforms primarily involved in drug metabolism.27 1-aminobenzotriazole reportedly inhibits the canine CYP2B11,28 and to our knowledge, ours is the first study to demonstrate that ABT inhibits all canine CYPs involved in ketamine biotransformation. In contrast, in equine liver microsomes, inhibition was less pronounced, suggesting that ABT does not inhibit all equine CYPs, yet metabolism of ketamine through equine cytosolic enzymes other than CYPs cannot be excluded. However, preliminary results showed that these enzymes were not involved in ketamine N-demethylation in human cytosol. It follows that ABT may not be feasible as a general CYP inhibitor in horses. Another study31 demonstrated that ABT is not a nonselective CYP inhibitor, with investigators cautioning that false conclusions may be made that remaining metabolic activity is non-P-450 mediated after ABT pretreatment.41

Ketoconazole at a concentration of 2µM inhibited ketamine N-demethylation completely in single human CYP3A4 and the canine ortholog CYP3A12. These findings agree with those of other investigators,25 who found ketoconazole is a potent and selective inhibitor for the canine single CYP3A12 at a concentration of 1µM. Unspecific inhibition of the single CYP3A4 and CYP3A12 by all other inhibitors was negligible at the 2µM concentration, indicating that results from other inhibitors did not reflect CYP3A inhibition. The highest inhibition in human liver microsomes was obtained with ketoconazole, which confirmed that CYP3A4 is predominantly responsible for ketamine N-demethylation.25,26 In canine and equine microsomes, ketoconazole inhibited ketamine N-demethylation to a much lesser degree. In dogs, ketoconazole can inhibit CYP3A12 completely, indicating this compound is a selective inhibitor for the canine CYP3A12.29 The amount of CYP3A12 in canine liver is reportedly lower (15%) than the amount of human ortholog in human liver (approx 50%),43 which might explain the species differences. In pooled canine microsomes in our study, the anti-CYP3A4 antibody resulted in a reduction of ketamine N-demethylation that was more pronounced than that with ketoconazole, suggesting that this antibody is not selective for the canine ortholog CYP3A12 and therefore blocks > 1 CYP. In equine microsomes, an inhibition of only 10% was obtained, which was lower than the 25% achieved with the chemical inhibitor and might have represented a low affinity or binding of this antibody or a low CYP3A content in equine liver. Evidence supports the hypothesis that CYP3A4 is responsible for ketamine biotransformation in various species.18 Experiments with single enzymes are necessary to evaluate the involvement of a CYP3A4 ortholog in equine liver microsomes.

According to our results, the human CYP2C9 and its canine ortholog CYP2C21 appear to be less involved in ketamine metabolism than CYP3A4 or its canine ortholog CYP3A12. Another study21 also demonstrated a minor contribution of CYP2C9 to ketamine N-demethylation. Furthermore, norketamine formation by CYP2C21 in our study was stereoselective, with a higher preference for S-norketamine than R-norketamine formation, as was reported elsewhere for canine microsomal preparations.18 Sulfaphenazole, which inhibits human CYP2C933 and canine CYP2C21,44 at a concentration of 2µM blocked ketamine metabolism nearly completely in human single CYP2C9, and the activity of the canine ortholog CYP2C21 was still approximately 60%. To inhibit the canine CYP2C21 completely, the higher concentration of sulfaphenazole was necessary, indicating a lower affinity for the canine enzyme. Our data showed that the canine single CYP2C21 was also blocked by clopidogrel, which is an inhibitor for CYP2B6, and nootkatone, which is an inhibitor for CYP2C19, whereas no inhibition was evident with the human ortholog. Evidence involving human single enzymes indicates clopidogrel also blocks CYP2C19 and CYP2C9.36 In dogs, CYP2C21 identifies 69% with the human CYP2C9 and 70% with CYP2C19 at the protein level, explaining why nootkatone also inhibited the canine CYP2C21 in our study.24 However, there was also evidence that the involvement of CYP2C21 in ketamine metabolism is minor because the norketamine peaks after 30 minutes were smaller than those of single CYP3A12 after 20 minutes or pooled canine microsomes after 8 minutes. A strong inhibition in ketamine N-demethylation in canine microsomes was seen with the CYP2C19 inhibitor nootkatone. S-mephentoin, which is a substrate for human CYP2C19, was strongly metabolized by canine microsomes in another study,24 whereas none of the canine single CYPs evaluated, including CYP2C21, appeared responsible for this effect.24 The conclusion was that another isoform might exist in dogs that is responsible for metabolizing the substrate for CYP2C19, which might explain the strong inhibition we observed.

In human liver microsomes, approximately 60% of norketamine formation was inhibited by anti-CYP2B6 antibody, in concordance with a previously reported 80% inhibition of norketamine formation at a ketamine concentration of 5µM.22 The antibody resulted in an inhibition similar to the chemical inhibitor clopidogrel in canine and equine microsomes. Despite this rather small inhibition observed, these findings considered together suggest that the CYP inhibited by anti-CYP2B6 is partly involved in ketamine N-demethylation.
Quinidine, an inhibitor of the human CYP2D6, caused a minor inhibition of ketamine biotransformation in human and equine liver microsomes, but none in canine microsomes, indicating that CYP2D6 is not a major contributor to ketamine metabolism. However, it remains possible that quinidine does not block equine and canine orthologs if it is, indeed, involved in ketamine N-demethylation.

In the study reported here, CYP3A4 was confirmed to be primarily involved in ketamine N-demethylation in humans. In contrast, the CYP2C family (particularly CYP2C19) and CYP2A6 appeared to be involved in ketamine biotransformation to a higher degree in dogs and horses than CYP3A4. Before definitive conclusions can be drawn regarding the possible orthologs in the animal species evaluated, investigations involving single CYPs are needed. 1-aminobenzotriazole might not be a feasible inhibitor of CYPs in horses, and clopidogrel and nootkatone are not ideal as selective inhibitors in dogs. Data obtained with ABT must be interpreted with caution even when human microsomes are used. Our findings suggest that species-specific data should be used to estimate drug-drug interactions.

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