Canine Cytochrome P-450 Pharmacogenetics

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INTRODUCTION

The cytochrome P-450 (CYP) drug-metabolizing enzymes are critical to the efficient elimination of many drugs used in clinical practice. Unfortunately in humans, and probably in all species of veterinary importance, there is considerable interindividual variability in the activity of these enzymes. Consequently, for a given drug dosage the effect can range from undetectable or suboptimal (with high enzyme activity, high drug clearance, and low plasma levels) to excessive or toxic (with low enzyme activity, low drug clearance, and high plasma levels). Causes of this variability can include concurrent exposure to CYP enzyme inhibitors or inducers in the diet or from coadministered medications (previously reviewed). Genetic variation is also a well-established

Disclosures: This work was supported by funds provided by the William R. Jones Endowed Chair in Veterinary Medicine at Washington State University. There are no conflicts of interest to report.
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KEYWORDS

- Dog
- Canine
- Genetic polymorphism
- Cytochrome P-450
- Pharmacokinetics

KEY POINTS

- Polymorphisms in genes encoding CYP enzymes could explain adverse drug effects or therapeutic failure in canine patients.
- A premature stop codon mutation in CYP1A2 is commonly found in certain dog breeds, including Beagle and Irish wolfhound.
- Although the CYP1A2 premature stop codon has shown large effects on the pharmacokinetics of some experimental compounds, effects on commonly used clinical drugs is currently unknown.
- Polymorphisms also exist in genes encoding canine CYP2C41, CYP2E1, CYP2D15, and CYP3A12 that have the potential to impact the metabolism of a large number of different drugs.
- Anesthetic drug hypersensitivity in Greyhounds may be the result of a genetic variant affecting canine CYP2B11 expression or function.
cause of CYP activity variability in humans, and current evidence suggests that it may be equally important in veterinary species, including dogs. Consequently, clinical assays for CYP gene variants that significantly impact drug disposition could be a useful tool to enable rational drug selection and dosage for the individual patient. This article reviews the current state of knowledge regarding the dog CYPs focusing on potentially clinically important genetic variants that could influence drug efficacy and toxicity.

DOG-HUMAN CYP SIMILARITIES AND DIFFERENCES

Much of the available published data on the CYPs so far concern the human CYPs. Indeed, the US Food and Drug Administration requires detailed label information regarding the involvement of specific human CYPs in the metabolism of all newly approved drugs intended for use in humans. Although much of the information can be applied in a general fashion to the dog CYPs, it is becoming increasingly apparent that there are important differences in the metabolism of drugs by human and dog CYPs, much of which have yet to be determined. Specific examples of some of the known similarities and differences are discussed next.

CYP Substrate Specificity

Table 1 lists common CYP drug substrates in humans compared with dogs. The CYPs are named according to gene sequence similarity and grouped according to family (number), subfamily (letter), and unique gene product (number), as in the canine CYP2B11 gene (family, 2; subfamily, B; 11th gene identified). Because of significant species differences in gene sequence of these enzymes, each species tends to have their unique CYP names, although orthologs (genes derived from the same ancestral gene that diverged after speciation) are found in most species. For example, CYP2B11 is considered to be the canine ortholog of human CYP2B6. Orthologs also tend to have roughly similar substrate specificities. For example, human CYP2B6 and canine CYP2B11 metabolize propofol. However, significant species differences exist. For example, midazolam is metabolized exclusively by human CYP3A4 and CYP3A5 (but not by human CYP2B6), whereas dog CYP2B11 (and not CYP3A12) primarily metabolizes midazolam. Similarly, dog CYP1A2 and dog CYP2A13 metabolize phenacetin, whereas only human CYP1A2 (and not human CYP2A6) metabolizes this drug. Although most dog CYPs have unique names, three of the drug-metabolizing CYPs (CYP1A1, CYP1A2, and CYP2E1) have identical names to those found in other mammalian species, in part because they have relatively conserved gene sequences between species, and in part because their naming preceded the convention to give unique names to the drug-metabolizing CYPs in different species.

CYP Abundance

Apart from differences in catalytic properties between dog and human CYPs, these enzymes also differ in the relative amount of each family and subfamily between dogs and humans. Fig. 1 shows the distribution of the different CYPs in liver and small intestinal mucosa of human and dog. The liver has the highest content of drug-metabolizing CYPs and is the most important organ for CYP-mediated drug elimination. The small intestine also has a high specific content of certain CYPs located within the mucosa and serves to decrease absorption of intact (unmetabolized) drugs thereby limiting systemic availability of orally administered drugs. Similarities between dog and human are apparent in that the CYP3A subfamily enzymes are the predominant isoforms in liver and intestines of both species. However, the CYP2D subfamily
enzyme CYP2D15 is more highly expressed (as a percentage of total CYPs) in the livers of dogs versus CYP2D6 in humans. Furthermore, the CYP2B subfamily enzyme CYP2B11 is more highly expressed in both livers and intestines of dogs than CYP2B6 in human livers and intestines. This difference could be a consequence of the many genetic mutations that have been associated with the CYP2D6 and CYP2B6 genes in humans. A clinical consequence is that drugs metabolized by CYP2D or CYP2B may have lower systemic levels in dogs than in humans.

**CANINE CYPS WITH KNOWN GENETIC POLYMORPHISM**

Table 2 summarizes published data regarding known genetic polymorphisms in the canine drug-metabolizing CYP enzymes including variant description, allele frequencies, and effects of the variant on enzyme function in vitro and in vivo.
The most comprehensively studied canine CYP genetic polymorphism is the premature stop codon mutation (c.1117C>T; R373X) located in the coding region of the CYP1A2 gene that results in complete loss of hepatic CYP1A2 protein and associated enzyme activity. This mutation was discovered independently by two Japanese pharmaceutical companies during preclinical testing of two unrelated investigational compounds (YM-6422712 and AC393313) that showed highly polymorphic pharmacokinetics of these compounds in their Beagle dog colonies. Both groups used genetic testing to screen a large number of their Beagle dog colonies and found that from 11% to 17% of their dogs had the homozygous mutant genotype and consequently did not express functional CYP1A2. The effect on the plasma drug levels of the investigational drugs was large with up to 17 times increased levels in deficient dogs (Fig. 2). However, animals that had at least one normal CYP1A2 copy (heterozygotes) did not have substantially different drug levels from the wild-type dogs. Several other investigational compounds including GTS-21 and BTP-2 have also been associated with variable CYP1A2 metabolism in Beagles.

The effect of this polymorphism on the pharmacokinetics and effects of clinically used drugs is unclear. In vitro studies using liver microsomes from CYP1A2-deficient and -expressing dogs indicated that phenacetin and tacrine (both selectively metabolized by human CYP1A2) were more slowly metabolized in deficient livers.
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Genetic Variant</th>
<th>Variant Allele Frequency</th>
<th>In Vitro Effect</th>
<th>In Vivo Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>SNP c.1117C&gt;T causing premature stop codon mutation at amino acid position 373 (R373X)</td>
<td>6% in 99 mixed-breed dogs from Brazil; 38% in 214 Beagles from Japan; see Fig. 3 for complete numbers</td>
<td>No functional enzyme</td>
<td>~50% lower phenacetin clearance after oral administration; no difference in phenacetin clearance after intravenous administration</td>
</tr>
<tr>
<td>CYP2C41</td>
<td>Partial or complete gene deletion</td>
<td>Gene absent in 24 of 28 (86%) dogs (Beagles and mixed breed)</td>
<td>No functional enzyme</td>
<td>Unknown</td>
</tr>
<tr>
<td>CYP2D15</td>
<td>S186G, I250F, I307V (WT2)</td>
<td>Unknown</td>
<td>Lower bufuralol hydroxylation than V1,*2 and *3; dextromethorphan demethylation and celecoxib hydroxylation same as for V1,*2 and *3</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>S186G, I250F, I307V, I338V, K407E (V1)</td>
<td>Unknown</td>
<td>No effect</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>S186G (CYP2D15*2)</td>
<td>Unknown</td>
<td>No effect</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>I250F, I307V (CYP2D15*3)</td>
<td>Unknown</td>
<td>No effect</td>
<td>Unknown</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>Y485D</td>
<td>15% in 100 mixed-breed dogs; 19% in 13 Beagles</td>
<td>No effect</td>
<td>Unknown</td>
</tr>
<tr>
<td>CYP3A12</td>
<td>T309S, R421K, K422E, N423K, M452T</td>
<td>Unknown</td>
<td>No effect</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Data from Refs. 12–19
However, other (human selective) CYP1A2 substrates including caffeine and melatonin were unaffected by the deficiency, implying that dog CYP1A2 does not selectively metabolize these latter drugs (unlike human CYP1A2). A recent study of phenacetin pharmacokinetics following oral and intravenous administration to CYP1A2 genotyped Beagles\textsuperscript{15} showed about twofold higher phenacetin exposure (based on area under the plasma concentration time curve) after oral administration in CYP1A2-deficient dogs, but there were much smaller (nonsignificant) differences in phenacetin levels after intravenous exposure. The authors concluded that phenacetin was not a selective or robust in vivo probe for CYP1A2 probably because of metabolism of phenacetin by other enzymes (eg, canine CYP1A1 or canine CYP2A13). These findings indicate that the effect of CYP1A2stop on a particular drug depends on the degree of importance of canine CYP1A2 in clearance and cannot be extrapolated directly from human data.

The prevalence of CYP1A2stop seems to vary considerably between and within dog breeds. Apart from research colony Beagle dogs in Japan,\textsuperscript{12,13} several other studies have surveyed this mutation in nearly 40 different dog breeds (and mixed-breed dogs) from the United States,\textsuperscript{24} Germany,\textsuperscript{25} and Brazil (Fig. 3).\textsuperscript{14} The Irish Wolfhound had the highest allele frequency (42%) followed by the Japanese Beagles (37%–39%) and Berger Blanc Suisse (28%). Interestingly, the beagles studied in Germany\textsuperscript{25} and the United States\textsuperscript{24} had less than half the allele frequency (15% and 13%, respectively) than the beagles from Japan,\textsuperscript{12,13} possibly reflecting colony founder effect differences. The remaining breeds studied had allele frequencies of 10% or less indicating that the likely frequency of the enzyme-deficient homozygous variant dogs would be 1% or less in the population (ie, relatively rare). Interestingly, many of the remaining affected breeds were herding dogs including Australian Shepherd, Collie, Shetland Sheepdog, Bearded Collie, Border Collie, and Old English Sheepdog.\textsuperscript{25}

Although this could be sampling bias, it might also indicate a common (although
perhaps more recent) ancestry of CYP1A2stop with the MDR1 gene deletion (MDR1del) mutation, which is commonly found in herding breed dogs. The latter results in drug sensitivity from deficiency of the P-glycoprotein transporter encoded by MDR1 (discussed elsewhere in this issue). Regardless, the clinical consequence is that herding breed dogs could be affected by multiple genetic defects (MDR1del and CYP1A2stop) influencing drug disposition and response.

**CYP2C41 Gene Deletion**

During initial attempts to clone canine CYP2C21 from dog liver RNA, a second CYP2C subfamily enzyme named CYP2C41 was discovered. This latter isoform was found to be present at the RNA and genomic DNA level in only about 16% (4 of 25) of dogs (2 of 10 mixed breeds and 2 of 18 Beagles). This contrasted with CYP2C21 that was...
found to be expressed in all dogs examined. This finding suggests the presence of a partial or complete deletion of the CYP2C41 gene in many dogs. This was confirmed (albeit with a lower deletion frequency) by a study in another laboratory that showed detectable CYP2C41 mRNA in 6 of 11 Beagle dogs. In vitro studies of recombinant canine CYPs indicate that CYP2C41 metabolizes many of the same substrates as CYP2C21 (including diclofenac and S-mephenytoin), although with much less efficiency. Consequently, the impact of the CYP2C41 deletion on canine drug metabolism or pharmacokinetics may be somewhat limited.

CYP2D15 Amino Acid Variants

Several studies have identified different CYP2D15 mRNA forms expressed in liver that vary in predicted amino acid coding sequence at three to five different residues (see Table 2). Although it is presumed that these changes are the result of single nucleotide polymorphisms (SNP), this has not yet been established, such as through genotyping of multiple dogs. In vitro studies of expressed amino acid variants suggest that the impact of these coding changes on enzyme function may be somewhat limited. The only exception was the WT2 form identified by Roussel and colleagues, which showed about 50% lower bufuralol hydroxylation compared with the other forms but unchanged celecoxib hydroxylation or dextromethorphan demethylation. The impact of these genetic variants on drug pharmacokinetics or effect has not been reported.

Paulson and colleagues originally undertook their study of CYP2D15 variants to explain polymorphic celecoxib clearance in vivo and celecoxib hydroxylation in vitro in a research colony of Beagle dogs. However, they did not directly address this hypothesis, such as through CYP2D15 genotyping of phenotyped dogs. Furthermore, although CYP2D15 was shown to be capable of hydroxyating celecoxib, a significant role for other CYPs was not excluded. Consequently, the mechanism underlying the celecoxib pharmacokinetic polymorphism remains unexplained.

CYP2E1 Amino Acid Variant

An SNP (1453T>C) resulting in a tyrosine for histidine substitution at amino acid position 485 (H485Y) was discovered during initial cloning of the CYP2E1 cDNA from canine liver RNA. Survey genotyping found an allele frequency of 15% in 100 mixed-breed dogs, and 19% in 13 Beagles. An in vitro study comparing expressed wild-type (485H) and variant (485Y) CYP2E1 isoforms showed no difference in chlorzoxazone hydroxylation activity. However, because only one substrate was evaluated, substrate-dependent effects cannot be excluded. In vivo effects of this SNP on drug pharmacokinetics have not been reported.

CYP3A12 Amino Acid Variants

A variant (called CYP3A12*2) that included five different nucleotide differences from the initial cloned sequence, and predicted to cause five unique amino acid changes, was also discovered during cloning of the CYP3A12 cDNA from canine liver RNA. In vitro experiments showed no effect of these amino acid changes on testosterone 6-β-hydroxylation by recombinant enzymes. Genotype frequencies or any association of genotype with drug metabolism phenotype measured in vivo have not been reported.

OTHER DOG CYPS ASSOCIATED WITH PHENOTYPIC VARIABILITY

CYP2B11 and Breed-related Anesthetic Drug Hypersensitivity

Severely delayed recovery has been reported for certain dog breeds (primarily Greyhounds and possibly other sighthounds) after use of injectable anesthetic agents.
including thiopental and thiamylal. Although initially attributed to decreased drug redistribution from the central compartment resulting from reduced body fat in Greyhounds, a series of studies demonstrated that the effect could be attributed to decreased drug clearance in Greyhounds compared with mixed-breed dogs, and was also prevented by pretreatment with a microsomal enzyme inducer (phenobarbital). A subsequent study showed that propofol (another short-acting anesthetic) was also cleared more slowly in Greyhounds, and pretreatment with chloramphenicol, a CYP inhibitor, decreased drug clearance even further (Fig. 4). In vitro mechanistic studies identified CYP2B11 as being the main enzyme responsible for CYP-dependent clearance of propofol. The molecular genetic basis for this breed-dependent difference in drug metabolism has not been reported.

**SUMMARY**

Published evidence indicates that variability in drug metabolism by CYP in dogs is likely to be considerable and is explained in part by the presence of genetic polymorphisms that vary between dog breeds. However, few CYPs (mainly CYP1A2) have been systematically investigated, and the influence of the discovered genetic variants on the pharmacokinetics of clinically used drugs and their effects is unclear. Predictions of genetic effects on particular drugs (eg, the effect of CYP1A2 stop codon mutation on phenacetin pharmacokinetics) from human data are complicated by human-dog differences in CYP substrate specificity and abundance. Consequently,
clinical studies confirming the impact of discovered variants on drug response in canine patients are essential.

REFERENCES


