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Canine cytochrome P450 (CYP) pharmacogenetics

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Synopsis

The cytochrome P450 (CYP) drug metabolizing enzymes are essential for the efficient elimination of many clinically used drugs. These enzymes typically display high interindividual variability in expression and function resulting from enzyme induction, inhibition, and genetic polymorphism thereby predisposing patients to adverse drug reactions or therapeutic failure. There are also substantial species differences in CYP substrate specificity and expression that complicate direct extrapolation of information from humans to veterinary species. This article reviews the available published data regarding the presence and impact of genetic polymorphisms on CYP-dependent drug metabolism in dogs in the context of known human-dog CYP differences. Canine CYP1A2, which metabolizes phenacetin, caffeine, and theophylline, is the most widely studied polymorphic canine CYP. A single nucleotide polymorphism resulting in a CYP1A2 premature stop codon (c. 1117C>T; R383X) with a complete lack of enzyme is highly prevalent in certain dog breeds including Beagle and Irish wolfhound. This polymorphism was shown to substantially affect the pharmacokinetics of several experimental compounds in Beagles during preclinical drug development. However, the impact on the pharmacokinetics of phenacetin (a substrate specific for human CYP1A2) was quite modest probably because other canine CYPs are capable of metabolizing phenacetin. Other canine CYPs with known genetic polymorphisms include CYP2C41 (gene deletion), as well as CYP2D15, CYP2E1, and CYP3A12 (coding SNPs). However the impact of these variants on drug metabolism *in vitro* or on drug pharmacokinetics is unknown. Future systematic investigations are needed to comprehensively identify CYP genetic polymorphisms that are predictive of drug effects in canine patients.

Keywords

Dog; Canine; Genetic polymorphism; Cytochrome P450; Pharmacokinetics

INTRODUCTION

The cytochrome P450 (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 inter-individual variability in the activity of these enzymes¹. Consequently, for a given drug dosage the effect can range from undetectable or suboptimal (with high enzyme activity, high drug clearance, and low

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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 and/or inducers in the diet or from coadministered medications (previously reviewed in ²). Genetic variation is also a well-established cause of CYP activity variability in humans, and current evidence suggests that it may be equally important in veterinary species, including dogs ¹. 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 chapter 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 United States FDA 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 as follows.

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 following speciation) are found in most species. For example CYP2B11 is considered to be the canine ortholog of human CYP2B6 ^{3,4}. Orthologs also tend to have roughly similar substrate specificities. For example both human CYP2B6 and canine CYP2B11 metabolize propofol ^{4,5}. However significant species differences exist. For example midazolam is metabolized exclusively by human CYP3A4 and CYP3A5 (but not by human CYP2B6) while dog CYP2B11 (and not CYP3A12) primarily metabolizes midazolam ⁶. Similarly, both dog CYP1A2 and dog CYP2A13 metabolize phenacetin ^{7,8}, while only human CYP1A2 (and not human CYP2A6) metabolizes this drug. Although most dog CYPs have unique names, 3 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. Figure 1 shows the distribution of the different CYPs in liver and small intestinal mucosa of human ^{9,10} and dog ¹¹. 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 limit 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. On

the other hand, 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 both the CYP2D6 and CYP2B6 genes in humans. A clinical consequence is that drugs metabolized by CYP2D15 and/or CYP2B11 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.

CYP1A2 premature stop codon (CYP1A2stop) polymorphism

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^{12, 13}. 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 relatively 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^{12, 13}. The effect on the plasma drug levels of the investigational drugs was large with up to 17 times increased levels in deficient dogs (see Figure 2)^{20, 21}. 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-21²² and BTP-2²³ have also been associated with variable CYP1A2 metabolism in Beagles.

As yet the effect of this polymorphism on the pharmacokinetics and effects of clinically used drugs is unclear. In vitro studies⁸ 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. 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 genotype Beagles¹⁵ showed about 2-fold higher phenacetin exposure (based on area under the plasma concentration time curve; AUC) after oral administration in CYP1A2 deficient dogs, but there were much smaller (non-significant) 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 (such as canine CYP1A1 and/or canine CYP2A13). These findings indicate that the effect of CYP1A2stop on a particular drug will depend on the degree of importance of canine CYP1A2 in clearance and cannot be extrapolated directly from human data.

The prevalence of CYP1A2stop appears to vary considerably both between and within dog breeds. Apart from research colony Beagle dogs in Japan^{12, 13}, several other studies have surveyed this mutation in nearly 40 different dog breeds (and mixed breed dogs) from the USA²⁴, Germany²⁵, and Brazil¹⁴ (see Figure 3). 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 Germany²⁵ and the USA²⁴ had less than

half the allele frequency (15% and 13%, respectively) than the beagles from Japan^{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 (i.e. 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 Sheepdog²⁵. 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 dogs²⁶. The latter results in drug sensitivity from deficiency of the P-glycoprotein transporter encoded by MDR1 (see Chapter7). 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 discovered¹⁶. This latter isoform was found to be present at both 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 dogs²⁷. 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^{28, 29}. 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 3 to 5 different residues (Table 2). Although it is presumed that these changes are the result of SNPs, 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 et al (1998) that showed about 50% lower bufuralol hydroxylation compared with the other forms but unchanged celecoxib hydroxylation¹⁸ or dextromethorphan demethylation¹⁷. As yet the impact of these genetic variants on drug pharmacokinetics or effect has not been reported.

Paulson et al (1999) originally undertook their study of CYP2D15 variants in order 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 hydroxylating celecoxib, a significant role for other CYPs was not excluded. Consequently, the mechanism underlying the celecoxib pharmacokinetic polymorphism remains unexplained.

CYP2E1 amino acid variant

A 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 RNA¹⁹. 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, since 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 5 different nucleotide differences from the initial cloned sequence, and predicted to cause 5 unique amino acid changes was also discovered during cloning of the CYP3A12 cDNA from canine liver RNA¹⁸. *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 have been reported for certain dog breeds (primarily Greyhounds and possibly other sighthounds) following use of the injectable anesthetic agents, including thiopental and thiamylal^{30–33}. 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)^{30, 31}. A subsequent study showed that propofol (another short acting anesthetic) was also cleared more slowly in Greyhounds³⁴, and pretreatment with chloramphenicol, a CYP inhibitor, decreased drug clearance even further³⁵. *In vitro* mechanistic studies identified CYP2B11 as being the main enzyme responsible for CYP-dependent clearance of propofol^{4, 36}. As yet, the molecular genetic basis for this breed-dependent difference in drug metabolism has not been reported.

CONCLUSIONS

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 to date, 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 (such as 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 will be essential.

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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.

Figure 1.

Comparison of relative CYP isoform protein levels in dog and human liver and small intestines. Protein amounts were determined by quantitative immunoblotting for human liver (Shimada et al, 1994) and intestines (Paine et al, 2006) or by quantitative mass spectrometry for Beagle dog tissues (Heikkinen et al, 2012). Note that CYP2A forms were not assayed in dog tissues and CYPs other than CYP2B11 and CYP3A12 were below the detection level in dog intestines.

Figure 2.

Effect of the CYP1A2 premature stop codon mutation (R373X) on the pharmacokinetics of the investigational compound YM-64227 (left) and the analgesic drug phenacetin (right) after oral administration to genotyped Beagle dogs. Data for YM-64227 are from Tenmizu et al (2006) and for phenacetin are from Whiterock et al (2012). The effect of the CYP1A2 premature stop codon on YM-64227 (about 25 times greater mean plasma AUC) was much greater than on phenacetin (about 2 times greater AUC).

Allele frequency (%) of the CYP1A2 stop codon mutation in different dog breeds

Figure 3.

Allele frequency (number of variant alleles as percent of total alleles) of the CYP1A2 stop codon mutation (R373X) in different dog breeds. Shown after each breed are the numbers of individual dogs that were sampled. Data are from dogs located in Brazil (*Scherr et al, 2011), USA (**Whiterock et al, 2007), Japan (**Mise et al, 2004 and ****Tenmisu et al, 2004), and Germany (Aretz and Geyer 2011). Only data from breeds in which DNA from at least 10 different dogs in that breed were sampled are shown. Other breeds in which at least one dog had the deficient allele included Whippet, Deerhound, Dalmation, and Jack Russell Terrier (Aretz and Geyer 2011).

Figure 4.

Greyhounds show slower clearance (left) and lower liver metabolism (right) of propofol compared with mixed breed and Beagle dogs. The left panel (from (Zoran et al. 1993)) shows mean propofol concentrations in 10 Greyhounds and 10 mixed breed dogs given propofol intravenously at a dose of 5 mg / kg body weight. The arrows indicate time of return of the righting reflex (filled arrow) and ability to stand (open arrow). The right panel shows propofol hydroxylation rates measured by HPLC with in vitro incubations of liver microsomes prepared from male Greyhounds, beagles and mixed breed dogs (5 each). The points represent data from individual dog livers, while the horizontal lines indicate the mean values of each group. *Adapted from Court MH, Hay-Kraus BL, Hill DW, et al. Propofol hydroxylation by dog liver microsomes: assay development and dog breed differences. Drug Metab Dispos 1999;27(11):1293.*

Table 1

Examples of drugs that are known substrates for specific human^a and/or dog^b CYPs

Human CYP	Substrates	Dog CYP	Substrates
CYP1A2	Caffeine Phenacetin Tacrine Theophylline	CYP1A2	Caffeine Phenacetin Theophylline
CYP2A6	Nicotine	CYP2A13	Phenacetin
CYP2B6	Bupropion Efavirenz Propofol	CYP2B11	Atipamezole Diclofenac Ketamine Medetomidine Midazolam Propofol Temazepam
CYP2C8	Amodiaquine	CYP2C21/CYP2C41	Diclofenac
CYP2C9	Diclofenac Tolbutamide S-warfarin		
CYP2C19	Omeprazole S-mephenytoin		
CYP2D6	Bufuralol Codeine Debrisoquine Dextromethorphan	CYP2D15	Celecoxib Debrisoquine (poor) Desipramine Dextromethorphan Imipramine
CYP2E1	Chlorzoxazone	CYP2E1	Chlorzoxazone
CYP3A4	Midazolam Triazolam Terfenadine Nifedipine	CYP3A12	Diazepam Diclofenac Eplerenone Imatinib Midazolam

^aData from P450 Drug Interaction Table. Indiana University Department of Medicine, 2009. Available at: <http://medicine.iupui.edu/clinpharm/ddis/table.aspx>. Accessed April 20, 2013.

^bData from Martinez MN, Antonovic L, Court M, et al. Challenges in exploring the cytochrome P450 system as a source of variation in canine drug pharmacokinetics. *L Drug Metab Rev* 2013;45(2):218–30.

Both sources also list other dog and human CYP substrates, inhibitors, and inducers.

Table 2

Summary of known canine CYP genetic polymorphisms

Enzyme	Genetic variant	Variant allele frequency	In vitro effect	In vivo effect
CYP1A2	SNP c.1117C>T causing premature stop codon mutation at amino acid position 373 (R373X)	6% in 99 mixed breed dogs from Brazil; 38% in 214 Beagles from Japan; see Figure 3 for complete numbers.	No functional enzyme	~50% lower phenacetin clearance after oral administration; no difference in phenacetin clearance after IV administration
CYP2C41	Partial or complete gene deletion	Gene absent in 24 of 28 (86%) dogs (Beagles and mixed breed)	No functional enzyme	Unknown
CYP2D15	S186G, I250F, I307V (WT2)	Unknown	Lower bufuralol hydroxylation than V1,*2 and *3; dextromethorphan demethylation and celecoxib hydroxylation same as for V1,*2 and *3	Unknown
	S186G, I250F, I307V, I338V, K407E (V1)	Unknown	No effect	Unknown
	S186G (CYP2D15*2)	Unknown	No effect	Unknown
	I250F, I307V (CYP2D15*3)	Unknown	No effect	Unknown
CYP2E1	Y485D	15% in 100 mixed breed dogs; 19% in 13 Beagles	No effect	Unknown
CYP3A12	T309S, R421K, K422E, N423K, M452T	Unknown	No effect	Unknown

Data from Refs 12–19.