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Review

Implications of hepatic cytochrome P450-related biotransformation processes in veterinary sciences

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ABSTRACT

Cytochrome P450 enzymes (CYP450) represent a superfamily of monooxigenases that play a pivotal role in drug metabolism. In contrast to the extensive database available for human and rodent CYP450 enzyme activities, the data related to animal species that are regular patients in veterinary medicine, are far from being complete. The major obstacles are the significant inter-species and intra-species differences. With the aim to provide an overview of the current knowledge, key data for important species, such as dogs and cats, horses, pigs and ruminants, are presented, and compared with findings from humans. Analysis of these data shows, that currently no links can be established between certain physiological traits, such as herbivorous and carnivorous species, monogastric animal and ruminants, nor within a given species, as for example cattle. This implies that for all new pharmaceutical entities individual assays are needed for every animal species or even every individual breed. It can be anticipated, however, that investigations into the upstream transcriptional regulation of CYP450 enzymes will provide more insight into the observed expression levels, thus allowing to modulate kinetic parameters of old and new drugs, as the same transcription factors control also the expression of prominent drug transporters.

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1. Introduction

The group of cytochrome P450 (CYP450) enzymes represents a large superfamily of membrane bound heme containing monooxigenases, which catalyse the incorporation of one oxygen atom from molecular oxygen into a substrate. The electrons required for the catalytic cycle are delivered by NADPH. This reaction can be observed in virtually all living organisms, from bacteria, in which cytochromes are plasmatic enzymes to mammalian species, in which cytochromes are bound to membranes of the endoplasmatic reticulum. The biological significance of mammalian cytochrome P450 enzymes has been recognised already in the 1964, when Omura and Sato first described the differential spectrum of dithionite reduced and carbon monoxide-complexed cytochromes. In 1996, Nelson et al. presented the first systematic approach in allocating individual cytochromes to phylogenetic families and subfamilies (Omura and Sato,1964). Within the complex group of cytochromes, the enzyme families 1–4 are

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primarily involved in the biotransformation of drugs and toxins, and hence are referred to as drug metabolising enzymes.

Despite the highly conserved functions of CYP450s, significant species differences exit in the level of expression of individual enzymes and their substrate specificity. These divergences are thought to be due to the fact that members of the CYP450 family diverged from each other as early as 2 billion years ago, resulting in less than 40% similarities in the amino acid sequences within the same P450 family. The CYP450 subfamilies emerged more than 400 million years ago. Within a subfamily, the amino acid similarities are normally greater than 55% (Nelson et al., 1996). A recent re-appraisal of the development of the CYP450 genes in vertebrates proposes a hypothesis of genomic instability, as it could be shown that human and mouse CYP450 genes reside in dense gene clusters. This is thought to explain the diversity in the expression and function of xenobiotic-metabolising enzymes (Thomas, 2007).

The phylogenetic divergence has been studied for example in species that became isolated, like the Australian marsupials, hypothesising that the evolution of CYP450s is correlated with geological and biological events (Olkowski et al., 1998). Folivorous marsupials often feed exclusively on *Eucalyptus* species, which are native to Australia. These plants contain a high level of phenolics and essential oils, the latter containing complex and variable mixtures of monoterpenes. In turn, it was hypothesised that Koalas (*Phascolarctos cinereus*) and the bushtail opossum (*Trichosurus vulpecula*) exhibit unique enzyme expression patterns to detoxify phenols and terpenoids (Stupans et al., 2001). However, no specific patterns were detected and it remains unclear whether or not the measurable CYP activities reflect acquired traits in response to the daily diet, or an evolutionary process.

In contrast to the extensive data base available for human and rodent CYP450 enzymes (the PubMed data base cites nearly 27,000 articles), the characterisation of the CYP450 system in animals that are common veterinary patients is still very incomplete (Sharer et al., 1995; Shimada et al., 2001; Gusson et al., 2006; Ioannidis, 2006). Moreover, the few cross-species investigations conducted by individual research groups remain difficult to compare, as the methods applied differ, particularly in terms of the model substrates used. These substrates were selected on the basis of the available knowledge from rodent or human studies, but enzyme velocity has not been characterised in the individual target animals, and enzyme kinetic data such as k_m and V_{max} values have been determined only in very few studies. The same applies for inhibitors and inducers, used to further characterize individual CYP450 in animals (Graham et al., 2002). Therefore it is the aim of the current review to present a compilation of the available data on CYP450 expression and substrate specificity in animal species relevant to veterinary medicine and to illustrate the motivation for CYP450-related research in veterinary sciences.

2. Species differences in CYP450 activity in species relevant to veterinary medicine

Despite the fact that apparently no distinct inherited patterns of CYP450 expression seem to exist between groups of animals that share other physiological features, such as being herbivorous or carnivorous species, or being phylogenetically closely related (such as different dog breeds), various attempts have been made to compile normal patterns of hepatic CYP450 enzymes with the aim to allow a general comparison between animal species (Fink-Gremmels and van Miert, 1994; Mahmood, 2001; Ioannides, 2006). This is of importance in veterinary medicine, as many drugs are used in more than one animal species and in consideration of the use of human drugs for certain indications in companion animals, such as dogs and cats, but also in horses. Moreover, comparative data in animals might allow to predict the toxicity of natural and industrial xenobiotics in animals (Nebbia, 2001). Generally, these investigations are conducted with liver microsomes, which were used to measure the conversion of known model substrates for individual CYP450-mediated reactions. Comparing different (laboratory) animal species the catalytic activity was described to be rather consistent for CYP2E1 > CYP1A2, CYP4A > CYP2D, and CYP3A (Guengerich, 1997). Major inconsistencies were reported in the extrapolation of substrate specificity regarding CYP2A, 2B and 2C (for details see Table 1).

Comparative investigations of the overall CYP450 activity (measured as differential spectrum between carbon monoxide-complexed and -non-complexed microsomal suspensions) in liver microsomes, isolated under standardised conditions, showed that the activity could be found in rabbits (1.77 ± 0.13 nmol/mg protein). Horses and pigs (means were 0.53 and 0.57 nmol/mg protein, respectively) had significantly lower levels of activity. Rodents (rats) liver microsomes used in the same assay showed an overall enzymatic activity of 0.85 nmol/mg protein and chickens had the lowest activity with 0.25 ng/ml protein (Nebbia et al., 2003).

3. Genetic polymorphisms in animal species relevant to clinical veterinary medicine

In humans, CYP P450 genes are highly polymorphic (see www.cypalleles.ki.se). Functional CYP450 polymorphisms may originate from gene deletion, gene duplication and deleterious mutations creating inactive gene products. Furthermore, amino acid changes might be introduced which can change substrate specificity (for a recent review see Ingelman-Sundberg et al., 2007). Although it is conceivable that all these types of polymorphisms exist in all animal species, they have not been investigated in detail as yet. There are only three prominent examples, not related to CYP450, for which genetic analyses were conducted. Cats were known for many decennia to be very sensitive to phenolic compounds as well as some drugs, such as acetaminophen. Later on it was noted that this sensitivity was attributable to a low glucuronidation capacity, but only in 2000, Court and Greenblad, conducted a genetic analysis and found that cats (and other felines) have a very low glucuronidation capacity due to a mutation in the UDP (uridine-diphosphate)-glucuronosyl transferase 1A6 gene, resulting in the expression of a non-functional protein. A comparable mutation can be found in the ferret that lacks apparently functional enzyme expression as well. Dogs, on the other hand, fail to express functional *N*-acetyl-transferases (NAT-1 and NAT-2), which are essential for the excretion of for example sulfonamides. Like NAT-2 slow acetylators in the human population (Hengstler et al., 1998) dogs are very susceptible to sulfonamides, and an iatrogenic conjunctivitis sicca is a common undesirable side effect of long-term sulfonamide therapies. Certain dog breeds carry also a mutation in the ATP-binding cassette (ABC) gene ABCB1, encoding for the efflux transporter P-

Table 1
Species-specific expression of major drug-metabolising P450 subfamilies (modified according to Guengerich, 1997; Thomas, 2007)

Cytochrome	Principle reaction	Comparison of the level of activity
CY1A2	7-Ethoxy-4-trifluor-methyl-coumarin-*O*-dealkylation	Dog >> rabbit, monkey > micropig > human > mouse > rat
CYP2A6	Coumarin-7-hydroxylase	Monkey > human > rabbit > micropig > dog > mouse >> rat
CYP2C	Diclofenac-4'-hydroxylase	Human > monkey, rat > rabbit > mouse > micropig > dog
CYP2C19	*S*-mephenytoin-4-hydroxylation	Monkey > human >> dog > rabbit > rat > mouse
CYP2C6	Amphetamine-hydroxylation	Rat >> mouse >> guinea pig, human, rabbit
CYP2E	Chlorzoxazone-6-hydroxylation	Similar in all tested species, despite different k_m values
CYP3A	Testosterone-6β-hydroxylation	Similar in all tested species, with the exception of dogs, having a very low activity