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MICROBIAL P450: DOES IT EXIST, AND WHAT CAN IT MEAN?

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SUMMARY

P450 enzymes play important physiological and patho-physiological roles in the complex interplay between a host and his intestinal microflora. The need for more information is underlined.

NOMENCLATURE

The cytochrome P450s (CYP) constitute a superfamily of haem-thiolate enzymes and their Fe-carbon complexes show an absorption spectrum with a maximum near 450. There are now over 1000 different P450s that have been identified from the whole biological kingdom. Due to this large number a standardised nomenclature has been developed (*Nelson* et al., 1996). In short, the P450 superfamily is subdivided into families. An individual P450 within a family are defined as having less than 40% sequence identity with a P450 in another family. The families are divided into sub-families and enzymes within a sub-family are more than 55% identical in sequence. A P450 enzyme is designated by the root symbol "CYP" (describing cytochrome and P450), an Arabic number denoting the family, a letter designating the subfamily and a Arabic numerals representing individual enzymes. It should be kept in mind that this system is based only on sequence similarity among the P450s and - unfortunately does not indicate the function(s) of individual P450s.

P450s AND METABOLISM OF XENOBIOTICS

Nowadays, it is generally recognised that multicellular organisms, including humans, are continuously exposed to foreign chemicals, collectively known as xenobiotics. They are found in our environment and include a vast range of compounds such as drugs, industrial chemical, pollutants, pesticides, plant products, alkaloids and toxins. Most of them are rather lipophilic, and due to their lipophilicity, many xenobiotics can be - and are - absorbed through our surfaces (intestine, lungs, and the skin). Also due to their lipophilicity – if the xenobiotics are not metabolised in the body - they will be concentrated in the tissue and sooner ore later they might be toxic for the host. Therefore, in order to be eliminated, many xenobiotics have to be converted into more water-soluble compounds, thereby influencing upon their excretion in the urine or faeces.

The enzymes catalysing these reactions, i.e. the xenobiotic-metabolising enzymes, are – for convenient reasons - often divided into two groups refereed

Table 1: Major reactions and groups of enzymes in xenobiotic biotransformation

Reaction	Type of enzyme
Phase I	
	Cytochrome P450
	Alcohol dehydrogenase
	Aldehyde dehydrogenase
Oxidation	Xanthine oxidase
	Monoamino oxidase
	Flavin mono-oxidase
Reduction	Quinone reduction
	Reductive dehalogenation (P450)
Hydrolysis	Epoxide hydrolase
Phase II	
Glucuronide conjugation	UDP-glucuronosyltransferase
Glutathione conjugation	Glutathione S-transferase
Sulphate conjugation	Sulphotransferase
Acetylation	N-acetyltransferase
Methylation	Methyltransferase

to as phase I and phase II (Table 1) As summarised by *McLellan* (2000), enzymes involved in phase I reactions, most of which represent P450s, expose or introduce a function group (-OH, -NH_2, -SH or -COOH) on the compounds by oxidation, reduction or hydrolysis reactions among others. In general, these alterations increase hydrophilicity to a minor extent. It is evident from Table 1 that phase II mainly involves conjugation of the compound, with molecules such as gluthatione, glucuronic acid, sulphate, taurine, glycine and other amino acids.

It has to be kept in mind that the biotransformations included in phase I may change the pharmacokinetic behaviour of many drugs. In fact, some drugs have to undergo biotransformation before exerting their effects. On the other side, however, biotransformation included in phase I may convert many xenobiotics to more reactive electrophilic metabolites that can form protein and DNA adducts, thereby exerting their toxic or tumourigenic effect (*Nebert* et al., 1996) It should also be kept in mind that important co-factors for phase II reactions are functional groups that are either present on the xenobiotics or have been introduced during phase I reaction. Thus, phase II biotransformation may or may not be preceded by phase I biotransformation

P450s AND EVOLUTION

It is generally believed that P450s are very old enzyme, probably occurring before the divergence of prokaryote and eukaryote. A early eukaryotic mitochondrial P450 (influencing upon the metabolism of cholesterol to steroids) is found in both plants and animals, indicated that animals diverged from plants around 1400 millions years ago (giving rise to P450s localised in the mitochon-

Table 2: Major human P450 families and primary function(s)

Family	Catalytic function
CYP1	Xenobiotic metabolism
CYP2	Xenobiotic catabolism
CYP3	Xenobiotic catabolism
CYP4	Fatty acid hydroxylation
CYP5	Thromboxane A2 synthase
CYP7A	Cholesterol 7-alpha-hydroxylase
CYP8A	Prostacyclin synthase
CYP8B	Sterol 12-alpha-hydroxylase
CYP11A1	Cholesterol side-chain cleavage
CYP11B1	Steroid 11-beta-hydroxylase
CYP11B2	Aldosterone synthase
CYP21	Steroid 21-hydroxylase
CYP27A1	Sterol 27-hydroxylase
CYP46	Cholesterol 24-hydroxylase

dria and endoplasmatic reticulum. A major evolutionary steps seems to have taken place around 900 millions years ago, resulting in one lineage continuing as endogenous P450s and the other began a new function, i.e. xenobiotic metabolism (*Nelson* and *Strobel*, 1997).
In this respect, it is a fascinating theory that the xenobiotic-metabolising enzymes have involved due to a continuous interaction or "evolutionary fight" between plants and animals (*Gonzales* and *Nebert*, 1990; *Nebert*, 1997). Plants are continuously evolving biosynthetic pathways in order to synthesise secondary metabolites for their reproductive cycles and to defend themselves from insect and animal predators (*Schuler*, 1996). Going back in history, it seems reasonable to assume that when animals started to consume plants, the plants responded by evolving new genes to synthesise toxic metabolites. In order to defend themselves from these plant toxins, animals developed new enzymes to cope with these new plant toxins (*Gonzales* and *Nebert*, 1990). Thus, the P450s have – and have played – a crucial role in the ecological balance in Mother Nature.

In this respect, it is neither surprising that many prokaryotes may contain P450s nor that many of our currently used drugs, often derived from natural plant metabolites, are metabolised by the P450 superfamily of enzymes.

XENOBIOTIC METABOLISM AND SUBSTRATE SPECIFICITY

As shown in Table 2, the CYP families 1,2 and 3 are primarily associated with xenobiotic metabolism. Most of the enzymes belonging to these 3 families have an extremely broad substrate specificity. It has also to be mentioned that many substances are metabolised – to varying degrees – by several different P450s and that one single enzyme can metabolise numerous, structurally diverse chemicals. Taken together, the enzymes, including in these three families have a collective capacity to metabolise – most often detoxify - an

enormous number of substances that we may be exposed to.

It should be mentioned that, in general, enzymes belonging to other families have a higher degree of substrate specificity and they are usually acting more upon endogenous compounds than xenobiotics. On the other hand, enzymes belonging to the three first families may also metabolise some endogenous compounds, as steroids.

LOCALISATION OF P450s

By far, liver is the main site of expression of xenobiotic-metabolising P450s. However, some of them may be found in extrahepatic tissue. It is known that that extrahepatic tissue may contribute to the xenobiotic-metabolising capacity of the body. In turn, this might results in a high local turnover of a drug, thereby influencing upon the local effect of the drug. Such extra-hepatic metabolism might even compensate to some extent for reduced hepatic elimination in cases of severe liver cirrhosis (*Krishna* and *Klotz*, 1994). This can be exemplified as follows. The CYP3A enzymes are involved in the metabolism of around 50% of clinically useful agents and have a very wide substrate specificity. CYP3A4is one of the major enzymes within this family. It is found at relatively high levels in enterocytes in the small intestine. Similar to what found in the liver, it can be induced by rifampin (*Kolars* et al., 1994).

P450s AND MICROORGANISMS

Over the years, it has been found that cytochrome P450s are not uncommon in prokaryotes and it has been found to be present in a number of bacterial strains (*Fulco*, 1991; *Nelson* et al., 1996). The huge enzymatic capacity of the intestinal flora also indicate metabolic reactions similar to those carried out by mammalian P450s It is indeed well known that the intestinal microflora is able to carry out enzymatic reactions involved in phase II, and previous investigations also indicate that the flora may (*Bakke* and *Midtvedt*, 1970) – but not always (*Borud* et al., 1971, 1973), play a role in phase I type of reactions. Comparative studies in germfree and conventional animals have shown that presence of an intestinal microflora induce and/or repress certain isoforms of hepatic P450s (*Nugon-Baudon* et al., 1998). However, the mechanism(s) behind these modulating effects of the intestinal microflora are not well understood. Additionally, it should be mentioned that several microbial enzymes might act upon metabolites formed during phase II reactions. Indeed, deconjugation of bile acids (*Midtvedt*, 1974) and steroid are solely a bacterial event and so is nearly also deconjugation of glucuronides (*Roed* and *Midtvedt*, 1977) and some drugs (*Peppercorn* and *Goldman*, 1972)

P450s AND SPECIFIC MEMBERS OF THE INTESTINAL FLORA

This possible enzyme-modulating effect of the intestinal microflora was the background for a resent study concerning presence of P450s in 18 bacterial strains, selected among the major group of species known to be present in the

human intestinal microflora (*John* et al., 2001). As summarised by the authors, "the amino acid identity, Southern blot and CO difference spectrum data all suggest the presence of a cytochrome P450-like gene in *Eubacterium aerofaciens*". They claim that *Eubacterium* might be found in the human intestine in a density of more than log 10 organisms per ml of content, and that their findings, "demonstrating the presence of cytochrome P450 or P450-like proteins in microflora will help us to gain a better understanding of the role specific microbes, like *E. aerofaciens*, may play in metabolising xenobiotics. In addition, it will allow us to understand its influence on hepatic cytochrome P450 expression and its overall association with tumour suppression and/or formation."

Surely, work like this should be extended. It goes without saying that if probiotics express P450 activity, they may influence upon the metabolism – and efficacy - of several drugs. It is indeed easy to predict that intake of some probiotics may influence upon the efficacy of contraceptives. A scenario that should be taken into consideration is that a long-term intake of a probiotic in a child might influence upon the development of the normal spectrum of P450s in the liver. To the best of my knowledge, investigations along these lines are not included in any of the long-term studies of intake of probiotics in cohorts of children. Additionally, in groups of individuals with an increased number of microbes in the small intestine (elderly people, patients using antacida, etc.), a microbial metabolism of drugs might easily take place before they are absorbed.

On the other hand, however, by accepting that microbes may have the possibility of exerting these types of enzymatic reactions it should be a future goal to select specific bacterial strains with specific action(s) on the (pro)drug given, thereby creating an increased local concentration of active drug. A more distant goal might be to profiling the levels of P450 iso-enzymes in the liver.
The answers of the initial questions are: Microbial P450 does exist, but we do not know what it really means.

LITERATURE

Borud, O., Midtvedt, I., and Gjessing L.R.: Urinary phenolic compounds in gnotobiotic and conventional rats on a free Diet, and before and after L-DOPA loading on a milk diet. Acta Pharmacol. Toxicol. 31, 540-549 (1971).

Borud, O., Midtvedt, T., and Gjessing, L.R.: Phenolic metabolites in urine and faeces from rats given radioactive 14C-L-DOPA. Acta Pharmacol. Toxicol. 33, 308-316 (1973).

Fulco, A.J.: P450Bm-3 and other inducible bacterial P450 cytochromes: Biochemistry and regulation. Ann. Rev. Pharmacol. Toxicol. 31,177-203 (1991).

Gonzalez, F.J. and Nebert, D.W.: Evolution of the P450 gene superfamily: Animal-plant "warfare", molecular drive and human genetic differences in drug oxidation. Trends Genet. 6, 182-186 (1990).

John, G.H., Walls, S, Keith, R. Goodfox-Jones, J., Tucker, K., and Abraham, K.J.: The presence of a cytochrome P450-like protein in the human intestinal flora *Eubacterium aerofaciens*. Microb. Ecol. Health Dis. 13, 3-8 (2001).

Kolars, J.C., Lown, K.S., Schmiedlin-Ren, P., Ghosh, M., Fang, C., Wrighton, S.A., Marion, R.M., and Watkins, P.B.: CYP3A gene expression in human gut epihelium. Pharmacokinetics 4, 247-259 (1994).

Krishna, D.R. and Klotz, U.: Extrahepatic metabolism of drugs in humans. Clin. Pharmacokin. 26, 144-160 (1994).

McLellan, R.A.: Interindiviual differences in xenobiotic-metabolising enzymes: The hu-

man genetic factor. Thesis, Karolinska Institute, Stockholm, Sweden, 2000.
Midtvedt, T.: Microbial bile acid transformation. Amer. J. Clin. Nutr. 27, 1341-1347 (1974).
Nebert, D.W., KcKinnon, R.A., and Puga, A.: Human drug-metabolizing enzyme polymorphisms: Effects on risk of toxicity and cancer. DNA Cell. Biol. 15, 273-280 (1996).
Nebert, D.W.: Polymorphisms in drug-metabolizing enzymes: What is their clinical relevance and why do they exist? Am. J. Hum. Genet. 60, 265-271 (1997).
Nelson, D.R., Koymans, L., Kamataki, T., Stegeman, J.J., Feyereisen, R., Waxman, D.J., Waterman, M.R., Gotoh, O., Coon, M.J., Estabrook, R.W., Gunsals, I.C., and Nebert, D.W.: P450 superfamily: Update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics 6, 1-42 (1996).
Nelson, D.R. and Strobel, H.W.: Evolution of cytochrome P-450 proteins. Mol. Biol. Evol. 4, 572-593 (1987).
Nugon-Baudon, L., Robot, S., Flinois, J.P., Lory, S., Beaune, P.: Effects of the bacterial status of rats on the changes in some liver cytochrome P450 (EC 1.14.14.1) apoprotein consequent to a glucosinolate-rich diet. Brit. J. Nutr. 80, 231-234 (1998).
Peppercorn, M.A. and Goldman, P.: The role of intestinal bacteria in the metabolism of salicylazosulfapyridine. J. Pharmacol. Exp. Ther. 18, 55-562 (1972).
Roed, T.O. and Midtvedt, T.: Origin of intestinal beta-glucuronidase in germfree, monocontaminated and conventional rats. Acta Path. Microbiol. Scand. (B) 85, 271-276 (1977).
Schuler, M.A.: The role of cytochrome P450 mono-oxygenases in plant-insect interacts. Plant Physiol. 112, 1411-1419.