ROLE OF XENOBIOTIC METABOLISM IN DRUG DISCOVERY AND DEVELOPMENT

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Keywords: drug metabolism, cytochrome P450(CYP)

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Summary

Drug metabolism is a major determinant in pharmacokinetics and hence regulates drug action. The vast majority of small molecule and biotechnology drugs are metabolised with very few drugs being excreted as unchanged parent drug. Drug metabolism contributes substantially to interindividual differences in drug response and is also often involved in drug interactions, resulting in either therapeutic failure or adverse effects. Consequently, metabolic features are among the main characteristics to be determined in a molecule that is being developed as a potential drug. Knowledge about the metabolism of a new chemical entity (NCE) and its affinity to certain drug-metabolising enzymes helps in the drug development process by providing important information for the selection of a lead compound from among a number of substances pharmacologically equally effective in their therapeutic response. Most information is available on cytochrome P450 (CYP) enzymes, which are in practice the most important group of drug metabolising enzymes. In modern drug development protocols, metabolic characteristics are assessed very early during the development process. This has been made possible by the advances made especially in modelling (*in silico*) and *in vitro* technology. This paper describes the overall role of drug metabolism studies in drug development with a focus on the emerging *in vitro* and modelling (*in silico*) methodology used to predict *in vivo* drug metabolism and kinetic parameters.

1. Introduction

Drug metabolism is an important and often crucial determinant of the pharmacokinetic behaviour of the majority of drugs. Very few drugs are actually eliminated solely by direct excretion of the unchanged parent drug. Drug metabolism contributes substantially to interindividual differences in drug response and is also often involved in drug interactions, resulting in either therapeutic failure or adverse effects. It should be emphasised that drug metabolism and pharmacokinetics are interrelated concepts but nonetheless distinct from each other.

The launch of a new drug is the result of a long process in which a large number of compounds are screened. To speed up the development time and to avoid failure due to pharmacokinetic liability, it is of great importance to be able to predict the metabolism in humans of a new chemical entity (NCE). Early knowledge about the metabolism of a

new NCE and its affinity to certain drug-metabolising enzymes helps in the drug development process by providing important information for the selection of a lead compound from among a number of substances pharmacologically equally effective in their therapeutic response. Information on metabolic properties of a NCE is needed also at later stages of drug development, especially during human clinical studies. Even for a drug already on the market metabolic studies may be required in specific situations.

The art of applying drug metabolism studies in drug development has advanced from a stage in which pharmaceutical companies did not pay any attention to the metabolic fate of an NCE to the present-day situation in which relatively accurate predictions on metabolism can be made very early in the drug development process. A historical perspective on the evolution of drug metabolism studies in industrial drug discovery and development has been written by White in 1998.

This article will analyse how metabolic aspects are taken into consideration during drug development programs. Emphasis is placed on the most important group of drug metabolising enzymes, the cytochrome P450 (CYP) enzymes, and on the rapidly developing field of applying modelling (*in silico*) and *in vitro* methods in drug metabolism studies. Metabolic aspects of traditional small-molecule drugs are used as examples, since CYP mediated metabolic pathways of biotechnological drugs are largely unknown.

2. General Aspects of Xenobiotic Metabolism

2.1. Role of Metabolic Studies during Drug Development

There are three distinct phases of a drug development program: discovery, preclinical development, and clinical development. In the drug development process, a large number of molecules are tested sequentially and, as often is the case today, in a parallel manner. Figure 1 presents some of the key studies to be made during this process.

Preclinical studies start at the very beginning of a lead compound selection and continue up to the time of the first phase I clinical studies. In the discovery phase, the primary goal is to identify molecules with efficacy towards the selected target (e.g. a receptor of enzyme). Proof-of-principle needs to be demonstrated in predictive *in vivo* animal models. Often this stage is delayed due to poor efficacy of the lead compound in the models, despite good *in vitro* potency. One of the reasons that the desired effect is not expressed in the animal model is metabolic instability of the compounds, resulting in their low exposure of the target tissue. Nowadays it is a fairly straightforward process to identify routes of metabolism and the structural site of chemical modification of the test compound. After identification of the metabolic site, new congeners can be designed and synthesised that are more metabolically stable than the original lead compound.

During human clinical studies, there are three major reasons to determine the metabolism of the test compound:

- Comparison of human metabolic pathways with those in preclinical animal studies
- Detection of active metabolites of the parent test compound
- Elucidation of to what extent the metabolism is mediated via CYP enzymes

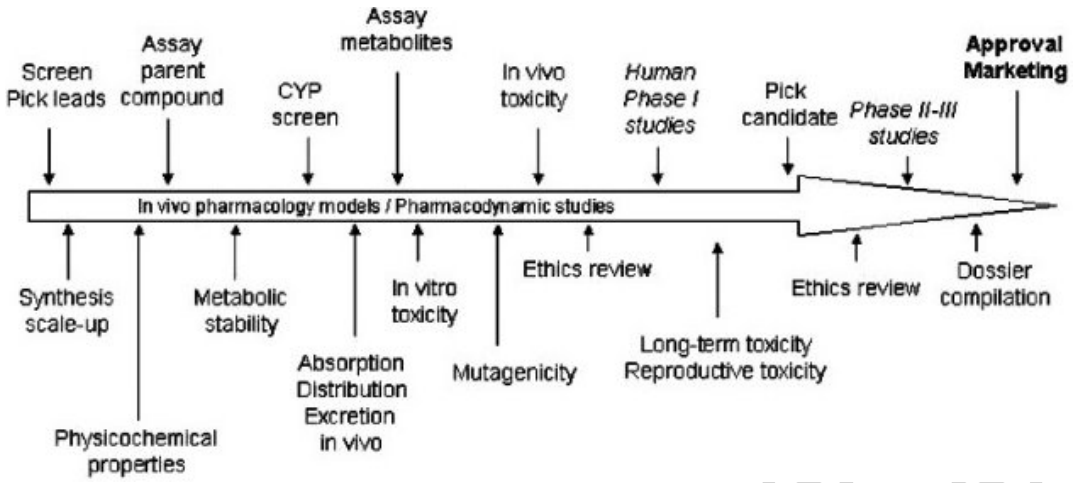

Figure 1. Key studies to be carried out with a molecule on its way to a drug.

In Phase I studies, ADME properties are studied using the test drugs, which makes it possible to compare the human metabolic pathways to those determined in animal *in vivo* toxicology studies. Traditionally the compounds to be studied are labelled with e.g. ^{14}C, but recently novel techniques such as positron emission tomography are being increasingly applied.

In many cases, active parent drug molecules produce active metabolites that may account for the majority of therapeutic effects (e.g. losartan). In the case of terfenadine, the parent compound turned out to be responsible for the cardiotoxic effects, while the metabolite had the desired histamine H_1 receptor blocking activity without major deleterious activity. CYP mediated metabolism is responsible for numerous drug-drug interactions. It is therefore highly desirable to determine whether a CYP dependent pathway is responsible for the elimination of the test drug. If the elimination turns out to be heavily mediated by CYP enzymes, as often is the case, elucidation of the specific CYP forms involved gives clues to potential interactions.

Due to species differences in metabolic pathways, previously unknown metabolites are often found in humans, meaning that the human subjects are exposed to compounds with unknown toxic potential. This may lead to a delay in clinical testing until an appropriate animal species can be tested with the synthesised metabolite. Metabolic stability assays employing different test species and human liver make it possible to select the species that best represent the human metabolic fate of an NCE. These results can be utilised in selecting most appropriate test species for further toxicological tests.

Because of the problems in extrapolating the results of animal studies to humans, various *in vitro* methods have been developed by employing human tissue-derived systems. Also regulatory authorities have begun to demand increasingly that the issues concerning metabolism and toxicity in test species compared to humans should be actively clarified in early preclinical tests. This is done by utilising liver preparations from humans and trying to find the test species that most closely resemble human metabolism and the production of toxic intermediates. It is important to elucidate the *in vitro* metabolism and the putative interactions at the time of planning other preclinical

and early clinical studies.

2.2. Overview of Drug Metabolising Enzymes

Most clinically used drugs are chemical compounds that do not belong to the normal composition of the human body, i.e. xenobiotics. The principal route of elimination of xenobiotics [see also - *Biodegradation of xenobiotics*] from the body is biotransformation [see also - *Biotransformations*]. They are eliminated by the so-called phase I and phase II drug-metabolising enzymes. These enzymes add functional groups to make lipophilic molecules more hydrophilic and hence easier to eliminate (Figure 2).

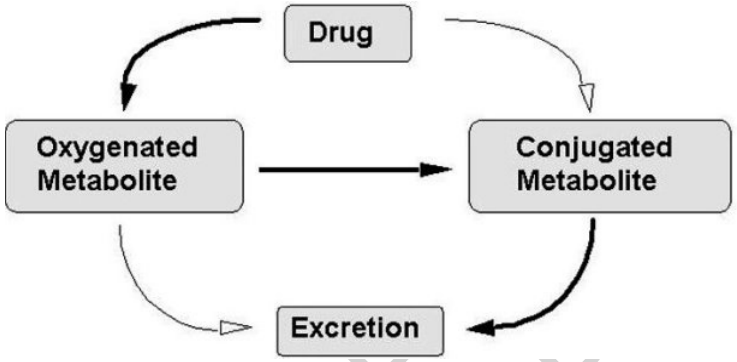

Figure 2. Main pathways of drug metabolism. The most common route is oxygenation followed by conjugation. A drug can be also excreted after being onlyoxygenated or conjugated.

The oxidative reactions are mainly catalysed by cytochrome P450 (CYP) enzymes (phase I metabolism) and, after that, by conjugating enzymes (phase II metabolism). Especially glucuronidation, catalysed by the several UDP- glucuronosyltransferase isoforms is an important route of phase II drug metabolism in humans. Some drugs (prodrugs) need to be metabolically activated before they are pharmacologically active. This activation usually occurs via CYP or hydrolytic enzymes [see also - *Enzyme production*].

The CYPs constitute in practise the most important group of drug-metabolising enzymes. For detailed descriptions of Phase II conjugating enzymes the reader is referred to the articles by Tukey and Strassburg on human glucuronosyltransferases, Weinshilboum and colleagues on drug methylating enzymes, Salinas and Wong on glutathione S-transferases, and Falany on sulfotransferases.

The CYP superfamily of microsomal hemoproteins catalyses the monooxygenation of a large number of endogenous and exogenous compounds. They play a key role in the metabolism of a wide variety of xenobiotics, including most drugs. The CYP superfamily is divided into families and subfamilies on the basis of their nucleotide sequence homology. Members of the subfamilies exhibit quite strict specificity in metabolising xenobiotics with a wide variety of substrates as a whole family. Some CYPs play a role in both the formation and the elimination of endogenous compounds, while some other CYPs, especially those belonging to the families 1-3, seem to be there principally for xenobiotic metabolism purposes [see also - *Biodegradation of*

xenobiotics].

2.3. CYP Enzymes involved in Xenobiotic Metabolism

The superfamily of CYPs consists of microsomal hemoproteins that catalyse the oxidative, peroxidative and reductive metabolism of a wide variety of endogenous and exogenous compounds. This superfamily is divided into families and subfamilies according to homologies in their nucleic acid sequences. Most biotransformation of xenobiotics is carried out by enzymes in the families CYP1, CYP2 and CYP3. Other families are mainly involved in the metabolism of endogenous compounds, such as fatty acids, bile acids, and hormones. In the human, the most important CYPs metabolising drugs are CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. About 70 percent of the CYP enzymes in the human liver belong to the families that participate in drug metabolism. Of these, CYP3A4 represents about 30 percent and CYP2C about 20 percent of the total CYP enzymes. These enzymes are the major P450 forms in human liver microsomes. Table 1 summarises the main features of xenobiotic-metabolising human hepatic CYPs.

Expression of CYP enzymes varies between individuals due to genetic and environmental factors and some diseases. These factors produce inter-individual variation in the rate and metabolic pathways of xenobiotics. One example of genetic factors influencing the inter-individual variation is the polymorphic expression of at least CYP2A6, CYP2C9, CYP2C19 and CYP2D6 enzymes in the population. The frequency of poor metabolisers (PMs) varies between races and ethnic groups. Some dietary compounds, cigarette smoking, alcohol and drugs may cause induction or diminution of the expression of certain CYPs.

CYP	Relative amount in liver (%)	Substrates (reaction in parenthesis)	Selective inhibitors	Other characteristics
1A2	~10	Ethoxyresorufin (*O*-deethylation) Phenacetin (*O*-deethylation)	Furafylline	Inducible
2A6	~10	Coumarin (7-hydroxylation)		Polymorphic
2B6	~1	*S*-Mephenytoin (*N*-demethylation)	Orphenadrine	
2C8	<1	Paclitaxel (6α-hydroxylation)	Quercetin	
2C9	~20	Tolbutamide (methyl hydroxylation) Diclofenac (hydroxylation) *S*-Warfarin (7-hydroxylation)	Sulfaphenazole	Polymorphic

2C19	~5	*S*-mephenytoin (4'-hydroxylation) Omeprazole (oxidation)		Polymorphic
2D6	~5	Dextromethorphan (*O*-demethylation) Debrisoquine (4-hydroxylation) Bufuralol (1'-hydroxylation)	Quinidine	Polymorphic
2E1	~10	Chlorzoxazone (6-hydroxylation) Aniline (4-hydroxylation)	Pyridine	Inducible
3A4	~30	Midazolam (1'- and 4-hydroxylation) Testosterone (6β-hydroxylation) Nifedipine (dehydrogenation)	Azole antimycotics	Inducible

Data adapted from Pelkonen & Breimer (1994) and Pelkonen *et al.* (1998; 2000).

Table 1. Summary of xenobiotic-metabolising human hepatic CYPs.

Experimental animals represent genetically homogenous populations; i.e. they do not exhibit large inter-individual variation in the activities of drug-metabolising enzymes, which is typical of the human population. The use of animal-derived *in vitro* models in preclinical drug research is restricted by the fact that laboratory animals often employ different enzymes than humans for the same metabolic pathway. Even orthologous CYP enzymes usually have quantitative and qualitative differences. Therefore, an evaluation of human tissue-derived *in vitro* systems is of paramount importance.

2.4. CYP3A4

The CYP3A4 enzyme deserves particular attention, since it participates in the metabolism of about 50 percent of the drugs in use today. CYP3A4 has the broadest catalytic selectivity of any CYP enzyme. The known substrates of CYP3A4 vary in size from small molecules, such as acetaminophen (M_r 151), to cyclosporin A (M_r 1201). For example, testosterone 6β-hydroxylation, midazolam hydroxylation, and erythromycin *N*-demethylation are catalysed by this enzyme. A list of approximately 100 drug substrates of CYP3A4 is found in the 1999 review article of F. Guengerich. Recent studies have shown that several endogenous oligopeptides are ligands of CYP3A4. Several tetra- and pentapeptides have affinities to CYP3A4 in the low micromolar range, especially some with an attached C-terminal amino group. However, it is not yet known whether oxidation of these peptides is actually catalysed by the CYP3A4 enzyme.

The CYP3A subfamily represents about 30 percent of the total CYP content in the

human liver, although the levels of the protein may vary 40-fold among individuals. CYP3A4 is the most abundant CYP enzyme in the human liver and it is expressed in several tissues, but the expression in the liver and in the small intestine is of major interest. CYP3A is inducible by many drugs, for example, rifampicin, dexamethasone, carbamazepine and phenobarbital. The induction of CYP3A has an effect on interindividual variation and affects both bioavailability and drug-drug interactions. Inhibitors of CYP3A have a wide variety of chemical structures. For example, the azole fungicides ketoconazole and itraconazole are potent inhibitors. Gestodene, a progesterone analog with a steroid structure has been long known as a mechanism-based CYP3A inhibitor.

The substrate specificity and catalytic features of CYP3A4 have recently been a target of active research. Due to the unique properties of CYP3A4, the enzymatic processes catalysed by it do not always follow the typical competitive inhibition kinetics. A substrate can either inhibit or stimulate the *in vitro* metabolism of another substrate, or activate its own metabolism. The kinetics can be either cooperative or allosteric, depending of the binding sites of the two substrate/inhibitor molecules or one molecule of two substrates each or one molecule of the substrate and an effector.

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