# 4. Induction and inhibition of cytochrome P-450*

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## RESUMEN

La inducción y la inhibición de los enzimas dependientes del citocromo P-450 (CYP) son fenómenos biológicos interesantes, de tal manera, que su estudio es muy importante en los ensayos de toxicidad de los agentes químicos, incluyendo los medicamentos, y en la terapéutica relacionada con las interacciones de fármacos. Esta revisión representa una visión breve y sistemática de la inducción y la inhibición, que comienza con la recopilación de sustratos típicos y reacciones, los cuales pueden ser utilizados *in vitro* para estudiar la actividad de isoenzimas del citocromo P-450 en animales y humanos. La inducción puede caracterizarse desde varios puntos de vista: descripción y clasificación en base a inductores, enzimas y receptores nucleares. Se presentan los inhibidores selectivos del CYP junto con los mecanismos de inhibición, y finalmente se discuten brevemente interacciones debidas a inhibición con significación clínica. Esta revisión finaliza con un esbozo de cómo estudiar la inducción y la inhibición durante el descubrimiento y desarrollo de un nuevo fármaco.

## 1. ABSTRACT

Induction and inhibition of cytochrome P-450 (CYP) enzymes is an interesting biological phenomenon as such, but it is of utmost importance in toxicity testing of chemicals, including pharmaceuticals, and in drug therapy presenting as drug-drug interactions. This review represents a short and systematic view of induction and inhibition, starting with com-

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pilation of typical substrates and reactions, which can be used in *in vitro* studies to study activities of cytochrome P-450 enzymes in animals and humans. Induction is then characterized from various viewpoints: description and classification of induction on the basis of inducers, enzymes, and nuclear receptors. CYP-selective inhibitors are then presented, together with mechanisms of inhibition and finally some well-characterized clinically significant inhibitory interactions are briefly discussed. This review ends with an outline how to study induction and inhibition during drug discovery and development.

## 2. INTRODUCTION

Detailed knowledge of metabolism of drugs is important, because 1) metabolism determines, to a large extent, pharmacokinetic behaviour, interindividual variability and interactions of a drug, all matters of great importance in drug treatment, and 2) differences in metabolism are also often behind the difficulties in the extrapolation from animals to humans, which is a serious obstacle in drug testing and development. Factors affecting drug metabolism are usually classified into genetic and non-genetic host and environmental factors (1). In the last category, chemical exposures, including drug treatment, exposure to occupational and industrial chemicals or environmental pollutants, can lead either to induction or inhibition of drug metabolism. Drug-drug interactions, based on induction and inhibition of drug metabolism, are among the most important factors complicating drug therapy and causing adverse reactions.

Induction is defined as the increase in the amount and activity of a drug metabolising enzyme, which is a long-term (hours and days) consequence of a chemical exposure (2-3). Inhibition of drug metabolism in general may mean either an acute decrease of metabolism of a particular substrate by another simultaneously present chemical or a time-dependent decrease in the amount of a drug-metabolising enzyme by several factors, such as a chemical injury or a disease process (2,4). In this review, we will deal only with inhibition at the level of enzymes.

The cytochromes P-450 (CYP) are a superfamily of heme-thiolate enzymes, some of which play major roles in the metabolism of drugs

and other xenobiotics, although endogenous compounds can also be their substrates (5,6). In humans and other mammalian species, CYP1, CYP2 and CYP3 families are primarily associated with the Phase 1 metabolism of exogenous compounds. Over 90% of all drug metabolism in man is P-450-mediated (5-9).

## 3. PREREQUISITES TO STUDY INDUCTION AND INHIBITION OF CYP ENZYMES

Previously, the study of induction and inhibition of drug metabolism was largely empirical and phenomenological and prediction beyond the compounds under actual study was very difficult, if possible at all. During the last decade or so, however, and particularly as a consequence of the detailed knowledge obtained about CYP enzymes and their regulation by endogenous and exogenous chemicals, both induction and inhibition can be understood on a detailed mechanistic basis and predictability of pharmacological and toxicological consequences has become possible and even a highly desirable part of early drug development.

As to clinical consequences of induction and inhibition, the nature of the metabolites produced by induced or inhibited enzymes determine the outcome. If the reaction to be studied leads to inactive product(s), induction results in attenuation and inhibition results in exaggeration of the effects of a drug. If the product is active, either pharmacologically or toxicologically, the reverse outcome is observed.

To be able to study induction and inhibition of CYP enzymes, there is a need of well-characterized «selective» or «diagnostic» substrates for each enzyme. A list of substrates which are claimed to be useful as *in vitro* (and sometimes as *in vivo*) probe drugs for various purposes is given in Table 1. In this table, also some information on CYP selectivity has been indicated, although here we do not try to give more detailed and quantitative information about this important characteristic of any model drug (2,8,9). It is of considerable importance to analyse in a detailed and quantitative manner the applicability and usefulness of the various proposed model drugs. Further information on CYP substrates (and inducers and inhibitors) can be found in several earlier and recent reviews (5,6, 11-16).

TABLE 1

*Compounds and reactions claimed to demonstrate a high degree of human CYP selectivity (for CYP nomenclature, see 10; for substrates, see 5-7; for selectivity, see 8-9)*

| *Enzyme(s)* | *Preferred substrate and reaction* | *Selectivity and other remarks* |
|---|---|---|
| CYP1A2 | phenacetin O-deethylation | selective |
| | ethoxyresorufin O-deethylation | selective (extrahepatic CYP1A1) |
| CYP2A6 | coumarin 7-hydroxylation | selective (all other CYPs studied negative) |
| CYP2B6 | bupropion hydroxylation | selective |
| CYP2C8 | taxol hydroxylation | selective |
| CYP2C9 | tolbutamide methylhydroxylation | fairly selective (other CYP2Cs) |
| | diclofenac hydroxylation | selective |
| | S-warfarin 7-hydroxylation | selective |
| CYP2C19 | S-mephenytoin 4-hydroxylation | selective |
| | omeprazole sulphoxidation | selective |
| | | N-demethylation by CYP3A4 |
| CYP2D6 | debrisoquine 4-hydroxylation | selective |
| | dextromethorpan O-deethylation | selective |
| | | N-demethylation by CYP3A4 |
| | bufuralol 1'-hydroxylation | selective |
| CYP2E1 | chlorzoxazone 6-hydroxylation | selective (also by CYP1A1) |
| | aniline 4-hydroxylation | selective |
| CYP3A4 | testosterone (steroid) 6ß-hydroxylation | selective (CYP3A5) |
| | midazolam 1-hydroxylation | selective (CYP3A5) |
| | nifedipine dehydrogenation | selective (CYP3A5) |
| CYP3A5 | as CYP3A4 substrates | no selective substrate known |

## 4. INDUCTION OF CYP ENZYMES

Classically, the definition of induction is the *de novo* synthesis of new enzyme molecules as a result of an increased transcription of the respective gene after an appropriate stimulus. However, in drug metabolism research the term induction has been used as a generic term, describing an increase in the amount and/or activity of a drug metabolising enzyme as a result of an exposure to an «inducing chemical», whatever the underlying mechanism. In the usual sense of induction, there is a certain lag period before an increase in enzyme activity can be observed. This lag period is due to the fact that it takes time to increase the amount of enzyme molecules, either as a result of increased transcription and translation or as result of the stabilisation of an enzyme by a substrate, which leads to a new steady-state level between synthesis and degradation.

The basic assumption is usually that induction leads to an increased amount of an existing enzyme (or enzymes) and not to a qualitatively different enzyme. This means that in the quantitative analysis the only changing measure is $V_{max}$. Obviously, when more than one enzyme is induced, calculations will become more complicated, but still there are no «new» enzymes present. The overall effect *in vivo* will still depend on the affinities and rates of metabolism of various enzymes participating in the metabolism of a compound under study.

An increase in enzyme activity, due to activation, is not usually included under the term induction, although the functional outcome is similar. Some examples include the effect of alpha-naphthoflavone and a number of other substrates on the activity of CYP3A4-catalyzed reactions, due to the peculiar substrate binding site and cooperative interactions of CYP3A4 enzyme (17) and a rapid enhancement of antipyrine elimination by heme arginate in porphyric patients (18), probably is due to the restoration of holoenzyme by heme in the presence of intact apoenzyme.

As potential consequences of enzyme induction for drugs that are active in their parent form, induction may increase the drug's elimination and decrease its pharmacological effect. For prodrugs, compounds that require metabolic activation and whose effects are produced by the active metabolites, enhanced pharmacodynamic effects may be expected. The toxicological implications of enzyme induction have been discussed by Park et al. (19).

Advances in molecular and cell biology during the past decade or so have helped to elucidate the major mechanisms by which drugs and xenobiotics induce the expression of CYP genes. This occurs mainly via receptor proteins that upon inducer binding are transformed into transcriptionally active DNA-binding forms. These active receptors then bind to their DNA response elements present in the regulatory region of the CYP genes and greatly speed up the production of CYP mRNA, up to 50-fold or more (20). In few instances, there are alternative mechanisms for CYP induction. The best-characterized example is the induction of CYP2E1 by ethanol and other small solvent molecules which appears to involve stabilization of CYP2E1 protein through binding of the inducer to the CYP2E1 active site (21). The role of other regulatory mechanisms, including those involving the 3'-areas of mRNA molecules, have been presented recently (22).

## 4.1. Spectrum of induction

Based on mostly animal experiments, inducers have been categorised into several classes (Table 2), which can be characterised mainly on the basis of the spectrum of enzymes induced and the potency of induction. This table gives only a qualitative view of the spectrum of induction and in the following paragraphs more background is given on mechanistic details and quantitative aspects of induction in man or human-derived systems.

Several individual agents that induce CYP enzymes have been identified in man, and the list of drugs whose pharmacokinetics and pharmacodynamics are affected by induction is rather long. For comprehensive updates on such drugs the reader is referred to recent relevant reviews, in which most original references of the subsequent chapters can be found (5,6,21,23,24).

TABLE 2

*Traditional classification of inducers of drug-metabolising enzymes. This classification is based mainly on animal studies, and the types of induction are not as clear-cut in humans. (For reviews, see ref. 3,23-24)*

| *Class* | *Prototype inducer* | *Principal enzymes (also non-CYP) affected* |
|---|---|---|
| PAH-type | 2,3,7,8-Tetrachlorodibenzo-p-dioxin | CYP1A, UGT1A, GSTM |
| Omeprazole-type | Proton pump inhibitors | CYP1A2 |
| Ethanol-type | Ethanol | CYP2E1 |
| Rifampicin-type | Rifampicin | CYP3A |
| Phenobarbital-type | Phenobarbital | CYP1A, CYP2A, CYP2B, CYP3A, UGT, GST |
| Glucocorticoid-type | Dexamethasone | CYP3A |
| Peroxisome proliferator-type | Clofibrate | CYP4 |

Abbreviations: UGT, UDP-glucuronosyltransferase; GST, glutathione transferase.

### 4.1.1. *Cigarette smoking and PAH-like inducers*

Cigarette smoking (the inducing effects of cigarette smoking is attributed to the polycyclic aromatic hydrocarbon (PAH) class of compounds) induces the metabolism of substances, which are metabolised by CYP1A forms. These include theophylline, caffeine, antipyrine, tacrine, melatonin, imipramine, paracetamol (acetaminophen), and phenacetin (6). The metabolism of these drugs is mediated predominantly by CYP1A2, one of the relatively liver-specific CYP enzymes. CYP1A1 is mainly an extrahepatic enzyme. It is highly induced in the lung, mammary gland, lymphocytes, and placenta by PAHs and cigarette smoke (25).

The regulatory mechanisms of CYP1A induction have been thoroughly elucidated (26,28,30). CYP1A inducers interact with the so-called Ah (Aryl hydrocarbon) receptor, which upon ligand binding is activated and translocated to the nucleus as a complex which includes also the ARNT (aryl hydrocarbon nuclear translocator) protein. The complex binds to specific regions in the regulatory areas of the *CYP1A* genes, the Ah-receptor regulatory elements (AhRE), also known as xenobiotic or drug responsive elements. This interaction leads to increased transcription of the *CYP1A* genes and the *de novo* production of CYP1A protein. Increased amounts of CYP1A enzymes may have two different types of consequences: increased toxicity due to more efficient activation of toxicants and procarcinogens that are substrates of these enzymes (toxic response), or decreased toxicity as a result of enhanced inactivation reactions (adaptive response) (28).

The regulation of CYP1A2 is not as well characterised as that of CYP1A1. It is inducible by smoking, charbroiled food, cruciferous vegetables, omeprazole and even vigorous exercise (31,32). Although the CYP1A-inducing capacity of omeprazole and other proton pump inhibitors in the human liver and alimentary tract has been clearly demonstrated, the mechanism is still somewhat unclear. However, the Ah receptor is involved in the induction mechanism by omeprazole. It is of interest that the inducing effect is strictly species specific, since the CYP1A1 gene is activated in human but not in mouse hepatocytes. The overall omeprazole-dependent increases in CYP1A activities in the liver and gut *in vivo* are rather low (usually less than two-fold) and high doses and/or prolonged treatments are needed to produce the inducing effect. Taken to-

gether, the evidence suggests that the induction caused by omeprazole is unlikely to have practical consequences (3).

### 4.1.2. *Ethanol*

Ethanol induces liver drug metabolism in humans as measured by both *in vivo* and *in vitro* parameters. The most important CYP enzymes induced by ethanol is CYP2E1, which serves as a mediator of the inducible oxidation of ethanol and a wide variety of toxic chemicals, including several procarcinogens (6). Numerous agents are capable of CYP2E1 induction in the rat, but only a few, including isoniazid, appear to be an inducer in humans (21). Isoniazid is also an inhibitor of the CYP2E1 enzyme and therefore the outcome of exposure is dependent on the specific regimen of isoniazid administration.

There is still some controversy as to whether the induction of CYP2E1 by ethanol and other substances and conditions (diabetes, ketonemia, fasting etc) is transcriptional, post-transcriptional, translational or post-translational. It is probable that different mechanisms are operative in different situations and conditions (21).

### 4.1.3. *Phenobarbital and other antiepileptic drugs*

Phenobarbital is the archetypal inducer of drug metabolism (33). Phenobarbital has long been known to be a strong and broad-spectrum *in vivo* inducer of drug metabolism. As an example of the potency of induction, the dose of warfarin required for the anticoagulant effect can be increased up to ten-fold during phenobarbital treatment. Also other antiepileptic drugs, especially phenytoin and carbamazepine, have been shown to induce drug metabolism in humans. Antiepileptics are broad-spectrum inducers, enhancing the metabolism of numerous drugs, including warfarin, cyclosporin A, theophylline, oral contraceptives and their own metabolism, leading to autoinduction (34).

In rodents, phenobarbital induces CYP forms in several subfamilies, including CYP1A, CYP2A, CYP2B and CYP3A, the members in the CYP2B subfamily reacting most sensitively (33). Several lines of evidence suggest

that in humans, the CYP3A forms are the ones most affected by phenobarbital and other antiepileptic drugs, but also other CYP enzymes, CYP2B6, members of the CYP2C subfamily (CYP2C8, CYP2C9 and CYP2C19) and CYP2A6, respond to antiepileptic drug treatment (26,29,33).

The mechanism mediating phenobarbital is characterised in a great detail during the last few years (26). A specific nuclear receptor termed *CAR* (constitutive androstane or constitutively active receptor) mediates CYP2B induction by phenobarbital and other similar compounds. Endogenous ligands for CAR are androstanes, for example androstanol, and it is peculiar to this receptor, that endogenous ligands are inhibitory. In the presence of phenobarbital, however, the constitutively active receptor is released and receptor activity is thereby de-repressed (26).

### 4.1.4. *Rifampicin and corticosteroids*

Rifampicin induces CYP3A enzymes in the liver, although weak induction of other CYP enzymes, including CYP2A6, CYP2C and CYP2B6, have also been noticed. Consequently, rifampicin accelerates the elimination of a large number of substances, although most of them are substrates for CYP3A4, such as midazolam, quinidine, cyclosporine A and many steroids (6). CYP3A enzymes are also present in the gut, and rifampicin is able to induce the gut enzymes, leading to an enhanced first pass effect for CYP3A4 substrates.

It is now known that rifampicin induces CYP3A4 and some other enzymes by binding to a nuclear receptor called *PXR* (pregnane X receptor) (27,29). PXR dimerizes with another nuclear receptor called RXR (retinoid X receptor) and this dimer binds to an appropriate binding element in the 5'-region of the gene to be induced. Endogenous ligands for the PXR receptor include pregnenolone derivatives and a number of other steroids. Also several glucocorticoids, dexamethasone as a prime example, also bind to PXR, causing CYP3A4 induction.

### 4.1.5. *Peroxisome proliferators*

It is well established that several agents that cause peroxisome proliferation in the liver, such as clofibrate and nafenopin, are potent hepato-

carcinogens and inducers of the CYP4A subfamily forms in rodents (35). A specific nuclear receptor called *PPAR* (peroxisome prolifarator activated receptor) binds peroxisome proliferators and certain polyunsdaturated long-chain fatty acids as endogenous ligands, forms a heterodimer with the RXR, and causes the activation of CYP4A genes (36), as other nuclear receptors.

Humans seem resistant to the peroxisome proliferating effects produced by this class of compounds, and they are not considered to pose a hepatocarcinogenic hazard to humans. Due to the very low abundance of CYP4A protein in the human liver and paucity of relevant drug substrates, its role in the overall pharmacokinetics of commonly used drugs must be considered as negligible.

## 5. INHIBITION: MECHANISMS AND QUANTITATION

The in-depth treatment and formal derivation of equations to characterise various modes of inhibition can be found in appropriate textbooks and handbooks. A good introduction to the basic phenomena of inhibition of drug metabolism is a book chapter of Boobis (4). Further readings on various basic and applied aspects of inhibition of drug metabolism can be found in several fairly recent reviews (37-43). The present treatment of the subject is restricted to CYP enzymes.

### 5.1. Inhibitory spectrum

In terms of understanding and predicting the outcome of inhibitory interactions, selectivity of a particular inhibitor towards CYP enzymes is of utmost importance. Many substances are relatively non-specific and even those claimed to be enzyme-specific usually have affinity to other enzymes, although this occurs only at higher concentrations (see table 3). One good example is cimetidine, a well-known inhibitor of P-450-linked reactions and one of the first inhibitors of drug metabolism to attract a lot of attention in clinical drug therapy (44). It has been shown that cimetidine interacts with at least human hepatic CYP1A, 2C, 2D, 2E and 3A forms, but with widely variable affinities (43).

## 5.2. Inhibitory potency in vitro

The most important single measure for inhibitory potency of a given compound is the $K_i$ value, or inhibition constant, which expresses an affinity of a compound to an enzyme. It should be stressed here, that a $K_i$ value is characteristic for each particular inhibitor and enzyme, and it is not dependent on any particular substrate used for the quantitation of an enzyme. With respect to human hepatic CYP enzymes, this value can be easily measured with standard *in vitro* approaches, in which various concentrations of a substance are incubated with human liver microsomes and an inhibition of a CYP-specific model reaction is quantitated. A subs-

TABLE 3

*Induction of major human CYP enzymes by various inducers and their regulatory factors. (For reviews of different induction mechanism, see ref 26-30)*

| *Gene family* | *Typical inducers* | *Major regulator activated by inducer* | *Comments* |
|---|---|---|---|
| CYP1A | Polycyclic aromatic hydrocarbons, cigarette smoking, omeprazole | *Ah* receptor | Modulated by protein kinases<br>Omeprazole mechanism still unclear |
| CYP2A | Rifampicin, barbiturates | Unknown (CAR, PXR ?) | Modulated by cAMP<br>mRNA stabilization |
| CYP2B | Barbiturates, antimycotics, phenothiazines, etc. | CAR, PXR | Modulated by glucocorticoids and protein kinases<br>Nuclear translocation<br>Species differences |
| CYP2C | Barbiturates, antimycotics, phenothiazines, etc. | CAR, PXR | |
| CYP2E | Ethanol, isoniazid, acetone | Stabilization of CYP2E1 | Multiple mechanisms in rodents |
| CYP3A | Rifampicin, barbiturates, antimycotics, phenothiazines, etc. | PXR, CAR | Species differences<br>PXR and CAR share ligands and target genes |
| CYP4A | Fibrates | PPARα | low PPAR α levels in humans |

tance may have affinity for an enzyme without being metabolised by the same enzyme (e.g. quinidine for CYP2D6) or it may be an alternative substrate of the enzyme and serve as an inhibitor on this basis. In both cases the $K_i$ value is derived from an *in vitro* experiment, but for an alternative substrate, a $K_i$ value should be the same as its $K_m$ value.

It may be worth of stressing here that assay conditions such as protein concentration, buffer, ions, pH and so on, may critically affect the inhibitory potency of the compound (45,46) and should be thoroughly investigated.

## 5.3. Inhibition of clearance

For any substrate, the ratio $V_{max}/K_m$ is a measure of intrinsic clearance, which relates to the efficacy of an enzyme to metabolise a substrate. Usually, in clinical usage, drug concentrations are far below their $K_m$ values, and in this situation it can be demonstrated that the intrinsic clearance is decreased dependent on the ratio between the concentration of an inhibitor to its $K_i$ value $[I]/K_i$. This statement is true for whatever the mechanism of inhibition may be. Assuming competitive inhibition and the substrate concentration far below its $K_m$ value (i.e. $[S] << K_m$ ), the percentage inhibition can be simply calculated according to the equation $I/(I+Ki) \times 100$. It has to be stressed that the number achieved is a very crude "first guess" and depends on a number of other factors which have been discussed in, for example, Boobis (4) and Pelkonen et al (2).

However, when substrate concentrations approach and exceed $K_m$ values, the mechanism of inhibition becomes important. In a competitive mode of inhibition, increasing substrate concentration abolishes inhibition because the inhibitor is increasingly removed from the active site of an enzyme. In this case, the denominator of the above mentioned simple equation should contain the term $(1-[S]/ K_m )$; the higher the substrate concentration [S], the lower the percentage inhibition. However, in a non-competitive mode of inhibition, a certain proportion of an enzyme, which is determined by the ratio $[I]/K_i$, is «inactivated» for a more prolonged period of time, being unavailable for catalysis, and the inhibition cannot be abolished by increasing the substrate concentration.

## 5.4. Mechanism based inhibition

For the CYP enzymes, the inhibitory species may not be a the substrate itself, but a metabolite, which is then complexed or covalently bound to a metabolising enzyme itself («suicide inhibition») or to other enzymes nearby. The consequence is a removal of a variable proportion of an enzyme from active catalysis, i.e. a non-competitive mode of inhibition. However, the detection of mechanism-based inhibition requires specific incubation conditions. A preincubation of liver microsomes in the presence of an inhibitor under the metabolising conditions is necessary, because the presence of a substrate might competitively inhibit the metabolism of a mechanism-based inhibitor. A specific case of mechanism based inhibition is the situation in which an enzyme is inactivated very slowly during *in vivo* conditions. In this case it is difficult to reveal inhibition in *in vitro* experiments.

## 5.5. Concentration of the inhibitor

Whatever the exact value of $K_i$ is, it does not directly tell us inhibition will be observed during the *in vivo* use of a compound. The critical factor in the term $[I]/K_i$ is the concentration of an inhibitor, which ideally means the concentration at the active site or a modulatory site. Obviously, this particular concentration is not known and surrogate values are usually used, such as total or free concentration in the plasma. Most authors think that the unbound, (i.e. free concentration) is the most appropriate to use, because it is only free drug that is able to transfer to hepatocytes and to the vicinity of CYP enzymes. However, it is conceivable —and for some drugs even shown— that many lipid-soluble drugs are concentrated in hepatocytes and consequently the actual concentration in the liver far exceeds that in plasma. Even the measurement of the partition between liver and plasma does not necessarily indicate the available portion of a drug to an enzyme, because a drug may be very tightly bound inside hepatocytes and may not be available to the active site of the enzyme. A detailed and extensive treatment of modelling and predicting interactions of drug metabolism, including factors affecting partition between liver and plasma, can be found in Leemann and Dayer (38).

### 5.6. Clinical significance of an interaction

Affinity and CYP selectivity can be studied *in vitro* and thus a potential of a drug to cause interactions can be revealed. However, this does not yet mean that the compound would cause clinically significant interactions. For such interactions to ocuur, two prerequisites have to be fulfilled: 1) The concentration of the drug in clinical situation should be high enough, so that inhibition would be manifested *in vivo*. 2) The therapeutic index of the drug should be narrow, such that a change caused by an interacting drug would cause side effects. The clinical significance of a drug interaction involves also a judgemental component, which in most cases is rather large. The judgmental components involve the severity of potential harm to the patient, assessment of decreased therapeutic outcome and so on. This makes it difficult to say unequivocally whether an interaction is «clinically significant». Semiquantitative classifications have been constructed, such as that of Preskorn (47) using the terms «Substantial», «Moderate», «Mild», «Unlikely», «Not clinically significant». However, in the end clinical assessment and judgment is the final arbiter as to the clinical and therapeutic significance of an interaction and this assessment may be difficult to put into exact numbers and may cause disagreement even between experts. However, a sort of yardstick to aid in the assessment of clinical significance of interactions is to keep in mind a list of interactions that have been demonstrated to be clinically significant (Table 4).

## 6. MEASURING INDUCTION AND INHIBITION IN DRUG DISCOVERY AND DEVELOPMENT

"Flaws" in metabolic and pharmacokinetic behaviour of a new chemical entity have been some of the major reasons for the termination of development and also withdrawal from the clinical use. Because drug development is hugely expensive, appropriate metabolic and kinetic characteristics of molecules and candidates should be screened as early as possible during the process. Obviously, before the first introduction of a new chemical entity (NCE) into a living human, only in vitro systems or animals could be used for screening purposes. Due to extensive interspecies differences (see Pasanen's chapter), use of animal in vitro or in vivo systems has severe restrictions and human-derived systems are clearly preferable. Below, a short outline of induction and inhibition screening systems available early in drug development is described.

TABLE 4

*A compilation of inhibitors, which have claimed to demonstrate some CYP enzyme selectivity. (For original references, see ref 2 and 6)*

| *CYP* | *Inhibitor* | *Mechanism of inhibition* | *Selectivity* | *Effect in vivo* |
|---|---|---|---|---|
| **1A2** | Furafylline | mechanism-based | very selective | +++ |
| | Fluvoxamine | competitive | also 2C19, 3A5 | +++ |
| **2A6** | Methoxalen | mechanism-based | also 1A2, 2E1 | + |
| | Trancylcypromine | competitive | reasonably selective | + |
| **2B6** | Ticlopidine | partially mechanism-based | reasonably selective | + |
| | Thiotepa | competitive | very selective | ? |
| **2C8** | No known | | | |
| **2C9** | Sulfaphenazole | competitive | very selective | +++ |
| | Tienilic acid | mechanism-based | ? | ++ |
| **2C19** | Fluvoxamine | competitive | also 1A2, 3A4 | + |
| **2D6** | Quinidine | competitive | very selective | +++ |
| **2E1** | Diethyldithiocarbamate | mechanism-based | also 2A6 | ++ |
| | 4-Methylpyrazole | competitive | also 2A6 | ++ |
| | Disulfiram | mechanism-based | also 2A6 | ++ |
| **3A4** | Troleandomycin | metabolic intermediate complex | selective | +++ |
| | Erythromycin | metabolic intermediate complex | selective | ++ |
| | Ketoconazole | competitive | reasonably selective | +++ |
| | Gestodene | mechanism-based | ? | +? |

## 6.1. CYP specificity of metabolism and interactions of a NCE

A prerequisite for rational study and prediction of metabolic interactions is the knowledge of CYP specificity of metabolism or affinity of a NCE. Currently there are a number of approaches available to study of the role of known CYPs in the metabolism and affinity of any xenobiotic.

If the principal metabolic routes of a NCE have been elucidated and a method is available for their quantitation in *in vitro* incubations, it is possible to employ «diagnostic» inhibitors (Table 5) and look which of them, and at which concentrations, inhibit metabolic routes. It is also possible to use enzyme-specific antibodies and to test which metabolic routes are inhibited and to what extent by a particular anti-CYP antibody. In a panel of human liver microsomes it is possible to correlate the metabolism of a compound under study with the activities of CYP-specific model reactions and thus get an idea about enzyme(s) catalysing the reaction. Practically all major CYP enzymes have been expressed in various host cells, such as bacteria, yeast and mammalian cells, and it is relatively straightforward to study either the metabolism of, or inhibition by, a compound under study in a cell system expressing a particular CYP enzyme. More extensive coverage of these approaches can be found in recent reviews (48-51).

TABLE 5

*Examples of drugs that have considered to cause clinically significant drug-drug-interactions*

| *Inhibitor(s)* | *Target CYP* | *Drugs affected* | *Remarks* |
|---|---|---|---|
| furafylline | CYP1A2 | caffeine, theophylline | furafylline withdrawn from development |
| fluvoxamine | CYP1A2, 2C19 | CYP1A2 substrates | warnings in PDRs |
| Sulfaphenazole, other CYP2C9 inhibitors | CYP2C9 | warfarin | warnings in PDRs |
| Quinidine | CYP2D6 | all CYP2D6 substrates (antidepressants, antipsychotics etc) | warnings in PDRs |
| Azole antimycotics (ketoconazole, itraconazole) | CYP3A4, many others | most statins, calcium channel blockers, cyclosporin A etc | warnings in PDRs, |
| | | Mibefradil | withdrawn |
| | | Terfenadine, Astemizole | withdrawn |

It is possible to make a number of predictions on the basis of the known characteristics of each CYP enzyme and on the basis of the known CYP-specificity of the metabolism of a NCE. For example, if it is known that the CYP3A4 enzyme participates in the metabolism or interactions of a particular substance (as is often the case), it is possible to identify some matters of concern on the basis of what is generally known about CYP3A4. The following list of predictions is from the review articles of Watkins (52) and Wilkinson (53).

a) CYP3A4 is induced by rifampicin, antiepileptics, dexamethasone etc and consequently, the elimination of a NCE might be enhanced in situations involving administration of these drugs. b) CYP3A4 levels are inhibited by ketoconazole, itraconazole and a large number of other compounds, as well as by grapefruit juice. The metabolism of a NCE might be inhibited by these substances. c) CYP3A4 is activated by several flavones and endogenous steroids. The flavones, which are constituents of food, may enhance the metabolism of substrates of CYP3A4. d) CYP3A4 is very variable between individuals. Also, the elimination of a NCE may be variable. e) CYP3A4 is present in intestinal epithelium. This fact may lead to a first-pass effect, i.e. reduced bioavailability of a NCE. f) CYP3A4 displays an age-related reduction in activity. The elimination of a NCE may show the same phenomenon. g) CYP3A4 activity is decreased in liver cirrhosis. The elimination of a NCE is expected to be decreased in severe liver disease. However, it has to be stressed that the significance of the above predictions are highly affected by principally quantitative considerations. For example: what is the proportion of metabolic clearance through the specific enzyme? It is clear that a 90 % portion of metabolic clearance through, say, CYP3A4 means a very high likelihood of predictions to be fullfilled than in the case, where only 20 % of metabolic clearance is through CYP3A4.

## 6.2. Assay systems for induction

Induction of human CYP enzymes is difficult to study because there are no human liver cell lines that express the full complement of CYP enzymes nor reproduce the induction observed in vivo, apart from the Ah receptor-mediated induction of CYP1A1 gene. Problems in tissue availa-

bility, interindividual differences, reproducibility, and ethical issues preclude the efficient large-scale use of human primary hepatocytes for induction screening (20, 57). Therefore, there is an increasing interest in the development of mechanism-based test systems for CYP induction, based on the characterized nuclear receptors AHR, CAR, PXR, and PPAR((29). The interaction of inducers with the particular receptor is exploited in an attempt to predict CYP induction at least in the following ways:

1) Direct binding assays rely on the ability of the putative inducer to displace a radioactively labelled receptor ligand.
2) Indirect binding assays measure the association of fluorescently labeled coactivator peptide with the inducer-bound receptor. The receptor can also be linked to another fluorophore to utilise the fluorescence resonance energy transfer (FRET) phenomenon in the assay.
3) Cell-based reporter gene assays measure the ability of inducer-bound receptor to interact with endogenous cellular coactivators after binding to its DNA response element that drives the expression of an easily assayable reporter gene such as luciferase or CAT enzyme.

### 6.3. Assay systems for inhibition

The most widely used in vitro system to study CYP inhibition is to measure affinities of a NCE for CYPs in CYP-selective model substrate assays (2,4,56,58). From the therapeutic point of view, it is important to know which drug-metabolising enzymes the substance under development has affinity to. The effects of a NCE on metabolite formation in the selected model system, usually human liver microsomes, are evaluated by incubating the substrate and the studied compound with the enzymes and observing the potential inhibition of metabolite formation in incubations with the studied compound. Testing the effects of an NCE on CYP-specific model activities and the effects of CYP-specific reference inhibitors on the metabolism of an NCE in human liver microsomes in vitro gives information about the affinity of an NCE for CYP enzymes. By comparing the effects of an

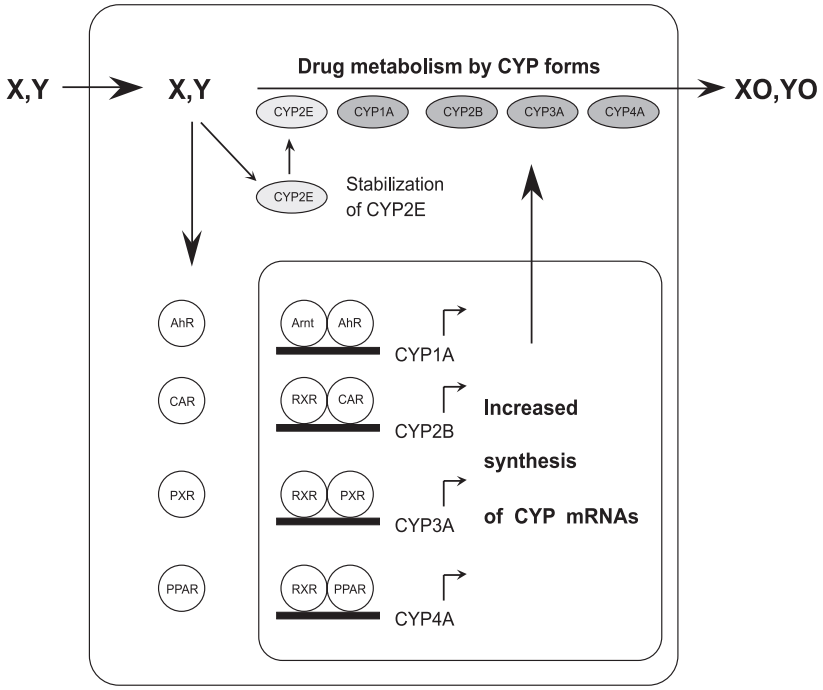


FIGURE 1. *Induction of drug metabolism by CYP enzymes are predominantly receptor-mediated.*

The xenobiotic compounds (X, Y) can increase their own metabolism to more hydrophilic metabolites (XO, YO) through binding to any of the ligand-activated nuclear receptors (CAR, PXR, PPAR) or the aryl hydrocarbon receptor (AhR). The activation of these receptors by X, Y then results in increased CYP gene transcription. CYP2E1 inducers usually stabilise the CYP2E1 protein without any gene activation (modified from Pelkonen *et al* 2002, ref. 3).

NCE on the CYP specific-activities with the respective effects of diagnostic inhibitors, a tentative prediction of the in vivo situation can be made. This approach also permits in vivo predictions about the behaviour of a NCE in man (metabolic pathways, intrinsic clearance, etc.), which helps to design in vivo studies for revealing possible interactions.

After the above studies, it is possible to plan further preclinical, molecular, toxicological and clinicopharmacological studies, with focussed consideration of those CYP enzymes which are of importance for the metabolism and kinetics of an NCE.

In clinical and clinicopharmacological studies, it is important to find suitable model substances, which can be used in in vivo conditions. In Table 6, some substrates and inhibitors are listed the in vivo usefulness in mind. It is clear that the in vivo use places very thorough requirements for drugs

TABLE 6

*Probe drugs/substances claimed to be useful in vivo in man (modified from Pelkonen et al 2002, ref. 3)*

| *CYP* | *Probe drug* | *Methods available*[1] | *Remarks*[2] |
|---|---|---|---|
| 1A2 | Caffeine (N-demethylations) | pm/u | Wide recreational use |
| | Furafylline | adm po | W; selective |
| | Fluvoxamine (inhibitor) | adm po | Inhibits also CYP2C19 and CYP3A4 |
| 2A6 | Coumarin (7-hydroxylation) | pm/u,(pm/b) | W; kinetically unsuitable |
| | Tranylcypromine (inhibitor) | adm po | C; not validated *in vivo* |
| 2B6 | Bupropion (hydroxylation) | pm/b, pm/u | C; not validated |
| 2C9 | Tolbutamide (methylhydroxylation) | pm/b, pm/u | W; well validated; safety? |
| | Diclophenac (4-hydroxylation) | pm/u, pm/b | C; Under validation |
| | Sulfaphenazole (inhibitor) | adm po | C; well validated |
| 2C19 | Mephenytoin (1-hydroxylation) | pm/u | W; extensively used; safety? |
| 2D6 | Debrisoquine (4-hydroxylation) | pm/u | W; extensively used; safety? |
| | Dextromethorphan (O-demethylation) | pm/u | C; well validated |
| | Quinidine (inhibitor) | adm po | C; well validated |
| 2E1 | Chlorzoxazone (6-hydroxylation) | pm/b, pm/u | C; well validated |
| | Disulfiram (inhibitor) | adm po | C; inhibits also CYP2A6 |
| 3A4 | Midazolam (1'-hydroxylation) | pm/b, pm/u | C; well validated |
| | Ketoconazole (inhibitor) | adm po | C; peroral use not common |
| | Itraconazole (inhibitor) | adm po | C; well validated; selective |

[1] symbols: p, parent drug; m, metabolite(s); b, blood (plasma, serum); u, urine; s, saliva; (r), radioactive label; ex, exhaled air; adm iv, administered intravenously; adm po, administered perorally.

[2] C, clinically used drug; W, withdrawn from the market or not been used as a pharmaceutical.

[3] for references concerning each probe drug, see section IIA in Rendic (6). A review of Streetman et al (59) presents a critical appraisal of CYP substrate probes.

to be used, because safety and ethical aspects are of utmost importance. It is also quite clear that only very few inducers can be used in clinicopharmacological studies —probably rifampicin is the only example— when the use is not associated with the treatment of the disease. One of the approaches to overcome these problems could be the use of microdosing.

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