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LABORATORY–CLINIC INTERFACE

Individualization of cancer therapy based on cytochrome P450 polymorphism: a pharmacogenetic approach

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Introduction

Most xenobiotics, including pharmaceutical agents, are metabolized to some extent. Metabolism results in detoxification and elimination of the drug or activation of the prodrug to the biologically active substance. When the pharmacological activity of a drug or toxin is linked to the catalytic activity of a specific enzyme, factors that influence the activity of the enzyme will affect the clinical response to the agent. Enzymes responsible for the activation and metabolism of drugs and other compounds in humans show wide interindividual variation in their protein expression or catalytic activity, resulting in unique drug metabolism phenotypes (1).

This variation can be due to transient causes such as enzyme inhibition and induction, or to permanent causes such as genetic mutations or gene deletion. An insertion, deletion or rearrangement mutation in either the regulatory or structural sequence of the gene responsible for a drug metabolizing enzyme may decrease the intracellular concentration or eliminate the enzyme protein, or may structurally alter the enzyme with consequent changes in enzyme function. A defect in transcription, RNA processing or RNA stability may occur. At the protein level, either a decreased intracellular concentration or total absence of the protein may occur secondary to a diminished rate of synthesis or an accelerated degradation. Finally, an enzyme may be structurally intact, but exhibit decreased affinity or maximal velocity for substrates or an alteration in stereoselectivity (2).

When specific gene mutations or deletions are maintained within the population, the gene is considered to be polymorphic or having multiple forms

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(1). Pharmacogenetics is the study of the linkage between an individual's genotype and that individual's ability to metabolize a foreign compound (1). Genetic polymorphism was originally defined by Ford in 1940 as 'a type of variation in which individuals with sharply distinct qualities co-exist as normal members of a population' (3). Biologically, genetic polymorphism can be thought of as an organizational arrangement whereby variability is structured into the population to give it a better chance of survival should changes occur in the environment (4).

Genetic polymorphism has been linked to three classes of phenotypes based on the extent of drug metabolism. Extensive metabolism (EM) of a drug is characteristic of the normal population; poor metabolism (PM) is associated with cumulation of specific drug substrates and is typically an autosomal recessive trait requiring mutation and (or) deletion of both alleles for phenotypic expression; and ultra-extensive metabolism (UEM) results in increased drug metabolism and is an autosomal dominant trait arising from gene amplification. The latter class of phenotypes is rare and has only been described for certain polymorphisms. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug (1, 5). It has been shown that significant ethnic differences exist in the frequency of the PM phenotype (6). There are, for example, significant interethnic differences in the prevalence of polymorphism of the 2D6 subfamily of cytochrome P450 enzymes (CYP450; P450). In Northern American and European Caucasian populations, the prevalence of the PM phenotype is 5–10% (1). However, the prevalence in American blacks is 1.8%, in native Thai 1.2%, 1.0% in Chinese, 2.1% in native Malay population, and apparently absent in the Japanese population (1).

Polymorphism in metabolism will generally only have serious consequences if the drug has a narrow therapeutic window: at low concentrations, the efficacy of the drug is minimal, whereas at high concentrations, the drug may be toxic. Therefore, a narrow therapeutic window requires a clinical detection method for the existence of a possible polymorphism in metabolism of a particular drug (7, 8).

An identical dosage could result in widely different concentrations of the therapeutically active compound (9). Besides variation due to polymorphism, other factors in the pharmacokinetic phase (such as absorption, distribution and clearance) and the pharmaco-dynamic phase (such as host sensitivity) are responsible for the interpatient variation. The possibility that some anticancer drugs are metabolized by enzymes exhibiting a genetic polymorphism (e.g. certain CYP450 enzymes) should be considered (10–13). This could be of clinical relevance with anticancer drugs because these drugs are normally administered at a dose that results in maximal tolerable toxicity. Poor metabolizers would face an increased risk of adverse effects, while extensive metabolizers might exhibit subtherapeutic plasma concentrations (provided that no important active metabolites are formed) (9).

The role of polymorphism in the metabolism of the family of antidepressant drugs, for example, has been, and still is, heavily investigated (14–17). CYP2D6 catalyses the metabolism of various antidepressant drugs, and knowledge of the drug metabolism status of a patient has shown to be helpful in the selection

Table 1. Selected anticancer agents studied for a possible role of P450 polymorphism

Bleomycin	Ifosfamide
Busulfan	Melfalan
Chlorambucil	6-Mercaptopurine
Cyclophosphamide	Methotrexate
Cytarabine	Paclitaxel (taxol)
Daunorubicin	Tamoxifen
Doxorubicin	Teniposide
Epirubicin	Thio-TEPA
Etoposide	Vinblastine
5-Fluorouracil	Vincristine

of a drug and dosage regimen, as well as in the prediction of clinically relevant drug interactions (14–17). Phenotyping of patients prior to drug therapy can result in important information about these issues. It is the opinion of some physicians that phenotyping of patients for CYP2D6 activity should be routine practice prior to the commencement of treatment with antidepressant agents. However, the relevance of this practice has been the subject of much debate, and other approaches such as limited sampling pharmacokinetic studies, which allow a direct assessment of interpatient variability, provides a feasible alternative (14). Currently, mutant alleles and other alleles are being cloned and sequenced, which has resulted in the development of allele-specific polymerase chain reaction (PCR) assays that can be used for the routine screening of patients for CYP2D6 activity and therefore for their ability to metabolize antidepressant agents (14).

The goal of this review is to examine the role of specific P450 enzymes in the metabolism of a group of frequently used anticancer drugs in humans (Table 1), and to discuss the possibility of utilizing pharmacogenetic information as a tool to individualize chemotherapy. This could contribute to the prevention of altered effectiveness and severe toxicity experienced by some patients treated with these drugs.

Materials and methods

The literature used for this review was obtained after a thorough search using Medline (1966-03/1997), Current Contents (06/10/1996-06/09/1997) and the search-option at the Internet site of the National Library of Medicine (PubMed; <http://www.ncbi.nlm.nih.gov/PubMed>), combined with articles arising from the literature found by this search. The keywords used for this search were: *cytochrome P450*, *biotransformation* and *elimination*, individually combined with the selected anticancer drugs (Table 1). This search produced a total number of 255 articles from which a selection was made for this review of the interesting and useful material.

Enzymes involved in drug metabolism

Drug metabolizing enzymes are classically divided into two broad categories. 'Phase I' enzymes include reductases, oxidases and hydrolases. This category involves almost exclusively cytochromes P450, which function by the insertion of one atom of atmospheric oxygen into a relatively inert substrate (7). 'Phase II' enzymes act on the oxygenated intermediates by conjugation with various endogenous moieties (glucuronide, glutathione, sulphate) to produce extremely hydrophilic products that are easily excreted from the cell (18–20). The reactive intermediates formed by P450 enzymes can be carcinogenic, mutagenic and/or toxic (7, 8). The coordinate regulation of phase I and phase II genes and the architectural arrangement of phase I and II enzymes in each cell are, therefore, important factors ensuring metabolic clearance of foreign substances from the body with a minimal risk of accumulation of the oxygenated intermediates that might lead to disease (7).

Among drug-metabolizing enzymes, the family of cytochrome P450 enzymes are often involved, and most clinically used drugs are metabolized to some degree by P450 enzymes.

The cytochrome P450 superfamily

The major route of phase I drug metabolism is oxidation by cytochrome P450 mixed-function mono-oxygenases located within the endoplasmic reticulum (1). These microsomal enzymes are found in most tissues especially in liver cells. All have characteristic ferrous-carbon monoxide complex Soret peaks near 450 nm, have monomeric molecular weights of about 50 000 and accept electrons from the flavoprotein NADPH-450 reductase (21). Small variations in the absorbance peak provided the first clue that P450 might actually consist of more than one protein. Since the 1980s, the powerful tools of modern molecular biology elucidated many details of this enzyme system. P450 is phylogenetically extremely conserved and is found in bacteria, plants and animals. The bacterial form is water soluble and has yielded a crystallographic structure (22). Mammalian P450s are thought to contain a similar hydrophilic domain but also to have an intramembranal structure (23). The characteristic optical properties of P450 were shown to arise from the haem iron's coordination chemistry, which differs from that in the respiratory cytochromes. In P450, the iron is ligated to the sulphur of a cysteinyl residue, technically making the enzyme a 'haem-thiolate protein', rather than a true cytochrome (24).

Most animal species, including humans, have several different forms of P450, each of which can catalyse a distinct repertoire of reactions. To date, about 40 forms of human P450 and many more forms from other species have been identified and sequenced (24, 25). A standard nomenclature has been adopted that categorizes the P450 superfamily into families and subfamilies, based on sequence homology. An individual protein is designated by the letters 'CYP' followed by an Arabic numeral indicating the family, a letter indicating the subfamily, and a second Arabic numeral indicating the particular protein. The name of the corresponding gene is the same, but is italicized. Recently, Daly

Table 2. The major drug-metabolizing P450 enzymes

P450 enzyme	Substrate
CYP1A2	Imipramine, caffeine, phenacetin
CYP2C8	Benzphetamine, diazepam, diclofenac
CYP2C9	Hexobarbital, ibuprofen, imipramine
CYP2C18	Omeprazol, oxiam drugs, proguanil, propranolol, retinoic acid, S-mephenytoin, S-warfarin, naproxen, tolbutamide, tetrahydrocannabinol
CYP2D6	Anti-arrhythmic agents, antihypertensives, β -blockers, monoamine oxidase inhibitors, morphine derivatives, antipsychotics, tricyclic antidepressants
CYP2E1	Chlorzoxazone
CYP3A3	Aldrin, benzphetamine, cyclosporin
CYP3A4	Erythromycin, lidocaine (lignocaine)
CYP3A5	Lovastatin, midazolam, quinidine
CYP3A7	Ethinylestradiol, terfenadine, triazolam, various 1,4-dihydropyridines
CYP4A11	Leukotriene receptor antagonists (long chain fatty acid hydroxylase)

Adapted from: Gonzalez, F. J. and Idle, J. R. (1994). Pharmacogenetic phenotyping and genotyping. Present status and future potential. *Clin. Pharmacokinet.* **26**: 59–70.

et al. (26) proposed a new allele nomenclature, since the number of reported alleles is increasing rapidly and the currently used nomenclature will become inadequate.

Estimates of the total number of functional P450 genes in mammalian species range between at least 60 to more than 200 (27). All of these genes are believed to have arisen by divergent evolution, i.e. from a common ancestral probably more than 2.5 billion years ago. A major driving force for the large number of gene duplications is believed to be animal–plant ‘warfare’ (19, 28–30).

Between two species, there are already numerous examples of different P450 genes, and, in some cases, different catalytic activity among members of the same P450 gene subfamily (19, 30). It is possible that a particular test compound will produce an entirely different response, with regard to toxicity or carcinogenicity, between a human and a laboratory animal, due to the presence or absence of a particular P450 gene in either species (7).

P450 enzymes of the CYP2C, CYP2D and CYP3A subfamilies are the most active in metabolizing known clinically used drugs (Table 2; Ref. 8). CYP1A2 and CYP2E1 metabolize only a few drug substrates. Polymorphism has been described for P450s in the CYP2C and CYP2D subfamilies, particularly in the latter (1, 2, 7–9). The CYP3A subfamily consists of four distinct enzyme forms and is among the most important in drug metabolism.

CYP1A1 metabolizes polycyclic aromatic hydrocarbons, but is not involved in drug oxidation. CYP1A2 is the primary enzyme responsible for activating heterocyclic arylamine food mutagens. CYP2E1 activates a number of low molecular weight suspected carcinogens including benzene and *N*-nitrosodimethylamine (8).

Phenotyping vs genotyping

The possibility of distinguishing the three classes of metabolizers (PM, EM or UEM) prospectively would allow appropriate modifications in patient treatment.

The specific drug metabolism may be identified by either phenotyping or genotyping.

Phenotyping is a procedure whereby at first a test drug is administered of which metabolism is known to be solely dependent on the function of a specific drug metabolizing enzyme, followed by measurement of the metabolic ratio (MR). The MR is the ratio of drug dosage or unchanged drug to metabolic measured in serum or urine. Each specific CYP450 enzyme corresponds with its own specific test drug which can be used to define the activity of the CYP450 enzyme. Gonzalez *et al.* (31) showed that is possible to predict the drug metabolic phenotype of the substrates of a distinct CYP450 enzyme if the individual's phenotype corresponding with the specific enzyme has been assessed relative to a reference substrate.

Phenotyping allows discovery of interactions between drugs or in the case of a pharmacokinetic study possible defects in the complete process of drug metabolism. Genotyping does not enable detection of these anomalies. Phenotyping offers the possibility to determine the overall metabolism of the drug under investigation in an individual, whereas with genotyping only specific mutations can be detected. Besides this advantage of phenotyping over genotyping, there are also some drawbacks of phenotyping mentioned in the literature. Co-administration of drugs (32, 33) or confounding effects of disease (34, 35) can result in incorrect phenotype assignment. Furthermore, the procedure can lead to problems since the protocols for testing are often complicated (1). Finally, there is always the risk of an adverse drug reaction occurring (36).

Genotyping offers the possibility to identify specific genetic mutations which cause certain drug metabolic phenotypes. There are two approaches currently used for the detection of specific mutations within genes associated with altered drug metabolism. With the first method, the specific region of a certain gene is amplified by using the technique of polymerase chain reaction (PCR) followed by digestion of the produced DNA with specific restriction endonucleases (1). The differences in size of the DNA fragments obtained as a result of endonuclease digestion are referred to as restriction fragment length polymorphism (RFLP) (8), and the exact size of the digestion products are determined by agarose gel electrophoresis. The second approach for the detection of possible mutations within a gene is by means of allele-specific PCR amplification (1). In this procedure, oligonucleotides specific for hybridizing with the common or variant alleles are utilized in parallel amplification reactions.

These two possible methods for genotyping have a few interesting advantages compared to phenotyping: there is no disturbance from underlying disease or drugs taken by the investigated individual, rapid intervention is possible since results are provided within 48–72 h and only small amounts of blood or tissue are necessary for these genotyping methods (1, 37). The fact that genotyping is probably less of a burden for the individual patient (e.g. only small amounts of blood are required) and that the required information obtained is more rapidly available compared to phenotyping, makes this technique very interesting since the question of practicality of phenotyping vs genotyping will probably play a major role in the clinical setting.

Anticancer drugs, cytochrome P450 and genotyping

It was found after thorough examination of the available literature that from the selected anticancer drugs (Table 1) CYP450 plays a major role in metabolism of cyclophosphamide, ifosfamide, etoposide, teniposide, paclitaxel (taxol), tamoxifen, thio-TEPA, vinblastine and vindesine, and the results of the studies conducted in the field of these antineoplastic drugs will be elucidated separately in detail.

However, for a large number of the investigated anticancer drugs, little or no information is available with regard to the detailed pathways of drug metabolism, or it has not yet been shown that CYP450 makes a contribution to the metabolism of these anticancer drugs.

Cyclophosphamide and ifosfamide

Cyclophosphamide and ifosfamide both belong to the family of the oxazaphosphorines and are isomeric prodrug alkylating agents. Both these drugs are devoid of alkylating activity and must first undergo bioactivation by hepatic cytochrome P450 (38, 39).

Cyclophosphamide is used in the treatment of a variety of human tumours, it has significant immunosuppressive activity and is clinically used in the treatment of malignancies, auto-immune diseases and in patient conditioning for renal and bone marrow transplantations (40). Cyclophosphamide is also a widely used antineoplastic drug in the treatment of childhood malignancies (41). Ifosfamide, the isomeric analogue of cyclophosphamide, has been shown to be effective against a wide spectrum of solid tumours both as a single agent or as a part of combined therapy, and is used in the treatment of both paediatric and adult malignancies (42, 43). The structure and metabolism of cyclophosphamide and ifosfamide show much resemblance, but ifosfamide produces less myelosuppression than cyclophosphamide and also exhibits little cross-resistance (44).

There are two pathways of metabolism of these drugs which are important and should be discussed: firstly the activation step and secondly an important route of detoxification.

The activation of both cyclophosphamide and ifosfamide is initiated with the ring oxidation at C4 (4-hydroxylation) (39). The formed 4-hydroxy metabolite (4-hydroxyphosphamide and 4-hydroxyifosfamide, respectively) equilibrates with the ring-opened aldophosphamide, which undergoes chemical decomposition to yield a mustard derivative (phosphoramidate mustard or ifosphoramidate mustard) and acrolein (39). The formed mustard possesses DNA-alkylating activity and is generally considered to be the therapeutically significant metabolite (39, 45). Acrolein on the other hand is an electrophilic aldehyde which lacks cytotoxic activity but is highly reactive and binds covalently to proteins, including cytochrome P450 reductase, leading to enzyme inactivation (46, 47). Clarke *et al.* (40) showed that CYP2B1 is the major enzyme involved in the 4-hydroxylation of cyclophosphamide in phenobarbital-induced rat liver microsomes. However, in uninduced liver microsomes, CYP2C6 and -2C11 were the predominant catalysts of cyclophosphamide activation (40).

Weber *et al.* (44) found, using P450 induction studies and antibody- and chemical-inhibition experiments, that the same three rat P450 enzymes also catalyse the ifosfamide activation. They also demonstrated that the involvement of a dexamethasone-inducible CYP3A subfamily, probably CYP3A1 or a form (6 β -2) which has not yet been characterized at the cDNA level, contributes in the metabolic activation of ifosfamide but not of cyclophosphamide (44). It was shown by Chang *et al.* (48) that CYP450 enzymes are the major catalysts of cyclophosphamide and ifosfamide activation in human liver, and that a subset of these enzymes carries out a great fraction of drug activation of these antineoplastic prodrugs. They showed that in the majority of the examined human liver samples, cyclophosphamide was activated at a higher rate than ifosfamide and that CYP2B6 contributes substantially to the metabolism of cyclophosphamide in some of the human liver microsomal preparations. However, this enzyme had little or no effect on microsomal ifosfamide activation (48). Chang *et al.* (48) also reported that CYP3A enzymes contributed significantly to liver microsomal ifosfamide 4-hydroxylation but that it only contributes at a low level to the corresponding microsomal cyclophosphamide hydroxylation. Weber *et al.* (44) obtained analogous results using rat liver microsomes, where a dexamethasone-inducible rat CYP3A accounted for the majority of ifosfamide 4-hydroxylase activity but did not contribute to cyclophosphamide 4-hydroxylation. Walker *et al.* (49) identified CYP3A4 as the major CYP450 responsible for the 4-hydroxylation of ifosfamide in the human liver. Chang *et al.* (48) also found that CYP1A1, -1A2, -2D6 and -2E1 are probably not participating in the activation of either cyclophosphamide or ifosfamide, and that CYP2A6 only plays a minor role in the activation of these anticancer drugs. Finally, this study showed that CYP2C8 and CYP2C9 are both competent in catalysing cyclophosphamide and ifosfamide hydroxylation (48). It was also reported by Walker *et al.* (49) that CYP2C9 and CYP2C18 play no major role in ifosfamide metabolism in human liver. Chang *et al.* (48) summarized their findings and concluded that CYP2A6, -2B6, -2C8, -2C9 and -3A4 enzymes contribute in the activation of cyclophosphamide and ifosfamide, whereas CYP1A1, -1A2, -2D6 and -2E1 play no role as catalysts in the activation of these antineoplastic drugs. A patient who is a poor metabolizer (PM) for any one of the mentioned CYP450 enzymes which play a role in the activation of cyclophosphamide and ifosfamide will probably experience decreased drug efficacy, since the activation of these antineoplastic drugs takes place at a lower rate.

An important route of detoxification of cyclophosphamide and ifosfamide is the side-chain N-dechloroethylation, which leads to the formation of inactive, monofunctional metabolites that are therapeutically inactive (39, 50, 51). The products resulting from this reaction are dechloroethylcyclophosphamide or dechloroethylifosfamide and chloroacetaldehyde (39, 50). The latter product has been shown to be a neurotoxic substance and could possibly be responsible for the neurotoxicity caused by treatment with these oxazaphosphorines (51, 52). Ruzicka *et al.* (51) showed that the N-dechloroethylation of cyclophosphamide and ifosfamide is catalysed by CYP450 enzymes. They also stated that this information is clinically interesting since N-dechloroethylation is only modest in the human cyclophosphamide biotransformation but ifosfamide is extensively metabolized by this pathway (51, 52). Walker *et al.*

(49) showed that CYP3A4 was the major cytochrome P450 enzyme for the N-dechloroethylation of ifosfamide in the human liver but they were unable to exclude the minor involvement of cytochrome P450 other than CYP3A4 in ifosfamide metabolism. Bohnenstengel *et al.* (53) found that CYP3A4 was also the major CYP450 enzyme responsible for the N-dechloroethylation of cyclophosphamide.

Etoposide and teniposide

The naturally occurring podophyllotoxins have been used therapeutically for over 1,000 years, and teniposide and etoposide belong to the family of semisynthetic podophyllotoxin derivatives (54). Teniposide and etoposide were first introduced in 1967 and 1971, respectively, and since this period, these drugs have become established in the treatment of several malignancies (54). Etoposide is frequently used for the treatment of small-cell lung cancer, germ cell tumours and lymphomas, and more recently Kaposi's sarcoma associated with AIDS (54, 55). Teniposide is mainly used in paediatric oncology, especially for neuroblastoma, but it is also active in the treatment of acute lymphatic leukaemia, lymphomas, brain tumours and small-cell lung cancer (54, 56).

It has been reported that the topoisomerase-inhibitors teniposide and etoposide induce a premitotic block in the cell-cycle; they are most cytotoxic in the late S- or early G₂- phases both *in vitro* and *in vivo* and therefore are classified as cell-cycle phase-specific cytotoxic drugs (54). It has also been shown that etoposide can induce both single- and double-strand DNA breaks in several tumour cell lines *in vitro* and in isolated tumour cell nuclei (55).

A great deal of the described human metabolites of teniposide and etoposide such as the hydroxyacid, aglycone glucuronides, other conjugates and the cis and picro forms of these drugs are only recovered as a small percentage of the administered dose and have only limited or no cytotoxicity (57). *In vitro* studies by Haim *et al.* (58, 59) using rodent liver microsomes and peroxidases showed O-demethylation of the dimethyloxyphenolic pendant ring of teniposide and etoposide to their catechol and quinone forms. Relling *et al.* (57) were the first to demonstrate the cytochrome P450-catalysed O-demethylation of teniposide and etoposide by human liver but they also showed that the reaction was highly variable. They stated that although this route of metabolism is possibly not of quantitative importance in relation to drug elimination, it might have qualitative impact on the variable formation of metabolites. It has been shown using rodent microsomes and peroxidase preparations that O-demethylated podophyllotoxin metabolites bind irreversibly to DNA and to microsomal protein and possess intrinsic cytotoxicity (55, 57). Therefore, this reaction may play a critical role in clinical efficacy and toxicity of teniposide and etoposide.

Paclitaxel (taxol)

Bark extracts of the Pacific yew tree *Taxus brevifolia* were first shown to be effective in the National Cancer Institute disease-oriented *in vitro* anticancer drug screening program in the early 1960s (60). Wani *et al.* (61) soon thereafter

identified and isolated taxol (paclitaxel) as an active constituent of these extracts. Taxol appears to inhibit cellular growth by promoting and stabilizing microtubule assembly by non-covalent interaction with tubulin, thereby blocking cell replication in the late G₂ mitotic phase of the cell cycle (62).

Taxol has been shown to be effective in advanced drug refractory ovarian carcinoma, previously treated patients with metastatic breast carcinoma, advanced non-small-cell lung cancer, advanced small-cell lung cancer and advanced head and neck cancer (63, 64).

There have been several studies on the metabolism of taxol (60, 64–75). Monsarrat *et al.* (65, 66) showed that the primary route of systemic elimination of taxol in humans and rodents proceeds via hepatic metabolism and biliary excretion.

Investigations of paclitaxel metabolism in rats and in human revealed that nine and five metabolites can be detected in the bile of the respective species (65, 66). From *in vitro* experiments using human liver microsomes, only three metabolites of paclitaxel have been detected (60, 67). So far investigations have shown that 6 α -hydroxytaxol is the primary metabolite detected both in human bile and after human adult liver microsomal incubations (60, 66, 67, 70). The formation of this metabolite is likely to be due to a single stereospecific hydroxylation at the '6' position of the taxane ring (68, 70). However, in a recent study, Sonnichsen *et al.* (72) found, using 49 human liver microsome preparations, that more than three paclitaxel metabolites were frequently formed, and that 6 α -hydroxytaxol was not always the predominant metabolite. They also demonstrated marked interindividual variability in the relative predominance of individual paclitaxel metabolites (72). Importantly, in 27% of the preparations used in this study, the metabolites named 'B' and 'C' were the predominant metabolites rather than 6 α -hydroxytaxol.

In both the studies by Rahman *et al.* (69) and Sonnichsen *et al.* (72) it has been shown that the formation of 6 α -hydroxytaxol is catalysed by human CYP2C8, and that it is the result of region-specific hydroxylation at position six of the taxane ring, with the 6-hydroxyl group *trans* to the 7-hydroxyl group. However, a few studies showed that several CYP3A substrates inhibited formation of 6 α -hydroxytaxol (60, 67, 71, 72).

Metabolite B is more polar than 6 α -hydroxytaxol and has been identified in human bile, after human liver incubations and in rodents (60, 65, 68, 70). This metabolite is formed by a single hydroxylation at the *para*-position of the C3 phenyl group on the C13 side chain of paclitaxel, and is catalysed by CYP3A4 (60, 67, 70), which was concluded from chemical inhibition, metabolic correlation and immuno-inhibition studies as well as using heterologously expressed P450.

Metabolite A is also a more polar substance than 6 α -hydroxytaxol and it has been shown that this dihydroxylated metabolite is formed by stepwise hydroxylations by CYP2C8 and -3A4 at the two sites previously described for 6 α -hydroxytaxol and metabolite B (60).

Sonnichsen *et al.* (72) described a paclitaxel metabolite, named 'C' with elution characteristics which had not been described previously. Based on the results of high correlations with the formation of metabolite B and the

midazolam 4-hydroxylation which are both substrates of CYP3A4 they stated this metabolite was formed by CYP3A4.

In summary, it can be concluded that 6 α -hydroxytaxol, metabolites B, C and A are formed by CYP2C8, -3A4, -3A4 and both -2C8 and -3A4, respectively, but that 6 α -hydroxytaxol is not the predominant paclitaxel metabolite in all individuals. The CYP3A4 isozyme may play a role in the metabolism and substantial interpatient variability in the clearance of paclitaxel. A poor metabolizer (PM) for CYP2C8 or CYP3A4 will probably experience increased toxicity if paclitaxel is administered, since these two enzymes play a major role in the elimination of this antineoplastic drug.

Tamoxifen

Tamoxifen was developed in the late 1960s as an anti-oestrogen. The drug inhibits oestrogen-stimulated cell division, but in some tissues it can also exhibit oestrogen-like activities (76). Tamoxifen is of proven efficacy in inhibiting the growth of oestrogen-receptor-positive breast cancers (76–79). Tamoxifen has also a number of beneficial effects apart from its primary action on breast cancer cells. In treated women, it has caused a significant reduction in serum cholesterol, and possibly in the incidence of fatal myocardial infarction (76).

However, tamoxifen has only limited efficacy, with only one-third of patients gaining benefit from the drug (80). It has been suggested that tamoxifen can also be used as a preventing agent for breast cancer for women at risk for developing this disease (81). However, in this regard, there is some concern being expressed over the potential of tamoxifen to cause hepatic and endometrial cancers (82–84).

The metabolism of tamoxifen shows a large interindividual variation, which might be a determinant of the efficacy and toxicity of this drug (85). The primary route of metabolism is N-demethylation, and the minor routes include N-oxidation, and 4- and 4'-hydroxylation. It has been shown in a few studies that CYP3A plays a role in catalysing the N-demethylation of tamoxifen (85–89). Jacololet *et al.* (86) were the first to show that the CYP3A family plays a significant role in the tamoxifen N-demethylation but they were unable to distinguish between closely related CYP3A forms involved in this metabolism of tamoxifen. The findings of Daniels *et al.* (87) confirmed the role of CYP3A with regard to the N-demethylation but their results also implicated that CYP2C catalyses this pathway of tamoxifen metabolism. The collective evidence reported by Mani *et al.* (88) demonstrated that the CYP3A enzyme catalyses N-demethylation. They found CYP3A1 to be the major catalyst of this reaction although they were unable to reconstitute the N-demethylation activity with purified CYP3A1 (88). The study by Mani *et al.* (88) also showed that CYP2B1 was not involved in the N-demethylation reaction, and that CYP1A1 also does not contribute significantly. Both Crewe *et al.* (85) and Simon *et al.* (89) reported that this reaction is catalysed predominantly by CYP3A4 with a possible contribution from CYP1A (89).

It is likely that a poor metabolizer (PM) for any one of the CYP450 enzymes which play a role in the N-demethylation of tamoxifen (e.g. CYP1A, -2C, 3A1, 3A4) will experience increased toxicity when tamoxifen is administered, since

in that case tamoxifen will be eliminated at a lower rate than expected by this route of metabolism.

Although plasma and tumour concentrations of 4-hydroxytamoxifen are only about 2% of those of the parent compound, 4-hydroxylation also is an important pathway of tamoxifen metabolism because the product of this reaction is intrinsically 100 times more potent as an oestrogen antagonist than the parent drug and thus may contribute to the clinical efficacy (85). Some authors have indicated that CYP2C might play a role in this reaction (87, 90). However, these results were obtained using inhibitory antibodies raised against rat CYP2C, which have questionable specificity compared to the human forms of CYP2C. Recently, the first step has been made in the identification of the CYP isoform specificity of tamoxifen 4-hydroxylation in humans by Crewe *et al.* (85). The findings of the investigators showed, by using the microsomes of 10 human livers, the involvement of CYP2D6, -2C9 and -3A4 in the 4-hydroxylation of tamoxifen (85). Furthermore, they found that the whole 4-hydroxylation activity could be accounted for by the sum of these activities and they had strong indications that metabolism by CYP2D6 was the most important determinant of the rate of 4-hydroxylation of tamoxifen in human livers (85). However, the authors of this study suggest that the marked interindividual variation in the contribution of these isoforms underlines the need for study of metabolic reactions in a sufficient number of livers that are characterized with respect to a range of cytochrome P450 activities.

Thio-TEPA

Thio-TEPA (N,N,N''-triethylenethiophosphoramidate) is an alkylating anticancer drug which is currently used for chemotherapy of a number of malignancies, including metastatic carcinoma of the breast, ovarian, bladder and meningeal carcinoma (91–93). The precise mechanism of action of thio-TEPA remains unknown, but DNA is believed to be the primary target of this cytotoxic agent (94).

A major route of thio-TEPA metabolism in both rodents and humans is the oxidative desulphuration to the pharmacologically active TEPA (N,N,N''-triethylenephosphoramidate) (94–99). In this reaction which was shown to be catalysed by cytochrome P450 (100, 101). In this reaction there is also the formation of a diffusible, chemically reactive species which has been shown to be elemental sulphur (95), and this substance can inactivate liver CYP and other enzymes (97, 98) and thus dramatically enhance the cytotoxicity of thio-TEPA in culture (94). It has been reported that this oxidative desulphuration of thio-TEPA to TEPA is carried out by CYP2B1, -2C6 and -2C11 in rat liver (94, 95).

Although TEPA has pharmacologic activity *in vitro*, the contribution of this metabolite to the overall *in vivo* cytotoxicity is still unclear (95). For example, the activity of TEPA as a cytotoxic agent in cell culture is not more active than thio-TEPA (94). Chang *et al.* (95) found that induction of hepatic thio-TEPA oxidation reduces tumour growth delay effects, whereas inhibition of the liver CYP-catalysed thio-TEPA metabolism both enhances antitumour activity and exacerbates systemic toxicity. They postulated that the therapeutically

significant cytotoxic metabolites *in vivo* are distinct from TEPA or any other metabolite derived from the oxidative desulphuration pathway (95). Ng *et al.* (94) also reported that TEPA is not the source of cytotoxic activity but rather results from the associated production of reactive metabolites which are distinct from TEPA. They also stated the possibility that the cytotoxic effect of the thio-TEPA-derived reactive metabolites may result from their action at the cell surface (94).

Egorin *et al.* (102) reported that in addition to the CYP-dependent oxidative desulphuration pathway, thio-TEPA can also be converted to N,N'-diethylenethiophosphoramidate and aziridine by a route that is probably CYP-independent. Chang *et al.* (95) suggested that one or both of these metabolites may be important mediators of the thio-TEPA cytotoxicity *in vivo*, and that the formation of these reactive species occurs either non-enzymatically or is catalysed by enzymes distinct from CYP2B1 and CYP2C11.

Vinblastine and vincristine

Vinblastine and vincristine are two naturally occurring *Vinca* alkaloids extracted from the *Catharanthus* plant species (103). They belong to the *Vinca* alkaloid family together with vindesine and navelbine. Vinblastine and vincristine are currently applied as very effective anticancer drugs both as single agent and in combination with other drugs (103, 104). Vinblastine is frequently applied in the treatment of Hodgkin's and non-Hodgkin's lymphoma as well as some solid tumours such as testicular and breast cancer (103, 104), whereas vincristine is particularly effective against acute lymphatic leukaemia, Wilms' tumour, rhabdomyosarcoma and various lymphomas (103).

Although these substances are structurally related, there have been observed marked differences in their antitumour activity as well as in their toxicity and pharmacokinetic behaviour (105, 106). The clinical pharmacokinetics of the *Vinca* alkaloids present large inter- and intra-individual variation (103). The suggestion has been made that the interpatient pharmacokinetic variability may be dependent upon individual differences in hepatic drug disposition (106).

To date, there has been only one study investigating the role of cytochrome P450 enzymes in the metabolism of vinblastine (104). In this study, Zhou-Pan *et al.* showed that vinblastine was metabolized to one main metabolite by human liver microsomes. However, after incubation of vinblastine with freshly isolated hepatocytes in suspension, the drug showed a biotransformation pattern characterized by at least four metabolites. Therefore it is concluded that both cytosolic and microsomal enzymes must be involved in the metabolism of vinblastine (104). The authors showed a wide interindividual variation in the metabolism of vinblastine and they found it to be mediated by the CYP3A subfamily (104).

There have not been any reports of P450 mediated or catalysed vincristine metabolism. However, Zhou *et al.* (107) demonstrated the involvement of CYP3A isozymes in the metabolism of vindesine of vindesine by using a bank of human liver microsomes and they also reported large interindividual variations in the metabolism of vindesine.

Since CYP3A4 plays a role in the elimination of vinblastine and vindesine, it

Table 3. The P450 isozymes which play a role in the human metabolism of the selected anticancer drugs and the expected effect in the case of a poor metabolizer (PM)

CYP450 enzyme	Antineoplastic drug (reaction)	Role	Expected effect for PM	Reference
1A	Tamoxifen (N-demethylation)	Elimination	Increased toxicity	(80, 84)
2A6	Cyclophosphamide/ifosfamide (4-hydroxylation)	Activation	Decreased efficacy	(43)
2B6	Cyclophosphamide/ifosfamide (4-hydroxylation)	Activation	Decreased efficacy	(43)
2C	Tamoxifen (N-demethylation)	Elimination	Increased toxicity	(82)
2C8	Cyclophosphamide/ifosfamide (4-hydroxylation)	Activation	Decreased efficacy	(43)
2C9	Taxol	Elimination	Increased toxicity	(64, 67)
	Cyclophosphamide/ifosfamide (4-hydroxylation)	Activation	Decreased efficacy	(43, 44)
	Tamoxifen (4-hydroxylation)	Elimination	Increased toxicity	(80)
2D6	Tamoxifen (4-hydroxylation)	Elimination	Increased toxicity	(80)
3A	Tamoxifen (N-demethylation)	Elimination	Increased toxicity	(80–84)
	Vinblastine/vindesine	Elimination	Increased toxicity	(99–102)
3A1	Tamoxifen (N-demethylation)	Elimination	Increased toxicity	(83)
3A4	Cyclophosphamide/ifosfamide (4-hydroxylation)	Activation	Decreased efficacy	(44)
	Cyclophosphamide/ifosfamide (side chain N-dechloroethylation)	Elimination	Increased toxicity	(44, 48)
	Taxol	Elimination	Increased toxicity	(55, 62, 65–67)
	Tamoxifen (N-demethylation)	Elimination	Increased toxicity	(80, 84)
	Tamoxifen (4-hydroxylation)	Elimination	Increased toxicity	(80)

is likely that administration of either one of these antineoplastic drugs to a poor metabolizer (PM) of CYP3A4 will lead to increased host toxicity.

Miscellaneous

5-Fluorouracil was reported to suppress some P450-mediated drug metabolizing reactions in the rat (108), whereas methotrexate was found to induce a decrease in cytochrome P450 level and activities in rats (109). Furthermore, it has been shown that the activation of the prodrug 6-mercaptopurine occurs intracellularly, whereas the contribution of the three major metabolic pathways involved (thiopurine methyltransferase, xanthine oxidase and hypoxanthine phosphoribosyltransferase) in the individual variations in the metabolism of 6-mercaptopurine are still the subject of pharmacogenetic studies (110). It has been shown that after a standard dosage of 6-mercaptopurine, there is a large interindividual variation in the formation of the 6-thioguanine nucleotide metabolites, due to the thiopurine methyltransferase genetic polymorphism (110). In children with high inherited activities of thiopurine methyltransferase, low concentrations of 6-thioguanine nucleotides are formed after a standard dose of 6-mercaptopurine, resulting in a high risk of disease relapse (110, 111).

In the case of the remaining selected anticancer drugs, there is no information about the possible involvement of P450 in their metabolism or elimination.

The different P450 isozymes which play a role in the activation or elimination of the investigated anticancer drugs, including the expected effects of polymorphism, are summarized in Table 3.

Discussion and conclusion

Examination of the available literature revealed that for the antineoplastic drugs cyclophosphamide, ifosfamide, etoposide, teniposide, paclitaxel, tamoxifen, thio-TEPA, vinblastine and vindesine, cytochrome P450 plays a major role in metabolism. Therefore, patients with cytochrome P450 polymorphism receiving these drugs may be prone to altered drug efficacy or toxicity. Structured investigations on metabolism are not yet a part of (pre-)clinical studies on antineoplastic drugs, whereas these studies would greatly improve the understanding of the interindividual variability in pharmacokinetics and pharmacodynamics.

Pharmacodynamic and pharmacokinetic studies are necessary to elucidate more about the metabolism of these antineoplastic drugs, especially on the possible involvement of P450 isozymes. This review might give a key to the selection of antineoplastic drugs for which it would be worthwhile to perform a pilot study on the clinical significance of cytochrome P450 polymorphism. For example, it could be interesting to investigate the genotype of a number of patients treated with the selected anticancer drugs by using standard PCR techniques and correlate the findings to toxicity and efficacy data.

Individualization of chemotherapy based upon DNA genotyping for cytochrome P450 polymorphism has become technically feasible, but is yet unexplored. On the basis of this review, pilot studies will be planned to apply these techniques to the field of oncology. Hopefully, this results in more effective and less toxic chemotherapeutic treatment, from which the individual patient eventually benefits.

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