Br J Clin Pharmacol 1995; **40**: 523–530

The role of human cytochrome P450 enzymes in the metabolism of anticancer agents: implications for drug interactions

KARI T. KIVISTÖ, HEYO K. KROEMER & MICHEL EICHELBAUM
Dr Margarete Fischer-Bosch-Institut für Klinische Pharmakologie, Stuttgart, Germany

1 Little information is available about the pharmacokinetic interactions of anticancer drugs in man. However, clinically significant drug interactions do occur in cancer chemotherapy, and it is likely that important interactions have not been recognized.
2 Specific cytochrome P450 (CYP) enzymes have been recently shown to be involved in the metabolism of several essential anticancer agents. In particular, enzymes of the CYP3A subfamily play a role in the metabolism of many anticancer drugs, including epipodophyllotoxins, ifosphamide, tamoxifen, taxol and vinca alkaloids. CYP3A4 has been shown to catalyse the activation of the prodrug ifosphamide, raising the possibility that ifosphamide could be activated in tumour tissues containing this enzyme.
3 As examples of recently found, clinically significant interactions, cyclosporin considerably increases plasma doxorubicin and etoposide concentrations. Although cyclosporin and calcium channel blockers may influence the pharmacokinetics of certain anticancer agents by inhibiting their CYP3A mediated metabolism, it is more likely that these P-glycoprotein inhibitors inhibit P-glycoprotein mediated drug elimination.
4 Appropriate caution should be exercised when combining P-glycoprotein inhibitors and potential CYP3A inhibitors with cancer chemotherapy.

Keywords cytochrome P450 anticancer agents drug interactions P-glycoprotein

Introduction

The pharmacokinetics of most anticancer drugs are highly variable from patient to patient [1–3]. This variability, together with the narrow therapeutic range of anticancer drugs, makes pharmacokinetic optimization of therapy difficult. It can therefore be difficult to obtain the expected benefit from cancer chemotherapy in individual patients and, at the same time, minimize adverse effects. Oxidative metabolism of drugs, for example, displays marked interindividual variations, resulting mostly from variability in the expression of different cytochrome P450 (CYP) enzymes in the liver and extrahepatic tissues. Several-fold differences between patients in the clearance of many anticancer drugs are common [2].

Drug interactions are likely to occur in the setting of cancer chemotherapy; many drugs inhibiting or inducing specific P450 enzymes are already known [4, 5]. In general, little information is available concerning the pharmacokinetic interactions of anticancer drugs with each other and with other drugs in man. It is, however, clear that clinically significant drug interactions do occur in cancer chemotherapy [6–8]. Moreover, it is likely that important interactions have not been recognized. There are of course many problems associated with characterization of interactions with anticancer drugs [6, 9]. For example, obtaining meaningful data from cancer patients is difficult since most chemotherapeutic regimens involve a combination of drugs.

Potential interactions of any new drug entity with other drugs should be identified early in the development of the drug, ideally before marketing [10, 11]. Clinically significant and potentially harmful interactions are often

Correspondence: Dr K. T. Kivistö, Dr Margarete Fischer-Bosch-Institut für Klinische Pharmakologie, Auerbachstrasse 112, D-70376 Stuttgart, Germany.

uncovered only after the drug has been in use for several years. Recent examples include inhibition of the metabolism of terfenadine and midazolam by erythromycin, itraconazole and other CYP3A inhibitors [5, 12, 13].

It is not feasible to study all potentially important drug interactions in volunteers or patients, but the P450 enzymes involved in the oxidative metabolism of a new drug should be identified in the development programme, using experiments with human liver microsomes and other suitable techniques [10, 11, 14, 15]. The relative contribution of each enzyme to the overall metabolism should also be determined [14, 15]. The likelihood for significant metabolic interactions is increased when a major metabolic step is catalysed by a single P450 enzyme. Regarding drugs with a rather wide therapeutic range, we may assume that at least 30% of the dose should be metabolized by a specific enzyme for an interaction to be potentially important. The case may, however, be different with anticancer drugs.

Until recently, knowledge of specific P450 enzymes catalysing the oxidative metabolism of different antineoplastic agents in man was very limited [16]. However, specific P450 enzymes involved in the metabolism of cyclophosphamide, ifosfamide, etoposide, teniposide, tamoxifen, taxol (paclitaxel) and vinca alkaloids have recently been identified. Furthermore, several competitive inhibitors of the metabolism of these drugs were found in these *in vitro* studies. The K_i value (inhibition constant) for competitive inhibition measured *in vitro*, together with its relationship to free (unbound) plasma concentrations of the inhibitor achieved with therapeutic doses *in vivo*, can be used as a rough guide to predict the likelihood of a significant *in vivo* interaction. In general, inhibition will occur also *in vivo* only if the steady-state concentration of the inhibitor is similar to the K_i value, or greater (see also Conclusions).

These recent data allow prediction of potentially clinically significant interactions and help to explain the pharmacological basis of some reported interactions. It should therefore be possible to identify, at an earlier stage, potential interactions affecting the metabolism of anticancer agents that might influence drug efficacy or toxicity. Knowing which isoenzymes are involved in the metabolism of a drug (and its active metabolites), together with other available *in vitro* data such as K_i values, also forms a solid basis for planning relevant *in vivo* drug interaction studies.

In this review, we examine the role of specific P450 enzymes in the metabolism of anticancer drugs in humans and discuss some significant interactions that often *appear* to result from inhibition of anticancer drug metabolism. The available evidence, however, strongly suggests that certain drugs influence the pharmacokinetics of anticancer agents also (and perhaps primarily) by acting as P-glycoprotein inhibitors, thereby inhibiting P-glycoprotein mediated drug elimination. For more detailed accounts of known interactions in the field, the reader is referred to recent reviews [6–8].

The role of pharmacokinetics in optimization of anticancer therapy

In view of the substantial interindividual variation in the expression of the P450 enzymes, metabolism probably plays a considerable role in the between-patient variability of the pharmacokinetics of anticancer agents. Thus, an identical dosage regimen can result in widely different concentrations of the therapeutically active component [1, 3, 17, 18]. The possibility that some anticancer drugs are metabolized by enzymes exhibiting a genetic polymorphism (e.g. CYP2D6) should also be considered [19–22]. This would be of major clinical relevance with drugs such as anticancer agents that have a narrow therapeutic range. 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). Recent work indicates that some anticancer agents, including doxorubicin and vinblastine, can competitively inhibit CYP2D6 in human liver microsomes [19, 22]. This does, however, not necessarily imply that these drugs are also substrates for this polymorphic enzyme.

Although attempts to correlate anticancer drug concentrations to pharmacological effects have not been very successful, systemic exposure to some anticancer drugs is correlated with either their toxicity, efficacy, or both [2]. There are a number of problems with therapeutic drug monitoring in cancer chemotherapy [9]. First, the drugs are often administered intermittently and a steady-state situation may not be reached. Second, some drugs are pro-drugs (cyclophosphamide and ifosfamide) and need to be metabolized (in the liver, extrahepatic tissues or in the target tissue) before becoming cytotoxic. Third, plasma drug concentrations may not directly reflect the concentrations achieved in the tumour tissue. Finally, most patients with cancer are given combination chemotherapy, together with other drugs such as anti-emetic agents as needed; the risk for a drug interaction under these conditions is high.

Accepting these difficulties, it is still striking, considering the narrow therapeutic range of anticancer agents and the often marked between-patient variability in their pharmacokinetics, that therapeutic drug monitoring has not found a more widespread use in oncology. Currently, drug concentration measurements are not used routinely in clinical practice to predict toxicity or efficacy of antineoplastic agents, except following high-dose methotrexate administration [23]. However, therapeutic monitoring of other drugs may also prove to be clinically useful, as demonstrated, for example, by studies on busulfan [24, 25]. High-dose busulfan is an essential component of many bone marrow transplantation (BMT) preparative regimens, and Grochow [25] has shown that the toxicity of BMT preparative regimens can be decreased by therapeutic monitoring. It should be noted that in this instance there is only one chance to provide a safe, therapeutic dose.

Studies have shown that P450 enzymes are expressed not only in the liver and extrahepatic tissues but also in different kinds of tumours [26–30]. The pro-drugs

cyclophosphamide and ifosfamide need to be activated by specific P450 enzymes to produce cytotoxic compounds, and, if these enzymes were also found in the tumour, local activation of the drug could be important for efficacy [31]. In selected cases, it might even be possible to enhance drug activation through modulation of the appropriate P450 enzyme(s) [32]. If bioactivation in the liver is predominant, the question arises whether the active metabolite(s) can achieve effective concentrations in the tumour. The delivery of the drug or the active metabolites into the tumour may be inadequate, for instance, due to the physicochemical nature of the compound or poor vascularization of the tumour.

When evaluating the potential significance of drug interactions with anticancer drugs, the key question is whether such interactions could affect, at least in selected cases, the overall clinical response or toxicity of the drug. In general, the pharmacological properties of anticancer drugs (e.g. steep dose-response curves and low therapeutic indices) suggest that even small changes in the pharmacokinetic profile could significantly alter toxicity or efficacy. On the other hand, the metabolism of many anticancer agents is rather complex and often results in formation of active metabolites. Therefore, changes in the clearance of the parent drug may not necessarily affect the response to the drug [33].

Specific anticancer agents

Anthracyclines

Kerr *et al.* [34] have examined the effect of verapamil on the pharmacokinetics of doxorubicin in five patients. The AUC and elimination half-life for doxorubicin increased and the clearance decreased with co-administration of verapamil, suggesting that verapamil inhibited the metabolism of doxorubicin. However, in a recent study in 17 patients (conducted without a control group), high-dose verapamil did not appear to modify the steady-state pharmacokinetics of doxorubicin [35]. Further work is required on the effects of verapamil and other calcium antagonists on doxorubicin pharmacokinetics.

On the basis of a preliminary study, cyclosporin was suggested to decrease the metabolism or excretion of doxorubicin in patients with cancer, resulting in a lower clearance than would be expected when doxorubicin is given alone [36]. Bartlett *et al.* [37] have recently studied the effect of cyclosporin on the pharmacokinetics of doxorubicin in 12 patients. Addition of cyclosporin increased the dose-adjusted AUC of doxorubicin by 55% and that of doxorubicinol by 350%. The total clearance of doxorubicin decreased about 50% in the presence of cyclosporin. Similar results were obtained by Rushing *et al.* [38] in seven patients: cyclosporin increased the dose-adjusted AUC(0, 36 h) of doxorubicin by 48% and the dose-adjusted AUC(0, 36 h) of doxorubicinol by more than 400%, and reduced doxorubicin clearance by about 40%.

The steady state plasma concentrations of taxol (paclitaxel; a substrate for CYP3A) and doxorubicin were not altered when the drugs were administered as simultaneous 3-day infusions compared with single-agent administration [39]. However, the steady-state concentration of doxorubicinol was significantly elevated with the combination regimen, suggesting that taxol inhibited its metabolism to the aglycones.

Doxorubicin is metabolized to doxorubicinol and the aglycones, doxorubicinone and 7-deoxydoxorubicinone [40]. Doxorubicinol is cytotoxic but the aglycones are not. Metabolism to doxorubicinol occurs by aldoketoreductases in cytoplasm, while the aglycones are formed by the microsomal enzyme NADPH cytochrome P450 reductase [40]. Doxorubicinol is also transformed into aglycones [41].

Since cyclosporin and, to a lesser extent, verapamil, both substrates for CYP3A, increased doxorubicin plasma concentrations, it is possible that one or more enzymes of the CYP3A subfamily plays a role in doxorubicin metabolism. More studies about the effects of CYP3A inducers and inhibitors on the pharmacokinetics and pharmacodynamics of doxorubicin are needed, and care should be taken when combining doxorubicin with such agents. Moreover, cyclosporin and calcium channel blockers may influence the pharmacokinetics of anthracyclines and other anticancer agents not only by inhibiting their metabolism but also by acting as P-glycoprotein inhibitors, thereby inhibiting, for example, P-glycoprotein mediated biliary drug elimination (see Inhibition of P-glycoprotein).

The interaction between cyclosporin and doxorubicin is of major clinical importance. In the study of Bartlett *et al.* [37], addition of cyclosporin to doxorubicin increased nausea and vomiting, and similar myelosuppression was observed when the dose of doxorubicin with cyclosporin was about 60% of the dose of doxorubicin without cyclosporin. Rushing *et al.* [38] also observed greater drug-related toxicity with the cyclosporin-modulated course of doxorubicin, and suggested that cyclosporin should be used for modulation of multiple drug resistance (MDR) only in clinical trials. Furthermore, caution should be exercised in giving doxorubicin to cancer patients on cyclosporin therapy [42].

Cyclophosphamide and ifosfamide

Cyclophosphamide and ifosfamide are alkylating anticancer agents that require biotransformation to produce pharmacologically active, cytotoxic compounds. Chang and colleagues [43] demonstrated that CYP2B6 and CYP3A4 are the major isoforms catalyzing cyclophosphamide and ifosfamide 4-hydroxylation (that is, activation), respectively, in human liver. Walker *et al.* [44] recently showed that CYP3A4 makes a significant contribution to both the activation and *N*-dechloroethylation of ifosfamide in human liver (Figure 1). The side chain *N*-dechloroethylation of ifosfamide is a pathway

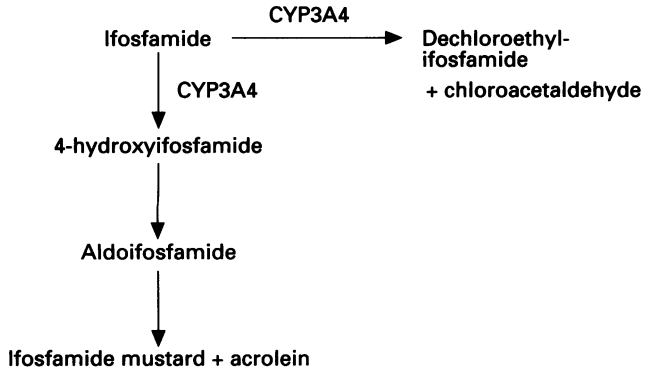

Figure 1 A simplified scheme for ifosfamide metabolism in man [43, 44].

leading to formation of the therapeutically inactive but supposedly neurotoxic metabolite chloroacetaldehyde [45]. Other dechloroethylated metabolites of ifosfamide have also been linked with neurotoxicity [46].

Drugs inducing or inhibiting the P450 enzymes catalyzing the activating step, 4-hydroxylation, of cyclophosphamide (CYP2B6) and ifosfamide (CYP3A4) might thus alter the pharmacological activity of these agents. However, selective inhibition of the *N*-dechloroethylation of ifosfamide to reduce toxicity and perhaps increase efficacy, by shunting more ifosfamide to the activating pathway, would not be possible without interfering with the activation step.

In animals the hepatic P450 2B proteins are highly inducible by, e.g. phenobarbitone, and CYP2B6 is probably induced by phenobarbitone in humans [47]. However, little is known about the human hepatic 2B6 protein, and few clinically relevant substrates for this enzyme have been identified. Mimura *et al.* [48] have recently characterized the CYP2B6 enzyme in human liver microsomes. In an immunoblotting analysis of 50 human liver samples, the protein band considered to be CYP2B6 was found in only 12 samples. The results obtained by Mimura *et al.* [48] suggest that human CYP2B6 catalyses activation of certain promutagens, but has low activities toward a number of typical substrates for P450 enzymes (e.g. benzo(*a*)pyrene, 7-ethoxycoumarin, ethoxyresorufin, ethylmorphine and aniline). The implications of the finding, that CYP2B6 is not expressed at all (or is expressed in very small amounts) in many subjects, for cyclophosphamide therapy are not clear, since cyclophosphamide may also be activated by other P450 enzymes [43].

The known drug interactions of cyclophosphamide have been discussed by Moore [33] and Wagner [49]. Only a few interactions have been reported in man, but phenobarbitone and allopurinol may influence cyclophosphamide pharmacokinetics. There appear to be no clinical data about the effects of other drugs on ifosfamide metabolism. A recent study showed that both cyclophosphamide and ifosfamide are competitive inhibitors of human hepatic CYP3A [50].

Many widely used drugs can induce or inhibit CYP3A4 in liver and extrahepatic tissues. Concomitant therapy with inducers of CYP3A4 (e.g. rifampicin, carbamazepine, phenobarbitone and phenytoin) enhances the expression of this enzyme and might increase the metabolic activation of ifosfamide, possibly resulting in enhanced efficacy and toxicity. In contrast, inhibitors and substrates of CYP3A4 (e.g. erythromycin, ketoconazole, itraconazole, diltiazem, verapamil and cyclosporin) could interfere with the activation of ifosfamide. It should be noted here that CYP3A4 is also expressed in small bowel enterocytes where it seems to be the major cytochrome P450 form present, having an important role in the prehepatic (first pass) drug metabolism [51].

Ifosfamide plays an important role as a component of chemotherapeutic regimens for lung cancer [52]. In a recent immunohistochemical study, eight of the 32 cases of primary pulmonary carcinoma showed expression of CYP3A [30]. The presence of CYP3A in pulmonary carcinomas may lead to local activation of ifosfamide in the tumour; thus, this pro-drug might be effective *in situ*, provided that it reaches the tumour. CYP3A has been found in different kinds of tumours [27–29], and the significance of activation of ifosfamide in tumours as compared with that in the liver is an issue that deserves further study. In the future, expression of CYP3A4 in the tumour might even be used as a marker for the prediction of response to ifosfamide therapy [44].

Epipodophyllotoxins

Relling *et al.* [53] recently showed that catechol formation by *O*-demethylation from teniposide and etoposide is primarily mediated by CYP3A4 in human liver. Several substrates for CYP3A4 (e.g. midazolam, erythromycin and cyclosporin) were identified as strong inhibitors of catechol formation from both etoposide and teniposide. The extent of contribution of *O*-demethylation to the overall *in vivo* elimination of these agents is not known, but catechol formation appears to play only a relatively small role in the metabolism of the epipodophyllotoxins [54]. However, the catechols of epipodophyllotoxins are cytotoxic [55], and it has been suggested that cytotoxic concentrations of the catechol metabolites might be achieved clinically [54].

Clinically relevant drug interactions mediated by the CYP3A subfamily have been found, although the available data are scanty. Concurrent therapy with anticonvulsive drugs (phenobarbitone or phenytoin) has been shown to significantly increase the clearance of etoposide and teniposide [56, 57]. Cyclosporin has a marked, concentration-dependent effect on the pharmacokinetics of etoposide [58]. Concomitantly administered cyclosporin produced, for example, an 80% increase in the AUC of etoposide and a 40% decrease in clearance in the 10 patients with plasma cyclosporin concentrations greater than 2000 ng ml^{-1} (measured by a nonspecific assay). The elimination half-life increased about twofold. Another substrate for CYP3A4, nifedipine, however, did not interfere with the pharmacokinetics of etoposide [59].

Tamoxifen

Tamoxifen is an antioestrogenic agent that is used in the treatment of breast cancer, especially in postmenopausal patients with oestrogen receptor positive tumours. The major metabolite of tamoxifen in plasma is the *N*-desmethyl derivative. Other metabolites have also been found in man, and it has been suggested that the metabolites of tamoxifen might contribute to its clinical activity [60, 61]. The major P450 enzyme catalysing tamoxifen *N*-demethylation in human liver belongs to the CYP3A subfamily and is most likely CYP3A4 [61, 62]. In the *in vitro* study of Jacolot *et al.* [61], erythromycin, cyclosporin, nifedipine, and diltiazem competitively inhibited *N*-demethylation of tamoxifen.

Aminoglutethimide has been shown to induce tamoxifen metabolism [63]. Otherwise, no clinical data about interactions of tamoxifen with inducers or inhibitors of CYP3A seem to be available, but such interactions are likely to occur.

Taxol (paclitaxel)

Harris *et al.* [64] have studied the metabolism of taxol in human hepatic microsomes. Their findings suggest that CYP3A4 is the major catalyst of the formation of a minor metabolite of taxol, whereas the identity of the enzyme(s) responsible for 6-α-hydroxytaxol formation could not be assigned with certainty. 6-α-hydroxytaxol is the major, but inactive metabolite of this antitumour drug in humans [65]. The results of Kumar *et al.* [66] suggested that taxol 6-α-hydroxylation in human liver is mediated by CYP3A, but apparently not CYP3A4. Cresteil *et al.* [67], however, reported that 6-α-hydroxytaxol formation can be assigned to the CYP2C subfamily, a finding later confirmed by Rahman *et al.* [68]. These investigators showed that, of several human P450 enzymes studied, only CYP2C8 formed detectable 6-α-hydroxytaxol [68].

As discussed above, there was no interaction between doxorubicin and taxol [39]. However, interactions resulting from induction or inhibition of P450 enzymes, especially CYP3A4 and CYP2C8, can be anticipated to occur in clinical practice (Figure 2). More studies about the effects of other drugs on taxol pharmacokinetics are awaited.

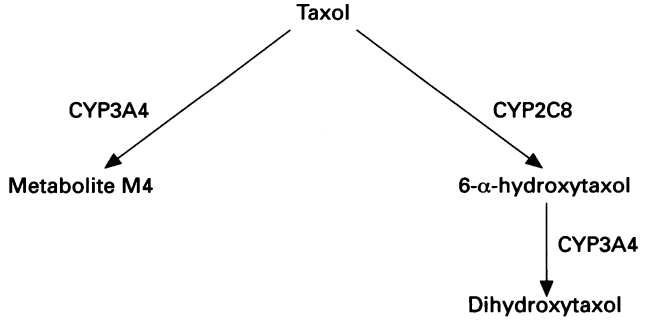

Figure 2 Pathways of taxol metabolism in man [64, 67, 68].

Vinca alkaloids

Zhou *et al.* [69] showed, using a bank of human liver microsomes, that vindesine was biotransformed into one major metabolite by the enzymes of the CYP3A subfamily. The structure of this metabolite was unknown. Other vinca alkaloids (vinblastine, vincristine and navelbine) had a marked inhibitory effect on vindesine metabolism, suggesting that the subfamily CYP3A plays a role in the hepatic metabolism of all these agents. Several other anticancer drugs, among them etoposide, teniposide (both known CYP3A4 substrates) and doxorubicin strongly inhibited vindesine biotransformation. Known inhibitors of CYP3A such as troleandomycin and erythromycin substantially inhibited vindesine metabolism.

Zhou-Pan *et al.* [70] recently also investigated vinblastine metabolism by using human liver microsomes. Vinblastine was converted into one major (unidentified) metabolite, and the results of careful experiments showed that vinblastine metabolism is mediated by the CYP3A subfamily, most likely the isoform CYP3A4. The vinca alkaloids vindesine, vincristine and navelbine considerably inhibited the metabolism of vinblastine. Likewise, other anticancer drugs such as doxorubicin, etoposide and teniposide were found to inhibit vinblastine metabolism [70]. Known inhibitors of the CYP3A subfamily such as ketoconazole and erythromycin also inhibited the metabolism of vinblastine in this *in vitro* study.

Knowledge of the metabolites of vinca alkaloids produced in man, their pharmacokinetics and possible contribution to the clinical response is limited. However, vinblastine is metabolized to the biologically active desacetylvinblastine.

The effect of nifedipine on the pharmacokinetics of vincristine has been evaluated by Fedeli *et al.* [18]. Concomitant treatment with nifedipine considerably decreased the clearance of vincristine, and the elimination half-life was about four times as long in the nifedipine treated group than in the control group [18]. Other clinically relevant interactions between vinca alkaloids and CYP3A inducers or inhibitors are likely to occur.

Inhibition of P-glycoprotein

It is of interest to note that the CYP3A substrates shown to influence the pharmacokinetics of some anticancer agents, namely cyclosporin, verapamil and nifedipine, can modulate multi-drug resistance (MDR) to cancer chemotherapy. In MDR, tumour cells become cross-resistant to a wide range of chemically dissimilar agents after exposure to a single (natural product) drug. The cells contain markedly increased levels of P-glycoprotein, a transmembrane drug efflux pump participating in the transport of these drugs out of the cells. MDR is a major factor in the resistance to drugs such as the anthracyclines, epipodophyllotoxins, taxol and vinca alkaloids. Calcium channel blockers and cyclosporin can reverse MDR by inhibiting the active

efflux of anticancer agents from tumour cells by the P-glycoprotein, hence overcoming the resistance of the tumour. These combinations are currently under intense clinical investigation.

Thus, calcium channel blockers and cyclosporin may increase the exposure to anticancer agents not only by inhibiting their metabolism but also acting as P-glycoprotein inhibitors. P-glycoprotein is expressed also in normal tissues, and compounds inhibiting P-glycoprotein and increasing intracellular drug retention in tumour cells may increase retention of the drug in normal tissues, resulting in delayed elimination of the drug from the body and enhanced pharmacological effects. Inhibition of P-glycoprotein mediated biliary drug elimination could, for example, play a role in these interactions. The relative contribution of P-glycoprotein inhibition and inhibition of anticancer drug metabolism by the same compound to the pharmacokinetic and pharmacodynamic consequences of the interaction is difficult to assess. However, inhibition of P-glycoprotein may be the predominant mechanism of these interactions.

Conclusions

The most important and best characterized enzyme of the CYP3A subfamily, CYP3A4, metabolizes many essential drugs. As discussed above, the CYP3A subfamily plays a role in the metabolism of several anticancer agents. It should, however, be emphasized that the metabolism of many essential anticancer agents remains poorly characterized (mainly due to analytical difficulties) and not all P450 enzymes participating in their metabolism have been identified. Therefore, it is usually not known (with the possible exception of ifosfamide and taxol) to which extent CYP3A enzymes contribute to the overall metabolism of specific anticancer agents. However, the role of CYP3A4 may be crucial in many cases since it is the most abundant P450 enzyme in human liver and it is also inducible [71].

Since many inhibitors and inducers of CYP3A are widely used in clinical practice, the potential for interactions between these agents and anticancer drugs is considerable. Furthermore, if anticancer agents that are substrates for CYP3A are used together in combination chemotherapy, the efficacy and toxicity of one or more of the components (or any other concomitantly used CYP3A substrate) may increase as a result of competitive inhibition of metabolism.

It should also be recognized that many anticancer agents have active metabolites which, depending on their potency and pharmacokinetics, may contribute to the clinical response. Inhibition of the metabolism of the (active) parent drug might result in reduced production of an essential active metabolite, and the effects of the parent drug might not be enhanced as much as the increased plasma concentrations would suggest. Therefore, it is difficult to evaluate the clinical significance of pharmacokinetic interactions without pharmacodynamic data.

It is now well accepted that *in vitro* approaches can be used to predict human drug metabolism and drug interactions. Several approaches are available to determine the contribution of different P450 enzymes to each known metabolic pathway of a drug [72, 73]. One important consideration for assessing the possible clinical significance of inhibition of drug metabolism found in *in vitro* studies is the K_i value of the competitive inhibitor (the compound's potency as an inhibitor) and its relationship to clinically achievable free (unbound) plasma concentrations of the inhibitor. If the K_i of an inhibitor is considerably higher than steady-state concentrations in patients, inhibition observed *in vitro* may not occur *in vivo*. In many cases, this approach would enable ruling out a clinically important interaction. However, it should be noted that the degree of inhibition caused by a competitive inhibitor depends not only on the K_i and concentration of the inhibitor but also on the concentration of the substrate and K_m (a constant representing the affinity between drug and enzyme). Furthermore, it is the drug concentration at the enzyme, rather than that in plasma, which is important.

In the light of the role of the CYP3A subfamily in the metabolism of several anticancer agents and the effects of P-glycoprotein inhibitors on their pharmacokinetics, appropriate caution should be exercised when combining other drugs with cancer chemotherapy. Interestingly, many CYP3A4 substrates are P-glycoprotein inhibitors. More clinically important interactions between anticancer drugs and CYP3A inhibitors as well as P-glycoprotein inhibitors are likely to emerge, and further study is required in this field.

The authors' own work has been generously supported by the Dr Mildred Scheel Foundation (grants W87/91/To1 and W90/92/Ei2) and the Robert Bosch Foundation. K.T.K. is supported by the Alexander von Humboldt Foundation.

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(Received 9 May 1995, accepted 23 August 1995)