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ORIGINAL ARTICLE

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Preclinical approach for identifying drug interactions

Abstract Pharmacokinetic drug interactions may be divided into two categories: induction and inhibition of enzymes involved in the metabolism of a drug. The induction and inhibition of such enzymes result in decreases or increases in the blood concentrations of the drug, causing drug effects to be altered. Cytochrome P450 (P450 or CYP) is an enzyme responsible for the metabolism of a wide variety of drugs, including some anticancer agents. If a drug with a high affinity to bind to a specific form of P450 is given to a patient in combination with other drugs mainly metabolized by this enzyme, the former may potentiate the pharmacological actions of the latter by preventing their metabolism and thus increasing their serum concentration. Alternatively, if a drug inhibits or inactivates essentially all forms of P450 nonspecifically, it may be possible that the pharmacological effects of other drugs used in combination with it will be enhanced. CYP3A4 is one of the major forms of P450 in human liver microsomes. In previous studies using human liver microsomes, docetaxel was determined to be metabolized mainly by this isozyme. Thus it was assumed that inducers and inhibitors of CYP3A4 might affect the pharmacokinetics of docetaxel. In our studies, administration of dexamethasone, a known inducer of CYP3A, to mice resulted in decreases in serum docetaxel concentrations. In contrast, ketoconazole, an inhibitor of CYP3A, is assumed to increase the serum and hepatic concentrations of docetaxel. As an example of a drug which might inhibit the metabolism of other drugs, we found that bis-aceto-ammine-dichloro-cyclohexylamine platinum(IV) (JM216), which is currently being developed as a potential anticancer agent, inhibits essentially all major forms of P450 present in human liver microsomes. Since its inhibition potency is relatively high, careful assessment of the effects of this drug on the metabolism of other drugs appears to be necessary.

Key words Cytochrome P450 · Docetaxel · JM216 · Inhibition · Induction

Introduction

Unlike pharmacodynamic interactions, pharmacokinetic drug interactions have been regarded as causing undesired drug effects. In most patients, multiple drugs are prescribed in combination to potentiate the desired effects or to diminish the side effects of other drugs used in the combination. Thus pharmacodynamic drug interactions are in principle necessary for efficient drug treatment. However, pharmacokinetic drug interactions also occur in some of the combinations of drugs prescribed.

Factors affecting pharmacokinetic drug interactions include drug absorption, metabolism, and excretion. Among these, the induction and inhibition of enzymes involved in drug metabolism have been regarded as major factors causing significant alterations to drug effects. For example, sorivudine, an antiviral agent, has been widely used to treat cancer patients also receiving 5-fluorouracil (5-FU) derivatives. Sorivudine irreversibly binds to and inhibits dihydropyrimidine dehydrogenase, an enzyme which detoxifies 5-FU [5]; therefore coadministration of sorivudine with 5-FU inhibited detoxification of 5-FU causing 5-FU to accumulate in the body. Fifteen cancer patients died in Japan due to this interaction [16].

Cytochrome P450 (P450 or CYP) is an enzyme responsible for the metabolism of a wide variety of foreign

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Table 1 P450 isozymes involved in the metabolism of anticancer drugs

CYP	Anticancer drug metabolized
2A6	Tegafur
2B6	Ifosfamide, cyclophosphamide
2C8	Paclitaxel
2D6	Tamoxifen
3A4	Ifosfamide, etoposide, cyclophosphamide, tamoxifen, docetaxel, teniposide, paclitaxel, vinca alkaloids, retinoic acid

chemicals including drugs [3]. A number of isozymes of P450 are known to exist, and these are classified into families and subfamilies [10]. While only some of the classic anticancer agents are metabolized by this enzyme, increasing numbers of new anticancer agents are now known to be metabolized by P450. Administration of drugs which induce or inhibit P450 may affect the metabolism of any of these anticancer drugs if they are prescribed in combination, and thus pharmacokinetic drug interactions can be expected to occur. In this review, we focus on preclinical studies of drug interactions caused by the effects of drugs on the metabolism of an anticancer agent and by an anticancer agent.

Anticancer agents metabolized by P450

As shown in Table 1, only a few anticancer agents have been proven to be metabolized by P450. In vitro studies using human liver microsomes have indicated that tegafur is metabolized by CYP2A6 to yield 5-FU, although the binding affinity of this drug for CYP2A6 is low. Thus it remains to be elucidated whether this drug is metabolized by CYP2A6 in vivo. However, if tegafur is metabolized by CYP2A6 in vivo, caution is required when administering it because approximately 3% of the Japanese population is deficient in CYP2A6 activity [6, 11].

Cyclophosphamide is known to be metabolized by CYP2B6 to yield the active metabolite 4-hydroxycyclophosphamide. Thus inducers of this P450, such as phenobarbital, should affect the pharmacokinetics and the effectiveness of this drug. Clinical trials to clarify the effects of phenobarbital treatment on the effectiveness of cyclophosphamide have been performed and supported the theoretical conclusion [6]. However, more recent studies have indicated that phenobarbital is also a carcinogenic agent and therefore further studies could not be done [12].

Of the various P450 isozymes, CYP3A4 is known to be present in abundance in human liver microsomes [14]. Many important anticancer agents are metabolized by CYP3A4, which is induced by glucocorticoids [2, 17] and inhibited by antifungal azole derivatives such as ketoconazole [9]. Interestingly, CYP3A4 is induced and inhibited by 14-member macrolides. CYP3A4 induced by 14-member macrolides also metabolizes these agents, producing reactive metabolites which subsequently bind to the heme portion of the cytochrome, resulting in its inhibition [7].

Recent studies have revealed that CYP3A4 and p-glycoprotein have many common substrates [15]. Azole compounds, cyclosporin, calcium blockers, and many other drugs known to be substrates for CYP3A4 in human liver microsomes are also substrates of p-glycoprotein, which is known to cause drug resistance [13]. Further studies are needed to confirm that anticancer agents such as docetaxel, which are metabolized by CYP3A4, can be substrates for p-glycoprotein.

Docetaxel metabolism may be affected by drug interactions

Catalysis of docetaxel metabolite formation

Preliminary studies with human liver microsomes have indicated that docetaxel is metabolized mainly by CYP3A4 [8]. To confirm this result and to clarify the in vivo significance of the involvement of CYP3A in the metabolism of this drug, we performed experiments using mouse models. In vitro experiments using mouse liver microsomes showed that docetaxel is metabolized to produce two main metabolites, M1 and M2 [unpublished results]. The Km values for M1 and M2 were determined to be 0.38 μM and 4.14 μM, respectively, indicating that M1 formation occurs preferentially in mice. The Vmax values for M1 and M2 formation were determined to be 63.6 pmol/mg/min and 4.21 pmol/mg/min, lending support to the concept that M1 formation occurs preferentially.

To confirm that the formation of M1 and M2 is catalyzed by CYP3A isozymes in mouse liver, the effect of antibodies to CYP3A2, a form of CYP3A found in rats, was examined. According to the sequence homology between CYP3As in mice and rats, the antibodies to CYP3A2 were expected to bind to CYP3A in mice. M1 and M2 formation in mouse liver microsomes was inhibited by the antibodies [unpublished results]. Thus, we confirmed that M1 and M2 formation is catalyzed mainly by CYP3A in mouse liver microsomes.

To confirm that human CYP3A4 in human liver microsomes can catalyze M1 and M2 formation, the ability of human CYPs to metabolize docetaxel was examined using genetically engineered B-lymphoblastoid cells expressing human CYPs. The results showed that only CYP3A4 could catalyze M1 and M2 formation [unpublished results].

Drugs affecting docetaxel metabolism

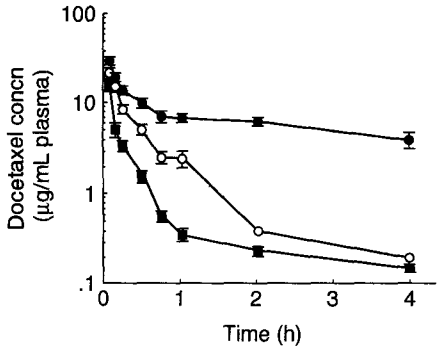
A number of inducers and inhibitors of CYP3A are known, with representative inducers being dexamethasone, erythromycin, and rifampicin, and inhibitors being 14-member macrolide antibiotics such as erythromycin and triacetyloleandomycin and azole antifungal agents such as ketoconazole and miconazole. As shown in Table 2, inhibitors of CYP3A inhibit the formation of the M1 and M2 metabolites [unpublished data]. Thus CYP3A in liver microsomes is the sole enzyme responsible for M1 and M2 formation.

Table 2 Effects of CYP inhibitors on M1 and M2 formation in mouse liver microsomes (*ND* not detectable, – not determined)

Inhibitor	CYP	M1 formation		M2 formation
		IC_{50} (μM)	Ki (μM)	IC_{50} (μM)
Caffeine	1A	>50	–	>50
Coumarin	2A	>50	–	>50
Sulfaphenazole	2B	>50	–	>50
7-ethoxycoumarin	2C	>50	–	>50
Propranolol	2D	>50	–	>50
Quinidine	2D	>50	–	>50
Chlorzoxazone	2E	>50	–	>50
Cyclosporin	3A	>50	–	>50
Erythromycin	3A	>50	–	>50
Ketoconazole	3A	0.54	0.18	0.32
Miconazole	3A	6.0	3.07	7.5
Midazolam	3A	7.5	2.40	ND
Troleandomycin	3A	0.45	0.85	0.20

Table 3 Inhibitory effects of various drugs on deocetaxel metabolism

Drug	M1 formation IC_{50} (μM)	M2 formation IC_{50} (μM)
Cisplatin	>100	>100
Cyclophosphamide	>100	>100
Doxorubicin	9.7	60.0
5-FU	>100	>100
Medroxyprogesterone	88.2	88.2
Mitomycin C	>100	>100
Tamoxifen	>100	>100
Dexamethasone	>100	>100
Indomethacin	>100	>100



Treatment	AUC	$t_{1/2\alpha}$	$t_{1/2\beta}$	MRT
	mg · h/mL	hL	hL	hL
None	8.0	0.24	0.92	0.63
Ketoconazole	29.5	0.32	1.17	1.84
Dexamethasone	3.3	0.16	1.08	0.35

Fig. 1 Changes in plasma docetaxel concentrations after pretreatment of mice with ketoconazole or dexamethasone. ○ untreated; ● ketaconazole treated; ■ dexamethasone treated

Because anticancer agents are often used in combination the possibility of drug interactions between docetaxel and other anticancer agents was examined. As shown in Table 3, of the anticancer agents tested only doxorubicin inhibited M1 and M2 formation. However, the IC^{50} value was 9.7 μM, suggesting that doxorubicin would probably have a minor effect if any interaction does occur.

The in vivo effects of the antifungal agent ketoconazole and the steroid dexamethasone on the pharmacokinetics of docetaxel in mice were studied because these agents significantly inhibit and induce docetaxel metabolism, respectively, in vitro [unpublished results]. As shown in Fig. 1, pretreatment of mice with ketoconazole and dexamethazone resulted in enhanced and decreased plasma AUCs for docetaxel, respectively. Thus it was confirmed that these agents which inhibit and induce CYP3A, respectively, also affect the pharmacokinetics of docetaxel. Similar increases and decreases in liver docetaxel concentrations were also seen (data not shown).

We also examined the effects of dexamethasone on the formation of M1 and M2 in vivo. Mice were administered dexamethasone 75 mg/kg i.p. and the plasma concentrations of M1 and M2 were measured periodically. The plasma concentrations of both M1 and M2 decreased after dexamethasone administration, indicating that M1 and M2 produced due to the activity of P450 are further metabolized by an enzyme induced in the mouse liver. In contrast, administration of ketoconazole 50 mg/kg ip resulted in increases in plasma M1 and M2 concentrations, suggesting that CYP3A in mouse liver probably further metabolizes M1 and M2 to an unknown metabolite(s).

The results described above indicate that CYP3A induction and inhibition affect the pharmacokinetics of docetaxel and alter the anticancer potency of this drug.

Nonspecific inhibition of P450 by bis-aceto-ammine-dichloro-cyclohexylamine platinum(IV), a novel oral anticancer agent: A possible drug interaction

Bis-aceto-ammine-dichloro-cyclohexylamine platinum(IV) (JM216) is a novel anticancer agent currently under development for clinical use [4]. The advantage of JM216 is that it can be administered orally. The purpose of our study was to examine whether JM216 interacts with other drugs that are likely to be administered in combination with it. To predict possible drug interactions, varying concentrations of JM216 were added to an incubation mixture containing human liver microsomes, a probe drug metabolized by a specific form of human P450, and other components, and incubated aerobically at 37 °C for 15 min. The effect of JM216 on the metabolism of paclitaxel is shown in Fig. 2. JM216 inhibited the metabolism of paclitaxel in a noncompetitive manner with an inhibition constant of 0.9 μM and an IC_{50} of approximately 2 μM, indicating that JM216 will potentially inhibit the metabolism of paclitaxel. The inhibition of P450 isozymes by JM216 and its effect on substrates of these isozymes of P450 are shown in Table 4. JM216 inhibited essentially all metabolic reactions examined, although the inhibition potency varied. These results suggest that JM216 inhibits the activity of P450 in a noncompetitive manner. Although the mechanism of inhi-

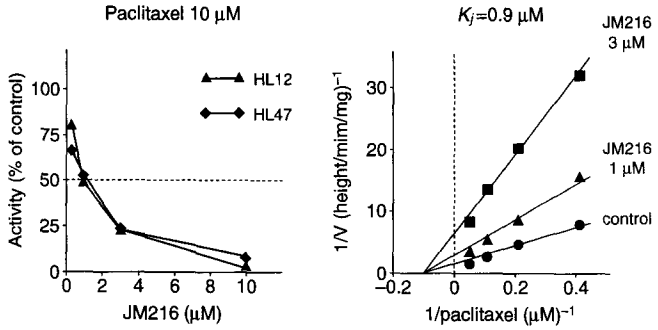


Fig. 2 Inhibition of paclitaxel metabolism in human liver microsomes by JM216. Liver microsomes from human subjects (HL12 and HL47) were incubated with paclitaxel in the presence of various concentrations of JM216. Paclitaxel activity with varying JM216 concentrations (*left*) and Lineweaver-Burk plots (*right*) are shown

Table 4 Nonspecific inhibition of P450 isozymes by JM216

CYP	Substrate	IC_{50} (μM)
1A	7-Ethoxyresorufin	3–10
2A6	Coumarin	1–3
2C8	Paclitaxel	1–3
2D6	(±)-Bufuralol	0.3–1
2E1	Aniline	1–3
3A4	Testosterone	0.3–1

bition is not known, JM216 may inhibit the activity of P450 by interacting with its heme moiety, which is critical to the activatation of molecular oxygen to oxidize substrates.

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