STRATEGIC PROPOSALS TO AVOID TOXIC DRUG INTERACTIONS DURING NEW DRUG DEVELOPMENT: MOLECULAR TOXICOLOGY PROPOSALS

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Introduction

Pharmacokinetic drug interactions can be caused by many factors including drug absorption, distribution, metabolism and excretion. Among these steps, metabolism is regarded as the most important factor which affects the concentration of drugs in the body.

While the pharmacokinetic drug interactions have been regarded as one of the main causes of drug interactions, it was not recognized as important in causing toxic drug effects until about 10 years ago. Looking at books on drug interactions (Am. Pharm. Asso., 1976; Kato *et al.*, 1979) published about 10 years ago, one sees that the pharmacodynamic rather than pharmacokinetic drug interactions were considered at that time: only a few examples caused by pharmacokinetic drug interactions were described.

There are several reasons why medical accidents caused by drug interactions occur: are clinical drug interactions due to 1) insufficient basic research ?, 2) lack of transfer of information ?, 3) insufficient knowledge and recognition of the importance of drug interactions ? Perhaps the most important thing to consider is the recognition of the importance of drug interactions. In the present paper, we would like to describe our current efforts looking at the basic methods to prevent possible medical accidents caused by drug interactions.

1) Implications of *in vitro* data for the *in vivo* situation with taxotere in mice

Taxotere is an antitumour agent currently under development for clinical use. The chemical structure of this drug is shown in Fig. 1. In a preliminary study (Francoise *et al.*, 1996) it was shown that this drug was metabolized by CYP3A in liver microsomes, while *in vivo* drug interactions were not examined. Thus, we initiated this study to discover if this drug was metabolized by cyp3a in mice *in vitro*, and if the inhibitors of cyp3a, such as ketoconazole and some other azole derivatives, interact with this drug to produce increased blood level. As shown in Fig. 2, antibodies to rat CYP3A2 inhibited potently the conversion of taxotere to metabolite M1 and M2 with amounts of antiserum. Although not shown, the representative inhibitors of CYP3A, such as ketoconazole and troleandomycin, also showed strong inhibition of the metabolism of taxotere at an order lower than μM . As expected, pretreatment of mice with ketoconazole prior to taxotere increased the AUC of this drug about 3.7-fold, and pretreatment of mice with dexamethazone, a known inducer of cyp3a reduced the AUC of taxotere by about 60%.

Whether CYP3A was also involved in the metabolism of taxotere in humans was examined. Taxotere was incubated with B-lymphoblast cells expressing human cytochrome P450. The results showed that only CYP3A expressed in B-lymphoblast cells was capable of metabolizing taxotere, suggesting that the same drug interaction seen in mice occurs in humans.

Fig. 1. Chemical structure of Taxotere.

Fig. 2. Immunoinhibition of Taxotere metabolism in mouse liver microsomes by rabbit anti-rat CYP3A2.

2) Possible drug interactions with histamine H1 antagonists

We found that promethazine, a histamine H1 antagonist, is metabolized mainly by CYP2D6 (Nakamura *et al.*, 1996). The major metabolic pathway of promethazine is shown in Fig. 3. Comparison of the K_m values for the major metabolic pathways revealed that the most important metabolic pathway was ring hydroxylation of promethazine. This ring hydroxylation was catalyzed almost exclusively by CYP2D6 as proved by experi-

ments using CYP preparations expressed in B-lymphoblast cells. These results suggest that promethazine will also be a drug which causes drug interactions when used with a drug metabolized mainly by CYP2D6 (Fig. 4). Many histamine H1 antagonists possessing chemical structures similar to promethazine have been widely used clinically (F. Estelle *et al.*, 1994). Thus, to know if promethazine inhibited the metabolism of other drugs and if histamine H1 antagonists, in addition to promethazine, were capable of inhib-

Fig. 3. Proposed metabolic pathways of Promethazine in human liver microsomes.

Fig. 4. Metabolism of promethazine by specific human CYPs expressed in B-lymphoblastoid cells.

iting metabolism we used as a probe bufuralol, known to be metabolized by CYP2D6. The results indicated that alimemazine, mequitazine and some other histamine H₁ antagonists inhibited the 1'-hydroxylation of the drug interaction between these histamine H₁ antagonists and a drug metabolized mainly by CYP2D6.

3) *In vitro* test system to predict drug interactions

Drug interactions can occur by many

mechanisms including drug absorption, distribution, metabolism and excretion. Thus, it is necessary to develop methods for predicting drug interactions caused by these mechanisms. Focusing on pharmacokinetic drug interactions, the *in vitro* test system is required to predict essentially all possible drug interactions. To predict drug metabolism and possible drug interactions in humans, most human liver samples being used in drug metabolism studies have to be imported from abroad. Human liver samples are a useful tool to pre-

dict drug interactions in humans. However, these samples should be available in Japan and should not need to be imported to avoid any international problems. The use of human enzymes generated by means of gene technology is recommended but this has limitations since there is no guarantee of the usefulness of enzymes produced for this purpose and such enzymes are expensive.

4) Recent progress in the methodology for predicting human drug metabolism

We are now developing a practical system to predict drug interactions in humans based on drug metabolism. As mentioned above, competition of substrates for the same enzyme is one of the major causes of drug interactions. Thus, we have developed a new method to determine the P450 form(s) involved in the metabolism of a drug, using recombinant *E. coli* in which a human P450 is expressed together with human P450 reductase. If we expect that a new drug under development is metabolized by the enzyme expressed in *E. coli*, the test compound will be added to the culture medium at low (probably 1 μ M or lower) and high (1 mM or higher) concentrations. The metabolites obtained from the culture containing the low concentration of a particular drug may give information that the same metabolite is formed in the body. The chemical structure of the same metabolite(s) can be determined by collecting the metabolite from the culture incubated with the high concentration of drug. The metabo-

lite-forming activity can be estimated using small amounts of human liver if needed.

Once the form of P450 involved in the metabolism of the drug is determined, then possible drug interactions can be predicted. This system will be completed within a year and will be used in the development of new drugs.

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