A PROPOSAL FOR AVOIDING TOXIC INTERACTIONS OF A DRUG UNDER DEVELOPMENT TO ESTIMATE INHIBITORY ABILITY ON THE DRUG METABOLIZING ENZYMES IN HUMAN LIVER

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Abstract – Drug interactions can be divided into those involving the pharmacokinetics of a drug and those affecting the pharmacodynamic response to it. Following strategies should be considered for avoiding pharmacokinetic drug interactions of new chemical entities under development.
1. To estimate the major metabolic pathways (including metabolic activation) using human liver preparations.
2. To identify the enzyme systems participating in the major metabolic pathways.
3. To examine metabolic inhibition for other drugs and by other drugs.
4. To confirm a pharmacokinetic effect for the combined drugs in vivo.

Introduction

It has been known that the combination of two or more drugs often produces therapeutic or toxic results which may be quite different from what would be expected knowing the pharmacological action of each of the single compounds involved. Thus, it has been recognized that certain drugs may interact with others in the organism, either enhancing or abolishing their beneficial or adverse effects. A great number of drugs have been shown to decrease hepatic drug metabolism by inhibiting the drug metabolizing enzymes. It is well documented that the dosing of such drugs may increase the plasma concentration of other drug. Some example of inhibited drug metabolism are of considerable clinical importance. Several cases of severe, even life-threatening, drug intoxication due to interactions have been observed. A number of drugs have been shown to increase hepatic drug metabolism by enzyme induction. The administration of such drugs may accelerate the metabolism of other drugs, and the therapeutic effects may abolished. Metabolic drug interactions are most likely to be important if elimination is by a single process; they are less relevant if two or more routes are available, unless the alternative pathways are saturable or give rise to toxic products. Drug metabolizing enzymes are present in multiple forms. Many drugs are metabolized by more than one form and the same metabolic step can be catalyzed by different isoenzymes. Thus, induction or inhibition of metabolism will occur only if both drugs bind to the active site of the same forms of the enzyme.

Enzyme Induction: The most powerful enzyme inducers in clinical use are the antibiotic rifampicin, and the anticonvulsants phenobarbitone, phenytoin, carbamazepine and pirimidone. As protein synthesis is required, the maximum effect is not seen for two or three weeks. The clinical result is increased metabolism of the target drug with concomitant attenuation of its pharmacological effect. Conversely, when the inducer is withdrawn the process goes into reverse with a decline in the number of enzymes, again over two- to three-week period, resulting in a gradual increase in the plasma concentration of the affected agent with potential risk of toxicity.
**Enzyme Inhibition:** Most, but not all, inhibitory interactions concern hepatic monoxygenase enzymes. Plasma concentration of the target drug are maximal about five half-lives after its metabolism has been partially inhibited when a new steady-state is achieved. Thus, potentiation of pharmacological effects can occur very quickly for drug with a short half-life but much more slowly for those with longer elimination periods. Commonly prescribed enzyme inhibitors include erythromycin, cimetidine, sodium valproate, dextroproproxyphene, oral contraceptives, propranolol and some tricyclic antidepressants, phenothiazines and sulphonamides. When an inhibitory drug is withdrawn, plasma concentrations fall over five half-lives with consequent reduction in therapeutic effect.

Theophylline

Phenytoin markedly stimulates theophylline elimination leading to increased dosage requirements\(^9\). Tobacco smoking also increases theophylline clearance\(^9\). The enzyme-inducing effect of hydrocarbons in burning tobacco appears to be exceptionally long lasting, persisting for up to three months after discontinuation of the habit. These changes are accompanied by a concentration-related reduction in clinical efficacy\(^9\).

In children, erythromycin may provoke unexpected nausea and vomiting, associated with a sudden increase in theophylline serum concentrations\(^9\). The similar macrolide antibiotic triacyctyleandomycin, produces 50% inhibition of theophylline clearance and corresponding increase in half-life\(^9\). As with phenytoin, non-linear metabolism at higher theophylline concentrations may account for its propensity to be involved in clinically relevant inhibitory interactions\(^9\).

Urinary metabolites of theophylline, 1,3-dimethyluric acid (32-55% of dose), 3-methylxanthine (9-30%) and 1-methyluric acid (14-26%), together with unchanged theophylline (8-17%), account for about 90% of the administered dose being excreted by human urine within 48 hours\(^7\). Salyers, Barr and Sipes\(^8\) were reported the urinary excretion of theophylline (27% of dose) and its metabolites, 1,3-dimethyluric acid (34%), 1-methyluric acid (21%) and uric acid (1.6%), after dosing to rat. They did not detected 3-methylxanthine in the rat urine. They also studied the metabolism of theophylline using precision-cut liver slice system from human and rat. Rat liver slices produced 1,3-dimethyluric acid, 1-methyluric acid and uric acid. Human liver slices produced the same metabolites, as well as 3-methylxanthine.

![Fig. 1. Metabolic pathways of theophylline in human.](image-url)
These results suggest that the in vitro metabolism studies using human liver preparations are very useful tools for the prediction of the metabolic pathways in human at the early phase of new drug development process.

Shimada, Green and Chiba\(^9\) characterized the enzyme catalyzed each metabolic pathway of theophylline in human. They used 4 approaches to reaction phenotyping. The first is correlation analysis of the formation rate of 1,3-dimethyluric acid, 3-methylxanthine and 1-methylxanthine by 14 samples of human liver microsomes and correlating reaction rates of acetaminophen (CYP1A2), 1'-hydroxybufuralol (CYP2D6), 6-hydroxychlorozoxzone (CYP2E1), 6β-hydroxytestosterone (CYP3A4). The second and third approaches are inhibition studies by specific antibody and specific chemical inhibitors for each enzyme. The fourth approach is metabolism by c-DNA-expressed human P450 enzymes: they measured the formation rate of each metabolite by specific cytochrome P450s expressed in lymphoblastoid cell lines (CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2D6, CYP2E1 and CYP3A4). Finally, they concluded the formation of 1,3-dimethyluric acid, 3-methyl-xanthine and 1-methyl-xanthine are mainly catalyzed by CYP1A2 in human liver.

Sorivudine

5-Fluorouracil is metabolized by dihydouracil dehydrogenase contained in liver cytosol fractions. Various inhibitors of the metabolism of 5-fluorouracil have been developed with a view to enhancing its therapeutic activity. These inhibitors include uracil analogues such as 5-ethyluracil, 5-iodouracil, 5-nitrouracil, 5-bromouracil and 5-cyanouracil. Most of these compounds are competitive inhibitors of 5-fluorouracil metabolism. Recently there have been deaths due to the coadministration of 5-fluorouracil with the antiviral drug sorivudine, 1β-arabinofuranosyl-(E)-5-(2-bromovinyl)uracil, whose major metabolite is 5-bromovinyluracil. 5-Bromovinyluracil is a potent inhibitor of 5-fluorouracil metabolism in rat but it is an irreversible inhibitor. Reigh et al.\(^10\) reported that the inhibitory effects of 5-bromovinyl uracil on the metabolism of 5-fluorouracil using human liver cytosol to clarify the inhibition of dihydouracil dehydrogenase.

![Fig. 2. Inhibitory effect of uracils on the metabolism of 5-fluorouracil.](image-url)
Table 1. Kinetics of 5-FU metabolism

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<th>Rat</th>
<th>Human</th>
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<tr>
<td>Km (µM)</td>
<td>59.2</td>
<td>25.6</td>
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<tr>
<td>Vmax (µM/min/mg)</td>
<td>0.877</td>
<td>0.275</td>
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<tr>
<td>Ki for 5-iodouracil (µM)</td>
<td>15.6</td>
<td>4.2</td>
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<tr>
<td>IC50 for BVU* (µM)</td>
<td>9.2</td>
<td>3.0</td>
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Rat liver cytosol (200 µl, 30mg protein/ml), human liver cytosol (200 µl, 44.4mg protein/ml) was incubated with 5-fluorouracil
* The cytosol was preincubated for 10 min with 5-bromovinyluracil

also occurs in the human liver preparation. In the case of sorivudine it appears to be it’s metabolite, rather than the drug itself, which is inhibiting the dihydrouracil dehydrogenase.

Cyclosporin

The immunoregulating agent cyclosporin is a lipid-soluble drug with dose-dependent nephrotoxic effects. Rifampicin has been shown to substantially accelerate its metabolism and may precipitate rejection episodes. Ketoconazole, diltiazem, erythromycin and danazol may all inhibit cyclosporin breakdown and put the patient’s renal allograft at risk. Cyclosporin itself may turn out to have inhibitory properties. Cyclosporin is oxidized in rabbit and human liver by CYP3A4. Pichard et al. demonstrated a screening for inducers and inhibitors of cyclosporin A oxidase in primary cultures of human hepatocytes and liver microsomes. They detected drugs that should interfere with cyclosporin A metabolism as inducers or inhibitors. Drugs detected as potential inducers of cyclosporin A oxidase included: rifampicin, sulfadimidine, phenobarbital, phenytoin, phenylbutazone, dexamethasone, sulfapyrazone and carbamazepine. Drugs detected as potential competitive inhibitors included: triacetyloleandomycin, erythromycin, josamycin, midecamycin, ketoconazole, miconazole, midazolam, nifedipine, diltiazem, verapamil, nicardipine, ergotamine, dihydroergotamine, glibenclamide, bromocryptine, ethynylestradiol, progesterone, cortisol, prednisone, prednisolone and methyl-prednisolone.

References


