MECHANISM-BASED INHIBITION OF CYP3A4 BY TAO AND MIFEPRISTONE, AND CYP2D6 BY PAROXETINE, IN HUMAN LIVER MICROSOMES

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Clinical drug-drug interactions (DDI) can lead fatal side effects, which have resulted in withdrawal of drugs from the market, or early termination of development, et cetera. We have previously developed in vitro DDI procedure, that meets the PhRMA Perspective (J Clin Pharmacol 2003,43:443), for seven CYPs. However, DDI predicted from in vitro data based on competitive models can underestimate the true interaction that could occur in vivo in the presence of a mechanism-based inhibitor, which, by the definition, is a compound that is metabolized by the enzyme into a species that acts as an affinity labeling agent, a transition state analogue, or a tight-binding inhibitor (either covalent or noncovalent) prior to release from the enzyme. Accumulated data on clinical DDI clearly indicate that such time-dependent inhibition can increase the co-medicated drug’s AUC more than 10 folds to result in a serious clinical incidence. We constructed the procedure for Mechanism-Based Inhibition (MBI) of CYP3A4 and CYP2D6 with human liver microsomes. In the current paper, we present MBI of CYP3A4 by troleandomycin (TAO) and mifepristone in time- and concentration-dependent manner, measuring both activities of midazolam 1'-hydroxylation and testosterone β-hydroxylation. Ketoconazole is used as a non-MBI control. MBI of CYP2D6 will be characterized measuring 1'-hydroxylation of bufuralol in the presence of paroxetine, using quinidine as a non-MBI control. $K_{\text{inact}}$ (min$^{-1}$) and $K_I$ ($\mu$M) values of TAO and mifepristone from MBI of CYP3A4, and paroxetine from MBI of CYP2D6 are calculated.

DANAZOL AND ITS METABOLITES ACT AS MECHANISM-BASED INACTIVATORS IN MICE.

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We have previously investigated the inhibition of CYP3A by danazol (DNZ), gonadotropin inhibitor, by using rat liver microsomes and found that not only DNZ but also its metabolites were mechanism-based inactivators of CYP3A. Objective of present study was to examine the effect of DNZ and its metabolites on the inhibition of CYP3A in vivo. DNZ and five metabolites of DNZ, namely, 2-aminomethylethisterone (M1), 2-formylethisterone (M2), 2-hydroxymethylethisterone (M3), $\Delta^1$-2-hydroxymethylethisterone (M4) and ethisterone were studied. DNZ or its metabolites (30mg/kg) were orally administered to male ddY mice (6-7w) and livers were excised at appropriate time after administration. CYP3A activity assessed by midazolam 1-OH hydroxylation was determined in microsomes prepared from the livers. CYP3A activity was decreased 70% at 6h after administration of danazol. The activity was also decreased to 50-90% at 6h after administration of these metabolites. Similar results were obtained from mice after intravenously administration (10mg/kg). Plasma concentration of DNZ was rapidly decreased after intravenously administration and only 5% of Cmax value at 2h. M1 was a major metabolite detected in plasma and its concentration became higher than danazol at 1h after administration. M3 and M4 were also detected in plasma. These results indicated that the metabolites of danazol had inhibitory effects on CYP3A and contributed to the danazol-induced decrease in CYP3A activity in vivo.