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The Quantitative Prediction of In Vivo Enzyme-Induction Caused by Drug Exposure from In Vitro Information on Human Hepatocytes

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Summary: There have been no reports of the quantitative prediction of induction for drug-metabolizing enzymes in humans. We have tried to predict such enzyme induction in humans from *in vitro* data obtained using human hepatocytes. The *in vitro* and *in vivo* data on enzyme induction by inducers, such as rifampicin, phenobarbital and omeprazole, were collected from the published literature. The degree of enzyme induction in humans was compared with that predicted from *in vitro* data on human hepatocytes. Using the *in vivo* data, we calculated the hepatic intrinsic clearance of typical CYP substrates, such as midazolam and caffeine, before and after inducer treatment and estimated the induction ratios of hepatic intrinsic clearance following treatment. In the *in vitro* studies, the amount of mRNA or enzyme and enzyme activity in human hepatocytes, with or without an inducer, were compared and the induction ratios were estimated. The unbound mean concentration was taken as an index of drug exposure and the induction ratios in the *in vivo* and *in vitro* studies were compared. The unbound mean concentrations of inducers used in the *in vitro* studies were higher than those in the *in vivo* studies. The maximum induction ratios by inducers in the *in vitro* studies were higher than those in the *in vivo* studies. The induction ratio for rifampicin, omeprazole, troglitazone, dexamethasone and phenobarbital increased as the unbound mean concentration increased to reach a constant value. The induction of CYP3A and 1A was analyzed by the Emax model. The maximum induction ratio (Emax) and the concentration at half maximum induction (EC50) for rifampicin, omeprazole, troglitazone, dexamethasone and phenobarbital were 12.3, 0.847 μmol/L, 2.36, 0.225 μmol/L, 6.86, 0.002 μmol/L, 8.30, 9.32 μmol/L, and 7.62, 58.4 μmol/L, respectively. The Emax and EC50 of omeprazole for CYP1A were 12.02 and 0.075 μmol/L, respectively. The predicted induction ratio of all those inducers, except for omeprazole, based on the Emax and EC50 values obtained from the *in vitro* data were similar to the observed values. On the whole, a good correlation between the observed and predicted induction ratio of omeprazole was observed ($r = 0.768$, $p < 0.05$), although the predicted induction ratio was higher than the observed value. In conclusion, the present study suggests that it is possible to predict quantitatively the CYP3A enzyme induction from hepatocyte data.

Key words: enzyme induction; hepatocyte; CYP3A; CYP1A

Introduction

Many drug-drug interactions caused by co-administration of drugs in clinical situations have been reported. These involve inhibition and induction of drug metabolizing enzymes. Enzyme inhibition causes adverse effects increasing the drug concentration while enzyme induction causes reduced efficacy by lowering the drug concentration. The prediction of drug-drug interactions during the drug discovery stage would help avoid such interactions. Although the use of human microsomes and recombinant CYP enables the prediction of enzyme inhibition,[1,2] no prediction method for enzyme induction has been established yet. Enzyme induction has been estimated using animals treated with the drug candidates and animal studies need relatively

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large amounts of candidates and, hence, such studies are not suitable for screening. In addition, it has been reported that there are species differences in enzyme induction.[3] Therefore, enzyme induction in humans has been estimated using primary cultured human hepatocytes.[4–7] Recently, it has become clear that the induction of CYPs contributes to a variety of nuclear receptors and a reporter gene assay of nuclear receptors has been developed for enzyme induction.[8–12] Although it is possible to estimate the enzyme induction potency of a candidate drug using human hepatocytes, quantitative prediction of enzyme induction in clinical situations has not been possible. Omeprazole and lansoprazole induce CYP1A and 3A in hepatocytes[13] and omeprazole is metabolized by CYP2C19 and CYP3A. The AUC of omeprazole in poor metabolizers of CYP2C19 is 4-fold greater than that in extensive metabolizers. Omeprazole induces CYP1A in poor metabolizers but not in extensive metabolizers at clinical dosages.[14,15] If a drug has the ability to induce an enzyme and there is low exposure to the drug in clinical situations, treatment with the drug will not induce the enzyme. The quantitative prediction of enzyme induction is important for successful drug development and the present study was carried out to investigate whether enzyme induction can be quantitatively predicted using human hepatocytes.

Methods

Data collection: Data on the human pharmacokinetics of a number of drugs metabolized by CYPs after treatment with inducers, rifampicin, omeprazole, lansoprazole, carbamazepine, dexamethasone, phenobarbital, troglitazone, phenytoin, and sulfinpyrazone, were collected[16–37] along with induction data of those drugs in human hepatocytes.[4–7, 38–45]

Calculation of *in vivo* data: The increase in the ratios of the hepatic intrinsic clearances of typical CYP substrates for CYP1A and 3A were calculated. In the case of intravenous data, the total body clearance of substrate was calculated from the AUC using equation 1. For oral administration, CL was calculated from the elimination half-life. The pharmacokinetic model for specific substrates was assumed to be the one compartment model and the distribution volume and renal clearance were assumed not to be affected by treatment with inducers. These drug parameters were obtained from Goodman & Gilman's textbook, The Pharmacological Basis of Therapeutics, 9th ed..[46]

$$CL = Dose/AUC \quad (1)$$

$$CL = 0.693\ Vd/T_{1/2} \quad (2)$$

The hepatic clearance (CLh) was estimated from the CL and renal clearance (CLr) using equation 3.

$$CLh = CL\text{-}CLr \quad (3)$$

Clint was calculated using the following equation based on the well-stirred model.

$$CLint = CLh\ Qh\ Rb/\{fp\ (Qh\text{-}CLh)\} \quad (4)$$

where Qh, Rb and fb are the blood flow rate, blood to plasma concentration ratio and blood unbound fraction, respectively. Rb and Qh were assumed to be 1 and 1610 mL/min, respectively.

The pharmacokinetic parameters of rifampicin, omeprazole, lansoprazole, carbamazepine, dexamethasone, phenobarbital and sulfinpyrazone in humans were also obtained from Goodman & Gilman's Textbook.[46] The parameters of troglitazone were obtained from the report by Izumi *et al.*.[47]

The mean unbound plasma concentrations (Css,u) were calculated from equation 5.

$$Css,u = fp\ dose/CL/T = fp\ AUC/T \quad (5)$$

where fp and T are the plasma unbound fraction and dosage interval.

Calculation of *in vitro* data: The induction ratios of mRNA (RT-PCR), enzyme activity (testosterone 6-beta hydroxylation activity etc.) and enzyme amount (Western blot) for CYP without an inducer to the values with an inducer were calculated. The mean unbound plasma concentrations (Css,u) in medium were calculated to compare the induction ratios at the same concentration under *in vivo* and *in vitro* conditions. The Css of an inducer was calculated by dividing the AUC by the medium change interval. The hepatic intrinsic clearance was calculated from equations 3 and 4. Body weight, liver weight, hepatic blood flow rate, cell number/g liver and Rb were assumed to be 70 kg, 1695 g, 0.95 mL/min/g, 120×10^6 cells/g liver and 1, respectively. These values were used and the intrinsic clearance per cell (CLint, cell) was obtained.

The intrinsic clearance per well (CLint,well) was calculated by multiplying CLint,cell by the cell number per well. The elimination rate constant (k) of an inducer from medium was calculated from equation 6.

$$k = fm\ CLint,well/V \quad (6)$$

where fm and V are the unbound fraction in the medium and medium volume.

It was assumed that there was no difference between the binding of an inducer to human albumin and bovine albumin. The nPt/Kd was calculated using equation 7 from the human plasma unbound fraction. Pt, n and Kd are the protein concentration, number of binding sites and dissociation constant, respectively. The human albumin concentration (M.W.:67,000) was assumed to be 500 μmol/L. The unbound fraction in medium was calculated by correcting human nPt/Kd by the ratio of the bovine albumin concentration in medium to the human albumin concentration in plasma (a) using