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

Motohiro Kato¹, Koji Chiba², Masato Horikawa³ and Yuichi Sugiyama⁴

¹Chugai Pharmaceutical Co. Ltd., Gotemba, Japan
²Pfizer Japan Inc., Tokyo, Japan
³Nissan Chemical Industries Ltd., Saitama, Japan
⁴Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan

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 vitro 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
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. Therefore, enzyme induction in humans has been estimated using primary cultured human hepatocytes. 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. 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 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. 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, and sulfinpyrazone, were collected along with induction data of those drugs in human hepatocytes. The pharmacokinetic parameters of rifampicin, omeprazole, lansoprazole, carbamazepine, dexamethasone, phenobarbital, and sulfinpyrazone in humans were also obtained from Goodman & Gilman’s Textbook.

The parameters of troglitazone were obtained from the report by Izumi et al.

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

\[
CSS,u = fp \times dose / CL_T = fp \times AUC / T
\]

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 x 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.

\[
k = fm \times CLint,well / V
\]

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
The unbound mean concentration in medium was calculated using equation 9.

\[ \text{CSS}_u = \frac{AUC \cdot fm}{T} \]

Where \( AUC \) is the area under the curve, \( fm \) is the unbound fraction in medium, and \( T \) is the medium change time.

Equations 10 and 11 were solved under steady-state conditions. Equation 12 was obtained.

\[ \frac{R_{ss}}{R_0} = \frac{E_{ss}}{E_0} = 1 + E_{max} \frac{CSS_u}{EC50 + CSS_u} \]

Where \( R_0 \) and \( E_0 \) are the initial amounts of mRNA and enzyme, respectively.

To estimate the EC50 and Emax values of inducers, the induction ratios (R) obtained from in vitro studies were fitted to equation 13 using the nonlinear regression program MULTI [43] in which each data point was not weighted. Bartlett analysis showed that there was no significant difference among the variance of the induction ratios at each concentration.

\[ R = 1 + E_{max} \frac{CSS_u}{EC50 + CSS_u} \]

The induction ratios in humans after inducer treatment were predicted using the EC50, Emax, and in vivo CSS_u values obtained by equation 13.

The method for predicting in vivo induction from in vitro data is shown in Fig. 1.

**Results**

Table 1 shows the induction ratio of CLint for specific CYP substrates after treatment with inducers. Treatment with rifampicin, 600 mg/day, caused a 1.47- to 5.35-fold induction of CYP3A. The induction ratio of CYP3A for treatment with phenobarbital, 100 mg/kg, was 3.08. The induction ratios for the other inducers were less than this value. Treatment with omeprazole, 40 mg/day, in CYP2C19 poor metabolizers caused a 1.62-fold induction of CYP1A. The unbound mean concentrations of 8 inducers in the in vitro studies were higher than those in the in vivo studies. The induction ratios for inducers at the highest concentration in the in vitro studies were also higher than those in the in vivo studies (Fig. 2). The induction ratio of rifampicin, omeprazole, troglitazone, dexamethasone and phenobarbital increased as the unbound mean concentration increased, reaching a constant value at high concentrations (Fig. 2). The induction of CYP3A and 1A was analyzed by the Emax model. Table 2 shows the maximum induction ratio (Emax) and the concentration
Table 1. Enzyme induction by various inducers in humans

<table>
<thead>
<tr>
<th>inducer</th>
<th>dose</th>
<th>period</th>
<th>substrate</th>
<th>CYP</th>
<th>induction ratio</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>rifampicin</td>
<td>600 mg/day</td>
<td>5 days</td>
<td>midazolam</td>
<td>3A</td>
<td>5.35</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>600 mg/day</td>
<td>7 days</td>
<td>quinidine</td>
<td>3A</td>
<td>4.84</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>600 mg/day</td>
<td>18 days</td>
<td>tacrolimus</td>
<td>3A</td>
<td>16.63</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>600 mg/day</td>
<td>7 days</td>
<td>nifedipine</td>
<td>3A</td>
<td>4.86</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>600 mg/day</td>
<td>9 days</td>
<td>propafenone</td>
<td>2D6,3A</td>
<td>18.25</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>600 mg/day</td>
<td>5 days</td>
<td>zolpidem</td>
<td>3A</td>
<td>1.75</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>600 mg/day</td>
<td>14 days</td>
<td>triazolam</td>
<td>3A</td>
<td>3.20</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>600 mg/day</td>
<td>14 days</td>
<td>6-OHC cortisol</td>
<td>3A</td>
<td>3.71</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>1200 mg/day</td>
<td>9 days</td>
<td>6-OHC cortisol</td>
<td>3A</td>
<td>3.90</td>
<td></td>
</tr>
<tr>
<td>carbamazepine</td>
<td>400–600 mg/day</td>
<td>3 weeks</td>
<td>omeprazole</td>
<td>3A4</td>
<td>2.44</td>
<td>26</td>
</tr>
<tr>
<td>sulfinpyrazone</td>
<td>800 mg/day</td>
<td>7 days</td>
<td>verapamil</td>
<td>3A</td>
<td>1.3</td>
<td>27</td>
</tr>
<tr>
<td>phenytoin</td>
<td>300–400 mg/day</td>
<td>10 days</td>
<td>cyclosporine</td>
<td>3A</td>
<td>2.79</td>
<td>28</td>
</tr>
<tr>
<td>dexamethasone</td>
<td>1.5 mg/day</td>
<td>4 days</td>
<td>triazolam</td>
<td>3A</td>
<td>1.24</td>
<td>29</td>
</tr>
<tr>
<td>troglitazone</td>
<td>400 mg/day</td>
<td>26 days</td>
<td>6-OHC cortisol</td>
<td>3A</td>
<td>2.2</td>
<td>30</td>
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<tr>
<td>phenobarbital</td>
<td>30 mg/day</td>
<td>6-OHC/17-OHCS</td>
<td>3A</td>
<td>1.09</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100 mg/day</td>
<td>14 days</td>
<td>6-OHC/17-OHCS</td>
<td>3A</td>
<td>1.59</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>40 mg/day</td>
<td>6-OHC/17-OHCS</td>
<td>3A</td>
<td>1.87</td>
<td>33</td>
<td></td>
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<tr>
<td>omeprazol</td>
<td>40 mg/day PM</td>
<td>7 days</td>
<td>caffeine</td>
<td>1A</td>
<td>1.62</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>40 mg/day EM</td>
<td>7 days</td>
<td>caffeine</td>
<td>1A</td>
<td>1.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>120 mg/day EM</td>
<td>7 days</td>
<td>caffeine</td>
<td>1A</td>
<td>1.26</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>120 mg/day EM</td>
<td>7 days</td>
<td>6-OHC cortisol</td>
<td>3A</td>
<td>1.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 mg/day EM</td>
<td>7 days</td>
<td>6-OHC cortisol</td>
<td>3A</td>
<td>1.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 mg/day PM</td>
<td>7 days</td>
<td>caffeine</td>
<td>1A</td>
<td>1.17</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>120 mg/day EM</td>
<td>7 days</td>
<td>caffeine</td>
<td>1A</td>
<td>1.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 mg/day PM</td>
<td>7 days</td>
<td>caffeine</td>
<td>1A</td>
<td>1.41</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 mg/day EM</td>
<td>7 days</td>
<td>caffeine</td>
<td>1A</td>
<td>1.39</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>20 mg/day EM</td>
<td>7 days</td>
<td>caffeine</td>
<td>1A</td>
<td>1.12</td>
<td></td>
</tr>
</tbody>
</table>

*: 6β hydroxy cortisol
**: 17-hydroxy corticosteroids
$: 2D6 poor metabolizer
#: 2C19 poor metabolizer
##: 2C19 extensive metabolizer

The Prediction of In Vivo Enzyme-Induction

Treatment reached a steady-state after 5 days. The inducers were administered for more than 5 days. The in vivo data appear to suggest that induction had reached a steady-state in every case. Enzyme induction is commonly estimated from this reduction in AUC. The AUC of midazolam and triazolam after multiple dosing of rifampicin fell to about 1/20 that before treatment. However, this phenomenon cannot be explained only by induction of hepatic CYP3A. It has been reported that rifampicin induces not only hepatic CYP3A but also intestinal CYP3A. Our previous report indicated that multiple administration of rifampicin caused a marked reduction in the intestinal availability of CYP3A substrates metabolized by intestinal first-pass metabolism, such as triazolam and midazolam. Therefore, in the present study, the change in hepatic CLint was estimated from the half-life at half-maximum induction (EC50) for rifampicin, omeprazole, troglitazone, dexamethasone and phenobarbital. The Emax and EC50 of omeprazole for CYP1A were 12.02 and 0.075 μmol/L, respectively (Fig. 2, Table 2). The predicted induction ratio of CYP3A for all the inducers, except for omeprazole, based on the Emax and EC50 obtained from in vitro data were similar to the observed in vivo values (Fig. 3A). The predicted induction ratio of omeprazole was higher than the observed value. However, a good correlation was found between the observed and predicted induction ratios of omeprazole (r=0.768, p<0.05) (Fig. 3B).

Discussion

Multiple administrations of inducers cause a reduction in the AUC of a drug. The induction by rifampicin
Fig. 2. Relationship between the unbound steady-state drug concentration and induction ratio of CYPs under in vivo and in vitro conditions. Open squares and closed circles represent the induction ratio in vivo and in vitro, respectively. The solid line represents the fitted line. The inducers of CYP3A are rifampicin (A), omeprazole (B), troglitazone (D), dexamethasone (E), phenobarbital (F) carbamazepine (G) and sulfinpyrazone (H). The inducer of CYP1A is omeprazole (C).

Table 2. The parameters for enzyme induction using human hepatocytes

<table>
<thead>
<tr>
<th>inducer</th>
<th>CYP</th>
<th>EC50 (µmol/L)</th>
<th>Emax</th>
</tr>
</thead>
<tbody>
<tr>
<td>rifampicin</td>
<td>3A</td>
<td>0.847 ± 0.749</td>
<td>12.3 ± 3.4</td>
</tr>
<tr>
<td>omeprazole</td>
<td>3A</td>
<td>0.225 ± 0.15</td>
<td>2.36 ± 0.92</td>
</tr>
<tr>
<td></td>
<td>1A</td>
<td>0.056 ± 0.074</td>
<td>13.6 ± 5.9</td>
</tr>
<tr>
<td>troglitazone</td>
<td>3A</td>
<td>0.002 ± 0.0005</td>
<td>6.86 ± 0.46</td>
</tr>
<tr>
<td>dexamethasone</td>
<td>3A</td>
<td>9.32 ± 2.29</td>
<td>8.30 ± 0.65</td>
</tr>
<tr>
<td>phenobarbital</td>
<td>3A</td>
<td>58.4 ± 95.8</td>
<td>7.62 ± 1.82</td>
</tr>
</tbody>
</table>

and not the AUC. The induction ratio of CYP3A estimated from the change in CLint for midazolam and triazolam after treatment with rifampicin, 600 mg/day, was 5.35 and 3.04, respectively. These values were comparable with the induction ratio estimated from the urinary 6-beta-hydroxy cortisol/cortisol ratio and other substrates, such as quinidine and nifedipine (Table 1). These results suggest that the contribution of intestinal

Fig. 3. Relationship between the predicted induction ratio in humans from in vitro data and the observed induction ratio of CYP3A (A) and CYP1A (B) in humans after inducer treatments. Open circles and vertical bars represent the mean and SD of induction ratios for rifampicin in Table 1. Closed circles, open squares, closed squares and closed triangles represent the induction ratio for dexamethasone, omeprazole, phenobarbital and troglitazone. The solid line indicates a 1:1 correspondence.
metabolism to the total body clearance might be minor although metabolism in the small intestine contributes markedly to the first-pass metabolism. The induction of hepatic CYP3A by treatment with rifampicin, 600 mg/day, was 2- to 5-fold. The marked reduction in the AUC of some substrates, such as midazolam and triazolam, could be due to the induction of intestinal metabolism.

In the present study, the induction ratio of mRNA, enzyme amount and enzyme activity were collected because good correlations were observed among these parameters.38,43) These correlations suggest that the induction of mRNA and enzyme reach a steady-state. The maximum induction ratio of CYP3A by rifampicin in in vitro studies was 32-fold. This was higher than that in in vivo studies (Fig. 2). To clarify whether the difference in enzyme induction under in vivo and in vitro conditions is due to exposure to the inducer, the degree of enzyme induction at the same unbound concentration was compared. In some in vitro studies, the medium contained bovine albumin. It was assumed that there was no species difference in bovine and human protein binding. The unbound fraction of all the inducers in the medium was more than 0.6 except for omeprazole. The effect of this assumption may be minor. The concentrations of inducers in all the in vitro studies were higher than those in the in vivo studies. Induction studies under conditions mimicking those in vivo might be needed to predict enzyme induction quantitatively.

The induction ratio for all inducers increased as the unbound mean concentration increased, reaching a constant value at high concentrations in the in vitro studies (Fig. 2). The induction of CYP3A and 1A was analyzed by the Emax model. The predicted induction ratio of those inducers of CYP 3A, based on parameters obtained from in vitro data, were similar to the observed values (Fig. 3A). This study suggests that enzyme induction in vivo may be predictable. Further studies are needed because this analysis involved a number of assumptions and there was considerable variation in the data used. The prediction of enzyme induction of CYP1A by omeprazole from human hepatocytes was overestimated (Fig. 3B). This overestimation might be due to the assumption that an inducer in the medium was reduced by metabolism. The CLint of omeprazole was the highest of all the inducers. Although the enzyme activity of hepatocytes in the absence of an inducer falls with time, the enzyme activity was assumed to be constant. In the case of rifampicin, the effect of this assumption may be minor. On the other hand, for omeprazole, this assumption may influence the exposure. The exposure of omeprazole might be underestimated because of the reduction in enzyme and the underestimation of exposure may be one reason for this. The influence of the metabolite may also be a factor as far as the difference between in vitro and in vivo is concerned. If a metabolite is a potential inducer, there may be a difference in the exposure of metabolite in vitro and in vivo. A correlation between the observed and predicted induction ratio for CYP1A by omeprazole was observed which suggested that 1A induction might be predictable following some corrections.

Marked individual and experimental differences in the in vitro studies were observed (Fig. 2). The prediction of enzyme induction from in vitro data depends on the Emax value. The Emax values of rifampicin, troleandomycin, dexamethasone and phenobarbital were 6.89–12.3 and, overall, similar. The coincidences between the observed and predicted induction ratio might be due to similar Emax values. Although some individual differences in the induction ratio for taxol and rifampicin were observed, there was a good correlation between the induction ratio for taxol and rifampicin. This result suggests the possibility of quantitative prediction following a correction using the Emax of a stable metabolic inducer, such as rifampicin or phenobarbital. The coincidences between the observed and predicted induction ratios in the present study suggest that the suitable Emax for CYP3A4 in human hepatocyte inducers might be approximately 10 to correct for the difference between in vivo and in vitro conditions.

Recently, the reporter gene assay has been used to determine enzyme induction instead of hepatocytes.11,12) The EC50 of rifampicin from human hepatocytes, which was 0.847 μmol/L, was comparable with the EC50 obtained from the reporter gene assay, which was 0.71 μmol/L.11) There was a good correlation between the induction ratios using human hepatocytes and the induction ratios obtained from reporter gene assay of fourteen inducers, except ritonavir and trocleandomycin (r = 0.864).12) The reporter gene assay might be useful for the future quantitative prediction of enzyme induction.

In the present study, many reports of enzyme induction in humans were subjected to detailed examination and most concerned rifampicin. The treatments with rifampicin, phenobarbital and carmabazepine markedly reduced the AUC of the co-administered drug. The induction effect of other inducers was weaker than that of rifampicin. Comparison with the induction effect of rifampicin might be important. A compound that has a stronger induction effect than rifampicin may not be suitable for use as a drug.

In conclusion, the present study suggests that it is possible to predict quantitatively CYP3A enzyme induction from hepatocyte data.

References
1) Ito, K., Iwatsubo, T., Kanamitsu, S., Ueda, K., Suzuki, H. and Sugiyama, Y.: Prediction of pharmacokinetic


