A Mechanism-Based Mathematical Model of Aryl Hydrocarbon Receptor-Mediated CYP1A Induction in Rats Using β-Naphthoflavone as a Tool Compound

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ABSTRACT:

β-Naphthoflavone (BNF) is a synthetic flavone that selectively and potently induces CYP1A enzymes via aryl hydrocarbon receptor activation. Mechanism-based mathematical models of CYP1A enzyme induction were developed to predict the time course of enzyme induction and quantitatively evaluate the interrelationship between BNF plasma concentrations, hepatic CYP1A1 and CYP1A2 mRNA levels, and CYP1A enzyme activity in rats in vivo. Male Sprague-Dawley rats received a continuous intravenous infusion of vehicle or 1.5 or 6 mg · kg⁻¹ · h⁻¹ BNF for 6 h, with blood and liver sampling. Plasma BNF concentrations were determined by liquid chromatography-tandem mass spectrometry. Hepatic mRNA levels of CYP1A1 and CYP1A2 were determined by TaqMan. Ethoxyresorufin-O-deethylation was used to measure the increase in CYP1A enzyme activity as a result of induction. The induction of hepatic CYP1A1/CYP1A2 mRNA and CYP1A activity occurred within 2 h after BNF administration. This caused a rapid increase in metabolic clearance of BNF, resulting in plasma concentrations declining during the infusion. Overall, the enzyme induction models developed in this study adequately captured the time course of BNF pharmacokinetics, CYP1A1/CYP1A2 mRNA levels, and increases in CYP1A enzyme activity data for both dose groups simultaneously. The model-predicted degradation half-life of CYP1A enzyme activity is comparable with previously reported values. The present results also confirm a previous in vitro finding that CYP1A1 is the predominant contributor to CYP1A induction. These physiologically based models provide a basis for predicting drug-induced toxicity in humans from in vitro and preclinical data and can be a valuable tool in drug development.

Introduction

Activation of the aryl hydrocarbon receptor (AhR) is known to mediate toxicity through the induction of genes in the “AhR gene battery,” including CYP1A1, CYP1A2, and CYP1B1 as well as NQO1 and UGT1A6 (Hankinson, 1995; Nebert et al., 2000). The activity of these genes contributes to the formation of reactive oxygen species that can subsequently lead to cellular oxidative stress, lipid peroxidation, and DNA fragmentation (Barouki and Morel, 2001; Bagchi et al., 2002). Kohn et al. (1993, 1994) developed a series of mechanistic models to describe the kinetics of binding of dioxin to the cytoplasmic AhR complex and the subsequent change in expression of some of the AhR-inducible genes, as well as the downstream effects on estrogen and thyroid hormones (Kohn et al., 1996). However, these authors only examined the dose responses at steady state after repeated dosing and did not characterize the temporal properties of their models.

In the present study, mechanism-based mathematical models of CYP1A enzyme induction were developed using β-naphthoflavone (BNF) as a tool compound. BNF is a synthetic flavone that selectively and potently induces CYP1A enzymes via AhR activation (Cutroneo et al., 1972; Guengerich and Liebler, 1985; Prochaska and Talalay, 1988). An earlier study showed that BNF clearance increases with time after continuous intravenous infusion to rats (Aidedoyin et al., 1993). Because BNF is also regarded as a substrate of these enzymes (Vyas et al., 1983), it was suggested that the time-dependent enhancement of BNF clearance was most likely a result of CYP1A induction.

The mathematical models presented here integrate BNF pharmacokinetics with the induction of CYP1A1 and CYP1A2 mRNA and CYP1A enzyme activity as measured by ethoxyresorufin-O-deethylation (EROD) and the subsequent enhancement of BNF clearance. The models simultaneously account for both the dose-dependent and the time-dependent relationship between all experimental data.

Physiologically realistic models such as these can potentially play an important role in integrated risk assessments by providing a scientific basis for extrapolating knowledge gained from in vitro, in situ, and in vivo animal experiments to predict the dose response and time course of drug-induced toxicity in humans (Heinrich-Hirsch et al., 2001; Conolly, 2002).

Materials and Methods

Animal Experiment. Male Sprague-Dawley rats (250–350 g) were cannulated in both the femoral artery and femoral vein. They received a 6-h...
continuous femoral vein infusion of vehicle (a 9:1 mixture by volume of polyethylene glycol 400 and propylene glycol) or 1.5 or 6 mg · kg⁻¹ · h⁻¹ BNF as a solution in vehicle. The rats were assigned to two groups, each dosed with vehicle or 1.5 or 6 mg · kg⁻¹ · h⁻¹ BNF. Group 1 rats (n = 3/time point/dose level) were sacrificed, and livers were excised at 0, 30, 75, 120, 240, and 360 min for mRNA and enzyme activity analysis. Blood was sampled from selected animals via the femoral artery at 0, 5, 15, 30, 45, 60, 75, 80, 100, 120, 160, 200, 240, 320, and 360 min (n = 3/time point/dose level). Group 2 rats (n = 3/time point/dose level) were sacrificed, and livers were excised at 0, 24, 48, 120, and 240 h for enzyme activity analysis.

Before their initiation, all animal study protocols were reviewed and approved by the GlaxoSmithKline Institutional Animal Care and Use Committee. Studies were performed in accordance with the recommendations found in the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996) and adhered to the U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (http://www.nal.usda.gov/awic/pubs/IACUC/vert.htm).

**Analysis of Plasma BNF Concentrations.** Rat plasma samples were assayed for β-naphthoflavone with a method based on protein precipitation with acetonitrile followed by liquid chromatography–tandem mass spectrometry analysis using positive ion TurboIonSpray ionization (lower limit of quantification, 50 ng/ml; 10-μl aliquot of rat plasma used).

**Microscopic Preparation and Enzyme Assay.** Rat liver microsomes were prepared by differential centrifugation (van der Hoeven and Coon, 1974). Protein content was determined by the BCA protein assay (Smith et al., 1985). Microsomal CYP1A activity was assessed using an ethoxyresorufin-O-deethylase assay as described by Burke et al. (1985).

**Measurement of CYP1A1 and CYP1A2 mRNA Levels.** Total RNA was extracted from the liver by tissue homogenization followed by column extraction using a SV 96 Total RNA Isolation System (Promega, Madison, WI). After quantification by RiboGreen assay, total RNA was used for cDNA synthesis using SuperScript II (Invitrogen, Carlsbad, CA). The resultant cDNA template was probed and quantified for CYP1A1 and CYP1A2 mRNA by TaqMan on an ABI Prism 7900 Sequence Detection System (Baldwin et al., 2006). The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase was run in parallel for each sample as an RNA quality control. Serially diluted rat genomic DNA was used as a standard for determining the relative copy number of the study genes.

**Enzyme Induction Model I.** Model I considers only the induction of CYP1A1 mRNA transcription by BNF and assumes that the CYP1A1 mRNA increase is the sole contributor to induction of CYP1A enzyme activity (as measured by EROD) (Fig. 1). A first-order single compartmental model was used to describe the plasma BNF pharmacokinetics using a Michaelis-Menten equation (eq. 1) to link enzyme activity to overall BNF plasma clearance:

\[
V_p \frac{dC_p}{dt} = R - \frac{V_{\text{max}} [\text{Enzyme}]_{1A}}{K_m + [\text{Enzyme}]_{1A}} C_p
\]  

(1)

where \( C_p \) is the BNF plasma concentration, \( R \) is the rate of intravenous infusion, \( V_p \) is the volume of distribution, \([\text{Enzyme}]_{1A}\) is the CYP1A1 activity, \( V_{\text{max}} \) is the BNF plasma clearance under conditions of maximum CYP1A1 induction, and \( K_m \) is the level of CYP1A1 activity at which BNF plasma clearance is half of the \( V_{\text{max}} \). Because CYP1A enzyme induction has been reported in several extrahepatic organs including the lung (Jones et al., 1982; Ioannides and Parke, 1990), the hepatic EROD is used as a surrogate for total CYP1A activity. The special linearized case in which \( K_m \gg [\text{Enzyme}]_{1A} \) was also tested.

The induction of the CYP1A1 mRNA level was modeled using a modified indirect pharmacodynamic response model (Sharma and Jusko, 1998). The BNF plasma concentration was assumed to induce hepatic CYP1A1 mRNA by increasing its transcription rate after either a saturable or a linear function and the rate of mRNA degradation was assumed to follow first-order kinetics as a function of the mRNA level.

Two competing induction scenarios proposed in Portier et al. (1993) and Kohn et al. (1994) were considered. The “independent” mechanism assumes that the baseline level of CYP1A1 mRNA is maintained independent of the AhR, whereas the “additive” mechanism assumes that an endogenous AhR ligand is responsible. In both cases, a maximum effect (\( E_{\text{max}} \) model) was used to describe the induction of the transcription rate of CYP1A1 mRNA by BNF plasma concentration:

(Independent) \[
\frac{d[\text{mRNA}]_{1A}}{dt} = k_{in,1A} \left( 1 + \frac{E_{\text{max},R1} \cdot C_p}{E_{\text{50},R1} + C_p} \right) - k_{out,1A}[\text{mRNA}]_{1A}
\]  

(2a)

(Additive) \[
\frac{d[\text{mRNA}]_{1A}}{dt} = \frac{E_{\text{max},R1}(C_p + C_i)}{E_{\text{50},R1} + (C_p + C_i)} - k_{out,1A}[\text{mRNA}]_{1A}
\]  

(2b)

where \([\text{mRNA}]_{1A}\) is the concentration of CYP1A1 mRNA, \( k_{in,1A} \) is the constant transcription rate independent of the AhR, \( E_{\text{max},R1} \) is the degradation rate of the mRNA, and \( C_p \) is the concentration of the endogenous AhR ligand.

Linear versions of these models (eqs. 2c and 2d) were derived assuming \( C_p \ll E_{\text{50},R1} \), and \( C_p + C_i \ll E_{\text{50},R1} \):

(Independent) \[
\frac{d[\text{mRNA}]_{1A}}{dt} = k_{in,1A} \left( 1 + S_{R1} \cdot C_i \right) - k_{out,1A}[\text{mRNA}]_{1A}
\]  

(2c)

(Additive) \[
\frac{d[\text{mRNA}]_{1A}}{dt} = S_{R1}(C_p + C_i) - k_{out,1A}[\text{mRNA}]_{1A}
\]  

(2d)

where \( S_{R1} \) is the proportionality coefficient between the BNF plasma concentration and CYP1A1 mRNA induction.

It is noteworthy that although the BNF-induced change in mRNA level is a complex chain of events that begins with the binding of BNF to the AhR, in the above equations these steps are lumped into a single step process by assuming the presence of a rate-limiting step.

Induction of CYP1A1 enzyme activity, based on EROD activity, was modeled using a modified indirect pharmacodynamic response model. The rate of \([\text{Enzyme}]_{1A}\) degradation was assumed to follow first-order kinetics:

\[
\frac{d[\text{Enzyme}]_{1A}}{dt} = k_{in,1A} + k_{E,\text{other} - k_{out,1A}[\text{Enzyme}]_{1A}}
\]  

(3a)

Two separate terms were used to model the rate of \([\text{Enzyme}]_{1A}\) production, one that is inducible by CYP1A1 mRNA \((k_{in,1A})\) and the other that is not inducible \((k_{E,\text{other}})\). An \( E_{\text{max}} \) model was used to describe the induction of \([\text{Enzyme}]_{1A}\) production rate by CYP1A1 mRNA:

\[
k_{in,1A} = \frac{E_{\text{max},R1}[\text{mRNA}]_{1A}}{E_{\text{50},R1} + [\text{mRNA}]_{1A}}
\]  

(3b)
$E_{\text{max},1}$ is the maximum rate of $[\text{Enzyme}]_{1A}$ induction contributed by CYP1A1 mRNA and $E_{\text{50},1}$ is the CYP1A1 mRNA level for 50% $[\text{Enzyme}]_{1A}$ induction.

Before induction, $[\text{Enzyme}]_{1A}$ is being produced at a baseline rate. It is assumed that the component of the baseline $[\text{Enzyme}]_{1A}$ production rate inducible by CYP1A1 mRNA, $k_{\text{in},1A}$, is due to the presence of a baseline level of CYP1A1 mRNA, $[\text{mRNA}]_{1A,1}$, and is governed by the same process as defined in eq. 3b. By assuming that $[\text{mRNA}]_{1A,1} \ll EC_{50},E$,

$$k_{\text{in},1A} = \frac{E_{\text{max},1}[\text{mRNA}]_{1A,1}}{EC_{50},E + [\text{mRNA}]_{1A,1}} = \frac{E_{\text{max},1}[\text{mRNA}]_{1A}}{EC_{50},E}$$

Combining eq. 3c with 3b yields

$$k_{\text{in},1} = k_{\text{in},1A}EC_{50},E \frac{[\text{mRNA}]_{1A}}{EC_{50},E + [\text{mRNA}]_{1A}}$$

Finally,

$$k_{\text{out},E} = (k_{\text{out},1A} + k_{\text{out},1A}) \frac{[\text{mRNA}]_{1A}}{[\text{mRNA}]_{1A}}$$

For the linear version of this model, by assuming that $[\text{mRNA}]_{1A,1} \ll EC_{50},E$,

$$k_{\text{in},1A} = k_{\text{in},1A}EC_{50},E \frac{[\text{mRNA}]_{1A}}{EC_{50},E + [\text{mRNA}]_{1A}}$$

**Enzyme Induction Model II.** Model II incorporates induction of the mRNA transcription rate of hepatic CYP1A1 and CYP1A2 mRNA, assuming that both CYP1A1 and CYP1A2 mRNA levels contribute to $[\text{Enzyme}]_{1A}$ induction (Fig. 2). The equations for BNF pharmacokinetics and induction of CYP1A1 mRNA are the same as those used in model I. The induction of CYP1A2 mRNA was modeled in the same fashion as described in eqs. 2a and 2b and their linear versions as in eqs. 2c and 2d. The induction of $[\text{Enzyme}]_{1A}$ (eq. 3d) was modified as below to account for contribution from both CYP1A1 and CYP1A2.

$$k_{\text{in},1} = k_{\text{in},1A}EC_{50},E \frac{[\text{mRNA}]_{1A}}{EC_{50},E + [\text{mRNA}]_{1A}} + k_{\text{in},2}EC_{50},E \frac{[\text{mRNA}]_{1A}}{EC_{50},E + [\text{mRNA}]_{1A}}$$

$$k_{\text{out},E} = (k_{\text{out},1A} + k_{\text{out},1A}) \frac{[\text{mRNA}]_{1A}}{[\text{mRNA}]_{1A}}$$

For a linear version of this model, by assuming that $[\text{mRNA}]_{1A,1} \ll EC_{50},E$ and $[\text{mRNA}]_{1A,1} \ll EC_{50},E$, eq. 4a is reduced to eq. 4c as follows:

$$k_{\text{in},E} = \frac{k_{\text{in},1A}EC_{50},E}{[\text{mRNA}]_{1A}(EC_{50},E + [\text{mRNA}]_{1A})} + \frac{k_{\text{in},2}EC_{50},E}{[\text{mRNA}]_{1A}(EC_{50},E + [\text{mRNA}]_{1A})}$$

$$k_{\text{out},E} = \frac{k_{\text{out},1A} + k_{\text{out},1A}}{[\text{mRNA}]_{1A}}$$

Model Development Approach. In general, all model parameters were optimized by fitting the models to all data simultaneously, including BNF plasma concentration, mRNA levels, and CYP1A activity. The only exception was the baseline degradation rate of CYP1A activity, which was determined separately by fitting a reduced model of CYP1A kinetics to the time course of CYP1A activity beyond day 2 of the infusion experiment.

It was assumed that the clearance of BNF from plasma is rapid and CYP1A1 mRNAs return to control levels faster than the degradation of CYP1A1 activity. Consequently, after enough time has passed at the end of infusion, the CYP1A1 mRNA levels would have returned to their respective preinduced baselines, and the time course of CYP1A activity will follow first-order degradation kinetics, which depends only on the production and degradation rate of CYP1A1 and is independent of CYP1A mRNA levels.

$$\frac{d[\text{Enzyme}]_{1A}}{dt} = k_{\text{in},E} - k_{\text{out},E} + k_{\text{out},E}$$

Once determined, the $k_{\text{out},E}$ was held constant in subsequent parameter optimization in which the models were fitted simultaneously to BNF, mRNA, and CYP1A activity data.

Parameter optimization was performed using the SAAM II program (version 2.0: SAAM Institute, Seattle, WA). Model discrimination was based on values for the Akaike information criterion (AIC) and the Schwarz-Bayesian information criterion as well as visual inspection of the goodness-of-fit plots. Simulation was carried out in SAAM II using the Rosenbrock integration method.

**Results**

Figure 3 illustrates the time profiles of BNF plasma concentration, hepatic CYP1A1 and CYP1A2 mRNA levels, and CYP1A1 activity after continuous intravenous infusion at 1.5 and 6 mg/kg/h (9 and 36 mg/kg) for up to 6 h. In vehicle-treated rats (not shown), BNF was not detectable in plasma, and hepatic CYP1A1 and CYP1A2 mRNA levels were close to basal levels.

Despite continuous infusion, plasma BNF concentrations reached a maximal level ($C_{\text{max}}$) at 80 to 120 min and then started declining toward a lower plateau value, showing a time-dependent increase in clearance. In both dose groups, hepatic CYP1A1 and CYP1A2 mRNA levels started to increase notably as early as 75 min after start of the infusion, reaching the maximal level at approximately 240 to 300 min.
The mRNA levels started declining toward the end of the infusion period.

In contrast, the increase in CYP1A activity lags behind the increase in mRNA levels, showing a notable increase 120 min after the start of the infusion and continuing to rise throughout the 6-h infusion period (Fig. 3). Figure 4 shows that CYP1A activity declined over time and returned to the predose baseline level over a 4-day period.

In summary, the induction of hepatic CYP1A1 and CYP1A2 mRNA and subsequently CYP1A activity occurred quickly after intravenous administration of BNF, causing increased metabolic clearance of BNF, which resulted in declining BNF concentrations during the remainder of the infusion. Overall, the time course of BNF plasma concentration, that of hepatic CYP1A1 and CYP1A2 mRNA levels, and CYP1A activity are closely interrelated. At doses of 9 and 36 mg/kg, the maximal fold increases for CYP1A1 mRNA were 1101- and 2356-fold, for CYP1A2 mRNA were 6- and 8-fold, and for CYP1A activity were 4- and 9-fold, respectively.

CYP1A Activity Degradation Rate Constant. The time course of CYP1A activity beyond day 2 of the infusion experiment is plotted in Fig. 4. The predicted time course of CYP1A activity best fitting to eq. 5 was also plotted. The best-fitting $k_{\text{out}}$ was 0.000611 min$^{-1}$, corresponding to a degradation half-life of 18.9 h. This was held as constant for all subsequent parameter optimization.

Model Fitting: Model I. Several versions of model I were evaluated, with either independent or additive mechanisms of CYP1A1 mRNA induction, using either linear or $E_{\text{max}}$ models to describe the CYP1A1 mRNA and activity induction and either linear or Michaelis-Menten models to describe the link between enzyme activity and BNF plasma clearance. The models were fitted to the time courses of mean BNF plasma concentration and hepatic CYP1A1 mRNA and CYP1A activity levels from both dose groups simultaneously. The degradation rate constant for CYP1A activity, $k_{\text{out, E}}$, was held constant as discussed above. In general, the models with the independent mechanism of CYP1A1 mRNA induction gave a better fit than the additive models, based on the Akaike information criterion (AIC <7 for the independent models versus AIC >7 for the additive models). Among the models with independent mechanism of CYP1A1 mRNA induction tested, the one that best fits the data uses a linear model to describe the induction of CYP1A1 mRNA by BNF and of CYP1A activity by CYP1A1 mRNA and a Michaelis-Menten equation to describe the enzyme activity-BNF plasma clearance relationship.

The time courses of BNF plasma, hepatic CYP1A1 mRNA, and CYP1A activity predicted by the best-fit model are plotted in Fig. 5. The model accurately predicts the initial rise and then fall of BNF plasma concentrations, peaking at approximately 100 min, which coincides with a noticeable increase in CYP1A1 activity at 120 min. Furthermore, the model predicted the slower rise of the hepatic CYP1A1 mRNA level, caused by the rise of the plasma BNF concentration and its return toward baseline as plasma BNF concentration declines. Finally, the model also adequately describes the even slower rise of CYP1A1 activity, which was at an elevated level even at the end.
of the infusion. The fitted parameter values for model I are summarized in Table 1.

Model Fitting: Model II. Several versions of model II, using either $E_{\text{max}}$ or linear models to describe the induction of CYP1A1 and CYP1A2 mRNA and CYP1A production rates and either linear or Michaelis-Menten models to describe the link between enzyme activity and BNF plasma clearance were also evaluated for their ability to simultaneously fit the time courses of BNF plasma concentration, hepatic CYP1A1 mRNA and CYP1A2 mRNA levels, and CYP1A activity from both dose groups. The degradation rate constant for CYP1A activity, $k_{\text{out}, C}$, was held constant, as for model I.

Among the versions of model II tested, the one that best fit the data uses linear models to describe the induction of CYP1A1 mRNA by BNF and consequently of CYP1A activity by CYP1A1 mRNA, linear models to describe the induction of CYP1A2 mRNA by BNF and of CYP1A activity by CYP1A2 mRNA, and a Michaelis-Menten equation to describe the enzyme activity-BNF plasma clearance relationship. The fitted parameter values are summarized in Table 1.

Overall, model II provided a slightly better fit to the data than model I. Although the value for total objective function is higher for model II than for model I, the values of the individual objective function for datasets common to both models are mostly lower for model II than for model I.

Discussion

Both models I and II (without or with CYP1A2) developed in the present study were able to capture the temporal interrelationship between BNF pharmacokinetics, CYP1A1 and CYP1A2 mRNA levels, and the increase in CYP1A1 activity data in vivo across doses.

The nonlinear relationship between CYP1A1 activity and BNF plasma clearance is evident from the modeling results. Whereas the CYP1A1 enzyme activity (as measured by EROD) was induced by 4-fold in the 9-mg/kg dose group, the model estimated clearance was only induced by 2.5-fold [from 67 ml/(min kg) at the onset of infusion to 168 ml/(min kg) at the end of the 6-h infusion]. Furthermore, additional CYP1A1 enzyme induction (10-fold increase in EROD activity) in the 36-mg/kg dose group only translated into a 3.6-fold induction in clearance.

It is worth noting that even though model I (without CYP1A2) can adequately describe the time course of induction, the CYP1A1 mRNA pathway alone cannot account for the entire baseline (preinduced) production of CYP1A1 activity as measured by EROD, requiring the inclusion of a separate, noninduced component of enzyme production in the model. This is expected, because EROD activity, which is used in the present study as a surrogate for CYP1A1 enzyme activity, has previously been shown to involve not only CYP1A but also other non-AhR-inducible P450 enzymes in untreated rat liver (Burke et al., 1994). Model I predicts that before induction CYP1A1 only accounts for approximately 3% of the total baseline production rate for EROD activity, whereas the separate, noninduced component contributes the remaining 97%. After the onset of BNF infusion at 36 mg/kg and as the CYP1A1 mRNA level increases, however, the contribution from
TABLE 1  
Estimated parameters from modeling using model I and II

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Definition</th>
<th>CYP1A1 Only (%CV)</th>
<th>CYP1A1 + CYP1A2 (%CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNF plasma PK</td>
<td>$V_{max}$ ml/(min·kg)</td>
<td>Plasma BNF clearance under conditions of maximal CYP1A induction</td>
<td>329.7 (58.3)</td>
<td>331.9 (57.7)</td>
</tr>
<tr>
<td></td>
<td>$K_m$ pmol/(min·mg protein)</td>
<td>CYP1A activity at which plasma clearance is half of the $V_{max}$</td>
<td>447.7 (112)</td>
<td>563.3 (97.6)</td>
</tr>
<tr>
<td></td>
<td>$V_p$ ml/kg</td>
<td>Volume of distribution</td>
<td>5041 (31.9)</td>
<td>5316 (44.9)</td>
</tr>
<tr>
<td>CYP1A1 mRNA</td>
<td>$k_{in, R_1}$ (copy/ng total RNA)·min$^{-1}$</td>
<td>Baseline zero-order CYP1A1 mRNA transcription rate constant</td>
<td>1.16 $\times 10^{-3}$ (37.7)</td>
<td>3.42 $\times 10^{-3}$ (41.6)</td>
</tr>
<tr>
<td></td>
<td>$k_{out, R_1}$ min$^{-1}$</td>
<td>First-order CYP1A1 mRNA degradation rate constant</td>
<td>7.24 $\times 10^{-4}$</td>
<td>6.16 $\times 10^{-3}$</td>
</tr>
<tr>
<td></td>
<td>$S_{R_1}$ (ng/ml)$^{-1}$</td>
<td>Proportionality coefficient between plasma BNF concentration and CYP1A1 mRNA induction</td>
<td>4.1 (27.2)</td>
<td>5.3 (23.0)</td>
</tr>
<tr>
<td>CYP1A2 mRNA</td>
<td>$t_{deg, 1A2}$ H</td>
<td>CYP1A1 mRNA degradation half-life</td>
<td>1.6</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>$k_{in, R_2}$ (copy/ng total RNA)·min$^{-1}$</td>
<td>Baseline zero-order CYP1A2 mRNA transcription rate constant</td>
<td>8.27 $\times 10^{-3}$</td>
<td>52.4 (31.2)</td>
</tr>
<tr>
<td></td>
<td>$k_{out, R_2}$ min$^{-1}$</td>
<td>First-order CYP1A2 mRNA degradation rate constant</td>
<td>2.13 $\times 10^{-2}$</td>
<td>14.9 (4.9)</td>
</tr>
<tr>
<td></td>
<td>$S_{R_2}$ (ng/ml)$^{-1}$</td>
<td>Proportionality coefficient between plasma BNF concentration and CYP1A2 mRNA induction</td>
<td>2.13 $\times 10^{-2}$</td>
<td>14.9 (4.9)</td>
</tr>
<tr>
<td>CYP1A (EROD)</td>
<td>$t_{deg, 1A2}$ H</td>
<td>CYP1A2 mRNA degradation half-life</td>
<td>2.65 $\times 10^{-3}$ (18.6)</td>
<td>1.6 $\times 10^{-3}$ (15.9)</td>
</tr>
<tr>
<td></td>
<td>$k_{in, E_1}$ (pmol/min/mg protein)·min$^{-1}$</td>
<td>Baseline zero-order EROD production rate constant contributed by CYP1A1 mRNA</td>
<td>8.57 $\times 10^{-2}$</td>
<td>8.73 $\times 10^{-2}$</td>
</tr>
<tr>
<td></td>
<td>$k_{in, E_2}$ (pmol/min/mg protein)·min$^{-1}$</td>
<td>Baseline zero-order EROD production rate constant contributed by CYP1A2 mRNA</td>
<td>8.73 $\times 10^{-2}$</td>
<td>8.73 $\times 10^{-2}$</td>
</tr>
<tr>
<td></td>
<td>$k_E$ (pmol/min/mg protein)·min$^{-1}$</td>
<td>Baseline zero-order EROD production rate constant</td>
<td>6.11 $\times 10^{-4}$ (fixed)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$t_{deg, EROD}$ h</td>
<td>EROD degradation half-life</td>
<td>18.9 (fixed)</td>
<td></td>
</tr>
</tbody>
</table>

PK, pharmacokinetics.

CYP1A1 mRNA quickly surpasses that of the noninduced pathway to become the dominating contributor of EROD activity production, reaching 90% by 35 min and exceeding 98% at the end of the 360-min infusion (Fig. 7).

The inclusion of the CYP1A2 pathway did not significantly improve the model fit, suggesting that CYP1A2 mRNA is a minor contributor to the induction of EROD activity. However, model II also predicts that CYP1A1 contributes minimally to the uninduced production of EROD activity. Before induction, the CYP1A1 pathway contributes only approximately 2% to the baseline production of EROD activity. After the onset of BNF infusion, however, the relative contribution shifted quickly in favor of CYP1A1 mRNA, contributing more than 80% to the production rate of EROD activity by the end of the 6-h infusion.

Among the models tested, the ones that produced the best fits to the data used a linear relationship between plasma BNF concentration and induction of CYP1A1 and CYP1A2 mRNA (model II only) and CYP1A enzyme activity. The in vitro EC50 values for BNF induction of CYP1A activity obtained in hepatocytes of dog (Graham et al., 2006), rat (Madan et al., 1999), and human (unpublished data cited in Graham et al., 2006) were 7.8, 1.5, and 4.2 μM, respectively. BNF plasma protein binding in the rat is 96% (Adedoyin et al., 1993). The highest mean unbound plasma concentrations in the rat were approximately 50 ng/ml (4% of 1200 ng/ml; 0.18 μM), well below the EC50 obtained in rat hepatocytes in vitro and in a range in which a linear relationship between BNF concentration and mRNA and activity induction could reasonably be expected.

The estimated in vivo degradation half-life of CYP1A by EROD activity predicted in the current study (18.9 h) is also in a range with previous reports either in vivo in rats treated with phenobarbital or BNF (Shiraki and Guengerich, 1984), in precision-cut human liver slices (Renwick et al., 2000), or clinically in heavy smokers after smoking cessation (Faber and Fuhr, 2004). Even though for the 36-mg/kg group, a 9-fold increase in CYP1A activity was observed within 6 h of treatment, simulation of prolonged infusion of 6 mg/kg/h using the best-fitting model II showed that the maximal induced enzyme activity is 40-fold above the baseline value at ~60 to 100 h (three to five half-lives). This result is consistent with previous studies in which we dosed BNF to rats by intraperitoneal injection at 80 mg/kg/day for 4 days and found that the induction of EROD activity was 48-fold over baseline in male rats (Chen et al., 2003). It is also consistent with the U.S. Food and Drug Administration’s current recommendation that to assess P450 induction hepatocytes should be treated for 2 to 3 days (Guidance for Industry: Drug Interaction Studies—Study Design, Data Analysis, and Implications for Dosing and Labeling, 2006, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072101.pdf).

A model that reproduces the dose-response relationships of experimental data might permit extrapolation of responses from one dose to another. Incorporation of known biochemical and physiological processes into the model also opens the possibility for extrapolation between dose routes, species, or altered physiological states. Both the current models and the models developed by Kohn et al. (1993, 1994) are mechanistic in nature. The models developed by Kohn et al. (1993, 1994) included a physiologically based pharmacokinetics model of dioxin as well as the kinetics of dioxin-AhR binding that leads to the induction of CYP1A enzyme and can potentially be used to predict the consequence of changes in these physiological and biochemical fac-
tors. Because of their complexity, however, these models also required the use of many model parameters obtained from the literature. The choice for the granularity of a model depends on the questions the model is constructed to address. The current models, although simpler, still capture the mRNA-P450 enzyme interrelationship and are well suited for extrapolation of in vitro data to predict in vivo responses. Additional work to further elucidate the in vitro-in vivo relationship of the model parameters, combined with development of mathematical models that connect induction of CYP1A activity to downstream toxicity such as reactive oxygen species elevation, DNA damage, or lipid peroxidation (Dewa et al., 2008) might eventually lead to scientifically sound approaches for estimating risks of adverse effects in human.

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References


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