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**Rifampin Induces the *In Vitro* Oxidative Metabolism, but Not the *In Vivo* Clearance of Diclofenac in Rhesus Monkeys**

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**Running title:** Diclofenac and rifampin interactions in rhesus monkeys

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WP 75-100

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**Number of text pages:** 15

**Number of table:** 1

**Number of figures:** 3

**Number of references:** 20

**Number of words:** 253 (Abstracts), 322 (Introduction), 1122 (Discussion)

**Abbreviations used are:** : DF, diclofenac; 4'-OH DF, 4'-hydroxy diclofenac; 5-OH DF, 5-hydroxy diclofenac; MDZ, midazolam; 1'-OH MDZ, 1'-hydroxy midazolam; AUC, area under plasma concentration-time curve;  $C_{max}$ , peak plasma concentration; CL, plasma clearance;  $F_h$ , hepatic availability;  $V_{dss}$ , volume of distribution at steady-state;  $t_{1/2}$ , half-life; i.pv., intra-hepatic portal vein; i.v., intravenous; IS, internal standard; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry.

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**ABSTRACT**

Effects of rifampin on *in vitro* oxidative metabolism and *in vivo* pharmacokinetics of diclofenac (DF), a prototypic CYP2C9 marker substrate, were investigated in rhesus monkeys. In monkey hepatocytes, rifampin markedly induced DF 4'-hydroxylase activity, with values for EC<sub>50</sub> of 0.2 - 0.4 μM and E<sub>max</sub> of 2 - 5-fold over control. However, pretreatment with rifampin did not alter the pharmacokinetics of DF obtained following either intravenous (i.v.) or intra-hepatic portal vein (i.pv.) administration of DF to monkeys. At the dose studied, plasma concentrations of rifampin reached 10 μM, far exceeding the *in vitro* EC<sub>50</sub> values. Under similar treatment conditions, rifampin was previously shown to induce midazolam (MDZ) 1'-hydroxylation in rhesus monkey hepatocytes (EC<sub>50</sub> and E<sub>max</sub> values ~0.2 μM and ~2 - 3-fold, respectively), and markedly affected the *in vivo* pharmacokinetics of midazolam, MDZ (>10-fold decreases in the i.pv. MDZ systemic exposure and its hepatic availability, F<sub>h</sub>) in this animal species. In monkey liver microsomes, DF underwent predominantly glucuronidation, and modestly oxidation; the intrinsic clearance (CL<sub>int</sub> = V<sub>max</sub>/K<sub>m</sub>) value for the glucuronidation pathway accounted for >95% (*vs.* about 75% in human liver microsomes) of the total (glucuronidation + hydroxylation) intrinsic clearance value. In monkey hepatocytes, the hydroxylation also was a minor component (≤10%) relative to the glucuronidation, supporting the liver microsomal finding. Collectively, our results suggest that the oxidative metabolism is not the major *in vivo* clearance mechanism of DF in either untreated or rifampin-treated monkeys, and conceivably also in humans, raising a question on the utility of DF as an *in vivo* CYP2C9 probe.

## Introduction

Induction of drug metabolizing enzymes, especially the cytochrome P450 (CYP) superfamily, by some drug molecules is a well known phenomenon and generally is undesirable since it can cause profound clinical effects, either by reducing therapeutic efficacy of drugs or enhancing toxicity from toxic or reactive metabolites (Thummel and Wilkinson, 1998). Accordingly, the potential for new chemical entities to cause CYP induction is usually assessed during lead optimization and identification in early drug discovery processes (Weaver, 2001; Worboys and Carlile, 2001). Currently, measurement of enzyme activities in cultured hepatocytes is the accepted and recommended method for studying CYP induction (LeCluyse, 2001; Bjornsson *et al.*, 2003). However, systematic and quantitative extrapolations of such *in vitro* enzyme induction data to *in vivo* situations have not been extensively studied, and studies to date, including our recent investigation on *in vitro* – *in vivo* drug interactions in rhesus monkeys (Prueksaritanont *et al.*, *in press*), have been limited to CYP3A, the most abundant of all of the human isoforms.

In a quest to expand the database, we subsequently evaluated a relationship between *in vitro* – *in vivo* induction of CYP2C9 activity by rifampin, using diclofenac (DF) as a functional probe, and the rhesus monkey as an animal model. Rifampin is a known human CYP2C9 inducer (Bjornsson *et al.*, 2003; Parkinson *et al.*, 2004). DF has been commonly used as a probe substrate for measuring *in vitro* and *in vivo* activity of CYP2C9 in humans (Tucker *et al.*, 2001; Bjornsson *et al.*, 2003). The rhesus monkey, which has recently been demonstrated to be a good animal model for studying CYP3A-mediated interactions in humans (Prueksaritanont *et al.*, *in press*), was selected as an animal model, based on several similarities between rhesus and human CYP3A and 2C isoforms (Tang *et al.*, 2005). This report describes apparently conflicting *in vitro* – *in vivo* results obtained from these studies, as well as those obtained subsequently to help

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explain the observed discrepancies. The latter studies included *in vitro* metabolism of DF in monkey liver microsomes and hepatocytes.

### **Materials and Methods**

**Materials.** DF, 4'-hydroxy (4'-OH) DF, Midazolam (MDZ), 1'-OH MDZ, diazepam and rifampin were obtained from Sigma (St. Louis, MO). All other reagents were of analytical or HPLC grade. Rhesus monkey and human liver microsomes (pooled from 10-20 individuals) were purchased from Xenotech (Kansas City, Kansas). Fresh rhesus monkey hepatocytes were prepared in-house according to the method of Moldeús *et al.* (1978).

***In Vitro* Induction Studies.** The study was conducted using rhesus monkey hepatocytes (n=3) as previously described (Prueksaritanont *et al.*, 2005; *in press*). In brief, hepatocyte cultures were treated, in triplicate for each treatment, for 2 days with culture media containing various concentrations of rifampin or vehicle control (DMSO, 0.1% v/v). At the end of the treatment (48 hr), DF 4'-hydroxylase activities were measured, in triplicate, by incubating DF (250 µM) with rhesus hepatocytes in 10 mM HEPES buffer, at 37°C, 95% humidity, and 5 % CO<sub>2</sub>, for 20 min. Samples from each well were transferred to a 96-well plate containing an equal volume of acetonitrile, and stored at 4°C until analysis by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) for DF and 4'-OH DF (Kumar *et al.*, 2002).

***In Vivo* Studies.** All studies were reviewed and approved by the Merck Research Laboratories Institutional Animal Care and Use Committee. The *in vivo* studies were carried out in a crossover fashion, with at least a 2-3-week washout period. Male rhesus monkeys (n=4, body weight = 4 – 7 kg) were pretreated orally with either vehicle (PEG400) or rifampin (18

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mg/kg, in PEG400), once daily for 5 days. On the morning of day 5, DF was administered intra-hepatic portally (i.pv.) to a cannulated hepatic portal vein at 0.1 mg/kg/hr for 4 hr to monkeys and blood samples were collected via a saphenous or femoral vein at predose, 60, 120, 150, 180, 200, 220, 240 (end of i.pv. infusion), 270, 300, 360, and 420 min after DF administration. Plasma samples were separated immediately at 10°C and kept frozen at -20°C. Additional studies also were conducted by intravenous (i.v.) administration of DF (0.1 mg/kg/hr for 4 hr) via a cephalic vein on day 5 in animals pretreated with the vehicle PEG400 and rifampin (18 mg/kg orally in PEG400) for 5 days.

***In Vitro* Metabolism Studies.** Studies to investigate the acyl glucuronidation pathway were conducted using rhesus monkey liver microsomes incubated with various concentration of DF in the presence of UDPGA, as previously described (Kumar *et al.*, 2002). For comparison purpose, a parallel study also was conducted using human liver microsomes. In brief, incubations (0.2-ml final volume) consisted of liver microsomes (0.1 mg/ml) previously preincubated with alamethicin (25 µg/mg microsomal protein) for 15 min, potassium phosphate buffer (100 mM, pH 7.4), MgCl<sub>2</sub> (10 mM), UDPGA (2 mM), and DF (0.5 – 150 µM final concentrations). After incubation time of 10 min, the reaction was quenched with 0.4 ml acetonitrile containing 3% formic acid, and the supernatant was separated for analysis by LC-MS/MS (Kumar *et al.*, 2002). Studies on kinetics of the oxidative metabolism of DF also were conducted in monkey and human liver microsomes, using conditions described previously by Kumar *et al.* (2002).

Subsequent metabolism studies were conducted using rhesus monkey hepatocytes (0.5 million cells/ml) incubated with 5 µM DF, in 10 mM HEPES buffer with a final incubation volume of 0.2 ml. The concentration of 5 µM is below K<sub>m</sub> values for both the glucuronidation and hydroxylation of DF estimated from the aforementioned liver microsomal studies. After incubation at 37°C, 95% humidity and 5 % CO<sub>2</sub>, the reaction was quenched, at various incubation

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times, with 0.2 ml acetonitrile containing 3% formic acid, and the supernatant was separated for analysis by LC-MS/MS (Kumar *et al.*, 2002).

**Analytical Procedures.** Concentrations of DF and rifampin in plasma were analyzed using LC-MS/MS. Plasma samples were spiked with the respective internal standard (tolbutamide for DF and diazepam for rifampin), and proteins were precipitated with acetonitrile (acetonitrile:sample = 2:1 (v/v)). After centrifugation, the supernatant were subjected directly onto LC-MS/MS analysis, and the analytes were quantitated by LC-MS/MS in selective reactions monitoring (SRM) mode using an AB/MDS SCIEX API 3000 tandem mass spectrometer (Concord, Ontario, Canada) interfaced with a SCIEX Turbo IonSpray source to a Perkin Elmer Series 200 liquid chromatography system. Chromatography was accomplished on a Synergi Fusion-RP column (2.0 x 50 mm, 4  $\mu$ M, Torrance, CA) for DF or a Betasil C<sub>18</sub> column (2.1 x 50 mm, 5  $\mu$ m, Keystone, Bellefonte, PA) for rifampin. The mobile phase consisted of 90% acetonitrile in water (solvent B) and 10% acetonitrile in 0.02% acetic acid (pH 4.5, solvent A), and was delivered at a flow rate of 0.5 ml/min. The elution of DF was achieved by a linear increase of solvent B from 5% to 25% over 0.5 min, from 25% to 53% over 3.5 min, and 53% to 80% over 0.5 min. Equilibration was allowed for additional 1.5 min, giving a total chromatographic run time of 6.0 min. The elution of rifampin was accomplished through a linear increase of solvent B from 0% to 90% over 0.6 min and held at that value for an additional 1.9 min. Equilibration was allowed for additional 1.5 min, giving a total chromatographic run time of 4.0 min. Selective reaction monitoring experiments in the positive ionization mode were performed using a dwell time of 150 ms per transition to detect ion pairs at  $m/z$  296/215 (DF), 271/155 (tolbutamide), 823/399 (rifampin) and 285/195 (diazepam). Calibration curves (5 - 5000 ng/ml) were prepared by plotting the appropriate peak area ratios against the concentrations of analyte in plasma using a weighted (1/x) quadratic regression. The concentration of the analyte in

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the unknown samples was determined by interpolation from the standard curve. For each analyte, standard curves showed satisfactory linearity and precision (<15% coefficient of variation).

**Data Analysis.** For the liver microsomal studies, apparent  $K_m$  and  $V_{max}$  values were estimated using a nonlinear regression program (Enfit from Biosoft, Ferguson, MO). The intrinsic clearance ( $CL_{int}$ ) values were estimated by dividing  $V_{max}$  by  $K_m$ . For the hepatocyte studies, the  $CL_{int}$  values were calculated by dividing initial metabolite formation rates obtained during the first 10-min incubation (nmol/min/million cells) by the substrate concentration used (5  $\mu$ M), which is below the  $K_m$  values determined for the glucuronidation and oxidation reactions.

The concentration of rifampin producing a 50% increase in DF 4'-hydroxylase or MDZ 1'-hydroxylase activity ( $EC_{50}$ ) was determined using non-linear regression analysis (PCNONLIN, Scientific Consulting, Cary, NC), as previously described (Prueksaritanont *et al.*, *in press*).

The area under the plasma concentration-time profile ( $AUC_{0-last}$ ) was calculated from time zero to the last detectable sampling time using the linear trapezoidal rule. The apparent terminal half-life ( $t_{1/2}$ ) was estimated by dividing 0.693 by the elimination rate constant determined using least-squares regression analysis of the log-linear portion of the DF plasma concentration-time data. Plasma clearance (CL) values for DF were calculated as the i.v. dose divided by their corresponding AUC from time zero to infinity ( $AUC_{0-inf}$ ). Hepatic availability ( $F_h$ ) was estimated by dividing AUC obtained following i.p.v. administration to that obtained by i.v. administration. Volume of distribution at steady-state ( $V_{dss}$ ) values were estimated by conventional moment analysis as i.v. dose multiplied by the first moment of the plasma concentration-time profile (AUMC) and divided by  $(AUC_{0-inf})^2$ . The peak plasma concentration ( $C_{max}$ ) was determined by observation.



## Results

***In Vitro* Induction Studies.** In rhesus monkey hepatocytes, rifampin markedly induced the formation of 4'-OH DF in hepatocyte preparations from three individual monkeys; values for  $EC_{50}$  ranged from 0.2 to 0.4  $\mu\text{M}$ , while those for  $E_{\text{max}}$  were about 2 – 5-fold, over control values (Fig. 1). A comparable magnitude of induction ( $EC_{50}$  and  $E_{\text{max}}$  values of 0.2  $\mu\text{M}$  and 2 – 3-fold over control values, respectively) was observed with MDZ 1'-hydroxylation in two of the same subjects (Prueksaritanont *et al.*, *in press*). In three additional subjects, about 2 – 3-fold increases in both enzyme activities also were obtained with 10  $\mu\text{M}$  rifampin (data not shown).

***In Vivo* Studies.** Upon i.v. administration of DF (0.1 mg/kg/hr), plasma concentrations of DF appeared to reach a steady-state level of about 0.8  $\mu\text{M}$  at about 2 hr post dose, and declined relatively quickly after the infusion stopped (Fig. 2A), with  $t_{1/2}$  of about 1-2 hr (Table 1). The CL of DF was moderate, with values of 10 ml/min/kg (Table 1). Treatment with rifampin (18 mg/kg) for 5 days did not significantly affect the i.v. plasma profiles (Fig. 2A) or pharmacokinetic parameters of DF; values for AUC,  $V_{\text{dss}}$ , CL and  $t_{1/2}$  of DF were comparable between the control and rifampin treated animals (Table 1). Effects of rifampin also were determined following i.p.v. administration of DF to monkeys; this route of administration provides advantages of maximizing the magnitude of interactions resulting from changes in both hepatic first pass and systemic clearance (Wilkinson 1978; Prueksaritanont *et al.*, *in press*). As shown in Fig. 2B and Table 1, rifampin also minimally affected the i.p.v. plasma levels of DF and its corresponding pharmacokinetic parameters; plasma levels of DF, although significantly lower than those observed following i.v. administration, were comparable between the control and rifampin treated animals. The hepatic availability,  $F_{\text{h}}$ , of DF was estimated to be about 30% in monkeys with or without rifampin pretreatment (Table 1).

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In rifampin-pretreated monkeys, rifampin plasma concentrations were maintained above 5  $\mu\text{M}$  over a period of 6 hr after the last dose (5<sup>th</sup> dosing day); values for  $C_{\text{max}}$  were about 16 and 10  $\mu\text{M}$ , and  $\text{AUC}_{0\text{-last}}$  were 67 and 42  $\mu\text{M}\cdot\text{hr}$  in the animals given i.v. and i.p.v. administration of DF, respectively (Table 1).

***In Vitro* Metabolism Studies.** Liver microsomal studies showed that DF underwent extensive glucuronidation in monkeys (Fig. 3A), with values for  $V_{\text{max}}$ ,  $K_{\text{m}}$  and  $\text{CL}_{\text{int}}$  ( $V_{\text{max}}/K_{\text{m}}$ ) of 7.5 nmol/min/mg, 12.2  $\mu\text{M}$ , and 613  $\mu\text{l}/\text{min}/\text{mg}$ , respectively. In contrast, the formation of 4'-OH DF was relatively minor (Fig. 3A); the  $V_{\text{max}}$ ,  $K_{\text{m}}$  and  $\text{CL}_{\text{int}}$  values were 1.2 nmol/min/mg, 72.2  $\mu\text{M}$ , and 16  $\mu\text{l}/\text{min}/\text{mg}$ , respectively. Accordingly, in monkey liver microsomes, the glucuronidation pathway represented about 95% of the total intrinsic clearances. In a parallel study using human liver microsomes, the glucuronidation pathway accounted for about 75% of the total intrinsic clearance. Values for the  $V_{\text{max}}$ ,  $K_{\text{m}}$ , and  $\text{CL}_{\text{int}}$  were 11.1 nmol/min/mg, 21.5  $\mu\text{M}$ , and 517  $\mu\text{l}/\text{min}/\text{mg}$  for the glucuronidation, and 1.9 nmol/min/mg, 10.3  $\mu\text{M}$ , and 184  $\mu\text{l}/\text{min}/\text{mg}$  for the oxidation pathways, respectively. These human liver microsomal results were in a comparable range to those reported previously by Kumar *et al.* (2002).

In rhesus monkey hepatocytes, formation of DF glucuronide was much higher than that of 4'-OH DF over the entire 1-hr incubation period (Fig. 3B). During the first 10-min of incubation, the sum of 4'-OH DF and DF glucuronide formed was approximately equal to the total DF consumption (Fig. 3B), suggesting minimal formation of other metabolites, including secondary products. The discrepancy between the combined DF glucuronide + 4'-OH DF formation and total consumption during the subsequent period (Fig. 3B) was suggestive of significant formation of secondary or sequential metabolites, likely to glucuronide and glutathione products of the hydroxyl DF (Tang *et al.*, 1999; Kumar *et al.*, 2002). The  $\text{CL}_{\text{int}}$  values (mean  $\pm$  SD) estimated during the initial incubation period from four individual subjects for the

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oxidation and glucuronidation pathways were  $2.0 \pm 1.6$  and  $43.2 \pm 6.0$   $\mu\text{l}/\text{min}/\text{million cells}$ , respectively. Thus, like the liver microsomal finding, the oxidative metabolism accounted for about 5%, and the glucuronidation represented approximately 95% of the total metabolism of DF in rhesus monkey hepatocytes.

## Discussion

In this study, we examined the *in vivo* consequence of CYP2C9 induction observed *in vitro* by rifampin, using DF as a functional probe. Comparative *in vitro* metabolism studies of DF in rhesus monkey and human liver microsomes, including immunoinhibition, chemical inhibition and activities of recombinant CYPs, consistently suggested the major contribution of CYP2C to the major oxidative pathway 4'-hydroxylation, and CYP3A to the minor 5-hydroxylation in both species (Leemann *et al.*, 1993; Shen *et al.*, 1999; Tang *et al.*, 2005). However, we observed an apparent disconnect between the *in vitro* and *in vivo* induction of DF metabolism in rhesus monkeys. *In vitro*, rifampin was a potent inducer of rhesus CYP2C; both  $EC_{50}$  and  $E_{\text{max}}$  values for the induction of 4'-OH DF formation in rhesus monkey hepatocytes were comparable to the respective values observed in this species for MDZ 1'-hydroxylase activity, a CYP3A marker (Prueksaritanont *et al.*, *in press*). In contrast to this *in vitro* observation, subsequent pharmacokinetic interaction studies in rhesus monkeys revealed that rifampin was not an inducer of DF clearance *in vivo*.

This apparent lack of *in vivo* effect of rifampin on the DF pharmacokinetics in the present monkey study is not likely due to an inadequate systemic exposure to rifampin. At the dose used in our *in vivo* studies,  $C_{\text{max}}$  values for rifampin were similar to those reported following a

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therapeutic dose in humans (Sanofi-Aventis, 2003), and well exceeded its *in vitro* EC<sub>50</sub> estimate for the induction of DF 4'-hydroxylase activity in monkey hepatocytes. In addition, rifampin, at comparable plasma levels, has been shown to cause a marked decrease (>10-fold) in the MDZ systemic exposure following i.p.v. administration of MDZ to monkeys (Prueksaritanont *et al.*, *in press*), and following its oral administration to humans (Niemi *et al.*, 2003). Rifampin is equally potent as an inducer of CYP3A-mediated MDZ 1'-hydroxylase activity in both rhesus monkey and human hepatocytes (Prueksaritanont *et al.*, *in press*).

The present negative *in vivo* interaction results between DF and rifampin, but the earlier positive observations between rifampin and MDZ also ruled out the possibility that the CYP3A-mediated oxidative metabolism is a major determinant of the *in vivo* clearance of DF in monkeys, as suggested by Tang *et al.* (1999). In contrast to earlier *in vivo* metabolism data which seemed to suggest that the oxidative metabolism of DF might account for the majority of *in vivo* clearance (Stierlin and Faigle, 1979; Stierlin *et al.*, 1979), Kumar *et al.* (2002) have recently proposed that the direct glucuronidation of DF is a more important component to the *in vivo* clearance than the oxidation pathway in rats, dogs and humans. This proposal was made based primarily on 1) *in vitro* liver microsomal metabolism data which showed much higher microsomal intrinsic clearance values for the acyl glucuronide (~70-90%) than the 4'-hydroxylation pathways, 2) a good agreement between *in vitro* and *in vivo* clearance data, in untreated subjects, when both the *in vitro* oxidation and direct glucuronidation were considered, and 3) *in vitro* liver microsomal data which showed that the glucuronide of DF could be further metabolized via oxidation, presumably leading to the *in vivo* formation of its 4'-hydroxy derivative which is eventually excreted to bile and urine. However, this proposal has not been corroborated by clinical drug interaction studies between DF and CYP2C9 inducers or inhibitors. Prior to our studies, there was no report on the glucuronidation of DF in monkeys.

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Our *in vitro* metabolism findings in both the liver microsomal and hepatocyte systems revealed that the direct glucuronidation was indeed the major ( $\geq 90\%$ ) and the hydroxylation was the minor ( $\sim 5\%$ ) metabolic pathways of DF in control rhesus monkeys. If the findings *in vitro* are scalable to *in vivo*, one would anticipate that the pharmacokinetics of DF in monkeys would not be affected appreciably by changes in its hydroxylation rate. This suggestion, which was in complete agreement with the present *in vivo* rifampin – DF interaction observations, was derived based on our preliminary study which showed a modest increase ( $< 30\%$ ) in DF glucuronide formation in rhesus monkey hepatocytes treated with  $10\ \mu\text{M}$  rifampin (data not shown), and an assumption that the intrinsic clearance value for the hydroxylation pathway was increased by 2 – 5 fold with rifampin treatment (i.e., from 5 out of 95 units to 10 out of 105 units – 25 out of 120 units), based on the present *in vitro*  $E_{\text{max}}$  value of  $\sim 2$  – 5-fold obtained for the 4'-OH DF formation in monkey hepatocytes.

In humans, the oxidative metabolism of DF, albeit more substantial and catalyzed by relatively higher affinity enzyme than in monkeys, also is likely a minor contributor ( $\sim 25\%$  based on the liver microsomal data) to its *in vivo* clearance. Similarly, it is expected that any changes in the DF hydroxylation rate, yielding  $< 50\%$  of its contributions to the total metabolic rate, would not have substantial impacts on the *in vivo* clearance of DF in humans. In this regard, there have been no clinical reports on the pharmacokinetic interactions of DF with known potent CYP2C9 inhibitors or inducers, including rifampin. In human hepatocytes, rifampin is an inducer of DF 4'-hydroxylase activity; a mean increase in the activity of about 2-fold over control has been reported in the presence of  $20\ \mu\text{M}$  rifampin (Parkinson *et al.*, 2004), and a comparable magnitude of increase also was obtained with  $10\ \mu\text{M}$  rifampin in our preliminary study (data not shown). By analogy, rifampin may also elicit modest effects on the DF pharmacokinetics, *via* induction of CYP2C9, in humans, as was the case in monkeys. It is worth noting that possible differences in the magnitude of drug interaction between DF and other potent enzyme inhibitors/inducers exist

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between monkeys and humans, based on the quantitative differences in the kinetics of DF metabolism observed in this study between the two species, and considering potential species differences, in general, in enzyme induction and inhibition reported in the literature.

In conclusion, using the rhesus monkey as an animal model, we showed that in contrast to significant induction of the *in vitro* oxidative metabolism, the *in vivo* pharmacokinetics of DF were not sensitive to rifampin treatment. Subsequent *in vitro* metabolism studies suggested that this apparent *in vitro* – *in vivo* discrepancy was due likely to the fact that the CYP2C-mediated oxidative metabolism of DF is not the major determinant for its *in vivo* clearance in monkeys, both untreated and pretreated with rifampin. Our data also substantiated a previous suggestion made based on an *in vitro* finding in rat, dog and human liver microsomes (Kumar *et al.*, 2002) that the direct glucuronidation of DF instead is a more important determinant for the *in vivo* clearance of DF in both animals and humans, thus raising a question on the utility of DF as a useful probe for measuring CYP2C9 activity *in vivo*.

### Acknowledgments

We thank Ms. J. Brunner, K. Michel and J. Adelsburger for animal experiments.

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Figure legends

*FIG. 1. Effect of various concentrations of rifampin on DF 4'-hydroxylase activity in rhesus monkey hepatocytes from 3 different individuals.*

Results are mean of triplicate determinations. DF 4'-hydroxylase activities were determined using 250  $\mu$ M DF. Rhesus monkey hepatocytes treated with vehicle (DMSO, control) or rifampin for 48 hr prior to enzyme activity determinations.

*FIG. 2. Mean plasma concentration-time profiles of DF following i.v. (A) or i.pv. (B) administration of DF (0.1 mg/kg/hr for 4 hr) to rhesus monkeys pretreated with vehicle or rifampin (18 mg/kg p.o. once daily) for 5 days.*

Results are mean  $\pm$  SD, n=4.

*FIG. 3. Kinetics of 4'-OH DF and DF acyl glucuronide formation in rhesus monkey liver microsomes (A), and time profiles of DF metabolism (DF disappearance and formation of its metabolites) in rhesus monkey hepatocytes (B).*

Results shown in Fig. 3A are mean  $\pm$  SD of triplicate determinations. Formation of 4'-OH DF and DF acyl glucuronide was determined following a 5-min (for glucuronidation) or 20-min (for oxidation) incubation with various concentrations of DF and liver microsomes (0.2 mg/ml for oxidation and 0.05 mg/ml for glucuronidation) in the presence of 1 mM NADPH and 5 mM UDPGA, respectively. Results shown in Fig. 3B are mean of two individual subjects, each determined in triplicate, using 5  $\mu$ M DF and rhesus monkey hepatocytes (0.5 million cells/ml).

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TABLE 1

Pharmacokinetic parameters of DF following intravenous (i.v.) or intraportal vein (i.pv.) administration of DF (0.1 mg/kg/hr for 4 hr) to rhesus monkeys pretreated with vehicle (PEG400) or rifampin (18 mg/kg p.o.) for 5 days

Compound measured	PK Parameters	Vehicle Control	Rifampin 18 mg/kg	
				<b>% of control</b>
<b><u>i.v. Dose</u></b>				
DF	AUC <sub>0-last</sub> , μM.hr	2.69 ± 0.58	2.22 ± 0.32	87 ± 26 (51 - 111)
	AUC <sub>0-inf</sub> , μM.hr	2.74 ± 0.54	2.27 ± 0.36	87 ± 26 (51 - 112)
	CL, ml/min/kg	8.5 ± 1.4	10.2 ± 1.7	126 ± 46 (89 - 198)
	V <sub>dss</sub> , L/kg	0.32 ± 0.05	0.29 ± 0.07	89 ± 11 (73 - 100)
	t <sub>1/2</sub> , hr	1.2 ± 0.7	1.2 ± 0.5	136 ± 47 (91 - 220)
	Rifampin	AUC <sub>0-last</sub> , μM.hr		67.0 ± 41.4
	C <sub>max</sub> , μM		16.1 ± 10.6	
<b><u>i.pv. Dose</u></b>				
DF	AUC <sub>0-last</sub> , μM.hr	0.69 ± 0.21	0.63 ± 0.19	92 ± 13 (73 - 103)
	AUC <sub>0-inf</sub> , μM.hr	0.73 ± 0.23	0.67 ± 0.21	92 ± 14 (71 - 101)
	F <sub>h</sub>	0.27 ± 0.09	0.31 ± 0.13	118 ± 55 (81 - 199)
	t <sub>1/2</sub> , hr	1.8 ± 1.3	1.6 ± 0.6	119 ± 64 (62 - 212)
Rifampin	AUC <sub>0-last</sub> , μM.hr		42.4 ± 21.8	
	C <sub>max</sub> , μM		9.8 ± 4.7	

Values are Mean ± SD, n=4.

Values in parentheses are range.

Fig. 1

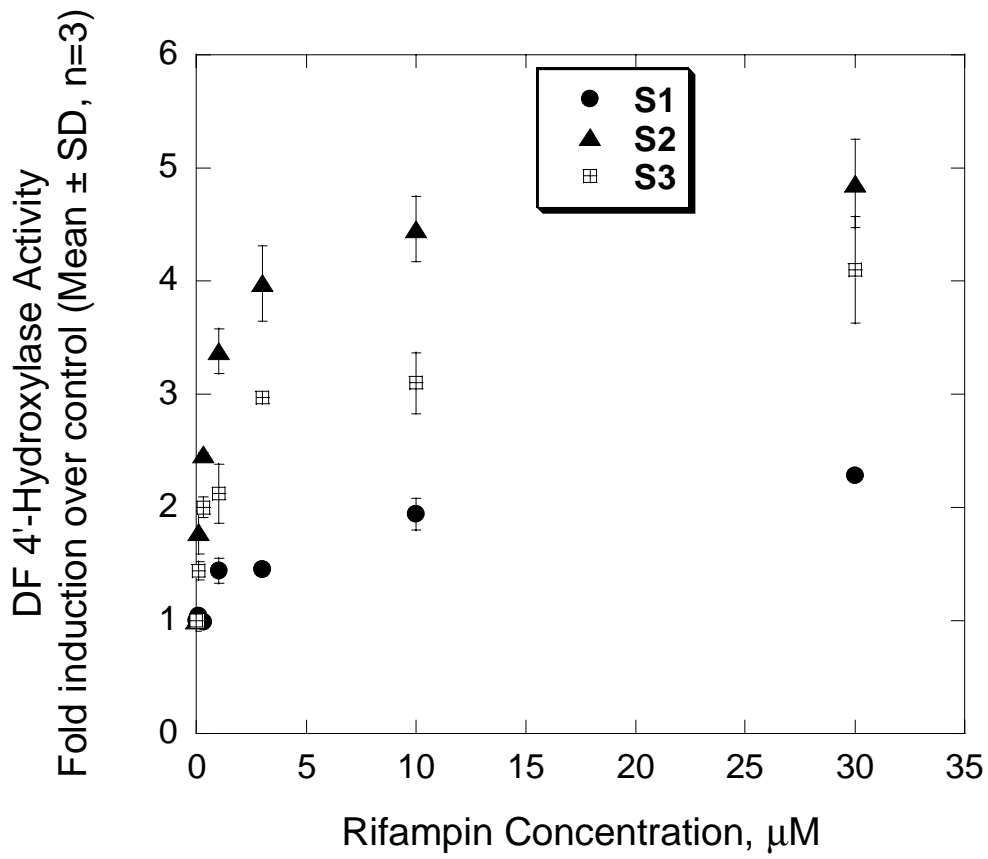


Fig. 2

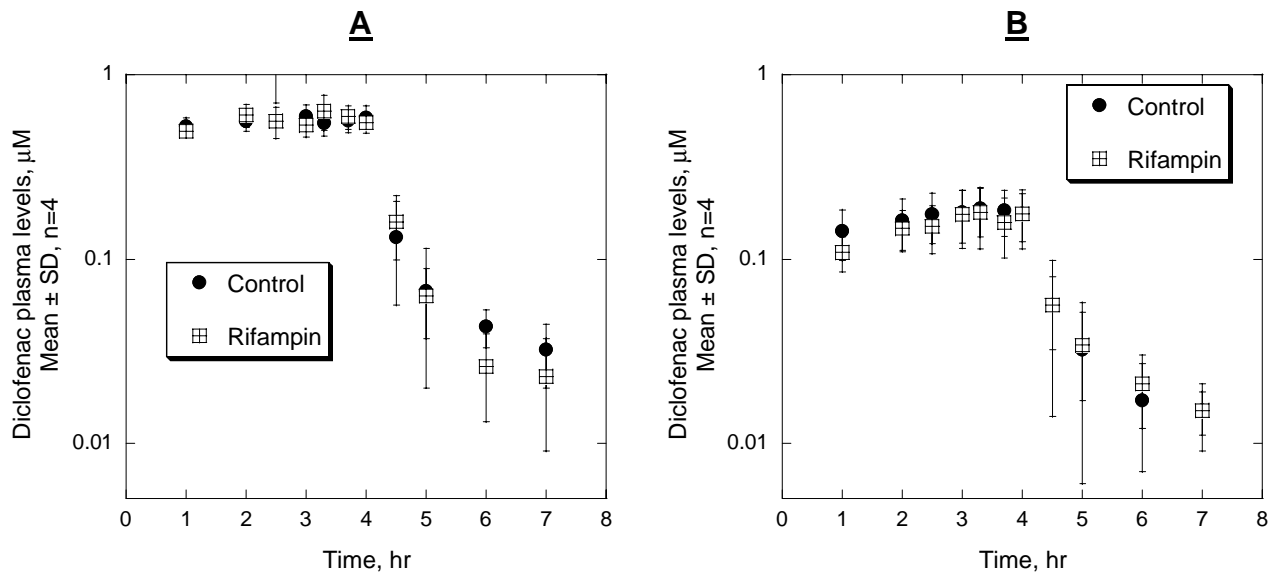


Fig. 3

