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Research Paper

Comparative Effects of Fibrates on Drug Metabolizing Enzymes in Human Hepatocytes

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Purpose. The induction potential of different fibric acid derivatives on human drug metabolizing enzymes was evaluated to help assess the role of enzyme induction on pharmacokinetic drug interactions.
Methods. Effects of gemfibrozil, fenofibric acid, and clofibric acid on expression levels of cytochromes P450 (CYPs) 3A4 and 2C8 and UDP-glucuronyltransferase (UGT) 1A1 were evaluated in primary human hepatocyte cultures. The potential for these fibrates to activate human pregnane X receptor (PXR) also was studied in a cell-based PXR reporter gene assay.
Results. All three fibrates caused increases in mRNA levels of CYP3A4 (2- to 5-fold), CYP2C8 (2- to 6-fold), and UGT1A1 (2- to 3-fold). On average, the effects on CYP3A4 were less than (≤30% of rifampin), while those on CYP2C8 and UGT1A1 were comparable to or slightly higher than (up to 200% of rifampin) the corresponding effects observed with rifampin (10 μM). Consistent with the mRNA results, all fibrates caused moderate (~2- to 3-fold) increases in CYP3A4 activity (measured by testosterone 6β hydroxylase), as compared to about a 10-fold increase by rifampin. Significant increases (3- to 6-fold) in amodiaquine *N*-deethylase (a functional probe for CYP2C8 activity) also were observed with clofibric acid, fenofibric acid, and rifampin, in agreement with the mRNA finding. However, in contrast to the mRNA induction, marked decreases (>60%) in CYP2C8 activity were obtained with gemfibrozil treatment. Consistent with this finding, co-incubation of amodiaquine with gemfibrozil, but not with fenofibric acid, clofibric acid, or rifampin, in human liver microsomes or hepatocytes resulted in significantly decreased amodiaquine *N*-deethylase activity (IC_{50} = 80 μM for gemfibrozil, >500 μM for fenofibric or clofibric acid, and >50 μM for rifampin). Similar to rifampin, all three fibrates caused a modest change in the glucuronidation of chrysin, a nonspecific substrate of UGTs. No significant activation on human pregnane X receptor (PXR) was observed with the three fibrates in a PXR reporter gene assay.
Conclusions. In human hepatocytes, both fenofibric acid and clofibric acid are inducers of CYP3A4 and CYP2C8. Gemfibrozil is also an inducer of CYP3A4, but acts as both an inducer and an inhibitor of CYP2C8. In this system, all fibrates are weak inducers of UGT1A1. The enzyme inducing effects of fibrates appear to be mediated via a mechanism(s) other than PXR activation. These results suggest that fibrates may have potential to cause various pharmacokinetic drug interactions via their differential effects on enzyme induction and/or inhibition.

KEY WORDS: clofibrate; clofibric acid; CYP2C8; CYP3A4; enzyme induction; enzyme inhibition; fenofibrate; fenofibric acid; fibrates; gemfibrozil; rifampin; statins; UGT1A.

INTRODUCTION

Derivatives of fibric acid or so-called fibrates have been used clinically to treat dyslipidemia, including hypertriglyceridemia (1). The mechanism of action for the hypotriglyceridemic action of fibrates, although not completely understood, is believed to be due to increased expression/activity of lipoprotein lipase and/or hepatic lipase, as well as decreased hepatic apoC-III synthesis. Clofibrate was the first fibrate to be prescribed. Gemfibrozil, fenofibrate, bezafibrate, and ciprofibrate are among currently available fibrates on the market, but in the United States only gemfibrozil and fenofibrate are prescribed. Of these fibrates, clofibrate and fenofibrate are available as ester prodrugs, which upon conversion *in vivo* yield pharmacologically active species, clofibric acid and fenofibric acid, respectively.

Fibrates are often used in patients with dyslipidemia, who frequently have other diseases that may require other drugs. Pharmacokinetic and/or pharmacodynamic interactions have been reported between fibrates and concomitant medications, including anticoagulants, antidiabetics and hypocholesterolemic agents (2,3). Recently, the interactions observed following a combination therapy between fibrates and hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (so-called statins), have been shown to have a phar-

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ABBREVIATIONS: CYP, cytochrome P450; LBD, ligand binding domain; MEM, minimum essential medium; PXR, human pregnane X receptor; UGT, UDP glucuronyltransferase.

macokinetic component (4–7). These interactions, which occurred to various degrees with different fibrates and statins, have been attributed, at least in part, to differential inhibitory effects of fibrates on CYPs and UGTs, as well as different clearance mechanisms among statins (8,9). Although both gemfibrozil and fenofibrate are not inhibitors of CYP3A4, gemfibrozil was shown to be a more potent inhibitor than fenofibrate of CYP2C8-mediated oxidative metabolism of cerivastatin and of the UGT1A1/1A3-mediated glucuronidation of several statins, including cerivastatin, simvastatin and atorvastatin (8,9). CYP2C8 is an important enzyme involved in the oxidation of cerivastatin, whereas CYP3A4 is the major enzyme for the oxidative metabolism of simvastatin or atorvastatin (8–10). These results are consistent with the higher magnitude of interaction observed with gemfibrozil and cerivastatin than other fibrate-statin combinations (6,9,11). However, in our previous studies, potential inducibility of drug metabolizing enzymes by fibrates was not incorporated into the model. Considering that fibrates are usually administered at relatively high doses with high systemic exposures for a relatively long treatment period (2,3,12) and that there have been several well documented clinically relevant drug interactions, resulting from enzyme induction and/or a combination of enzyme inhibition and induction (13–17), there is a possibility for enzyme induction to also occur with fibrates. Differential inducibility effects among fibrates, if exist, would complicate data interpretations and eventual conclusions. To date, there has been limited information regarding enzyme induction by fibrates on drug metabolizing enzymes in humans.

Thus, the aims of the present investigation were to compare the induction potential of gemfibrozil, fenofibric acid, and clofibric acid on CYP3A4, CYP2C8, and UGT1A1. These enzymes were selected based on availability of mRNA probes, and because they are known to be inducible by xenobiotics and to be involved in the metabolism of wide variety of marketed drugs, and especially statins (8,18–21). In this study, primary cultures of human hepatocytes were used as the model system, and effects of fibrates primarily on mRNA expression levels were characterized and compared with rifampin, a reference control. In order to relate results obtained from this *in vitro* study to *in vivo* clinical situations, all compounds were studied at or over their therapeutic plasma concentration range: 50 and/or 250 μM fibrates (3,4,12) and 10 μM rifampin (22). In addition, functional activities of CYP3A4 and CYP2C8 were determined in parallel using specific marker substrates. Chrysin glucuronidation also was measured to gauge possible effects of fibrates on activity of UGTs. To better understand an apparent lack of correlation between mRNA and functional activity observed for CYP2C8, inhibitory effects of fibrates on the CYP2C8 functional marker activity used in this study were compared. A possibility for the fibrates to activate human PXR, a mechanism recently shown to mediate induction of several drug metabolizing enzymes (20,23–25), also was studied in a cell-based PXR reporter gene assay.

MATERIALS AND METHODS

Materials

Clofibric acid, gemfibrozil, fenofibric acid, rifampin, testosterone, cortisone, amodiaquine, and chrysin were obtained from Sigma (St. Louis, MO, USA), and 6β-hydroxytestosterone was purchased from Steraloids (Wilton, NH, USA). Glutamine, nonessential amino acids, and penicillin-streptomycin were purchased from Invitrogen (Carlsbad, CA, USA). Insulin, transferrin, selenium, linoleic acid, and bovine serum albumin were purchased from BD Biosciences (Bedford, MA, USA) and hepatocyte culture media from JRH Biosciences (Lenexa, KS, USA). Minimum essential medium (MEM) and Dulbecco's modified Eagle medium (DMEM) with or without bovine serum were obtained from Invitrogen Corp. Glo-Lysis buffer and luciferase assay reagent were obtained from Promega (Madison, WI, USA). All other reagents were of analytical or HPLC grade. Human liver microsomes were obtained from Xenotech (Kansas City, KS, USA) and Gentest (Woburn, MA, USA). Human hepatocytes from 4–6 different donors (Caucasian males and females, age 14–69 years) were obtained from commercial sources (CellzDirect, Tucson, AZ, USA; *in vitro* Technologies, Baltimore, MD, USA; and Tissue Transformation Technologies, Edison, NJ, USA) in 24-well culture plates. Oligonucleotides were purchased from GIBCO BRL Life Technologies (Rockville, MD, USA), Midland Certified Reagent Company (Midland, TX, USA), or PE Applied Biosystems (Foster City, CA, USA).

Cell Culture Treatment

Hepatocyte cultures were maintained at 37°C, 95% humidity, and 5% CO_2 in hepatocyte culture media supplemented with L-glutamine (0.292 mg/ml), nonessential amino acid solution (10 μM), insulin (6.25 μg/ml), transferrin (6.25 μg/ml), selenious acid (6.25 ng/ml), bovine serum albumin (125 mg/ml), linoleic acid (5.35 μg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml), and 0.1 μM dexamethasone. Cells were maintained for 2 days post-plating, with medium changed daily. Hepatocyte cultures were treated, in triplicate for each treatment, for 2 or 3 consecutive days with culture media containing each fibrate (50 and 250 μM), rifampicin (10 μM) or vehicle control (DMSO, 0.1% v/v). Over the treatment period, dosing media were changed at 24-h intervals. At the end of the treatment (48 and 72 h), medium was aspirated from wells and plates were either stored at −70°C for later RNA isolation and quantitation, or replaced with 10 mM HEPES buffer and returned to the incubator for 10 min, after which the 10 mM HEPES buffer was aspirated and replaced with 0.5 ml of HEPES buffer containing enzyme marker substrates for measuring enzyme activities (see below).

RNA Isolation and Quantitation

Total RNA was isolated using an RNeasy 96 kit with a BioRobot 3000 (Qiagen, Inc., Valencia, CA, USA). The RNA was DNase I treated and eluted with a total of 110 μl water. The RNA was quantitated using a Ribogreen RNA Quantitation Reagent kit (Molecular Probes, Eugene, OR, USA).

A two-step reverse transcription-polymerase chain reaction (RT-PCR) was conducted by reverse transcribing 50 ng of total RNA to cDNA using TaqMan Reverse Transcription Reagents with random hexamer primers. cDNA (2 μl) was amplified using the Taqman Universal Master Mix and Ribosomal RNA Control kits according to the manufacturer's rec-

ommended protocol (PE Applied Biosystems, Foster City, CA, USA). Taqman primers and probes were designed using Primer Express Software v1.0 (PE Applied Biosystems) and Genbank accession numbers Y00498, M18907, and AF297093 for CYP2C8, CYP3A4, and UGT1A1, respectively. The primers and probes were optimized and validated using cDNA templates for the respective targets. Taqman primer/probe concentrations for specific targets CYP3A4, CYP2C8, and UGT1A1 were 300/200 nM, 300/200 nM, and 900/225 nM, respectively. The CYP3A4, CYP2C8, and UGT1A1 probes were labeled with the fluorescent dyes FAM (5′) and TAMRA (3′), and the 18S rRNA probe was labeled with VIC (5′) and TAMRA (3′). PCR amplification conditions were as follows: 1 cycle at 50°C, 2 min; 1 cycle at 95°C, 10 min; 40 cycles at 95°C, 15 s; 40 cycles at 60°C, 1 min PCR amplified cDNAs were detected by real-time fluorescence on an ABI PRISM 7700 Sequence Detection System (Perkin Elmer). Quantitation of the target cDNA in treated samples vs. DMSO (vehicle) control samples was calculated by correcting for the 18 rRNA in each sample (ΔCt) using the equation: Fold Change $= 2^{-\Delta\Delta Ct}$.

PXR Reporter Gene Assay

The ligand binding domain (LBD) of human PXR, nucleotides 622-1608 (GenBank AF061056), was amplified from commercially available Human Liver Quick-Clone cDNA (Becton, Dickinson and Company) using primers, LBD Forward 5′-ATGATCGAATTCGACGAGGCCGTG-3′and LBD Reverse 5′-GGGCCTCGAGTCAGCTACC-3′. The reporter construct was generated through insertion of 5 tandem copies of the upstream activation sequence for GAL4 into the luciferase reporter vector, pFR-LUC vector (Stratagene, La Jolla, CA, USA), as previously described in Hartley *et al.* (26). HepG2 cells (American Type Culture Collection, Manassas, VA, USA) grown to 90% confluence in T75 cm^2 flasks were transfected with Transfast (250 μl/flask, Promega Corp.), 14.4 μg/flask of the human PXR-GAL4, and 28.8 μg/flask of the 5 X Gal4 luciferase reporter plasmid all in 6 ml MEM without bovine serum. After a 3-h incubation, additional MEM media (10 ml/flask) was added to each transfection and the cells were allowed to incubate overnight. Transfected cells were split into 24-well plates (250,000 cells/well) on the following day. Immediately after plating, cells were treated with each fibrate, rifampin, or DMSO as vehicle (0.1%). Forty hours after treatment the experiment was terminated by aspiration of the media and addition of Glo-Lysis buffer (100 μl). An equal volume of cell lysate from each sample was combined with luciferase assay reagent, and luminescence was assessed in a Wallac Microbeta 1450 liquid scintillation/luminescence plate reader (Wallac, Turku, Finland).

Determination of Enzyme Activity

After 48 and 72 h of exposure to the test compounds, testosterone 6β-hydroxylase (CYP3A4) and amodiaquine *N*-deethylase (CYP2C8) activities, and chrysin glucuronide formation in cultured hepatocytes were measured using testosterone, amodiaquine and chrysin as marker substrates, respectively. Chrysin is an efficient substrate for UGT1A1, UGT1A9 (27), and UGT1A3 (28). Testosterone (250 μM), amodiaquine (25 μM), and chrysin 25 (μM) were incubated with human hepatocytes in 10 mM HEPES buffer, at 37°C, 95% humidity, and 5% CO_2, for 3, 1, and 1.5 h, respectively. Under these incubation conditions, rates of metabolite formation were linear with respect to protein concentration and incubation time. Samples from each well (400 μl) were transferred to a 96-well plate containing an equal volume of acetonitrile, and stored at 4°C until analysis by high performance-liquid chromatography (HPLC) or liquid chromatography coupled with mass spectrometry (LC/MS) (see below).

Inhibitory Effect of Fibrates on CYP2C8 Activity *in Vitro*

Activities of CYP2C8 (amodiaquine *N*-deethylase) were determined using amodiaquine (25 μM) as a marker substrate (29). Stock solutions of fibrates (1–25 mM) were prepared in 50% acetonitrile in water. Each fibrate (20–500 μM final concentration) and rifampin (1–50 μM final concentration) was co-incubated with the marker substrate and human liver microsomes (0.02 mg microsomal protein/0.2 ml incubation) or human hepatocytes (0.05 million cells/0.2 ml incubation) before the reaction was initiated with NADPH (1 mM). In a separate experiment, each fibrate was preincubated with human liver microsomes in the presence of NADPH for 30 min before the reaction was initiated with amodiaquine. The incubation was performed at 37°C for 20 min (liver microsomes) or 60 min (hepatocytes) and the reaction was terminated with the addition of acetonitrile. The formation of amodiaquine N-deethylated product was determined by LC/MS (see below).

HPLC and LC-MS/MS Analyses

HPLC analyses of incubates of testosterone and chrysin for determination of 6β-hydroxy testosterone and chrysin glucuronide, respectively, were performed using a reversed phase chromatography, as previously described (10,30). Due to unavailability of chrysin glucuronide synthetic standard, chrysin glucuronide was identified based on HPLC retention time of a product formed in human liver microsomal incubates with chrysin and UDPGA, and confirmed by mass spectral analysis. The samples were chromatographed on a Keystone BDS C_{18}-Symmetry column (150 × 4.6 mm, 5 μm), preceded by a C_{18} guard column, with a linear gradient of ACN and 25 mM ammonium acetate (0–70% acetonitrile in 15 min).

A PE Sciex API 3000 triple quadrupole mass spectrometer was used for determination of amodiaquine N-deethylation. The instrument was operated in a positive ionization mode using the Heated Nebulizer interface. Selected reaction monitoring (SRM) was used to determine specific precursor-ion to product-ion transitions for each analyte (LC-MS/MS). Chromatography was conducted using a Hypersil-C_{18}-BDS reversed-phase HPLC column (2.1 mm ID × 20 mm, 5 μm particle size) purchased from Phenomenex (Bellefonte, PA, USA). The mobile phase consisted of A: 10% methanol in deionized water with 0.05% formic acid, and B: 10% deionized water in acetonitrile with 0.05% formic acid. The analytes were eluted using a linear gradient from 5–60% B over 2.5 min at a flow rate of 0.5 ml/min. The precursor → product ions monitored were *m/z* 328 → *m/z* 283 (amodiaquine *N*-deethylated product) and *m/z* 325 → *m/z* 160 (quinidine, an internal standard).

Due to unavailability of the metabolite standards for amodiaquine N-deethylated product and chrysin glucuronide, no attempt was made to estimate the absolute concentrations of these metabolites. To determine the inhibitory effect of fibrates or rifampin, peak area ratios between each metabolite and the internal standard obtained in the presence of fibrates or rifampin were compared to those obtained without the test compounds. Preliminary studies showed that these peak area ratios were linear upon dilution over the studied range, and that various concentrations of fibrates did not affect the ionization signals of the ions monitored.

RESULTS

Effects of Fibrates on mRNA Levels of CYP3A4, CYP2C8, and UGT1A1

Under the experimental conditions used in this study, rifampin, a known inducer of CYP3A4, induced markedly (mean ~14-fold, Table I) the mRNA level of CYP3A4 in all five hepatocyte preparations (Fig. 1A). In addition, rifampin also significantly induced mRNA levels of CYP2C8 (mean ~3-fold) and UGT1A1 (mean ~2-fold) (Table I; Figs. 1B and 1C), similar to previous observations (19,20). Under the same conditions and in most subjects, fenobric acid and gemfibrozil, at 250 μM, significantly induced mRNA levels of CYP3A4, CYP2C8 and UGT1A1, with the lowest induction level observed with UGT1A1 (Figs. 1A, 1B, and 1C). On average, the effects of all three fibrates (250 μM) on CYP3A4 were much less than (≤30% of rifampin), while those on CYP2C8 and UGT1A1 were comparable to or slightly higher than (~60-200% of rifampin), the corresponding effects observed with rifampin (10 μM) (Table I). At 50 μM concentration, gemfibrozil or fenofibric acid exhibited lesser effects on mRNA levels of these enzymes (Table I). On an equimolar basis, clofibric acid appeared to have slightly lesser effects on all three enzymes than fenofibric acid or gemfibrozil (Table I). For CYP3A4 and UGT1A1, the magnitude of induction by all compounds appeared maximal following 48-h treatment, whereas for CYP2C8, there was a slight increase in mRNA levels with the 72-h (vs. 48-h) treatment (Table I). For all enzymes and all compounds studied, marked inter-subject variability in the magnitude of induction was observed (Figs. 1A, 1B, and 1C).

Effects of Fibrates on Catalytic Activities of CYP3A4, CYP2C8, and UGTs

In agreement with the aforementioned observation with mRNA levels of CYP3A4, testosterone 6β-hydroxylase activities were induced by rifampin, with a mean level of induction of approximately 10-fold (Fig. 2A). The effects of fibrates on CYP3A4 activity also were consistent with their effects on CYP3A4 mRNA expression; all three fibrates (at 250 μM) increased activities of CYP3A4 by ~2- to 6-fold (Fig. 2A). As was observed with the mRNA results, this induction effect on CYP3A4 activity was generally less with clofibric acid (mean ~2-fold or ~10% of rifampin) than with fenofibric acid or gemfibrozil (mean ~4-fold or ~40% of rifampin). In addition, the effect also was lower with 50 μM (~1.5- to 2-fold) than with 250 μM fibrates (data not shown). For all treatments and following both 48-h and 72-h incubations, a reasonably good correlation, with R^2 of > 0.8, was observed between CYP3A4 mRNA and its activity (data not shown).

Also in agreement with CYP2C8 mRNA induction, significant increases in amodiaquine N-deethylase activity by rifampin, clofibric acid and fenofibric acid were evident in a limited set of subjects, with fenofibric acid showing a greater effect (Fig. 2B). Mean increases in CYP2C8 activity following 72-h incubation were ~5-fold, ~3-fold, and ~7-fold for rifampin (10 μM), clofibric acid (250 μM), and fenofibric acid (250 μM), respectively. However, in the same set of subjects, gemfibrozil (250 μM) caused marked decreases (>60% of vehicle control) in amodiaquine *N*-deethylase activity (Fig. 2B), in contrast to the mRNA results. Similar results, but with slightly lesser effects, also were evident following 48-h pretreatment and at 50 μM fibrates (data not shown).

Rifampin and all three fibrates, at 250 μM, insignificantly induced the glucuronidation of chrysin, a substrate for UGT1A1, UGT1A3 and UGT1A9 (27,28), for the majority of subjects studied following the 72-h pretreatment (Fig. 2C). Similar observations were also obtained with 48-h incubations and at the 50 μM concentration (data not shown).

Inhibitory Effects of Fibrates on CYP2C8 Activity

In both human hepatocyte (Fig. 3A) and human liver microsomal (Fig. 3B) systems, gemfibrozil exhibited a much more pronounced inhibition on amodiaquine N-deethylase activity than did clofibric acid or fenofibric acid. The inhibitory effect of gemfibrozil was concentration dependent, with an IC_{50} value of ~80 μM in both systems. Preincubation of fibrates with human liver microsomes in the presence of NADPH did not increase their inhibitory activity on CYP2C8 (data not shown), suggesting that the inhibitory effect was not mechanism-based. Less than 40% inhibition of CYP2C8 activity was observed at the highest concentration (500 μM) tested for both fenofibric acid and clofibric acid. Rifampin, up

Table I. Effects of Rifampin and Fibrates on mRNA Levels in Primary Culture Human Hepatocytes

Treatment	Concentration (μM)	CYP3A4		CYP2C8		UGT1A1	
		48 h	72 h	48 h	72 h	48 h	72 h
Rifampin	10	14.2 ± 15.3 (100)	12.7 ± 7.7 (100)	3.1 ± 1.6 (100)	3.4 ± 1.2 (100)	2.4 ± 1.5 (100)	1.9 ± 0.4 (100)
Clofibric acid	250	1.8 ± 1.7 (<10)	2.5 ± 2.7 (10)	2.2 ± 1.0 (57)	3.2 ± 2.3 (79)	1.7 ± 0.7 (60)	1.6 ± 0.4 (66)
Fenofibric acid	50	1.4 ± 0.9 (<10)	1.6 ± 0.8 (<10)	1.8 ± 1.5 (137)	2.9 ± 1.8 (78)	1.1 ± 0.5 (—)	1.8 ± 0.9 (90)
Fenofibric acid	250	4.6 ± 3.2 (30)	4.3 ± 1.7 (32)	4.5 ± 4.6 (181)	6.3 ± 5.5 (198)	2.5 ± 1.2 (161)	2.9 ± 1.8 (210)
Gemfibrozil	50	1.4 ± 1.1 (<10)	1.5 ± 1.2 (<10)	1.1 ± 0.6 (10)	1.1 ± 0.7 (10)	1.5 ± 0.6 (40)	1.1 ± 0.4 (29)
Gemfibrozil	250	3.5 ± 3.9 (21)	3.8 ± 2.6 (25)	2.2 ± 1.1 (55)	4.9 ± 3.8 (142)	1.9 ± 0.6 (90)	2.2 ± 0.7 (128)

Results (average ± SD of 3–5 donor preparations) are expressed as fold increases over vehicle control [DMSO at 0.1% (v/v)]. Values in parentheses are mean percentage increases relative to rifampin (positive control).

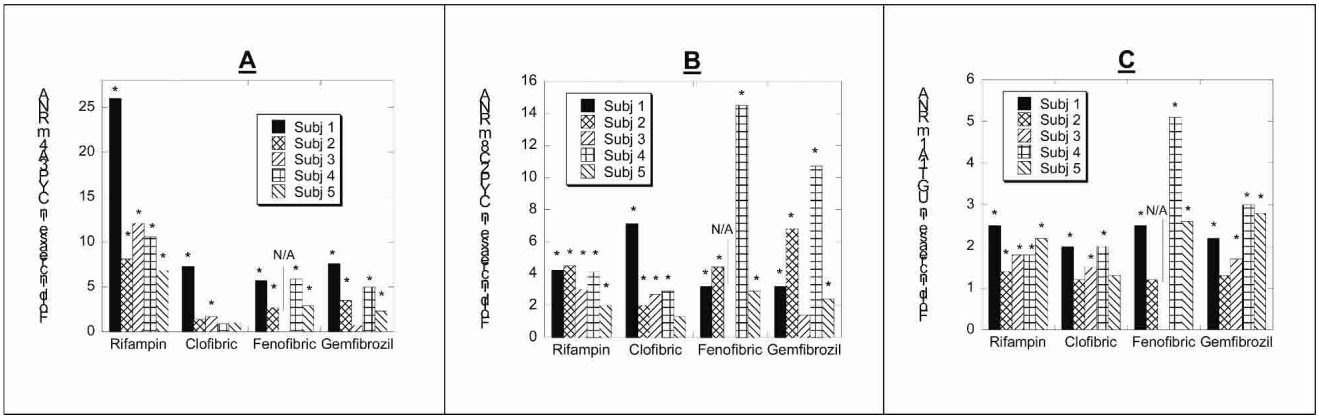

Fig. 1. Induction of CYP3A4 (A), 2C8 (B), and UGT1A1 (C) mRNA levels by rifampin, clofibric acid, fenofibric acid, and gemfibrozil in primary cultures of human hepatocytes. Hepatocyte cultures were treated for 72 h, in triplicate for each inducer concentration, with rifampin (10 μM) or each fibrate (250 μM). Following an aspiration of medium, total RNA was isolated and specific mRNA relative amounts were determined as described under "Materials and Methods." Results are expressed as fold increase over vehicle control [DMSO at 0.1% (v/v)]. N/A = data not available. *Indicates statistically significant difference from control ($p < 0.05$).

to 50 μM concentration, also did not cause significant inhibition (<15%) on the amodiaquine N-deethylase activity in human liver microsomes (data not shown).

PXR Activation

To determine whether the induction of CYP3A4, CYP2C8 and UGT1A1 by fibrates is mediated through direct activation of PXR, fibrates and rifampin, a positive control, was incubated with HepG2 co-transfected with a hPXR expression vector and a reporter gene. As shown in Fig. 4, PXR was activated strongly by rifampin (10 μM), consistent with previous findings (19,31). However, under the same experimental conditions, all three fibrates (up to 250 μM) failed to activate PXR to appreciable extent. At the highest concentration of fibrates tested, no evidence for cell toxicity was observed in this experiment.

DISCUSSION

The current results showed that all three fibrates were capable of inducing CYP3A4, CYP2C8 and UGT1A1, at the mRNA level. To our knowledge, this is the first report of induction of CYP3A4, CYP2C8, and UGT1A1 by fenofibric acid and gemfibrozil in human hepatocytes. Our finding that clofibrate induced CYP2C8 and CYP3A4 mRNA agreed with a previous report, based on a genechip microarray assay (32). Unlike rifampin which is a more potent inducer of CYP3A4 relative to CYP2C8, all three fibrates showed a trend for greater effects on mRNA of CYP2C8 than CYP3A4. Consistent with the fact that the human UGT family is generally much less susceptible than CYPs to induction by typical enzyme inducers (33,34), UGT1A1 mRNA was least induced by all compounds tested.

In the current study, the induction potential of fibrates on CYP3A4 also was demonstrated at the enzyme functional activity level. However, in the case of CYP2C8, a good agreement between mRNA and functional activity results, although limited, was observed only with clofibric acid and fenofibric acid, but not gemfibrozil. This disagreement was shown to be due to the inhibitory effect of gemfibrozil, but not the other two fibrates on CYP2C8 activity, as measured by amodiaquine N-deethylase. Our present results which

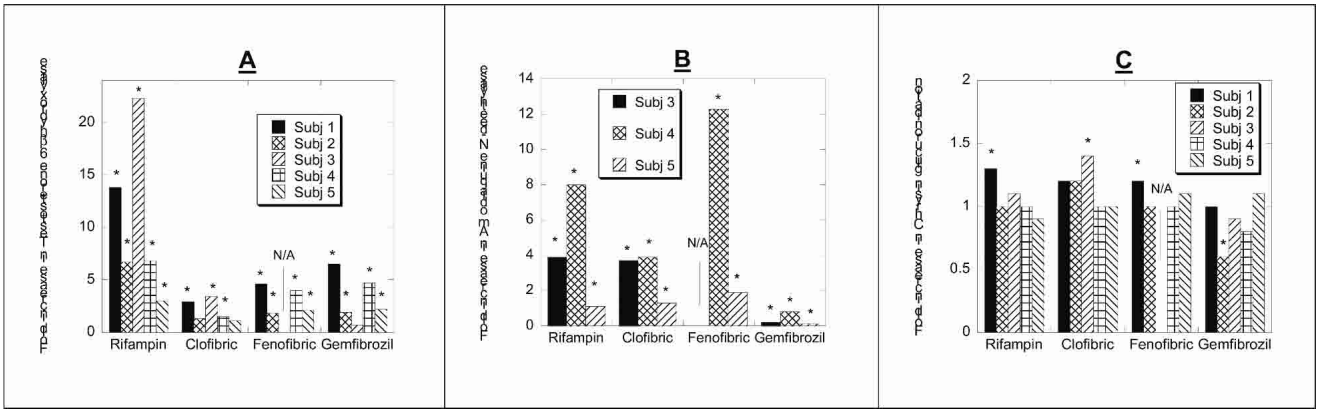

Fig. 2. Induction of testosterone 6β-hydroxylase (A), amodiaquine *N*-deethylase (B), and chrysin glucuronidation (C) activities by rifampin, clofibric acid, fenofibric acid, and gemfibrozil in primary cultures of human hepatocytes. Hepatocyte cultures were treated, in triplicate for each inducer concentration, for 72 h with rifampin (10 μM) or each fibrate (250 μM), and enzyme activities were determined as described under "Materials and Methods." Results are expressed as fold increase over vehicle control [DMSO at 0.1% (v/v)]. N/A = data not available. *Indicates statistically significant difference from control ($p < 0.05$).

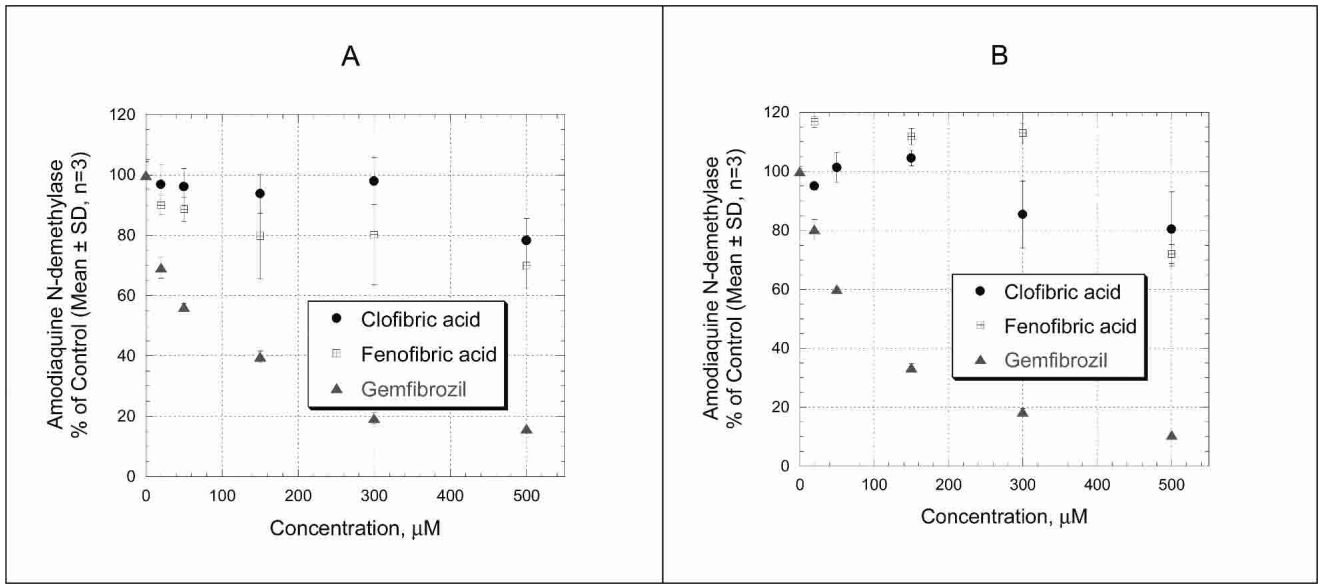

Fig. 3. Inhibitory effects of fibrates on CYP2C8 activity in human hepatocytes (A) or human liver microsomes (B). Incubations were conducted, in triplicate, by co-incubating amodiaquine (25 μM) and each fibrate (0–500 μM) with either human hepatocyte suspension (0.25 million cells/0.5 ml incubation) for 60 min or pooled human liver microsomes (0.02 mg microsomal protein/0.2 ml incubation) and NADPH (1 mM) for 20 min, as described under "Materials and Methods." Results (mean ± SD, n = 3) are expressed as % of vehicle control (50% acetonitrile in water).

showed that gemfibrozil is a relatively potent inhibitor of CYP2C8 activity in both the microsomal and hepatocyte system, suggest that the inhibitory effect observed in the induction experiment was primarily a result of direct inhibition of the enzyme. The results also agreed well with our previous finding using paclitaxel 6α-hydroxylase, another specific marker for CYP2C8 functional probe (9). It is noteworthy that a similar discrepancy between mRNA expression and functional activity has been reported previously with the hepatocyte model for CYP3A4 by ritonavir and troleandromycin, known inducers and mechanism-based inhibitors of CYP3A4, or for both CYP3A and CYP1A1/2 by bergamotin, an inducer and inhibitor of CYP3A and CYP1A1/2 (17,19). Based on chrysin glucuronidation, our results suggest that the three fibrates as well as rifampin are at best weak inducers of activity of UGT1A1 and possibly also other UGTs. It is noteworthy that the current finding of a slight decrease in chrysin glucuronidation observed with gemfibrozil, but not fenofibric acid, was not unexpected based on our earlier finding of the inhibitory effect of gemfibrozil, but not fenofibric acid, on the glucuronidation of statins, substrates for UGT1A1 and UGT1A3 (8,9,35).

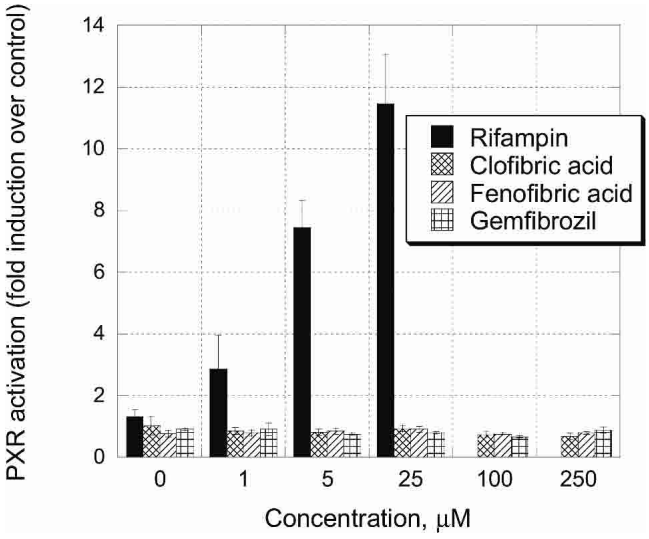

Fig. 4. Activation of human PXR by rifampin and fibrates. HepG2 cells transfected with the human PXR-GAL4, and Gal4 luciferase reporter plasmid were treated with each fibrate, rifampin, or DMSO (0.1%) as vehicle. Forty-eight hours after treatment, the experiment was terminated, cell lysate from each sample was combined with luciferase assay reagent, and luminescence was assessed. Results (means ± SD of four determinations) are expressed as % of vehicle control.

Thus, these *in vitro* results suggest that gemfibrozil and fenofibrate have potential to cause clinical drug interactions via their ability to alter CYP3A4, CYP2C8, and to a lesser degree UGT1A1. A recent clinical observation (36) that both gemfibrozil and fenofibrate did not cause a significant decrease, but in fact a modest increase (~20–45%) in the systemic exposure of pitavastatin, whose metabolism is mediated by UGT1A1, UGT1A3, and UGT2B7 (37), support the view that both fibrates did not substantially induce any of these UGTs. Also consistent with the CYP3A4 induction potential observed in this study, gemfibrozil was shown to increase the exposure (~4-fold) to a CYP3A4-mediated metabolite of cerivastatin in a clinical drug interaction study (6). In the same study, decreased exposure (>70%) to a CYP2C8-mediated metabolite of cerivastatin by gemfibrozil also was observed (6), suggesting that gemfibrozil exhibited net inhibitory effect on CYP2C8 *in vivo*. Interestingly, these clinical observations are in line with the present human hepatocyte results obtained based on enzymatic activity measurement, and not mRNA expression. It is also noteworthy that the markedly

increased exposure (~4- to 6-fold) to cerivastatin by gemfibrozil in humans (6), an observation consistent with net inhibition of cerivastatin total metabolism, suggested that the impact of CYP3A4 and CYP2C8 induction by gemfibrozil was masked by its inhibitory effect on CYP2C8 and likely also UGT1A1/3 (8,9). Additionally, the magnitude of interaction caused by gemfibrozil might have been higher if there was no complication from its induction potential. Furthermore, due to its opposing effects, the final clinical outcomes of gemfibrozil drug interaction studies would be dependent not only on relative potencies of induction vs inhibition of gemfibrozil, but also on relative contributions of the affected enzymes to overall metabolism of an interacted compound. In this regards, the relatively lower magnitude of interaction on the pharmacokinetics of simvastatin or pitavastatin than cerivastatin by gemfibrozil is consistent with the fact that UGT1A1 and/or CYP2C8 metabolism are relatively minor pathways for simvastatin acid or pitavastatin than for cerivastatin (37,38).

In contrast to gemfibrozil, there have been no published reports of significant drug interactions between fenofibrate and substrates of CYP3A4 or CYP2C8. The lack of reports on CYP3A4 substrates with fenofibrate might be attributable to the relatively low levels (<2-fold) of enzyme induction at the therapeutic concentration of 50 μM (12), as compared to ~10-fold induction by rifampin. Considering that fenofibric acid and rifampin, at their respective therapeutic concentrations, exhibited comparable potency in CYP2C8 induction (~3- to 4-fold), and that rifampin has been recently shown to substantially reduce (~65%) the exposure of rosiglitazone, a CYP2C8 substrate, in humans (39), it is possible that the lack of clinical drug interaction reports with fenofibrate is due to limited clinical studies with CYP2C8 substrates, or possibly to high plasma protein binding of fenofibric acid. However, in a clinical pharmacokinetic study, fenofibrate caused about 30% decrease in exposure of simvastatin acid with minimal change in exposure of simvastatin when co-administered with simvastatin (35). Theoretically, this observed decreased exposure could be attributable to the induction potential of either CYP2C8, or a combination of CYP2C8 and UGT1A1, by fenofibric acid, considering that both CYP2C8 and UGT1A1 contribute in part to the overall metabolism of simvastatin acid (21,38). This hypothesis, however, remains to be confirmed.

The present finding that fibrates caused enzyme induction with minimal PXR activation suggests that fibrates induced the transcriptional activation of these enzymes via non PXR activation mechanism(s). Because the regulation of CYP3A4, CYP2C8 and UGT1A1 expression has also been shown to be mediated through glucocorticoid receptor, and the constitutive androstane receptor (CAR) (25,40), further studies appear warranted with respect to the enzyme induction mechanism of fibrates.

To conclude, this report provides a direct evidence for induction and/or inhibition of human drug metabolizing enzymes by fenofibric acid and gemfibrozil and affords possible explanations for differential pharmacokinetic drug interactions observed with the fibrates and statins. Additionally, the results suggest a potential for these fibrates to cause various clinical drug interactions via their differential effects on drug metabolizing enzymes.

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REFERENCES

1. D. J. Rader and S. M. Haffner. Roles of fibrates in the management of hypertriglyceridemia. *Am. J. Cardiol.* **83**:30F–35F (1999).
2. A. Lozada and C. A. Dujovne. Drug interactions with fibric acids. *Pharmacol. Ther.* **63**:163–176 (1994).
3. D. B. Miller and J. D. Spence. Clinical pharmacokinetics of fibric acid derivatives (Fibrates). *Clin. Pharmacokinet.* **34**:155–162 (1998).
4. J. T. Backman, C. Kyrklund, K. T. Kivistö, J.-S. Wang, and P. J. Neuvonen. Plasma concentrations of active simvastatin acid are increased by gemfibrozil. *Clin. Pharmacol. Ther.* **68**:122–129 (2000).
5. C. Kyrklund, J. T. Backman, K. T. Kivistö, M. Neuvonen, J. Laitila, and P. J. Neuvonen. Plasma concentrations of active lovastatin acid are markedly increased by gemfibrozil but not by bezafibrate. *Clin. Pharmacol. Ther.* **69**:340–345 (2001).
6. J. T. Backman, C. Kyrklund, M. Neuvonen, and P. J. Neuvonen. Gemfibrozil greatly increases plasma concentrations of cerivastatin. *Clin. Pharmacol. Ther.* **72**:685–691 (2002).
7. C. Kyrklund, J. T. Backman, M. Neuvonen, and P. J. Neuvonen. Gemfibrozil increases plasma pravastatin concentrations and reduces pravastatin renal clearance. *Clin. Pharmacol. Ther.* **73**:538–544 (2003).
8. T. Prueksaritanont, J. Zhao, and B. Ma. Mechanistic studies on the metabolic interactions between gemfibrozil and statins. *J. Pharmacol. Exp. Ther.* **301**:1042–1051 (2002).
9. T. Prueksaritanont, C. Tang, Y. Qiu, L. Mu, R. Subramanain, and J. H. Lin. Effects of fibrates on metabolism of statins in human hepatocytes. *Drug Metab. Dispos.* **30**:1280–1287 (2002).
10. T. Prueksaritanont, L. M. Gorham, and B. Ma. *In vitro* metabolism of simvastatin in humans: identification of metabolizing enzymes and effect of the drug on hepatic P450s. *Drug Metab. Dispos.* **25**:1191–1199 (1997).
11. J. A. Farmer. Learning from the cerivastatin experience. *Lancet* **358**:1383–1385 (2001).
12. K. Rašlová, D. Dubovská, V. Mongiellová, and T. Trnovec. Relationship between plasma fenofibric acid levels and the effect of micronized fenofibrate on cholesterol, low-density-lipoprotein cholesterol and apolipoprotein B in patients with primary hypercholesterolemia. *Eur. J. Clin. Pharmacol.* **52**:101–106 (1997).
13. J. Sahi, M. A. Milad, and X. Zheng. Avasimibe induces CYP3A4 and multiple drug resistance protein 1 gene expression through activation of the pregnane X receptor. *J. Pharmacol. Exp. Ther.* **306**:1027–1034 (2003).
14. J. Sahi, C. B. Black, and G. A. Hamilton. Comparative effects of thiazolidinediones on in vitro P450 enzyme induction and inhibition. *Drug Metab. Dispos.* **31**:439–446 (2003).
15. D. J. Greenblatt, L. L. von-Moltke, J. P. Daily, J. S. Harmatz, and R. I. Shader. Extensive impairment of triazolam and alprazolam clearance by short-term low-dose ritonavir: the clinical dilemma of concurrent inhibition and induction. *J. Clin. Psychopharmacol.* **19**:293–296 (1999).
16. M. Pfister, L. Labbe, J. F. Lu, S. M. Hammer, J. Mellors, K. K. Bennett, S. Rosenkranz, L. B. Sheiner, and AIDS Clinical Trial Group Protocol 398 Investigators. Effect of coadministration of nelfinavir, indinavir, and saquinavir on the pharmacokinetics of amprenavir. *Clin. Pharmacol. Ther.* **72**:133–141 (2002).
17. Y. H. Wen, J. Sahi, and E. Urda. Effects of bergamottin on human and monkey drug-metabolizing enzymes in primary cultured hepatocytes. *Drug Metab. Dispos.* **30**:977–984 (2002).
18. J. K. Ritter, F. K. Kessler, M. T. Thompson, A. D. Grove, D. J. Auyeung, and R. A. Fisher. Expression and inducibility of the human bilirubin UDP-glucuronosyltransferase UGT1A1 in liver and cultured primary hepatocytes: evidence for both genetic and environmental influences. *Hepatology* **30**:476–484 (1999).
19. G. Luo, M. Cunningham, and S. Kim. CYP3A4 induction by drugs: correlation between a pregnane X receptor reporter gene assay and CYP3A4 expression in human hepatocytes. *Drug Metab. Dispos.* **30**:795–804 (2002).

20. S. Gerbal-Chaloin, J.-M. Pascussi, L. Pichard-Garcia, M. Daujat, F. Waechter, J. M. Fabre, N. Carrere, and P. Maurel. Induction of CYP2C genes in human hepatocytes in primary culture. *Drug Metab. Dispos.* **29**:242–251 (2001).
21. T. Prueksaritanont, R. Subramanian, and X. Fang. Glucuronidation of statins in animals and humans: a novel mechanism of statin lactonization. *Drug Metab. Dispos.* **30**:505–512 (2001).
22. *Physician's Desk Reference*. Thompson PDR, New Jersey, 2003, pp. 759.
23. B. Blumberg, W. Sabbaagh, H. Juguilon, J. Bolado, C. van Meter, E. Ong, and R. Evan. SXR, a novel steroid and xenobiotic-sensing nuclear receptor. *Gene Dev* **12**:3195–3205 (1998).
24. J. Lehmann, D. McKee, M. Watson, T. Wilson, J. Moore, and S. Kliewer. The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J. Clin. Investig* **102**:1016–1023 (1998).
25. J. Sugatani, K. Yamakawa, and E. Tonda. The induction of human UDP-glucuronosyltransferase 1A1 mediated through a distal enhancer module by flavonoids and xenobiotics. *Biochem. Pharmacol.* **67**:989–1000 (2004).
26. D. P. Hartley, X. Dai, and Y. D. He. Activators of rat PXR differentially modulate hepatic and intestinal gene expression. *Mol. Pharmacol.* **65**:1159–1171 (2004).
27. T. Walle, Y. Otake, A. Galijatovic, J. K. Ritter, and U. K. Walle. Induction of UDP-glucuronosyltransferase UGT1A1 by the flavonoid chrysin in the human hepatoma cell line Hep G2. *Drug Metab. Dispos.* **28**:1077–1082 (2000).
28. M. D. Green, C. D. King, B. Mojarrabi, P. I. Mackenzie, and T. R. Tephly. Glucuronidation of amines and other xenobiotics catalyzed by expressed human UDP-glucuronosyltransferase 1A3. *Drug Metab. Dispos.* **26**:507–512 (1998).
29. X.-Q. Li, A. Björkman, T. B. Anderson, M. Ridderström, and C. M. Masimirembwa. Amodiaquine clearance and its metabolism to N-desethylamodiaquine is mediated by CYP2C8: a new high affinity and turnover enzyme-specific probe substrate. *J. Pharmacol. Exp. Ther.* **300**:399–407 (2002).
30. A. Galijatovic. U. K. Walle, and T. Walle. Extensive metabolism of the flavonoid chrysin by human Caco-2 and Hep G2 cells. *Xenobiotica* **29**:1241–1256 (1999).
31. B. Goodwin, E. Hodgson, and C. Liddle. The orphan human pregnane X receptor mediates the transcriptional activation of CYP3A4 by rifampin through a distal enhancer module. *Mol. Pharmacol.* **56**:1329–1339 (1999).
32. L. Richert, C. Lamboley, and C. Viollon-Abadie. Effects of clofibric acid on mRNA expression profiles in primary cultures of rat, mouse and human hepatocytes. *Toxicol. Appl. Pharmacol.* **191**:130–146 (2003).
33. M. G. Soars, D. M. Petullo, J. A. Eckstein, S. C. Kasper, and S. A. Wrighton. An assessment of UDP-glucuronosyltransferase induction using primary human hepatocytes. *Drug Metab. Dispos.* **32**: 140–148 (2004).
34. V. Meunier, M. Bourrié, B. Julian, E. Marti, F. Guillou, Y. Berger, and G. Fabre. Expression and induction of CYP1A1/1A2, CYP2A6 and CYP3A4 in primary cultures of human hepatocytes: a 10-year follow-up. *Xenobiotica* **30**:589–607 (2000).
35. A. J. Bergman, G. Murphy, J. Burke, J. J. Zhao, R. Valesky, L. Liu, K. C. Lasseter, W. He, T. Prueksaritanont, Y. Qiu, A. Hartford, J. M. Vega, and J. F. Paolini. Simvastatin does not have a clinically significant pharmacokinetic interaction with fenofibrate in humans. *J. Clin. Pharmacol.* **44**:1054–1062 (2004).
36. P. Mathew, T. Cuddy, W. G. Tracewell, and D. Salazar. An open-label study on the pharmacokinetics (PK) of pitavastatin (NK-104) when administered concomitantly with fenofibrate or gemfibrozil in healthy volunteers. *Clin. Pharmacol. Ther.* **75**:33 (2004).
37. H. Fujino, I. Yamada, S. Shimada, M. Yoneda, and J. Kojima. Metabolic fate of pitavastatin, a new inhibitor of HMG-CoA reductase: human UDP-glucuronosyltransferase enzymes involved in lactonization. *Xenobiotica* **33**:27–41 (2003).
38. T. Prueksaritanont, B. Ma, and N. Yu. Human hepatic metabolism of simvastatin hydroxy acid is mediated primarily by CYP3A, not CYP2D6. *Br. J. Clin. Pharmacol.* **56**:120–124 (2003).
39. J. Y. Park, K. A. Kim, M. H. Kang, S. L. Kim, and J. G. Shin. Effect of rifampin on the pharmacokinetics of rosiglitazone in healthy subjects. *Clin. Pharmacol. Ther.* **75**:157–162 (2004).
40. Z. Dvořák, M. Modrianský, and L. Pichard-Garcia. Colchicine down-regulates cytochrome P450 2B6, 2C8, 2C9, and 3A4 in human hepatocytes by affecting their glucocorticoid receptor-mediated regulation. *Mol. Pharmacol.* **64**:160–169 (2003).