The mechanism by which inducers of drug metabolism and drug transport exert their effects in humans in vivo is poorly defined because sampling of tissues of interest, such as the liver, is by necessity restricted. However, use of the human mononuclear cell fraction (lymphocytes), a readily obtainable blood fraction, has been proposed as a surrogate tissue that may be valuable in quantifying induction events on an individual basis. For example, the induction of P-gp in transformed human cell lines and human immortalized keratinocytes has been correlated with increased hepatic P450 (P450) genes in the mononuclear fraction (lymphocytes) of human blood. A total of 50 healthy volunteers (age, 18–74) participated in two studies in which 600 mg of rifampin was administered orally once daily in the evening for 7 days. Twenty of these individuals also received fexofenadine before and after rifampin dosing. MDR1 and CYP2C8 mRNAs were expressed in 100% (50 of 50) and 95% (35 of 37) of individuals, respectively, at baseline. A significant \( P < 0.05; n = 37 \) increase in the expression of MDR1 mRNA from 176,900 \( \pm \) 122,000 to 248,500 \( \pm \) 162,300 molecules/\( \mu \)g of RNA was observed following rifampin administration in the human lymphocytes. There was no significant \( P > 0.05 \) difference in MDR1 mRNA expression between males and females at baseline. Interestingly, 58% of the individuals \( n = 29 \) demonstrated a 120% increase [95% confidence interval (CI); 120%; range, 81–153%; responders] in MDR1 mRNA expression. In contrast, the remaining 42% of individuals \( n = 21 \) exhibited a mean decrease of \(-5.2\%\) (95% CI; \(-5.2\%\); range, \(-15\%\) to \(+4\%\); nonresponders). Rifampin steady-state trough serum concentrations were not significantly different \( P > 0.05 \) between responders and nonresponders. Likewise, there was no relationship between the observed induction in MDR1 mRNA expression in lymphocytes and the observed increase in fexofenadine oral clearance in twenty volunteers. The mRNA of CYP2E1, CYP3A5, CYP3A7, CYP4A11, and CYP4B1 genes were variably expressed at baseline and following rifampin treatment. In contrast, CYP2C9 and CYP3A4 mRNAs were undetectable in lymphocytes both before and after rifampin dosing. Interindividual variability in baseline expression and inducibility of MDR1 and P450 mRNA in human lymphocytes appeared to be substantial and may not reflect the expression of these enzymes in other tissues.

The human MDR1 gene (ABCB1) encodes for P-glycoprotein (P-gp), an ATP-dependent transmembrane protein that transports numerous neutral and cationic compounds out of many cells. Consequently, induction of P-gp is at least partly responsible for the development of resistance to chemotherapeutic regimens, and it is involved in changes in both drug distribution and clearance (Chin et al., 1993). MDR-1 expression is increased in peripheral blood lymphocytes from patients with Crohn’s disease and ulcerative colitis who require bowel resection or proctocolectomy due to glucocorticoid treatment failure (Farrell et al., 2000). In many tissues, P-gp and CYP3A are coexpressed, and therefore, induction of P-gp may attenuate the induction of CYP3A by limiting the intracellular inducer concentration (Wacher et al., 1995; Fisher et al., 1996; Schuetz et al., 1996a,b). Thus, the ability to quantify MDR1 mRNA expression in the mononuclear fraction would be clinically useful in monitoring the development of resistance to certain therapeutic compounds, such as oncolytic agents and human immunodeficiency virus protease inhibitors, and could also be helpful in predicting drug disposition and drug-drug interactions involving CYP3A and P-gp substrates. The potent inducer of CYP3A4, rifampin, induced P-gp in transformed human cell lines and leukocytes, a CYP3A protein was induced by dexamethasone in both polymorphonuclear and mononuclear fractions, although it was not detectable at baseline (Mahnke et al., 1996). It is presently unknown whether human CYP3A expression is inducible in the mononuclear fraction of human blood.

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1 Abbreviations used are: P450, cytochrome P450; MDR1, multidrug resistance-1; P-gp, P-glycoprotein; RT-PCR, reverse transcription-polymerase chain reaction; F, forward; R, reverse; crsRNA, competitive reference standard RNA; PXR, pregnane X receptor.

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**ABSTRACT:**
Reverse transcription-polymerase chain reaction (RT-PCR) and quantitative, competitive RT-PCR were used to examine the capability of rifampin to induce the expression of mRNA derived from multidrug resistance-1 (MDR1) and drug-metabolizing cytochrome P450 (P450) genes in the mononuclear fraction (lymphocytes) of human blood. A total of 50 healthy volunteers (age, 18–74) participated in two studies in which 600 mg of rifampin was administered orally once daily in the evening for 7 days. Twenty of these individuals also received fexofenadine before and after rifampin dosing. MDR1 and CYP2C8 mRNAs were expressed in 100% (50 of 50) and 95% (35 of 37) of individuals, respectively, at baseline. A significant \( P < 0.05; n = 37 \) increase in the expression of MDR1 mRNA from 176,900 \( \pm \) 122,000 to 248,500 \( \pm \) 162,300 molecules/\( \mu \)g of RNA was observed following rifampin administration in the human lymphocytes. There was no significant \( P > 0.05 \) difference in MDR1 mRNA expression between males and females at baseline. Interestingly, 58% of the individuals \( n = 29 \) demonstrated a 120% increase [95% confidence interval (CI); 120%; range, 81–153%; responders] in MDR1 mRNA expression. In contrast, the remaining 42% of individuals \( n = 21 \) exhibited a mean decrease of \(-5.2\%\) (95% CI; \(-5.2\%\); range, \(-15\%\) to \(+4\%\); nonresponders). Rifampin steady-state trough serum concentrations were not significantly different \( P > 0.05 \) between responders and nonresponders. Likewise, there was no relationship between the observed induction in MDR1 mRNA expression in lymphocytes and the observed increase in fexofenadine oral clearance in twenty volunteers. The mRNA of CYP2E1, CYP3A5, CYP3A7, CYP4A11, and CYP4B1 genes were variably expressed at baseline and following rifampin treatment. In contrast, CYP2C9 and CYP3A4 mRNAs were undetectable in lymphocytes both before and after rifampin dosing. Interindividual variability in baseline expression and inducibility of MDR1 and P450 mRNA in human lymphocytes appeared to be substantial and may not reflect the expression of these enzymes in other tissues.

**INDUCTION OF MULTIDRUG RESISTANCE-1 AND CYTOCHROME P450 mRNAS IN HUMAN MONONUCLEAR CELLS BY RIFAMPIN**

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The mechanism by which inducers of drug metabolism and drug transport exert their effects in humans in vivo is poorly defined because sampling of tissues of interest, such as the liver, is by necessity restricted. However, use of the human mononuclear cell fraction (lymphocytes), a readily obtainable blood fraction, has been proposed as a surrogate tissue that may be valuable in quantifying induction events on an individual basis. For example, the induction of cytochrome P450 (P450) \( 2 \)E1 mRNA in human lymphocytes has been correlated with increased hepatic CYP2E1 activity, reflected in the oral clearance of chlorozoxazone in humans (Raucy et al., 1997). Conversely, CYP3A4, the most abundant drug-metabolizing enzyme in human intestine and liver (Bork et al., 1989; Kolars et al., 1992) has been reported to be absent in the human bone marrow fraction in noninduced individuals (Janardan et al., 1996). However, in rat
human duodenum cells in vivo, but it is unclear whether significant and coordinate induction occurs in all expressing tissues in vivo (Schuetz et al., 1996a; Greiner et al., 1999).

In this study, the presence or absence of mRNA for the drug-metabolizing P450s and MDR1 in human lymphocytes was examined before and after rifampin treatment by RT-PCR. Quantitation of MDR1 and P450 mRNA was achieved using specific RNA internal standards that were generated based on a deletion concept (Riedy et al., 1995) to examine whether the expression of these mRNAs within the lymphocyte is coordinately regulated by rifampin. Additionally, the relationship between lymphocyte MDR1 mRNA expression and the in vivo disposition of fexofenadine, a substrate of P-gp, was investigated before and after rifampin administration.

**Experimental Procedures**

**Materials.** All enzymes and cofactors used for the synthesis of internal standard RNA by reverse transcription and PCR amplification were purchased from Promega (Madison, WI), and Integrated DNA Technologies (Coralville, IA) synthesized all primers. The TRI Reagent for RNA isolation was obtained from Promega (Madison, WI), and Integrated DNA Technologies (Coralville, IA). All other general chemicals and drugs were purchased from Sigma Chemical Co. (St. Louis, MO). Where necessary, diethylpyrocarbonate-treated water was used to make solutions. Furthermore, all RT-PCR reactions were assembled on ice.

**Human Study Design.** Fifty healthy, nonsmoking volunteers (25 males and 25 females) between the ages of 18 to 75 and medication-free (with exception of combination oral contraceptives in females) participated in this study that was approved by Indiana University Hospital Institutional Review Board. Volunteers were determined to be healthy by physical examination and laboratory tests for complete blood count, urinalysis, and blood chemistry. Forty-six of the volunteers were Caucasian, two were Oriental, one was African American, and one was Hispanic. For 2 weeks before the study and during the week of study, the subjects neither consumed alcohol nor citrus fruits (e.g., grapefruit), citrus fruit-containing products, or cruciferous vegetables.

**Study 1.** Thirty-seven of the volunteers participated in the following study. A baseline blood sample (30 ml) was obtained, after which volunteers received 600 mg of rifampin once daily in the evening for 7 days. A second blood sample was obtained on day 7 of treatment. Seven females were receiving combination oral contraception during this study.

**Study 2.** Twenty volunteers, seven of which participated in the first study, participated in a study to investigate the effect of rifampin coadministration on the disposition of fexofenadine following oral administration. No females were receiving combination oral contraception during the study. The details and results of this study have been reported elsewhere (Hamman et al., 2001). Briefly, 30 ml of baseline blood was obtained for the isolation of lymphocytes before fexofenadine administration. Subsequently, a 60-μg dose of fexofenadine was administered orally, and serial blood samples were collected for 48 h. Then, the volunteers received 600 mg of rifampin once daily in the evening for 7 days. Blood was then collected for the isolation of lymphocytes, and the pharmacokinetics study was repeated. Fexofenadine serum concentrations were determined as previously described (Hamman et al., 2001).

**Isolation of Blood Mononuclear Fraction.** Lymphocytes were isolated from 25 ml of heparinized blood using 10 ml of Isolymph (Gallard-Schlesinger Industries, Carle Place, NY) as directed by the manufacturer. Mononuclear cells containing mainly lymphocytes (75%) and 12 to 25% monocytes were counted, and the remaining portion of pellet was dissolved in TRI Reagent and stored at −80 °C until RNA isolation.

**RNA Isolation and cDNA Synthesis.** The mononuclear fraction was dissolved in 1 ml of TRI Reagent solution composed of an acid-guanidinium system for the single-step isolation of total RNA, according to the protocol of the Molecular Research Center (Cincinnati, OH; Chomczynski, 1993). The first-strand cDNA was synthesized from total RNA by reverse transcription following oligo dT extension using avian myeloid leukemia virus-reverse transcriptase from Promega in a 20-μl final reaction volume. The reaction was allowed to proceed at 45 °C for 1 h before termination by heating to 95 °C for 5 min and was diluted to 50 μl. Two control reactions (one without RNA, the other in which cDNA synthesis was performed without the enzyme) were included to verify the absence of contamination.

**Polymerase Chain Reaction.** Gene-specific sense and antisense primers of MDR1, CYP2C8, CYP2C9, CYP2E1, CYP3A4, CYP3A5, CYP3A7, CYP4A11, and CYP4B1 (Table 1) were used, and optimal amplification conditions, particularly the annealing temperatures, were determined for each set of primers (Table 2). The specificity of these primers was assessed using the NCBI Blast program (NIH, Bethesda, MD) (Altschul et al., 1997). The primer set of MDR1, CYP2C8, CYP2C9, CYP2E1, CYP3A4, CYP3A5, CYP3A7, CYP4A11, and CYP4B1 (Table 1) were used, and optimal amplification conditions, particularly the annealing temperatures, were determined for each set of primers (Table 2). The specificity of these primers was assessed using the NCBI Blast program (NIH, Bethesda, MD) (Altschul et al., 1997). The polymerase chain reaction (PCR) results of this study have been reported elsewhere (Hamman et al., 2001). Briefly, 30 ml of baseline blood was obtained for the isolation of lymphocytes before fexofenadine administration. Subsequently, a 60-μg dose of fexofenadine was administered orally, and serial blood samples were collected for 48 h. Then, the volunteers received 600 mg of rifampin once daily in the evening for 7 days. Blood was then collected for the isolation of lymphocytes, and the pharmacokinetics study was repeated. Fexofenadine serum concentrations were determined as previously described (Hamman et al., 2001).
mide for visualization under UV irradiation. To confirm the specificity of the PCR product by hybridization with a labeled probe, the products from the agarose gel were transferred onto a nylon (Nytran; Schleicher & Schuell, Inc., Keene, NH) membrane by Southern technique and baked at 80°C for 1 h to fix the DNA fragment onto the membrane. Sequence-specific internal oligonucleotide probes (Table 1) were labeled with [γ-32P]ATP by a T4 polynucleotide kinase reaction purified by precipitation and counted on a liquid scintillation counter (LS 3801; Beckman Coulter, Inc., Fullerton, CA). Hybridization was performed according to the protocol of Schleicher and Schuell (Keene, NH).

**Design of Primers for Generating Competitive Reference Standard RNA.** The primers designed for the synthesis of specific internal competitive reference standard RNA (crsRNA) for MDR1, CYP3A4, and CYP3A5 mRNA quantitation are shown in Table 3. The F crs-primers (5′–3′) are chimeric in sequence to the respective mRNA strand with the exception of 23 bases at the 5′-end, which are derived from the bacteriophage of T7 RNA polymerase promoter (Riedy et al., 1995). The insertion of this sequence facilitated the synthesis of crsRNA by in vitro transcription of the crsDNA product. The R crs-primers (3′–5′) are complementary to the respective mRNA gene, except that the initial 20 to 25 bases at the 5′-end of these primers are ~75 to 80 bases downstream of the respective mRNA sequence from the next 20 bases at the 3′-end to create an internal 75 to 80 nucleotide deletion. The resulting competitive PCR product was short enough to be separated from the target mRNA product on agarose gels.

**In Vitro Synthesis of crsRNA.** Using 10 pmol R crs-primer (Table 3), the first-strand crs-cDNA was generated by reverse transcription from the total RNA solution, as described above. The first strand crs-cDNA was then amplified by PCR after adding PCR buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, and 1.5 mM MgCl2), 10 pmol of crs F primer (Table 3), and 5 U Taq DNA polymerase in a final volume of 100 µl and using a thermal profile of 94°C, 5′-1′ denaturation, 55°C, 1′ annealing, and 72°C, 2′ extension for 35 cycles. The PCR product was electrophoresed on 2% agarose gel (containing ethidium bromide) along with a DNA ladder of 100 base pairs to verify its size. The crsDNA band was excised from the gel under UV light, eluted using Wizard PCR Purification System (Promega), and the concentration was estimated using a spectrophotometer at 260 nm (DU 65 Beckman Spectrophotometer).

One microgram of crsDNA was transcribed to crsRNA by T7 RNA polymerase in a final volume of 100 µl at 37°C for 2 h (Vanden Heuvel, 1998). The crsDNA template was then degraded by addition of RNase-free DNase at 30°C for 1 h followed by extraction of crsRNA with TRI Reagent-chloroform, followed by precipitation from the aqueous phase of RNA-free DNase at 30°C for 1 h followed by extraction of crsRNA with TRI Reagent-chloroform, followed by precipitation from the aqueous phase of RNA-free DNase at 30°C for 1 h followed by extraction of crsRNA with TRI Reagent-chloroform. Then, 4 ml of ethyl acetate/hexane (75:25, v/v) was added, and the resulting mixture was mixed vigorously. The organic layer was transferred to a clean test tube and evaporated to dryness. The residue was reconstituted with 100 µl of mobile phase (20 mM ammonium acetate, pH 7/methanol, 20:80, v/v), and 50 µl was injected onto a 5-µm octyl column (100 x 4.6-mm i.d.; Brownlee Precision Co., San Jose, CA). The sample was introduced into the mass spectrometer after being eluted at 1 ml/min. Quantification of rifampin was achieved on a mass spectrometer (Finnigan Navigator; Thermo Finnigan, San Jose, CA) interfaced with a high-performance liquid chromatograph (1100 series; Hewlett Packard, Palo Alto, CA) operated in the positive ion mode-atmospheric pressure ionization with selective ion monitoring (M + H; ri-

### Table 2

**Polymerase chain reaction amplification conditions for each gene, including temperature, time (seconds), and number of amplification cycles**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A4</td>
<td>94°C, 35°C</td>
<td>55°C, 35°C</td>
<td>72°C, 35°C</td>
<td>35</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>94°C, 35°C</td>
<td>55°C, 35°C</td>
<td>72°C, 45°C</td>
<td>40</td>
</tr>
<tr>
<td>CYP3A7</td>
<td>94°C, 35°C</td>
<td>60°C, 35°C</td>
<td>72°C, 30°C</td>
<td>35</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>94°C, 35°C</td>
<td>62°C, 35°C</td>
<td>72°C, 30°C</td>
<td>35</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>94°C, 35°C</td>
<td>70°C, 35°C</td>
<td>72°C, 30°C</td>
<td>35</td>
</tr>
<tr>
<td>CYP4A11</td>
<td>94°C, 30°C</td>
<td>57°C, 35°C</td>
<td>72°C, 35°C</td>
<td>35</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>94°C, 40°C</td>
<td>50°C, 45°C</td>
<td>72°C, 45°C</td>
<td>40</td>
</tr>
<tr>
<td>CYP4B1</td>
<td>94°C, 30°C</td>
<td>57°C, 35°C</td>
<td>72°C, 30°C</td>
<td>35</td>
</tr>
<tr>
<td>MDR1</td>
<td>94°C, 35°C</td>
<td>60°C, 35°C</td>
<td>72°C, 35°C</td>
<td>35</td>
</tr>
</tbody>
</table>

Quantification of crsRNA by Competitive RT-PCR. In an initial experiment, logarithmic dilutions (10⁰ to 10⁷ molecules/µl) of crsRNA were used with a constant amount of target RNA to find the range in which the competitive product was formed with approximately equal intensity to that of the target mRNA. Reverse transcription was performed as previously described using 10 pmol of reverse primer of the respective gene (Table 1), 1 U of avian myeloid leukemia virus-reverse transcriptase, and 100 ng of RNA in a final volume of 10 µl. After terminating the RT reaction by heating to 95°C, 15 µl of PCR master mix was added to each sample with a final volume of 25 µl (Vanden Heuvel, 1998). The reaction was allowed to amplify for 32 cycles using the conditions as shown in Table 2. Control reactions, one without reverse transcriptase and one without internal standard mRNA, were run to verify the absence of contamination in cDNA and RNA, respectively.

The PCR product (20 µl) was separated on a 2% agarose gel containing ethidium bromide at 80 mA for approximately 45 min. The digital image of the gel was generated by the Doc 1000 UV fluorescent system (Bio-Rad Laboratories Hercules, CA), whereas the Molecular Analysis software (Bio-Rad) was used to quantify band volume (intensity). Molar ratios were computed by the dividing the band volume of target mRNA by that of crsRNA. Using the latter established range, another test was performed with serial dilutions of crsRNA and a constant amount of target mRNA to construct a standard curve. In addition, a series of test samples (100 ng of RNA) with only one concentration of crsRNA, which was previously determined as the approximate equivalent amount of PCR product to that of target mRNA in the initial trial, were also included in the assay.

The log ratio of target band volume/crsRNA band volume was plotted (y-axis) as a function of the log of crsRNA molecules on the x-axis to determine the number of target mRNA molecules in the samples. A straight line was fitted to the points using Statmost 32 software (Datamost Corp., Sandy, UT) using linear regression. The anti-log of the intercept divided by slope (b/m) represented the initial number of the target mRNA molecules present in the RNA sample (Vanden Heuvel, 1998). This value was used to compute the ratios of target mRNA molecules/crsRNA molecules in other serial dilutions, which were subsequently plotted as the log ratio of target mRNA molecules/crsRNA molecules on the x-axis and the log ratio of target band volume/crsRNA band volume on the y-axis. The regression parameters of this plot were used for computing the number of target mRNA molecules in other samples. The intraday precision (n = 5) for the quantitation of MDR1 mRNA was approximately 15% at 18,000 MDR1 mRNA molecules. The reported values for each sample’s mRNA content are the means of triplicate determinations.

**Rifampin Quantitation.** Serum rifampin trough concentrations were determined using high-performance liquid chromatography with a mass spectrometer detector. To 1 ml of serum, an equal volume of 1 M NaOH glycine buffer, pH 11.3, was added along with the internal standard clarithromycin. Then, 4 ml of ethyl acetate/hexane (75:25, v/v) was added, and the resulting mixture was mixed vigorously. The organic layer was transferred to a clean test tube and evaporated to dryness. The residue was reconstituted with 100 µl of mobile phase (20 mM ammonium acetate, pH 7/methanol, 20:80, v/v), and 50 µl was injected onto a 5-µm octyl column (100 x 4.6-mm i.d.; Brownlee Precision Co., San Jose, CA). The sample was introduced into the mass spectrometer after being eluted at 1 ml/min. Quantification of rifampin was achieved on a mass spectrometer (Finnigan Navigator; Thermo Finnigan, San Jose, CA) interfaced with a high-performance liquid chromatograph (1100 series; Hewlett Packard, Palo Alto, CA) operated in the positive ion mode at atmospheric pressure ionization with selective ion monitoring (M + H; ri-
fampin, 824.5 ml; clarithromycin, 749.2 ml. The minimum detectable rifampin concentration was 2 ng/ml, and the interday coefficient of variation was 8.0% at 10 ng/ml.

Results

The quantitative RT-PCR assays were validated using mRNA isolated from a human renal cell line, which expressed mRNAs of MDR1 and all of the P450s of interest. We confirmed specificity of the products by hybridization with 32P-labeled probes. The number of mRNA molecules of MDR1, CYP3A4, and CYP3A5 in the human renal cell line was determined to be 56,500, 765,000, and 33,500,000 per μg of RNA, respectively. This observation is consistent with our finding that CYP3A5 is the principle CYP3A enzyme expressed in the human kidney (Haehner et al., 1996).

We examined the expression of the rifampin-inducible MDR1, CYP2E1, and CYP3A4/5/7 enzymes and the nonrifampin-inducible (CYP2C8, CYP2C9, CYP4A11, and CYP4B1) P450s in the human mononuclear fraction before and after rifampin administration (600 mg every evening) to 37 healthy volunteers (Table 4). Only, MDR1 mRNA was found in all lymphocyte samples (n = 37) both before and after rifampin treatment. Although the mRNAs for many of the enzymes examined were detected, the concentrations were below the limit of quantification. Therefore, only the mRNA for MDR1 was quantitiated.

Quantitative RT-PCR revealed substantial interindividual variability in the expression of MDR1 mRNA. The mean number of MDR1 mRNA molecules per microgram of RNA at baseline was extremely variable [176,900 ± 121,900 (mean ± S.D.), ranging between 9,000–470,000 molecules/μg of RNA]. No significant (P > 0.05) difference in the baseline MDR1 mRNA expression between males (154,500 ± 114,500 molecules/μg of RNA; n = 25) and females (199,300 ± 127,200 molecules/μg of RNA; n = 25) was observed. The expression of MDR1 mRNA was significantly (P < 0.05) increased from 176,900 ± 122,000 to 248,500 ± 162,300 molecules/μg of RNA following rifampin administration to the 50 volunteers. In the seven female volunteers receiving oral contraception, the baseline MDR1 expression was 132,900 ± 132,000 molecules/μg of RNA, which was not significantly (P > 0.05) different to the baseline MDR1 expression in females not receiving oral contraceptives (225,100 ± 118,700 molecules/μg of RNA; n = 18).

It was clear from the data that some volunteers exhibited a greater response to rifampin administration compared with others. Therefore, we reasoned that a 50% increase in expression of MDR1 mRNA would be clinically important, and consequently, we stratified individuals as rifampin responders (n = 29; 50% or greater increase) and nonresponders (n = 21; less than 50% increase). A statistically significant (p < 0.05) increase in MDR1 mRNA expression of 120 ± 98% (95% CI; 120%; range 81–153%) occurred in responders (Fig. 1). In contrast, the nonresponders showed a −5.2 ± 22% decrease in their MDR1 mRNA expression (95% CI; −5.2%; range, −15 to +4%) after rifampin administration. We also noted that those individuals who exhibited lower levels of baseline expression tended to show the greatest induction of MDR1 gene expression. The mean number of copies (molecules per microgram of RNA) at baseline was 202,700 ± 139,500 molecules/μg of RNA for those not significantly induced versus 158,700 ± 106,000 for those that experienced significant induction of MDR1 mRNA. The rifampin serum trough concentration observed in responders (median, 1.2 μg/ml; range, 0.1–10 μM) compared with nonresponders (median, 0.9 μg/ml; range, 0.2–3.1 μM) was not significantly different (P ≥ 0.05), suggesting that the difference in response to rifampin observed is unrelated to the serum concentrations of rifampin.

In a separate investigation (study 2), the effect of rifampin administration on MDR1 mRNA expression in lymphocytes was examined in 20 individuals. The effect of rifampin on fexofenadine disposition has been previously reported in detail (Hamman et al., 2001). Before oral fexofenadine administration, lymphocytes were harvested from participants before and after 7 days of rifampin dosing (600 mg once daily in the evening). In agreement with the above observation, the mean MDR1 mRNA expression was significantly increased, approximately 30%, from 232,000 ± 91,000 to 305,000 ± 127,000 molecules/μg of RNA. The baseline MDR1 mRNA was not significantly different between males and females. Likewise, rifampin dosing significantly increased the oral clearance of fexofenadine 116%, from 130 ± 54 to 282 ± 80 l/h. There was no relationship between the percent change in MDR1 mRNA expression and the percent change in fexofenadine oral clearance (P > 0.05; n = 20; r = 0.02).

Seven individuals participated in both studies, which were con-
was no significant difference (P/h11022molecules/h9262both studies. Each point represents the mean of triplicate determinations.

20 individuals that participated in study 2. Seven of the volunteers participated in pated in study 1 (see text for details of study). Open and solid circles represent the 37 individuals that partici- pated in rifampin dosing. Open and solid squares represent the 37 individuals that partici- pated in rifampin dosing and 20 of 37 volunteers after rifampin dosing.

CYP3A7 mRNA was expressed in 9 of 37 individuals before rifampin treatment. Quantitative competitive RT-PCR was attempted, but due to very low levels of CYP3A5 mRNA expression, the exact number of mRNA copies could not be determined. Furthermore, CYP3A7 mRNA was expressed in 9 of 37 individuals before rifampin dosing and 20 of 37 volunteers after rifampin dosing.

CYP2E1 mRNA was present in 41% (15 of 37) of individuals at baseline and in 78% (29 of 37) of samples after rifampin administra- tion. This observation is contrary to the report of Hukkanen et al. (1997), who consistently detected CYP2E1 mRNA in eight of eight volunteers. In addition, only 4 of 15 individuals demonstrated a 2-fold or greater increase in the density of CYP2E1 mRNA bands following rifampin dosing. CYP4A11 mRNA was present in 60% (22 of 37) of participants before treatment, and about 27% of these individuals exhibited increased optical density (≥2-fold) of mRNA bands post-treatment. CYP4B1 was expressed in 19% (7/37) of individuals both before and after rifampin treatment. This is consistent with other investigators who detected CYP4B1 mRNA in a minority of samples (Hukkanen et al., 1997). Finally, there was no relationship between increased MDR1 mRNA expression following rifampin administra- tion and the baseline or increased expression of the various cytochrome P450 mRNAs in the lymphocytes.

Discussion

The human MDR1 gene encodes for P-glycoprotein, which is responsible for the ATP-dependent cellular efflux of many neutral and cationic compounds, including chemotherapeutic drugs such as the vinca alkaloids, anthracyclines, and epipodophyllotoxins. Thus, the induction of this transporter confers at least partial resistance to these agents, and recently it has been implicated in resistance to human immunodeficiency virus-protease inhibitors (Chin et al., 1993; Kim et al., 1998). However, the significance of P-gp extends far beyond resistance to chemotherapeutic agents. The commonality of tissue expression and substrate specificity between P-gp and CYP3A4 has implicated P-gp in drug clearance and drug-drug interactions at the levels of both gut wall and liver (Wacher et al., 1995; Fisher et al., 1996; Schuetz et al., 1996a; Hall et al., 1999). There is also evidence to suggest that induction of P-gp influences the expression and induc- ibility of CYP3A in cells that coexpress these proteins (Schuetz et al., 1996a). The importance of P-gp in steroid hormone synthesis in adrenal cortex and in the transport of cytokines by certain classes of lymphocytes raises the possibility of its role in immune surveillance (Lucia et al., 1995).

Predicting the development of drug resistance or susceptibility to the pharmacological effects of xenobiotics may be possible if an easily obtainable source, such as the human blood mononuclear frac- tion, can be used for screening relative P-gp expression. Although baseline expression is tissue-selective, induction at one locus, such as the mononuclear fraction, might reflect at least a degree of induction in all tissues when a potent inducer is considered. It has been shown previously that lymphocyte, monocyte, and polymorphonuclear neu- trophil fractions of blood leukocytes express MDR1 mRNA (Janardan et al., 1996). Expression of MDR1 mRNA in white blood cells varies from greatest in natural killer cells > CD8 T-cells > CD4 T-cells > polymorphonuclear leukocytes > B-cells to very little or none in monocytes (Klimecki et al., 1994). The number of MDR1 mRNA copies in leukemic blasts (of myeloid, not lymphocyte, origin) ranged from undetectable to 1,810,000 molecules/μg of RNA, the median being 55,000 molecules/μg of RNA (Lytton et al., 1994). In the present study, the mononuclear fraction exhibited a mean mRNA content of 176,900 molecules/μg of RNA, with a range of 9,000 to 470,000 molecules. It is likely that this primarily represents MDR1 mRNA content in lymphocytes since monocytes are found to contain little or no MDR1 mRNA (Klimecki et al., 1994). This difference between the amounts of MDR1 mRNA in lymphocyte fraction from healthy individual versus malignant myeloid population may, how- ever, reflect exposure to chemotherapeutic agents that can cause an increase in MDR1 expression.

We have demonstrated that MDR1 mRNA was not only expressed at baseline in all young healthy individuals that we examined but was also induced significantly by 7 days of rifampin treatment. There was

Fig. 1. The effect of rifampin administration on MDR1 mRNA expression in lymphocytes from 50 healthy volunteers.

Solid symbols represent individuals (n = 29) demonstrating a 50% or greater increase in MDR1 mRNA expression following oral rifampin dosing (600 mg once daily in the evening for 7 days). Open symbols represent individuals (n = 21) exhibiting a change in MDR1 mRNA expression of less than 50% following rifampin dosing. Open and solid circles represent the 37 individuals that participated in study 2. Seven of the volunteers participated in both studies. Each point represents the mean of triplicate determinations.
a 120% increase in MDR1 mRNA expression in 58% of individuals. The remaining 42% of volunteers experienced very little change in their MDR1 expression following rifampin administration. The mechanism underlying the observed bimodal distribution of lymphocyte MDR1 responsiveness to rifampin is unclear. Although it is possible that the nonresponders represent individuals with MDR1 variants, the primers used in this study recognize a region of the MDR1 mRNA, which is conserved between the MDR1 wild type and the currently identified variants (Cascorbi et al., 2001). Even though numerous variants of MDR1 have been described, there is no reason to believe that there is allele-specific induction by pregnane X receptor (PXR) ligands. Indeed, our data with fexofenadine indicate that, in contrast to the lymphocyte findings, 24 of 24 participants experienced a significant increase in the oral clearance of fexofenadine (Hamman et al., 2001). Similarly, others have demonstrated that all individuals receiving rifampin or St. John’s wort had increased P-glycoprotein and MDR1 mRNA expression in the intestinal wall (Greiner et al., 1999; Durr et al., 2000). Thus, it is surprising that we did not observe a relationship between lymphocyte MDR1 mRNA expression and fexofenadine oral clearance before or after rifampin administration.

In light of these observations, the induction of P-glycoprotein may be regulated in a tissue-selective manner. MDR1, as with CYP3A4, contains a PXR responsive element, and PXR is expressed in a tissue-selective pattern. For example, human kidney does not express PXR, and P-glycoprotein is not induced by rifampin in this tissue (Lehmann et al., 1998; Hamman et al., 2001). Thus, our observation of MDR1 responders and nonresponders may reflect individual differences in the expression of PXR in the lymphocytes of these individuals, but this hypothesis requires further investigation.

It is clear from the data presented that the quantitation of MDR1 mRNA in lymphocytes is not a suitable surrogate for P-gp activity in other tissues. An alternative strategy for assessing P-gp activity in other tissues may be to examine the activity of P-gp in lymphocytes. To this end, Hitzl and colleagues (2001) have demonstrated that the efficiency of the P-gp-mediated efflux of rhodamine 123 in CD56+ natural killer cells is related to the presence of the C3435T mutation in the MDR1 gene. However, it is unknown whether rhodamine 123 efflux in CD56+ natural killer cells directly reflects P-gp activity in other tissues and, therefore, whether it would be predictive of the in vivo disposition of P-gp substrates such as digoxin.

CYP3A4 and its mRNA are ubiquitously expressed in human liver and intestinal villi, whereas CYP3A5 and the corresponding mRNA are expressed in only a subset of individuals but ubiquitously expressed in human kidneys (Watkins et al., 1987; Wrighton et al., 1990; Haehner et al., 1996). Rifampin is known to strongly induce CYP3A4 transcripts both in vivo and in vitro (Kolars et al., 1992; Schuetz et al., 1996b) through the activation of a single orphan nuclear receptor, designated as the PXR, which binds to the AG(G/T)TCA motif in the 5′-promoter region of CYP3A4 as a heterodimer with the 9-cis retinoic acid receptor (Kolars et al., 1992; Schuetz et al., 1996b; Lehmann et al., 1998; Goodwin et al., 1999). The expression of these transcriptional enhancers in peripheral blood cells has not been examined.

Previous studies detected CYP3A5 in the polymorphonuclear fraction of leukocytes in six of six individuals but only one individual expressed CYP3A5 in the mononuclear fraction, and CYP3A4 mRNA was absent in all lymphocyte samples (Janardan et al., 1996). In agreement with Janardan and coworkers (1996), we found that CYP3A4 mRNA was not expressed at baseline in lymphocytes from any individual, and in addition, we demonstrate that the expression CYP3A4 mRNA in these cells could not be induced by rifampin. In contrast, Nakamoto et al. (2000) observed CYP3A4 mRNA induction in three individuals following rifampin administration. This conflicting observation most likely reflects differences in the primers used. Examination of the primers used by Nakamoto and colleagues (2000) for CYP3A4 (NCBI Blast program) suggests that their primers were not only specific for CYP3A4 mRNA but also recognized CYP3A5 and CYP3A7 mRNA (Altschul et al., 1997). Thus, the results reported by Nakamoto and coworkers (2000) concerning an induction of CYP3A4 mRNA in lymphocytes may actually reflect an induction of CYP3A7 mRNA in these cells.

In contrast to CYP3A4, we were able to detect expression of mRNAs corresponding to the other members of the human CYP3A subfamily, CYP3A5 and 3A7. It has been previously reported that CYP3A5 is not inducible by rifampin in cultured human hepatocytes and colon-derived cell lines (Combolbert et al., 1989; Schuetz et al., 1996a). Hepatic expression of CYP3A5 occurs in individuals who express significant CYP3A5 mRNA. This occurs in 60% of African Americans and 33% of Caucasians having at least one CYP3A5*/1 allele (Kuehl et al., 2001). In agreement with the finding of others (Kuehl et al., 2001), we found that CYP3A5 mRNA was detected in the lymphocytes from 30% of the Caucasian volunteers, and its expression was not induced by rifampin administration. This observation is consistent with the absence of a PXR binding domain in the 5′ promoter region of the CYP3A5 gene. Our results suggest that screening of lymphocytes for CYP3A5 mRNA expression may provide a simple approach to identifying individuals that express significant amounts of this protein at other sites of importance, such as the liver and intestine.

In contrast to CYP3A5, CYP3A7 contains a PXR binding element in the 5′ promoter regions of the gene and has been reported to be inducible by rifampin in primary human hepatocytes (Greuet et al., 1996). In the current study, a significant increase in the number of individuals expressing lymphocytic CYP3A7 mRNA was noted in response to rifampin treatment (24 versus 54%). It is unclear whether the expression of CYP3A7 mRNAs is accompanied by expression of the corresponding proteins. Although an immunoblotting assay by Ribrag et al. (1995) did not find the presence of CYP3A4 in normal lymphocytes, others recently were able to detect CYP3A protein in B-lymphocytes but not in T-lymphocytes (which constitute ~80–90% of the total lymphocytes fraction in blood), by using an antibody that recognized all human CYP3A enzymes (Sempoux et al., 1999). Thus, these immunochromatography protein bands may represent CYP3A5 and/or CYP3A7 in blood lymphocytes of some individuals rather than CYP3A4 per se. A change in the relative population of B- and T-lymphocytes in blood may also determine the detection of CYP3A5 and CYP3A7 in the mononuclear fraction.

Even though rifampin is thought to be a relatively specific inducer of CYP3A and MDR1, induction of other P450s, such as CYP2C8/9, has been noted in primary human hepatocyte cultures (Schuetz et al., 1996b), although this was not confirmed by other investigators (Morel et al., 1990; Runge et al., 2000). The present study also suggests that there is induction of CYP2C8 and CYP2E1 transcripts by rifampin in blood lymphocytes. The optical density of the CYP2C8 mRNA bands was increased 2-fold or greater in 14 of the 30 lymphocyte samples expressing CYP2C8 mRNA following rifampin administration. Furthermore, there was a dramatic increase in the number of individuals expressing CYP2E1 mRNA from 41% before to 78% after rifampin treatment. Unlike the other P450s, the expression of CYP2E1 is regulated at transcriptional and post-translational levels. Although CYP2E1 is not regarded as a highly polymorphic gene, several polymorphisms have been reported. Among the various identified alleles, only CYP2E1*7B, with an allele frequency of 0.05 in Caucasians, appears to have functional significance because its transcriptional
activity is 2-fold higher than that of wild allele (Daly et al., 1998). Thus, it is likely that differences in the CYP2E1 mRNA expression in the absence of medication reflect the influence of environmental and physiological modulators.

In conclusion, peripheral lymphocytes represent a readily accessible source of cells that have been advocated as reporters of protein induction that may occur in other inaccessible tissues of interest, such as the liver. For instance, lymphocytes fail to express CYP3A4 and CYP2C9 mRNA and, therefore, are not a suitable surrogate for monitoring the expression and activity of these enzymes in other tissues. Although MDRI mRNA was expressed in the lymphocytes from all individuals, there was no relationship between lymphocytic MDRI mRNA expression and the observed changes in oral fexofenadine clearance following rifampin administration, suggesting that lymphocytes are not a suitable surrogate for monitoring alterations in hepatic and intestinal MDRI expression and activity. In contrast to the MDRI finding, the proportion of individuals expressing lymphocytic CYP3A5 mRNA (30%) is similar to the proportion of liver samples demonstrating immunodetectable CYP3A5 (25–30%). Thus, lymphocytes may provide a noninvasive method for assessing the hepatic expression of CYP3A5 in human volunteers.

References