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Modulators and Substrates of P-glycoprotein and Cytochrome P4503A Coordinately Up-regulate these Proteins in Human Colon Carcinoma Cells

ERIN G. SCHUETZ, WILLIAM T. BECK, and JOHN D. SCHUETZ

Departments of Pharmaceutical Sciences (E.G.S., J.D.S.) and Molecular Pharmacology (W.T.B.), St. Jude Children's Research Hospital, Memphis, Tennessee 38105

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SUMMARY

Xenobiotics frequently induce proteins involved in their detoxification. Because many drugs that are metabolized by human cytochromes P450 (CYP) 3A4 and 3A5 are also transported by the drug efflux pump P-glycoprotein, we determined whether expression of these proteins was altered by a variety of drugs in a cell line derived from a human colon adenocarcinoma, LS180/WT, and its adriamycin-resistant subline, LS180/AD50. P-glycoprotein and CYP3A4 were constitutively expressed in both LS180/AD50 and LS180/WT cells, and both proteins were up-regulated after treatment with many drugs, including rifampicin, phenobarbital, clotrimazole, reserpine, and isosafrole. However, there were some exceptions because P-glycoprotein was up-regulated by midazolam and nifedipine, whereas CYP3A4 was not. CYP3A5, which is also constitutively expressed in these cells, remained unchanged with most drug treatments but was up-regulated by reserpine and clotrimazole. The apparent coordinated coexpression of the *CYP3A* gene family and P-glycoprotein in the LS180 cells suggests that for common orally administered drugs, P-glycoprotein may play an important role in net drug absorption and drug/drug interactions of shared CYP3A4/P-glycoprotein substrates.

Metabolism of drugs and other foreign compounds often involves sequential reactions with oxidation by cytochrome P450 phase I enzymes, followed by phase II reactions in which the hydroxylated metabolite is conjugated to a polar ligand. It is becoming increasingly clear that the drug efflux pump Pgp, associated with MDR and located on the cellular plasma membrane, is an additional component in the xenobiotic detoxification cascade (1). Humans have two *MDR* genes, with the one involved in drug transport being *MDR1* (2). Consistent with a role in xenobiotic detoxification, Pgp is most highly expressed in tissues that participate in xenobiotic detoxification, such as the intestine and liver. Analogous to the cytochromes P450 and phase II enzymes, Pgp expression is increased after treatment with drugs and other chemicals (2). Indeed, in rat liver Pgp is coordinately up-regulated with CYP1A1 and phase II enzymes after treatment with aromatic hydrocarbons (3). There are species differences in this response because aromatic hydrocarbons do not induce *mdr1* mRNA in mouse liver (4) and we have found polymorphic inducibility of human *MDR1* mRNA by aromatic hydrocarbons in primary human hepatocytes (5). However, the possible coordinate regulation of Pgp with other phase I drug-metabolizing enzymes remains unelucidated in humans.

Frequently drugs that up-regulate detoxification proteins are also substrates for these proteins. Many substrates or modulators of Pgp are also substrates or modulators of the *CYP3A* gene family. For example, taxol, the epipodophyllotoxins, CsA, nifedipine, tamoxifen, amiodarone, verapamil, terfenadine, and imidazole antimycotic agents such as ketoconazole are substrates or modulators of both CYP3A (6–9) and Pgp (10–13). CYP3A is the most prominent CYP450 in humans, comprising an average of 30% and 70% of total CYP450 in human liver and intestine, respectively (14, 15). Detailed studies on the regulation of CYP3A have also established that this gene family is induced in some species by glucocorticoids and rifampicin, phenobarbital, and imidazole antimycotic agents (8, 16). However, despite the obvious overlap in CYP3A and Pgp substrates and modulators, there has not been a simultaneous comparison of the effect of commonly administered clinical agents on their expression. Thus, this study was designed to test the hypothesis that the *MDR1* and *CYP3A* gene families might be coordinately regulated by similar substrates or modulators.

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ABBREVIATIONS: CsA, cyclosporin A; CYP, cytochrome P450; WT, wild-type; Pgp, p-glycoprotein; *MDR1*, human gene or mRNA encoding the drug transporting P-glycoprotein; PCR, polymerase chain reaction; bp, base pair(s); MDR, multi-drug resistance.

To test our hypothesis, we used a human colon carcinoma cell line, LS180/AD50 and its parent LS180/WT, because several drugs, including verapamil, nifedipine, reserpine, and CsA, have been documented to increase *MDR1* and Pgp expression in these cells (12, 17). Moreover, during the study, we found that the LS180 cells express both adult CYP3A4 and CYP3A5. This presented us with the unique opportunity to make a simultaneous comparison and to report for the first time that CYP3A and Pgp are companion detoxification components whose expressions are coordinately increased by many drugs in a human colon carcinoma cell line.

Materials and Methods

Cell cultures. The parent cell line designated LS180/WT was derived from a human colon adenocarcinoma (18) and was obtained from the American Type Culture Collection (Rockville, MD). LS180/AD50 cells were previously derived from LS180/WT by stepwise selection in adriamycin [some of its features have been described (12)] and were kindly provided by Dr. Antonio Fojo (National Cancer Institute, Bethesda, MD). Both cell lines were maintained in RPMI 1640.

Immunoblot analysis. LS180 cells growing in monolayer culture were removed from the plate by scraping in phosphate-buffered saline. The cells were pelleted at 10,000 × *g* and resuspended in storage buffer (100 mM potassium phosphate, pH 7.4, 1.0 mM EDTA, 20% glycerol, 1 mM dithiothreitol, 20 μM butylated hydroxytoluene, 2 mM phenylmethylsulfonyl fluoride), and lysates were generated by sonication. For immunodetection of Pgp, lysates (50 μg) were resuspended in Laemmli sample preparation buffer, loaded immediately onto a 6% Laemmli polyacrylamide gel, and electrophoresed. Proteins were transferred to nitrocellulose and then incubated sequentially with primary polyclonal rabbit anti-*mdr* (Ab-1) IgG (Oncogene Science, Uniondale, NY) and peroxidase conjugated anti-rabbit IgG and developed with the Amersham ECL detection system. For immunodetection of CYP3A4 and CYP3A5, 100 μg of lysate in Laemmli sample buffer was heated for 3 min at 90° and electrophoresed on a 12% polyacrylamide gel, transferred to nitrocellulose, and developed sequentially with monoclonal antibody to human CYP3A proteins (19) and peroxidase conjugated anti-mouse IgG and developed as described above. The relative amounts of Pgp and CYP3A proteins were determined by densitometric analysis.

Northern blot analysis. Total RNA was extracted from LS180/WT cells (20), and 10 μg was analyzed by Northern blotting (20). Equivalent loading of RNA samples and uniform transfer was ensured by analysis of ethidium bromide staining of the 18*S* and 28*S* ribosomal RNAs before and after transfer to membrane filter. The pADR1 cDNA (for human MDR1) (21) was kindly provided by Dr. Ken Cowan (National Cancer Institute, Bethesda, MD), and the pHFLA-A cDNA (for *CYP3A*) was isolated in this laboratory and represents nucleotides 458–846 of *CYP3A7* (22), which under normal stringency conditions will recognize all human CYP3A mRNAs.

cDNA synthesis. From 5 to 10 μg of total RNA from LS180 cells was reverse transcribed to cDNA by incubating Moloney murine leukemia reverse transcriptase (Life Technologies, Gaithersburg, MD) in a buffer containing 10 mM Tris·HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 1.0 mM of each dNTP.

PCR reactions. PCR reactions were performed in 100 μl final volumes [consisting of 10 mM Tris·HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% (w/v) gelatin, 0.2 mM of each dNTP, and 100 pmol of each oligonucleotide (Table 1)], 1 μg of first-strand cDNA or various amounts of CYP3A positive controls [full-length *CYP3A4* (23) (generously provided by Dr. Frank Gonzalez, National Cancer Institute, Bethesda, MD), *CYP3A5* cDNA (24), *CYP3A5P* pseudogene (25), or bp 404-1970 of *CYP3A7* (26)], and 2.5 units of *Taq* polymerase. After the addition of 100 μl of mineral oil, the reactions were initially denatured at 94° (2 min) and then cycled in an Omnigene thermocycler (National Labnet Co., Woodbridge, NJ) (Table 2). A final 72° extension step was performed for 10 min at the end of all of the cycles. We routinely analyzed 40 μl of the PCR reaction product by separation in a 0.5% NuSieve/0.5% agarose (w/v)-containing ethidium bromide for visualization. Sizes of the PCR products were estimated from the migration of DNA size markers (123-bp ladder) run concurrently. Transfer of gels to Nytran membranes was achieved through capillary blotting. The membranes were prehybridized overnight at 42° with 15% formamide (26) and hybridized overnight under the same conditions with a ^{32}P-labeled oligonucleotide targeted to a region internal to the amplified PCR product. The membranes were washed under low stringency for 1 hr [0.2× standard saline citrate and 0.2% sodium dodecyl sulfate (w/v) at 42° 1 × standard saline citrate = 0.15 M NaCl and 0.015 M sodium citrate]. Blots were visualized by autoradiography or with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). PCR reactions were performed twice to confirm the size of the amplified products. Control PCR reactions included water blanks as well as samples that had reverse transcriptase omitted.

Results

We first considered whether a series of known inducers of hepatic CYP3A, previously untested as substrates or modulators of Pgp, could regulate expression of either Pgp or CYP3A in colon carcinoma cells. Although the antibody used cannot discriminate among *MDR* family members, there is substantial evidence that *MDR1* is the primary, if not exclusive, Pgp subtype expressed in intestine (27–29). As previ-

TABLE 1
CYP3A oligonucleotides

Gene		Oligonucleotide sequence (5′ to 3′)	5′ base[a]	Size[b]	Ref.[c]
				bp	
CYP3A4	(S)	CCT TAC ACA TAC ACA CCC TTT GGA AGT	1353	381	(14)
	(AS)	AGC TCA ATG CAT GTA CAG AAT CCC CGG TTA	1734		
	(INT)	CAC CAG AGA CCT CAA ATT ACT TTG TGA A	1631		
CYP3A5[d]	(S)	CAA TGG GGT ATT CAT TCC CAA AGG GTC AAT	1236	310	(24)
	(AS)	GTT TTT CTG GTT GAA GAA GTC CTT GCG TGT	1546		
	(INT)	GTT CAG TAA GAA GAA GGA CAG CA	1341		
CYP3A7	(S)	ATT CCA AGC TAT GTT CTT CAT CAT	1189	554	(26)
	(AS)	AAT CTA CTT CCC CAG CAC TGA	1743		
	(INT)	CGT CTT CAT TTC AGG GTT CTA TTT GTA	1613		

[a] Number refers to 5′ nucleotide location in the oligonucleotide.
[b] Size of the PCR product.
[c] Reference.
[d] New *CYP3A5* oligonucleotides were developed because those used previously (26) were not specific for *CYO3A5*.
S, sense; AS, antisense; INT, internal oligonucleotide.

TABLE 2
CYP3A PCR amplification conditions

Primer	Sample	Anneal	Extend	Denature	Cycles
3A4	cDNA clone	69.5°, 30 sec	72°, 45 sec	90°, 1 min	30
	First-strand cDNA	68°, 30 sec	72°, 45 sec	90°, 1 min	40
3A5 or 3A7	cDNA clone	50°, 30 sec	72°, 45 sec	90°, 1 min	30
	First-strand cDNA	50°, 30 sec	72°, 45 sec	90°, 1 min	35

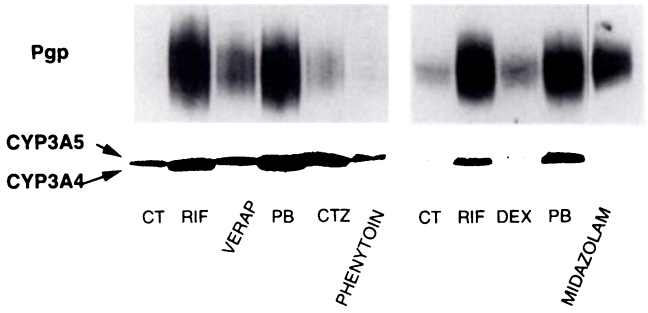

Fig. 1. Immunoblot analysis of Pgp and CYP3A in drug-treated LS180/AD50 cells. Confluent LS180/AD50 cells either untreated [control (*CT*)] or treated for 72 hr with 1 mM phenobarbital (*PB*) or 10 μM rifampicin (*RIF*), verapamil (*VERAP*), clotrimazole (*CTZ*), phenytoin (*PHENYTOIN*), dexamethasone (*DEX*), or midazolam (*MIDAZOLAM*) were harvested, and the amounts of Pgp, CYP3A4, and CYP3A5 proteins were quantified by immunoblot analysis (see Materials and Methods). *Right*, Pgp was developed for a longer time to visualize its level in untreated controls.

ously shown (12), treatment of LS180/AD50 cells with verapamil increased Pgp expression. Pgp was strongly upregulated (up to 29-fold) by treatment with phenobarbital, rifampicin, midazolam, and clotrimazole, whereas phenytoin caused only a modest change in expression of this protein (Fig. 1 and Table 3). Dexamethasone, a prototypical inducer of CYP3A and an inducer of Pgp in some hepatocyte cell lines (30), had a weak effect on Pgp expression.

Analysis of CYP3A proteins revealed both immunodetectable CYP3A4 and CYP3A5 in untreated LS180/AD50 cells; this result is consistent with expression of both isoforms throughout the human large and small intestine *in vivo* (14, 31). The identity of the CYP3A isozymes was confirmed by their comigration with authentic human liver CYP3A4 and CYP3A5 present in microsomes of human liver (see Fig. 3, *HL 14*). Immunoblot analysis of lysates from clotrimazole-treated LS180 cells (Fig. 2) further revealed two proteins that, based on their migration in sodium dodecyl sulfate-polyacrylamide gels and based on immunoreactivity with anti-CYP3A antibody, were identified as CYP3A4 (51 kDa) and CYP3A5 (52 kDa). Lysates from rifampicin-treated cells contained abundant CYP3A4 and a lesser amount of CYP3A5. Other studies confirmed that the CYP3A immunoreactive proteins in LS180/AD50 did not comigrate with CYP3A7 (51.5 kDa) present in human fetal liver (Fig. 2). Thus, LS180/AD50 cells express CYP3A4 and CYP3A5 but not CYP3A7.

It would have been informative to quantitatively compare the amount of CYP3A in human intestine with the amount of CYP3A in LS180 cells. However, we repeatedly found that CYP3A protein was not detectable in microsomes prepared from LS180 cells (whereas it was readily detectable in the lysate), making a direct quantitative comparison impossible with CYP3A content in human intestinal microsomes.

Immunoblot analysis revealed that the amount of CYP3A4 was increased by rifampicin and phenobarbital treatment, whereas there was no apparent effect on CYP3A5 protein expression. In contrast, clotrimazole increased expression of both CYP3A4 and 3A5 (Fig. 1). Because verapamil was a weak inducer of total CYP3A, we could not readily discriminate whether CYP3A4 or CYP3A5 was selectively induced. Midazolam and CsA, known substrates for CYP3A4 and CYP3A5 (8), were without effect on expression of these proteins, despite inducing Pgp (Fig. 1, Table 3, and not shown). Dexamethasone, the prototypical glucocorticoid inducer of hepatic CYP3A (16), failed to induce CYP3A4 and CYP3A5.

TABLE 3
Effect of various drugs on expression of P-glycoprotein and CYP3A in LS180 cells

Treatment	Fold-increase in Pgp	Rank order (Pgp)	Fold-increase in CYP3A4/5	Rank order (CYP3A)
Reserpine	29 ± 3.3[a]	1	7.6 ± 0.6	1
Rifampicin	16.2 ± 10.8 (range, 6.2–29.9)	2	3.2 ± 1.4	3
Phenobarbital	14.4 ± 3.6 (range, 6.0–22)	3	6.1 ± 3.3	2
Verapamil	10.8 ± 8.3	4	1.7 ± 0.4	7
Midazolam	5.9 ± 1.7	5	1.0	
Rapamycin	4.9 ± 2.1	6	1.0	
Clotrimazole	4.1 ± 1.3	7	4.5 ± 1.1	5
FK506	3.2 ± 0.7	8	1.3 ± 0.2	9
Isosafrole	2.7 ± 0.3	9	2.6 ± 0.1	6
Amiodarone	2.4 ± 0.7	10	1.0	
Triactyloleandomycin	2.3 ± 0.8	11	1.5 ± 0.2	8
Erythromycin	2.0 ± 0.6	12	5.3 ± 1.2	4
Dexamethasone	1.5 ± 0.3	13	1.0	
Nifedipine	1.3 ± 0.1	14	1.0	
Phenytoin	1.3 ± 0.1	15	1.0	

[a] The values are the mean of up to five separate determinations ± standard deviations; values of 1.0 = no change from untreated control. All cultures were treated for 48–72 hr with 10 μM drug, except for phenobarbital (1 mM).

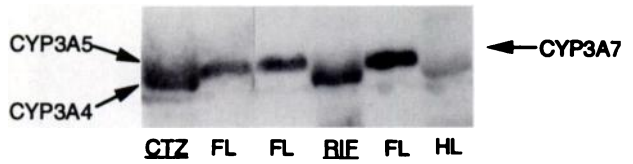

Fig. 2. Immunoblot analysis of CYP3A proteins in LS180 cells. LS180 cells were treated for 72 hr with clotrimazole (*CTZ*) or rifampicin (*RIF*), and 100 μg of total cell lysate was analyzed by immunoblot analysis. Adult human liver (*HL*) microsomes (1 μg) containing only CYP3A4 or fetal liver (*FL*) microsomes (0.5 μg) containing only CYP3A7 were loaded as controls.

Thus, Pgp and CYP3A4 are coordinately increased by many, but not all, xenobiotics in the LS180/AD50 cell line.

We next determined whether a Pgp "reversing agent" (10) not previously identified as a modulator of CYP3A might up-regulate this cytochrome. Consistent with previous results with LS180/AD50 cells (32), treatment with reserpine caused Pgp expression to increase as much as 30-fold (Fig. 3). Reserpine also caused up-regulation of both CYP3A4 and CYP3A5, with a maximal increase of CYP3A of 8-fold. Reserpine had a more potent effect on Pgp than on CYP3A, with an ED_{50} value of 1.2 μM for Pgp (versus 2.5 μM for CYP3A4), although these differences were not statistically significant.

We extended the study to include additional agents that are known substrates for or modulators of CYP3A to analyze their potential to regulate Pgp (Table 3). From these results, the drugs can be classified into two categories: (i) drugs that up-regulate both Pgp and CYP3As (typical examples being rifampicin, phenobarbital, reserpine, and clotrimazole), and (ii) drugs that up-regulate only Pgp, not CYP3A (typical examples being midazolam, rapamycin, phenytoin, and nifedipine). Although the rank order for induction of Pgp differed from that of CYP3A, linear regression analysis revealed a positive correlation ($r = 0.7$) between fold-increase of CYP3A and Pgp for the agents examined.

Because drug selection could have altered the characteristics of expression of Pgp and CYP3A, we examined whether the prototypical CYP3A inducer rifampicin could mediate expression of these proteins in the WT parental cells (LS180/WT). We found that these cells responded to rifampicin (Fig. 4) (and all other drug treatments; not shown) with enhanced expression of Pgp and CYP3A proteins. In contrast to reserpine, the ED_{50} values for the half-maximal effect of rifampi-

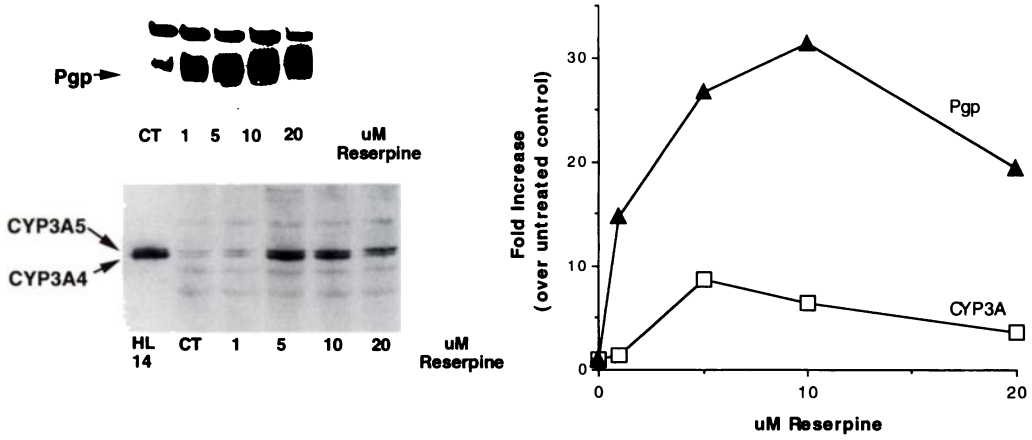

Fig. 3. Immunoblot analysis of Pgp and CYP3A in reserpine-treated LS180/AD50 cells. LS180/AD50 cells either untreated [control (*CT*)] or treated with 1, 5, 10, or 20 μM reserpine for 72 hr were harvested, and visualized by immunoblot analysis as described in the Materials and Methods. *Left*, 5 μg of microsomes from human liver 14 (*HL 14*) served as a CYP3A4 and CYP3A5 control. The immunoblots were quantified by densitometry, and the results are expressed graphically as fold-increase in CYP3A or Pgp protein in drug-treated cells divided by values in untreated control cells (*right*). *Points*, mean of duplicate experiments.

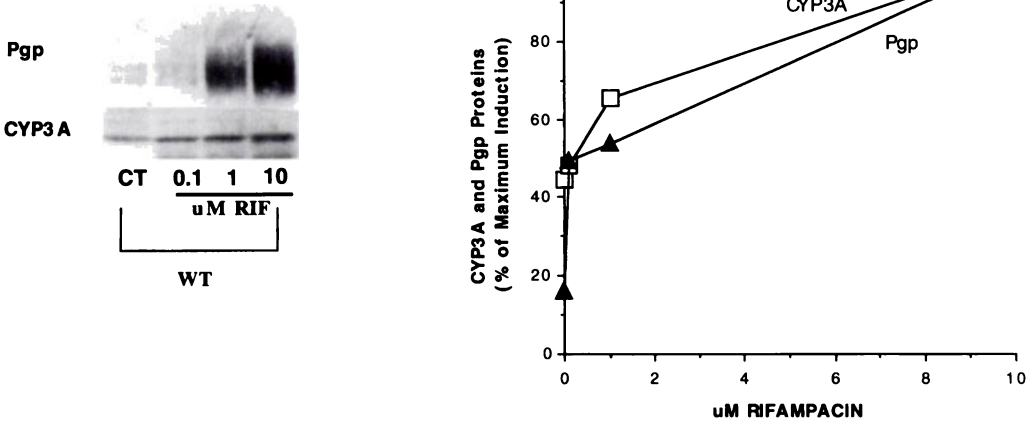

Fig. 4. Immunoblot analysis of Pgp and CYP3A in rifampicin-treated LS180/WT cells. Confluent LS180/WT cells either untreated [controls (*CT*)] or treated for 72 hr with 0.1, 1, or 10 μM rifampicin (*RIF*) were harvested and analyzed for CYP3A and Pgp proteins as described in the legend for Fig. 1 (left). The immunoblots were quantified by densitometry, and the results are expressed graphically as percent of maximum induction in drug-treated cells (*right*). *Points*, represents the mean of duplicate experiments.

cin on CYP3A and Pgp were similar in the LS180 cells (Fig. 4). Northern blot analysis of LS180/WT (Fig. 5) and LS180/AD50 cells (not shown) treated with rifampicin and reserpine demonstrated that these drugs increased expression of *MDR1* and CYP3A mRNAs, whereas treatment with dexamethasone did not (not shown).

To further confirm the identity of the CYP3As expressed in LS180/WT cells, we used PCR analysis and CYP3A4-, CYP3A5-, or CYP3A7-specific oligonucleotide primers (Table 1). We first verified the specificity of our primers by using the cloned cDNAs for *CYP3A4, CYP3A5, CYP3A7* and the CYP3A5 pseudogene, CYP3A5P, as templates. Each cDNA was subjected to stringent amplification conditions (Table 2) that only permit the oligonucleotides to amplify the corresponding cDNA (Fig. 6). CYP3A4-specific primers readily amplified a DNA fragment of appropriate size (381 bp) from the *CYP3A4* cDNA, whereas there was no amplification in the tubes containing cDNAs for *CYP3A5P, CYP3A5, CYP3A7,* or the water blank. Similarly, *CYP3A5* and *CYP3A7* primers selectively amplified products only from their corresponding cDNA templates. Verification of the

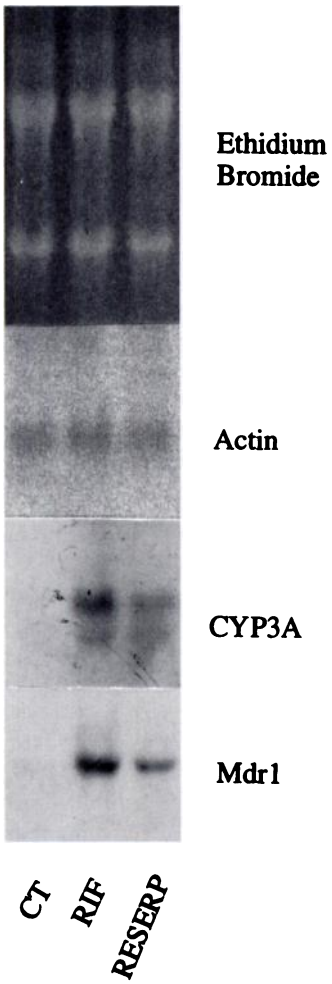

Fig. 5. Northern blot analysis of *MDR1* and CYP3A mRNAs in LS180/WT cells. Confluent LS180/WT cells either untreated [controls (*CT*)] or treated for 72 hr with 1 mM phenobarbital (*PB*) or 10 μM rifampicin (*RIF*) or reserpine (*RESERP*) were harvested, and 10 μg of total RNA was analyzed by Northern blot analysis. Gels were stained with ethidium bromide or hybridized with cDNAs for actin, *MDR1*, or CYP3A mRNAs.

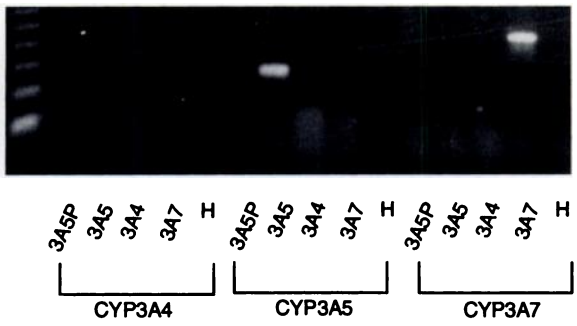

Fig. 6. PCR products obtained from *CYP3A* cDNA clones. Synthetic oligonucleotide primers complementary to hypervariable regions of *CYP3A4, CYP3A5,* and *CYP3A7* were used to amplify the respective products. The templates were cloned cDNAs for *CYP3A5P, CYP3A5, CYP3A4, CYP3A7,* or water (*H*). The amplification products were resolved on agarose gels stained with ethidium bromide.

CYP3A5 and *CYP3A7* amplified products was further confirmed by their size on agarose gel (310 and 554 bp, respectively) and hybridization with specific oligonucleotide probes targeted to sequences internal to the respective PCR products.

Total RNA from untreated control or rifampicin- or reserpine-treated LS180/WT cells was reverse transcribed, and each cDNA was subjected to PCR analysis under the conditions described in Table 2 to selectively amplify *CYP3A4, CYP3A5,* and *CYP3A7.* Analysis of the amplified products on agarose gels stained with ethidium bromide revealed that the CYP3A4 primers amplified a DNA fragment of appropriate size in the adult human liver sample and in the LS180/WT cells treated with rifampicin or reserpine but not in either the fetal liver sample or the untreated LS180 cells (Fig. 7). CYP3A5-specific primers amplified a DNA fragment of appropriate size in the adult human liver sample (identified on immunoblot analysis to be a CYP3A5 protein expressor) and in the LS180 cells treated with rifampicin or reserpine, whereas there was no signal detected in untreated LS180 cells. A faint band of appropriate size was observed in the fetal liver samples on longer exposure (not shown). The CYP3A7 primers consistently amplified cDNA only from fetal liver and the positive control *CYP3A7* cDNA. Each of the gels was then Southern blotted and hybridized with the corresponding CYP3A oligonucleotide specific to sequences internal to the PCR product. This hybridization analysis confirmed that CYP3A4 and CYP3A5 (but not CYP3A7) were present in LS180/WT cells. Although equivalent amounts of first-strand cDNA were added to each PCR reaction mixture, no attempt was made to quantify the PCR products. Nevertheless, these results qualitatively confirm the immunoblot analysis (Figs. 1–3) results that LS180/WT and LS180/AD50 cells express CYP3A4 and CYP3A5 but not CYP3A7.

Discussion

The present study was undertaken to determine whether human Pgp and CYP3A might be similarly regulated by CYP3A substrates and inducers. Our studies used the model system LS180/WT and its drug-selected subline, LS180/AD50, because the latter cells were documented to respond to the presence of several xenobiotics with increases in expression of Pgp and its mRNA, *MDR1* (12, 32) and, importantly,

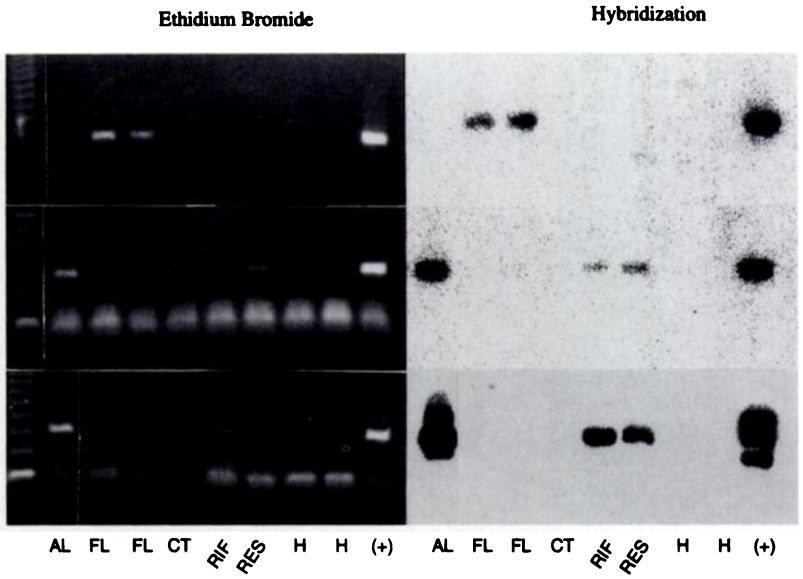

Fig. 7. Amplification of CYP3A4 and CYP3A5 from LS180 cells. First-strand cDNA was synthesized from total RNA prepared from human adult liver (*AL*), human fetal liver (*FL*), or LS180/WT cells; untreated controls (*CT*); or cells treated with rifampicin (*RIF*) or reserpine (*RES*). First-strand cDNA was amplified using primers specific for each of the indicated CYP3A cDNAs. Specific positive controls (+) for the CYP3A7, CYP3A5, and CYP3A4 primer amplifications were the cloned cDNAs for CYP3A7, CYP3A5, and CYP3A4, respectively (see Materials and Methods). The negative PCR control was water (*H*). PCR product (40 μl) was resolved on an agarose gel, visualized by ethidium bromide (*left*), and then Southern blotted to a Nytran membrane and hybridized with CYP3A4, CYP3A5, and CYP3A7 oligonucleotides (*right*) specific for sequences internal to the amplified PCR products (see Table 1).

because, as we report for the first time, these colon carcinoma cells also expressed human CYP3A4 and CYP3A5. Overall, our results revealed that the rank order of agents that effect Pgp expression is remarkably similar to that known for substrates or modulators of CYP3A4. Indeed, the effects of the agents on the expression of both CYP3A and Pgp were positively correlated ($r = 0.7$). For example, the most effective agents to up-regulate expression of Pgp were also the most efficacious for CYP3A4 (rifampicin, reserpine, phenobarbital, and clotrimazole). However, there were noteworthy differences, with selective up-regulation of Pgp (but not CYP3A4) by midazolam and CsA. There was a corresponding increase in expression of *MDR1* and CYP3A mRNAs in the LS180/WT cells by rifampicin and reserpine (Fig. 5), suggesting that these drugs do not modulate Pgp expression by post-translational mechanisms. Indeed, reserpine-mediated up-regulation of Pgp appears to be, in part, regulated at the transcriptional level as these treatments cause a dose- and time-dependent increase in *MDR1* mRNA expression (17, 32). Although studies are under way to understand the mechanistic basis for this phenomenon, we note that this is the first study to examine in parallel regulation of human CYP3A4 and Pgp and to demonstrate that human CYP3A and Pgp can be coordinately up-regulated by commonly administered therapeutic agents.

Because CYP3A represents 70% of total P450 in human intestine (33) and because intestinal CYP3A is important to first-pass metabolism of many drugs (34), a replicating cellular model that retains expression of CYP3A characteristic of human intestine *in vivo* would be advantageous for regulatory studies. Other than a report that rifampicin can induce CYP3A4 in human small intestinal enterocytes *in vivo* (34), virtually nothing is known about xenobiotic regulation of human intestinal CYP3A. Importantly, with this identification of CYP3A4 and CYP3A5 in the LS180 cells, it was possible, for the first time, to evaluate CYP3A regulation in human colon carcinoma cells derived from human intestine. A recent report suggested that Caco-2 cells, derived from a primary colonic tumor, might be an appropriate surrogate for studies of CYP3A regulation in human small intestine (35). However, Caco-2 cells express a CYP3A protein that does not comigrate with either CYP3A4 or CYP3A7 and that remains unidentified (35). Thus, except for primary human hepatocytes, no other cultured cell has been described that maintains expression of adult CYP3A4. In addition, because CYP3A5 is polymorphically expressed in only 20–25% of humans (36), its expression cannot be assessed in all preparations of primary human hepatocytes. A comparison of xenobiotic regulation of CYP3A4 and CYP3A5 in LS180 cells with their regulation in human hepatocytes revealed that most inducers of CYP3A4 in human liver, such as rifampicin and phenobarbital, were effective in increasing expression of CYP3A4 in this human colon carcinoma cell line as well. However, dexamethasone, a potent and robust inducer of human hepatic CYP3A4 (16), had no effect on CYP3A4 or CYP3A5 expression in LS180 cells. In addition, both clotrimazole and reserpine were able to up-regulate CYP3A5, a protein whose expression is not thought to be modulated by drugs in human liver (36). Thus, there are some tissue-specific differences between regulation of CYP3A4 and CYP3A5 in human hepatocytes versus the human colon carcinoma cell.

There is a growing awareness of the contribution of human intestinal CYP3A in first-pass drug metabolism (34). Less well defined is the influence of Pgp on intestinal drug absorption. However, Pgp is present in the epithelial lining throughout the human gastrointestinal tract (37), and there is evidence that intestinal Pgp may limit intestinal absorption of CsA (38, 39), benzo[*a*]pyrene (37), vinblastine, and docetaxel (40). If regulation of Pgp in a human colon adenocarcinoma cell line, LS180, reflects regulation of Pgp throughout the human intestine, then up-regulation of intestinal Pgp by rifampicin may have important clinical implications. There are reports of many drug interactions with medications given orally in combination with rifampicin. For example, rifampicin decreases the bioavailability of CsA and increases its oral clearance (41, 42). Rifampicin induction of intestinal and hepatic CYP3A (which metabolizes CsA) is believed to be responsible for the increased clearance and decreased bioavailability of CsA. However, because Pgp transports CsA (43), an alternate explanation is possible. Rifampicin-mediated increased expression of human Pgp, as

seen in the LS180 cells (Figs. 1 and 4 and Table 3), would result, if it occurs in normal intestine, in greater transport of CsA back into the intestinal lumen, leading to decreased net absorption and thus decreased bioavailability of CsA. Knowledge that Pgp is induced by rifampicin and phenobarbital raises the possibility that many clinically important drug interactions previously attributed to intestinal CYP3A4 may also be due, in part, to alterations in expression of *MDR1*/Pgp. Clearly, these drug interactions should therefore be reanalyzed in the context of the potential for interaction with rifampicin and phenobarbital at the *MDR1*/Pgp locus.

There is much effort to identify additional substrates, inhibitors, and modulators of Pgp to enhance chemotherapeutic efficacy. Not developed previously is a singular characteristic shared among these agents, that many are also substrates, inhibitors, and/or modulators of CYP3A. Indeed, knowledge that rifampicin and phenobarbital are inducers of CYP3A led to their being tested and identified, for the first time, as regulators of Pgp (Fig. 1). It is therefore possible that a survey of other CYP3A substrates or inducers may be prognostic for identifying additional Pgp modulators and candidate reversing agents for pharmacological intervention. Furthermore, it is likely that part of the enhanced cytotoxicity with current regimens of Pgp reversing agents (e.g., CsA) coadministered with chemotherapeutic agents (e.g., the epipodophyllotoxins) is due to CsA inhibition of CYP3A-mediated metabolism of these drugs (7, 44).

We conclude that Pgp and CYP3A4 are coordinately upregulated by many xenobiotics in LS180 human colon carcinoma cells. The availability of CYP3A and Pgp genomic clones should allow the identification of regulatory factors that may be common to these genes. Finally, with the identification in this study that Pgp is up-regulated by many clinically administered drugs, the role of Pgp within the context of the overall cellular pharmacology and interindividual differences in drug disposition of shared CYP3A4/Pgp substrates needs to be considered.

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Send reprint requests to: Dr. Erin G. Schuetz, St. Jude Children's Research Hospital, Department of Pharmaceutical Sciences, 332 N. Lauderdale, Memphis, TN 38105.