—Regular Articles—

Evaluation of Gene Induction of Drug-Metabolizing Enzymes and Transporters in Primary Culture of Human Hepatocytes Using High-Sensitivity Real-Time Reverse Transcription PCR

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In the present study, the induction of drug-metabolizing enzymes and transporters was evaluated by analyzing mRNA expression in human hepatocytes after exposure to various compounds. The compounds tested included typical enzyme inducers, rifampicin and omeprazole, and controls. All experiments were performed in the presence of 0.1% DMSO. Analysis was performed by the real-time reverse-transcription polymerase chain reaction method (RT–PCR) in the presence of TaqMan probes using an ABI PRISM 7700 Sequence Detector system. A new analytic method to quantify mRNA levels in small numbers of human hepatocytes has been developed for phase I enzymes, phase II enzymes, and transporters. The levels of CYP1A1, CYP2B6, CYP2C8, CYP3A4, CYP3A5, ADH3, and ABCG1 mRNA in human hepatocytes increased after exposure to rifampicin. The levels of CYP1A1, CYP2B6, CYP3A4, CYP3A5, and ABCG1 mRNA recovered after a change to media without rifampicin. The levels of CYP1A1, CYP1A2, CYP1B1, ALDH3, and ALDH6 mRNA increased after exposure to omeprazole, and recovered after a change to media without omeprazole. On the other hand, the levels of ADH3 and ABCB4 mRNA decreased after exposure to omeprazole, and recovered after a change to media without omeprazole. In conclusion, these results demonstrate the applicability of quantitative real-time RT–PCR to the evaluation of the gene induction and recovery of drug-metabolizing enzymes and transporters after exposure to drugs in human hepatocytes.

Key words——gene induction; human hepatocytes; quantitative real-time RT–PCR

INTRODUCTION

Drug-drug interactions are an important consideration in drug development. However, species differences in the inhibition and induction of drug-metabolizing enzymes are frequently observed in the metabolism of xenobiotics. Interestingly, the induction of CYP3A4 in human hepatocytes by several model compounds has been found not to correlate with the induction of CYP3A1/2 in rat hepatocytes.[1] Studies employing human tissues are useful in overcoming this problem. Primary culture of human hepatocytes represents a unique *in vitro* system for studying the potential of drugs to induce phase I and phase II enzymes involved in drug metabolism. It has been demonstrated that human hepatocytes can be cryopreserved and thawed without significant loss of the activities of the major cytochrome P450 isoforms or the major phase II enzymes,[2] suggesting that cryopreserved human hepatocytes may represent a useful experimental system for evaluation of the metabolism, inhibition, and induction of xenobiotics after exposure to various compounds.

Rifampicin is known to induce the metabolism of numerous drugs, including warfarin, digitoxin, ethynylestradiol, glucocorticoids, vitamin D, and thyroxine.[3] In addition, rifampicin and its analogue rifabutin increase their own metabolism after repeated administration to patients.[4] Rifampicin also causes an increase in the urinary ratio of 6β–hydroxycortisol to 17–hydroxycorticosterone, indicating the induction of CYP3A4 *in vivo*.[5,6] Rifampicin is a potent inducer of CYP3A in primary cultures of human hepatocytes,[7—9] and omeprazole is a potent inducer of CYP1A in primary cultures of human hepatocytes.[10—13]

There have been reports on the usefulness of the quantitative real-time reverse-transcription polymerase chain reaction method (RT–PCR) for the analysis of human CYP1A1 and CYP3A4 mRNA expression in primary cultures of human hepatocytes[10] and mouse cytochrome P450 mRNA expression in mouse liver.[14] However, methods for the quantitative, high-throughput, real-time RT–PCR of human

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phase I enzymes, phase II enzymes, and transporters have not been available. We have therefore developed a high-throughput, semiautomated, quantitative, single-tube RT–PCR assay to detect the mRNAs of human phase I enzymes, phase II enzymes, and transporters, and report a method for evaluating the gene induction of drug-metabolizing enzymes and transporters after exposure to model drugs such as rifampicin and omeprazole in primary culture of human hepatocytes.

MATERIALS AND METHODS

1. Materials Cryopreserved human hepatocytes (Lot 100, sex: female, age: 74 years, race: Caucasian) were purchased from In Vitro Technologies, Inc. (Baltimore, MD, USA). Trypan blue was purchased from Flow Laboratories, Ltd. (Irvine, UK). Hepatocyte Culture Medium (CC–3198) was purchased from BioWhittaker, Inc. (Walkersville, MD, USA). Adult human total RNA from the liver was purchased from CLONTECH Laboratories, Inc. (Palo Alto, CA, USA). An Rneasy® Mini Kit and QIAshredder™ were purchased from QIAGEN (Hilden, Germany). Yeast tRNA was purchased from Life Technologies, Inc. (Rockville, MD, USA). TaqMan One-Step RT–PCR Master Mix Reagents, TaqMan GAPDH Control Reagents, TaqMan (β-actin Control Reagents, Micro Amp® Optical 96–well Reaction Plates, Optical Adhesive Covers, and Optical Cover Compression Pads were purchased from Applied Biosystems (Foster City, CA, USA).

All other chemicals and reagents used were of analytical reagent grade.

2. Monolayer Culture of Hepatocytes The cryopreserved human hepatocytes were suspended in Hepatocyte Culture Medium. The hepatocytes were centrifuged (45 X g, 4℃) for 3 min and resuspended in the same medium. The number of cells was counted using a Coulter Counter. Cell suspensions with viability rates of 86% and 91% as assessed by trypan blue dye exclusion were used for the experiments. The cell suspensions were diluted to a final concentration of 2.5×10^5 viable cells/ml using Hepatocyte Culture Medium, and inocula of 1×10^5 viable cells/0.4 ml/well were introduced into 24–well plates that had been coated with type I collagen. The cells were cultured for 3 h after inoculation under 5% CO_2 and 95% air at 37℃. The medium was then replaced with fresh medium, and the cells were cultured for 21 h under 5% CO_2 and 95% air at 37℃. The medium was then replaced with fresh medium without human epidermal growth factor (hEGF), gentamicin, and amphotericin B, and the cells were cultured for 24 h under 5% CO_2 and 95% air at 37℃. The cells were used for experiments at 48 h after inoculation.

3. Experiments Using Monolayer Cultures of Hepatocytes After 48 h of inoculation, human hepatocytes were treated with a number of known inducers for an additional 24 or 48 h. Media with the inducers but without hEGF, gentamicin, and amphotericin B were changed daily during treatment. These media were then replaced with fresh media without inducers, hEGF, gentamicin, and amphotericin B, and the cells were cultured for 24 or 48 h under 5% CO_2 and 95% air at 37℃. The medium was changed daily. The effects of rifampicin and omeprazole were studied at doses of 0.2, 10, and 50 μM. All inducers were dissolved in DMSO to a final vehicle concentration of 0.1% (v/v). Controls with DMSO–untreated cells were included in the experimental design to assess the effect of DMSO on mRNA expression. Total RNA was extracted from the hepatocytes using the Rneasy® Mini Kit and QIAshredder™.

4. Oligonucleotides The forward and reverse primers and the TaqMan probes were designed using Primer Express software (Applied Biosystems) from the human mRNA sequence (Table 1). Each primer and/or probe was homology searched by an NCBI BLAST search to ensure that it was specific for the target mRNA transcript. The primers and TaqMan probes were synthesized by Sawady Technology Co., Ltd. (Tokyo, Japan). The TaqMan probes contained 6–carboxyfluoresencin (FAM) at the 5′ end and 6–carboxytetramethylrhodamine (TAMRA) at the 3′ end and were designed to hybridize to a sequence located between the PCR primers.

5. TaqMan RT–PCR Conditions Human total RNA was diluted with yeast tRNA at 50 μg/ml. The RT–PCR assay was performed in 50 μl of TaqMan One-Step RT–PCR Master Mix Reagents containing 300 nM forward primer, 900 nM reverse primer, 200 nM TaqMan probe, and 6.4 to 100,000 pg of total RNA. Amplification and detection were performed using the ABI PRISM 7700 Sequence Detector system (Applied Biosystems) with the following profile: 1 cycle of 48℃ for 30 min, 1 cycle of 95℃ of 10 min, and 50 cycles each of 95℃ for 15 s and 60℃ for 1 min.

Table 1. Primers and Probes Used for RT-PCR Analysis

mRNA	Sequence	Position
Phase I enzymes		
CYPs		
CYP1A1 (GenBank accession number NM_000499)		
Forward primer	GTCATCTGTGCCATTTGCTTTG	589–610
Reverse primer	CAACCACCTCCCCGAAATTATT	685–664
Probe	CGCTATGACCACAACCACCAAGAACT	616–641
CYP1A2 (GenBank accession number AF182274)		
Forward primer	TGTTCAAGCACAGCAAGAAGG	860–880
Reverse primer	TGCTCCAAAGACGTCATTGAC	951–931
Probe	CTAGAGCCAGCGGCAACCTCATCCCA	884–909
CYP1B1 (GenBank accession number NM_000104)		
Forward primer	ACCGTTTTCCGCGAATTC	766–783
Reverse primer	GTACGTTCTCCAAATCCAGCC	961–941
Probe	AGCAGCTCAACCGCAACTTCAGCAACTT	785–812
CYP2A6 (GenBank accession number AF182275)		
Forward primer	TTTTGGTGGCCTTGCTGGT	20–48
Reverse primer	GGAGTTGTACATCTGCTCTGTGTTCA	171–146
Probe	TGCCTGACTGTGATGGTCTTGATGTCTGTT	40–69
CYP2B6 (GenBank accession number AF182277)		
Forward primer	CCCCAAGGACACAGAAGTATTTC	1146–1168
Reverse primer	GATTGAAGGCGTCTGGTTTTTC	1228–1207
Probe	TGAGCACTGCTCTCCATGACCCACACTA	1175–1202
CYP2C8 (GenBank accession number NM_000770)		
Forward primer	GGACTTTATCGATTGCTTCCTG	783–804
Reverse primer	CCATATCTCAGAGTGGTGCTTG	926–905
Probe	TTGGCACTGTAGCTGATCTATTTGTTGCTGGA	863–894
CYP2C9 (GenBank accession number M61857)		
Forward primer	GACATGAACAACCCTCAGGACTTT	766–789
Reverse primer	TGCTTGTCGTCTCTGTCCCA	910–891
Probe	AAAACACTGCAGTTGACTTGTTTGGAGC	863–890
CYP2C18 (GenBank accession number M61856)		
Forward primer	AGGATATTGACATCACCCCCA	1394–1414
Reverse primer	TCAGACAGGAATGAAGCAGAGCT	1473–1451
Probe	AATGCATTTGGTCGTGTGCCACCCT	1420–1444
CYP2C19 (GenBank accession number NM_000769)		
Forward primer	GAACACCAAGAATCGATGGACA	748–769
Reverse primer	TCAGCAGGAGAAGGAGAGCATA	943–922
Probe	TAATCACTGCAGCTGACTTACTTGGAGCTGGG	863–894
CYP2D6 (GenBank accession number NM_000106)		
Forward primer	CCTACGCTTCCAAAAGGCTTT	720–740
Reverse primer	AGAGAACAGGTCAGCCACCACT	912–891
Probe	CAGCTGGATGAGCTGCTAACTGAGCACA	748–775
CYP2E1 (GenBank accession number AF182276)		
Forward primer	TTCAGCGGTTCATCACCCT	1070–1088
Reverse primer	GAGGTATCCTCTGAAAATGGTGTC	1146–1123
Probe	TCCAACCTGCCCCATGAAGCAA	1096–1117
CYP2J2 (GenBank accession number NM_000775)		
Forward primer	AGCTTAGAGGAACGCATTCAGGA	460–482
Reverse primer	CGAAGGTGATGGAGCAAATGAT	592–571
Probe	AGGCCCAACACCTCACTGAAGCAA	485–508

Table 1. continued.

mRNA	Sequence	Position
CYP3A4 (GenBank accession number AF182273)		
Forward primer	GATTGACTCTCAGAATTCAAAAGAAACTGA	825–854
Reverse primer	GGTGAGTGGCCAGTTCATACATAATG	973–948
Probe	AGGAGAGAACACTGCTCGTGGTTTCACAG	946–918
CYP3A5 (GenBank accession number NM_000777)		
Forward primer	CCTTACCCCAGTTTTTGAAGCA	684–705
Reverse primer	TCCAGATCAGACAGAGCTTTGTG	881–859
Probe	TTTCTTTCGAATTCTGGGAGTCAATCATC	850–822
CYP4A11 (GenBank accession number NM_000778)		
Forward primer	AGGAGCTACAACGGATTCAGAA	209–230
Reverse primer	ACGAACTTTGCCTCCCCATAG	288–268
Probe	ACATTCCCAAGTGCCTGTCCTCATTG	241–266
CYP27 (GenBank accession number M62401)		
Forward primer	AGAGGAGATTCCACGTCTAGGAC	171–193
Reverse primer	ACATCCACATTGGACCGTACTT	292–271
Probe	TGCGCTTCTTCTTTCAGCTGTTCGTTCA	197–224
Epoxide hydrolase		
EPHX1 (GenBank accession number NM_000120)		
Forward primer	TCGATAAGTTCCGTTTCACCC	200–220
Reverse primer	AATTCATTCCGCCAGTAGGAGA	302–281
Probe	ACCTTTGGAGGACAGCTGCTTCCACTA	222–248
EPHX2 (GenBank accession number NM_001979)		
Forward primer	GCCACTACCCGGCTTATGAAA	145–165
Reverse primer	TTTAGCGGTCTCGGAGCACTT	237–217
Probe	CACACTTTCCCAGTGGATACCACTCATGGA	174–203
Quinone oxidoreductase		
PIG3 (GenBank accession number NM_004881)		
Forward primer	GGCCCCTGTTTTCAAAGCTAC	743–763
Reverse primer	CAGAATTTGCTCCGTGAAAGC	858–838
Probe	TTAAGCGAGGAAGTCTGATCACCAGTTTGC	767–796
NMOR2 (GenBank accession number NM_000904)		
Forward primer	TCTATGCACACCAGGAACCCA	26–46
Reverse primer	CCCTGCCTGCTCAGTTCATCTA	101–80
Probe	CTTTCAACGGATCCTTGAAGAATGTGGC	50–77
Monoamine oxidase		
MAOA (GenBank accession number NM_000240)		
Forward primer	CCTTGACTGCCAAGATTCACTTC	827–849
Reverse primer	TGCACTTAATGACAGCTCCCAT	919–898
Probe	CCAGAGCTTCCAGCAGAGAGAAACCAGTT	853–881
MAOB (GenBank accession number NM_000898)		
Forward primer	CTTTTTGGAGAGACATTTGCCC	1440–1461
Reverse primer	TCACAAGTAGCCCCCTTTTGT	1555–1535
Probe	ATTGACCACCATCTTTTCAGCAACGGCTC	1491–1519
Prostaglandin endoperoxide synthase		
PTGS1 (GenBank accession number M59979)		
Forward primer	CTGCAGCTGAAATTTGACCCA	1066–1086
Reverse primer	ACCTTGAAGGAGTCAGGCATG	1187–1167
Probe	AGCTGCTGTTCGGTGTCCAGTTCCAATA	1088–1115
PTGS2 (GenBank accession number NM_000963)		
Forward primer	AGTCCCTGAGCATCTACGGTTT	792–813

Table 1. continued.

mRNA	Sequence	Position
Reverse primer	CCCATTCAGGATGCTCCTGTT	928–908
Probe	TCTTTGGTCTGGTGCCTGGTCTGATG	830–855
Dihydopyrimidine dehydrogenase		
DPYD (GenBank accession number NM_000110)		
Forward primer	CTTTGAGAGCTGTGACCTCCA	2321–2341
Reverse primer	TCAGCAGAGTCAATTCCACCA	2399–2379
Probe	CTCGTGCTCTGCCTGGATTTCCCATT	2345–2370
Aldehyde oxidase		
AOX1 (GenBank accession number NM_001159)		
Forward primer	AAGGCCAGCCCTTCGAATACT	3464–3484
Reverse primer	TATACTGCAGCCAACATCCATG	3585–3564
Probe	CCTGTTCCGAGGTTGAAATAGACTGCCT	3500–3527
Xanthene dehydrogenase		
XDH (GenBank accession number NM_000379)		
Forward primer	ACCGCTTCCACTACTTCAGCTAT	3446–3468
Reverse primer	TTAGACTGGAGCCAACATCCATG	3562–3540
Probe	AACAGGAGATCATAAGAACCTCCGCACAGA	3504–3533
Esterase		
CES1 (GenBank accession number NM_001266)		
Forward primer	CCAGAGAGAGTCAACCCCTTCT	935–956
Reverse primer	TCCTGCTTGTTAATTCCGACC	1061–1041
Probe	ATGCTGCTGCTGAAAACACCTGAAGAGCTT	976–1005
CES2 (GenBank accession number NM_003869)		
Forward primer	ACCGCAGTGGAGTCAGAGTTTC	298–319
Reverse primer	ATGCTGAGGTACAGGCAGTCCT	383–362
Probe	TTCAACATGACCTTCCCTTCCGACTCC	328–354
AADAC (GenBank accession number NM_001086)		
Forward primer	GCCTCTCCCAGATAACGTTGA	63–83
Reverse primer	TCAAAGCTCCCGACAACCTTAA	206–185
Probe	AGCCATGGAGAATGATGTGGATAAACGC	86–113
LIPA (GenBank accession number NM_000235)		
Forward primer	AAAGGTCCCAAACCAGTTGTCTT	226–248
Reverse primer	CACACGTCAAAACCAGCATCA	347–327
Probe	CAACATGGCTTGCTGGCAGATTCTAGTAAC	253–282
NTE (GenBank accession number NM_006702)		
Forward primer	GGCAACGTCATTGAGAAAATGC	3634–3655
Reverse primer	AATCTCTGCCAAGTCAGTGAAGC	3747–3725
Probe	TCTACAGACCTTAATGAGAGCCGCCGTG	3670–3697
UCHL1 (GenBank accession number NM_004181)		
Forward primer	CCTGTGGCACAATCGGACTTA	233–253
Reverse primer	AACTGATCCATCCTCAAATCCC	306–285
Probe	TCACGCAGTGGCCAATAATCAAGACA	255–280
UCHL3 (GenBank accession number NM_006002)		
Forward primer	CATCCTAACTGGCAATTCGTTG	76–97
Reverse primer	AGAAGTAAGACTGCACAGACTGGTC	164–140
Probe	TGGAATGGATCCTGAACTCCTTAGCATGGT	105–134
ESD (GenBank accession number M13450)		
Forward primer	CTTCCCCAACTCATAAATGCC	427–447
Reverse primer	ACAGATCAGAGCTCCATGACCTC	519–497
Probe	TTTCCAGTGGATCCCCAAAGGATGTC	451–476

Table 1. continued.

mRNA	Sequence	Position
Flavin-containing monooxygenase		
FMO3 (GenBank accession number NM_006894)		
Forward primer	GTCACTCGATTCGGAACCTTC	706–726
Reverse primer	GCTCTTTCCTCAGGACTCCATT	844–823
Probe	CAATTTACCGACAGCCATCTCTGACTGGT	735–763
FMO4 (GenBank accession number NM_002022)		
Forward primer	TACATGGATGATATCGCTGCC	1297–1317
Reverse primer	CATGAGGCGGTACTGATAAGGA	1422–1401
Probe	CACAAAGCCCAGCATCCCACTTCTGT	1326–1351
FMO5 (GenBank accession number NM_001461)		
Forward primer	AAGGTCTTCCCTCCTAACCTGG	1063–1084
Reverse primer	AGCGTCCTTGGAGCTCTGAAAT	1162–1141
Probe	TTGATTCAGCCCTTAGGAGCCATTATGC	1111–1138
Alcohol dehydrogenase		
ADH1 (GenBank accession number NM_000667)		
Forward primer	TGATAAAGTCATCCCACTCGC	261–281
Reverse primer	CCCTGAGGATTGCTTACATCG	365–345
Probe	AAATGCAGAATTTGTAAAAACCCGGAGAGCAACTAC	298–333
ADH2 (GenBank accession number NM_000668)		
Forward primer	CATTCACCACTTCCTTGGCAC	411–431
Reverse primer	ATGAGGCAGACTTTCTCCAGTTT	518–496
Probe	CTTCTCCCAGTACACGGTGGTGGATGAGAA	438–467
ADH3 (GenBank accession number NM_000669)		
Forward primer	TGAGGAGGTAGAGGTTGCACCT	72–93
Reverse primer	CCACTAACCACATGCTCATCTGAA	167–144
Probe	TCATGAAGTTCGCATTAAGATGGTGGCTGC	102–131
ADH4 (GenBank accession number NM_000670)		
Forward primer	TGCTGGTAGCAAAGGATTGACT	903–924
Reverse primer	CCAACCACCAAAGAATGTTCC	984–964
Probe	TTTTCCAGAGGAGCTAATAATCGGCCGTAC	927–956
ADH5 (GenBank accession number NM_000671)		
Forward primer	CCTTCTAGGTTGTGGCATTTCA	564–585
Reverse primer	TAACTGCCAATCCGACTCCTC	676–656
Probe	AAGTTGGAGCCTGGCTCTGTTTGTGC	616–641
ADH6 (GenBank accession number NM_000672)		
Forward primer	CCTGCCAGTGTTCAACTCA	889–910
Reverse primer	GATGTGCTGTCTGCTCTTCCA	987–967
Probe	CAGTGGCCAGTTGTTCTTCTCAGGACGT	912–939
ADH7 (GenBank accession number NM_000673)		
Forward primer	TCAGTCCCAAGGACTCTACCAA	722–743
Reverse primer	CTTCAAAGGTGTATCCCACGTT	802–781
Probe	CCCATCAGTGAGGTGCTGTCAGAAATGA	745–772
HADH2 (GenBank accession number NM_004493)		
Forward primer	ATCAACACTGCCAGTGTGGCT	451–471
Reverse primer	AGTGTCATGCCCACTATTCCC	539–519
Probe	TCAGGTTGGACAAGCTGCATACTCTGC	483–509
HEP27 (GenBank accession number NM_005794)		
Forward primer	CTGGTCTCTTCCATTGCAGCTT	505–526
Reverse primer	CAGTTTACCCGGATGTCCTTG	635–615
Probe	CCAGCTCCAATGCCAGTGTTCTAGTGA	610–584

Table 1. continued.

mRNA	Sequence	Position
Aldehyde dehydrogenase		
ALDH1 (GenBank accession number AF003341)		
Forward primer	TGAGTGATTTAGCAGGCTGCA	359–379
Reverse primer	TGGCCACATACACCAATAGGTTC	494–472
Probe	CAAAACATTGCGCTACTGTGCAGGTTG	381–407
ALDH2 (GenBank accession number NM_000690)		
Forward primer	TGGTTACTTCATCCAGCCCAC	1179–1199
Reverse primer	GCTCTCCCAACAACCTCCTCTAT	1307–1285
Probe	TTTGGAGATGTGCAGGATGGCATGA	1204–1228
ALDH3 (GenBank accession number NM_000691)		
Forward primer	AGCTGAGTGAGAACATGGCGA	422–442
Reverse primer	ATGGTCGAACCTCTCCTTGAGC	549–528
Probe	TCCCCCAGTACCTGGACAAGGATCTGTA	461–488
ALDH4 (GenBank accession number NM_003748)		
Forward primer	CAACATCATCCAGTTTGTGCC	768–788
Reverse primer	CGAAGTGGAAGTTCTTTCCGC	967–947
Probe	AGCTCAGAGCACCTCTGTGGCATCAA	823–848
ALDH5 (GenBank accession number NM_000692)		
Forward primer	GGTTTCTTCATCAAGCCTACTGTC	1183–1206
Reverse primer	CAGGCCCAAAGATCTCCTCTT	1261–1241
Probe	TGGCGTGCAGGATGACATGAGAATT	1212–1236
ALDH6 (GenBank accession number NM_000693)		
Forward primer	GAACGGTCTGGATCAACTGCTAC	1382–1404
Reverse primer	AGTTCTCTGCCATTTCCTGACA	1466–1445
Probe	CTATGCACAGGCTCCATTTGGTGGCTT	1413–1439
ALDH9 (GenBank accession number NM_000696)		
Forward primer	AGCATGGAACTACCCCTTTCA	459–479
Reverse primer	AAAGACCATGGCATTACCACAG	534–513
Probe	ATTGCCTCTTGGAAGTCGGCTCCA	481–504
ALDH10 (GenBank accession number NM_000382)		
Forward primer	GCAGCGATTTGACCACATTTTC	525–546
Reverse primer	TAACATGGACTTTTCCCTCCCA	644–623
Probe	CGGTTGGCAAAATTGTCATGGAAGCT	563–588
Phase II enzymes		
Methyltransferase		
TPMT (GenBank accession number NM_000367)		
Forward primer	TTGCGGAAAAGCGGTTGA	207–224
Reverse primer	CACTGATTTCCACACCAACTACAC	277–254
Probe	TGTCCCCGGTCTGCAAACCATTTCAT	251–226
HNMT (GenBank accession number NM_006895)		
Forward primer	AGGCTCAATACCCAGGAGTTTGT	224–246
Reverse primer	TTTACGTTCTCGAGGTTCGATGTC	335–312
Probe	TTGAGCCAAGTGCTGAACAAATTGCC	263–288
COMT (GenBank accession number M58525)		
Forward primer	TTCTCTTGGAGGAATGTGGCC	605–625
Reverse primer	AGGAACGATTGGTAGTGTGTGCA	743–721
Probe	ACAGTGCTACTGGCTGACAACGTGATCTGC	640–669
ASMT (GenBank accession number NM_004043)		
Forward primer	CTGGAAACCATCATTCTCAGCA	577–598

Table 1. continued.

mRNA	Sequence	Position
Reverse primer	AGCCCCACCTATGAGTGAGAAC	654–633
Probe	CGCAAGGACAGAAAACCAAACACCGC	605–630
GAMT (GenBank accession number NM_000156)		
Forward primer	ATCCTGTACGACACGTACCCACT	394–416
Reverse primer	ACTTGGACTTCATCAGCTCCC	544–524
Probe	ACACCAGTTCAACTTCATCAAGAACCACGC	438–467
NNMT (GenBank accession number NM_006169)		
Forward primer	AGACCTGCTGATTGACATCGG	168–188
Reverse primer	GTCAGTGACGACGATCTCCTTAA	255–233
Probe	TCTGGCCCCACTATCTATCAGCTCCTCTCT	190–219
PEMT (GenBank accession number NM_007169)		
Forward primer	ATCCTGGACAACCCCATGTACT	409–430
Reverse primer	CTTTCTGCCGGTAGATCTCAGC	574–553
Probe	TGGTGGCCCTCACCTACATAATGGCTCT	503–530
PNMT (GenBank accession number NM_002686)		
Forward primer	TGCAGCCACTTTGAGGACATC	271–291
Reverse primer	GGCATGTTGGCTGTACATGCT	390–370
Probe	ATGACAGATTTCCTGGAGGTCAACCGC	295–321
Acetyltransferase		
NAT1 (GenBank accession number NM_000662)		
Forward primer	TCAGCCTCAGGTGCCTTGT	426–444
Reverse primer	AGATTTTTCGGTATTTGCTGTCTTCT	568–543
Probe	TCTTCCGTTTGACGGAAGAGAATGGATT	446–473
NAT2 (GenBank accession number NM_000015)		
Forward primer	ACGTCTCCAACATCTTCATTTATAACC	631–657
Reverse primer	CCTCAGTGAGAGTTTTAAACTCGACC	781–756
Probe	CATCATTTTGTTCCTTGCAGACCCCA	659–684
ARD1 (GenBank accession number AF085355)		
Forward primer	TCTACCTACAATACCTCGCCCAC	92–114
Reverse primer	ACTGAGCCTTCTGCTTTACCCA	194–173
Probe	ATTGTTGCAGAGGCACCTGGTGGAGA	130–155
Rhodanese		
TST (GenBank accession number NM_003312)		
Forward primer	ACATCCCAGGTACCGTGAACA	608–628
Reverse primer	TGGCTTAGACAGGTCCACTTTCT	726–704
Probe	TGAGGAGATCCGCCATCTGTTCCA	675–698
Acyl–CoA: amino acid N–acyltransferase		
BAAT (GenBank accession number NM_001701)		
Forward primer	AGCCAGTGCATATCCGAGCT	44–63
Reverse primer	TTCATCTTCCAGTGATGCCTGA	117–96
Probe	AGGCCTGATTCCCTTTCAGATGGTGAGT	66–93
Glutathione S–transferase		
GSTP1 (GenBank accession number NM_000852)		
Forward primer	CTGGTGGACATGGTGAATGAC	265–285
Reverse primer	CGCCTCATAGTTGGTGTAGATGA	342–320
Probe	AGGACCTCCGCTGCAAATACATCTCC	293–318
GSTT1 (GenBank accession number NM_000853)		
Forward primer	AGAGTTGGATGTGACCCTGCA	414–434
Reverse primer	TCAGCTAAGGAGATGTGAGGACC	500–478
Probe	TTGCTCGAGGACAAGTTCCTCCAGAA	436–461

Table 1. continued.

mRNA	Sequence	Position
GSTM3 (GenBank accession number NM_000849)		
Forward primer	CGAGTGGACATCATAGAGAACCA	298–320
Reverse primer	AGCTCTTCCAAGTACTGAGGCTT	404–382
Probe	TAATGGATTTCCGCACACAACTGATAAGGCT	323–353
GSTM4 (GenBank accession number NM_000850)		
Forward primer	TTGGAGAACCAGGCTATGGAC	298–318
Reverse primer	TTCCCCAGGAACTGTGAGAAGT	431–410
Probe	TCCAATCAGCTGGCCAGAGTCTGCTACAGCCCT	322–354
GSTA2 (GenBank accession number M16594)		
Forward primer	CAGACCAGAGCCATTCTCAACT	199–220
Reverse primer	AAGGCTAGAGTCAAGCTCTTCCA	522–500
Probe	TACTCAACCTGAGGAACAAGATGCCAAGC	333–361
GSTA4 (GenBank accession number NM_001512)		
Forward primer	AGTTGGTACAGACCCGAAGCA	191–211
Reverse primer	AGTTCCAGCAGATCCAGTGTCC	314–293
Probe	TGGCAAGAACCTCAAGGAGAGAACCCTGA	246–274
MGST1 (GenBank accession number U46498)		
Forward primer	TATTCCTTGAGTGGTCCCGAC	unknown
Reverse primer	AATGGTGTGGTAGATCCGTGCT	unknown
Probe	ACAGCCATCCTGCACTTCAGACTATTTGTC	unknown
MGST2 (GenBank accession number NM_002413)		
Forward primer	CTGCTGGCTGCTGTCTCTATTC	19–40
Reverse primer	TTGTTGTGCCCGAAATACTCTC	162–141
Probe	CTCGGCCTGTCAGCAAAGTTATTTTGC	42–68
MGST3 (GenBank accession number NM_004528)		
Forward primer	TACAGCACGGACCCTGAAAAT	130–150
Reverse primer	ACTTCCAACGTGTTCTGGTGG	200–180
Probe	CACATCTTCAACTGCATTCAGCGAGC	154–179
GSTZ1 (GenBank accession number NM_001513)		
Forward primer	TCCTATTTCCGAAGCTCCTGC	28–48
Reverse primer	TTCAGTGCCTGGAAGTCCTTAG	161–140
Probe	CATGGAGAGTTCGAATTGCTCTGGCC	50–75
Sulfotransferase		
CHST1 (GenBank accession number NM_003654)		
Forward primer	AGGACTTCTCCAACTCCGTGTC	830–851
Reverse primer	CCGTAGATCTCCTCGGTCTTCTT	953–931
Probe	CAAGTACATGTTGGTGCGCTACGAGGA	885–911
CHST3 (GenBank accession number NM_004273)		
Forward primer	ACAAGCTGAAGCAGATTCCCC	149–169
Reverse primer	GAGAGATGCGTTCTCAGCTAAGATC	231–207
Probe	TCTAGCAGATGCCAACAGCACCGA	174–197
CHST4 (GenBank accession number NM_005769)		
Forward primer	CCCTTAATGTCTCCCAGGCTTG	980–1001
Reverse primer	CGGTAGCCCAGCAAATTCATG	1079–1059
Probe	CGCTGGTCTTTGCCCTATGAAAAGGTTTCT	1003–1032
CST (GenBank accession number NM_004861)		
Forward primer	AATGCCCACTACCTCCGAAAC	628–648
Reverse primer	AAGTAGAGCACGTCCTCCAGCT	827–806
Probe	TCCACCTGGTGCTCCTTCAAGAGTACTTC	737–765
SULT2A1 (GenBank accession number NM_003167)		

Table 1. continued.

mRNA	Sequence	Position
Forward primer	CCACGTTTATTCTCCTCCCAC	277–297
Reverse primer	AGCACAGTTCCTTGACAAAACC	476–455
Probe	TCCCCATCCAGTTATTCCCCAAGTCTT	299–325
TPST2 (GenBank accession number NM_003595)		
Forward primer	TTTGACCTCAGCAGCTACCGT	604–624
Reverse primer	AGGAAGTCGAGGATGAGCTTGA	767–746
Probe	ATCGAGGTGATGTACGCCCAGTGCAT	652–677
UDP-glucuronosyltransferase		
UGT1 (GenBank accession number NM_001072)		
Forward primer	CCTGGAGCATACATTCAGCAGAA	531–553
Reverse primer	AAGGAAGTTGGCCACTCGTTG	639–619
Probe	ACCCTGTGTCCTACATTCCCAGGTGCTA	560–587
UGT1A9 (GenBank accession number AF056188)		
Forward primer	CATGCCAGAGGTGAGTTGG	174–192
Reverse primer	CAAAAATGTCATTGTATGAACCCATT	352–327
Probe	TTCAACTTCATATACCCTGGAGGATCTGGA	231–260
UGT2B10 (GenBank accession number NM_001075)		
Forward primer	AAATGGACTACAGTTCTGCTGATACAA	10–36
Reverse primer	TCCAAAGGCTGTATTCTGCG	106–87
Probe	CATACCAGCACCTTTCCACAACTCCCA	83–57
UGT2B11 (GenBank accession number NM_001073)		
Forward primer	CTTCAGTTCTTCTGCTGATACATCTCA	17–43
Reverse primer	CGAATGTCTGACCATCTCTTAACC	302–279
Probe	TGATGCATCCACTCTTAAATTTGAAGTTTATCCT	201–234
UGT2B17 (GenBank accession number NM_001077)		
Forward primer	CTCAGTTGTTACTTTAGCTCTGGGAGT	40–66
Reverse primer	ACTGGCATTGACAAGAATAGAAGCC	201–177
Probe	AAAGGTGCTGGTGTGGCCCACAGAAT	72–97
Transporters		
ABC transporters		
ABCA1 (GenBank accession number NM_005502)		
Forward primer	AACAGCAGTTGGATGGCTTAGA	1169–1190
Reverse primer	CACAGAACCATTACTGGACTGGA	1263–1241
Probe	AAGACATCGTGGCGTTTTTGGCC	1202–1224
ABCA2 (GenBank accession number U18236)		
Forward primer	AACAAGCGGAAGCTCTCCA	unknown
Reverse primer	GTGTGATGTCAGCACCACTGA	unknown
Probe	TACCCAGCCTTCATCTTCCTGGACGA	unknown
ABCA4 (GenBank accession number NM_000350)		
Forward primer	GGTCAAGAGATTCCAACACACCA	4077–4099
Reverse primer	GGTATTCGCCAAAAGGAAGGA	4201–4181
Probe	TGCTCCCGGCTACCTTTGTGTTTTTGG	4133–4159
ABCA8 (GenBank accession number NM_007168)		
Forward primer	GCTTGCTTAGTCCCTTTGCCTT	1085–1106
Reverse primer	AAATCGCCAATGCCAGATAGAG	1252–1231
Probe	TTCCTCATCCATCGGACGGCTCAA	1163–1186
ABCB1 (GenBank accession number NM_000927)		
Forward primer	AGGAAGACATGACCAGGTATGC	323–344
Reverse primer	CCAACATCGTGCACATCAAAC	506–486

Table 1. continued.

mRNA	Sequence	Position
Probe	CCTGGCAGCTGGAAGACAAATACACAA	411–437
ABCB2 (GenBank accession number NM_000593)		
Forward primer	CGCCTCACTGACTGGATTCTAC	628–649
Reverse primer	TTGTTATAGATCCCGTCACCCA	752–731
Probe	ATGGCTCAGCCGATACCTTCACTCGAAA	653–680
ABCB3 (GenBank accession number NM_000544)		
Forward primer	CTGGTCGTGTGATTGACATCCT	518–539
Reverse primer	GCAAGTTGATTCGAGACATGGT	670–649
Probe	AGGTGATTTTGACCCCCATGCCTTT	543–567
ABCB4 (GenBank accession number NM_000443)		
Forward primer	GACTGCGGTCAATGGCTTTTA	2357–2377
Reverse primer	GCAAGTCTTGTAGAAAGTGCACC	2453–2431
Probe	TAAGACAGGACATGAGCTGGTTTGATGACC	2387–2416
ABCB6 (GenBank accession number AJ289233)		
Forward primer	CATTGTGTTCCTGTGCATGAGT	1245–1266
Reverse primer	TCTCGGCGTTGTAATACTTCACC	1408–1386
Probe	TTACCTCACCCTGACCATTGTGGTCACT	1269–1296
ABCB7 (GenBank accession number NM_004299)		
Forward primer	CGCTTCTATGAGCCTCAAAAGG	1558–1579
Reverse primer	CCAGCTAATTTTGCCACTGCAT	1757–1736
Probe	AAGATGTGAGCCTGGAAAGCCTTCGGA	1610–1636
ABCB8 (GenBank accession number NM_007188)		
Forward primer	CGTGAATGTACAGATCCCCCT	429–449
Reverse primer	CCTGGACACCATAGAGGATAAGC	565–543
Probe	ATGACTGAGTCCCAGAATCTCAGCACCCAC	511–540
ABCB9 (GenBank accession number AF216833)		
Forward primer	ATATGCCAGCAAAGTGGACCAT	1128–1149
Reverse primer	AGCCCAGTTGCTCCAAAGAAAC	1211–1190
Probe	AATGCAGTTAGCAAGGAAAGAGGCAGTCGC	1152–1181
ABCB11 (GenBank accession number NM_003742)		
Forward primer	CATAGTCCAAGCTGCCAAGGAG	1581–1602
Reverse primer	CTGGCGATAGCTACCCTTTGTT	1712–1691
Probe	CTACAACTTCATCATGGACCTGCCACAGC	1611–1639
ABCC1 (GenBank accession number NM_004996)		
Forward primer	ACTCATTCAGCTCGTCTTGTCC	546–567
Reverse primer	TCAACCCTGTGATCCACCAGA	682–662
Probe	AGATCGCTCACCCCTGTTCTCGGAAA	576–601
ABCC2 (GenBank accession number NM_000392)		
Forward primer	ACGGACAGCTATCATGGCTTCT	1176–1197
Reverse primer	TGGTCACATCCATGAGCTTCT	1306–1286
Probe	ACCCTATCCAACTTGGCCAGGAAGGAGT	1216–1243
ABCC3 (GenBank accession number NM_003786)		
Forward primer	CTTAAGACTTCCCCTCAACATGC	1731–1753
Reverse primer	GGTCAAGTTCCTCTTGGCTCA	1837–1817
Probe	TGTGTCTCTGAAACGGATCCAGCAATTC	1788–1815
ABCC5 (GenBank accession number NM_005688)		
Forward primer	TCACGCAGTCACCTTTCCTCTC	3134–3155
Reverse primer	CTTGGTTGTCATCCAGCAGCT	3256–3236
Probe	CCTTGCCACCATCCACGCCTACAATA	3180–3205
ABCC6 (GenBank accession number NM_001171)		

Table 1. continued.

mRNA	Sequence	Position
Forward primer	TTGGATTCGCCCTCATAGTCC	224–244
Reverse primer	CAGTAACCAAACAGCACTCCAGA	419–397
Probe	CCCAGAATTCCTCATTCATCCTACTGTGTGGC	303–334
ABCC9 (GenBank accession number NM_005691)		
Forward primer	GGTGGTCAGATTTGCAGTCAA	1770–1790
Reverse primer	ACAGGACTCAAAAGGAAGCGAA	1893–1872
Probe	ATGAGATTGGTGACGACAGTTGGCGAAC	1835–1862
ABCD1 (GenBank accession number NM_000033)		
Forward primer	ACTGCCACTGGCTACTCAGAGT	1057–1078
Reverse primer	TAGTGAAGGCTTCTGTGCGCT	1159–1139
Probe	CCGTGAAGAAGGCAGCCTTGGAAAA	1091–1115
ABCD3 (GenBank accession number NM_002858)		
Forward primer	GGAGATGTTTTGATCCGAGACC	1354–1375
Reverse primer	CCAAGAACACGGAAAAGTGAAC	1460–1439
Probe	ATTTGTGGTCCAAATGGCTGCGGAAA	1411–1436
ABCE1 (GenBank accession number NM_002940)		
Forward primer	CCACACATCGATATTGTGCCAA	248–269
Reverse primer	TCAGGAGGATCATCGTACTTTCC	416–394
Probe	TGCCTATCCCTCGTCCAGGTGAAGTT	290–315
ABCF1 (GenBank accession number AF027302)		
Forward primer	ACACTCCTCAAGCACATTGCC	916–936
Reverse primer	TGTCTCATCTGCTACCACCTCCT	1008–986
Probe	CCCTGAGCATCCCTCCCAACATTGAT	944–969
ABCF2 (GenBank accession number NM_005692)		
Forward primer	CAGCAGGTTGCACAGGAAATTT	18–39
Reverse primer	TTGGACTTGAGGTGCTCCTTGTA	112–90
Probe	TCTGTGAGAAGCAGACAATCACCAAGTGGC	43–72
ABCG1 (GenBank accession number NM_004915)		
Forward primer	CCTACTACCTGGCCAAGACCAT	1478–1499
Reverse primer	AGTACACGATGCTGCAGTAGGC	1555–1534
Probe	ACGTGCCCTTTCAGATCATGTTCCCAGT	1505–1532
ABCG2 (GenBank accession number NM_004827)		
Forward primer	GCACTCTGACGGTGAGAGAAAA	395–416
Reverse primer	CCAGACACACCACGGATAAACT	563–542
Probe	TCTCAGCAGCTCTTCGGCTTGCAA	425–448
Organic cation transporter		
SLC22A1 (GenBank accession number NM_003057)		
Forward primer	CTTCCATCGTCACTGAGTTCAAC	395–417
Reverse primer	ACAAGAAGCCCGCATTCAAAC	481–461
Probe	TGCTGACTCCTGGAAGCTGGACCTCTTT	426–453
SLC22A3 (GenBank accession number AF078749)		
Forward primer	GATGCTTTTGCCTGAAACCA	unknown
Reverse primer	ACAGGAATGTGGACTGCCAAGT	unknown
Probe	TATTGCCTTGCCAGAGACAGTGGATGATGT	unknown
SLC22A5 (GenBank accession number NM_003060)		
Forward primer	CTTCCTTTCAGCGATGGTTGA	1128–1148
Reverse primer	GGTACCAGCTGCATGAAGAGA	1262–1242
Probe	TGGCCTGGCTGCTGCTGCAATATTT	1166–1190
Organic anion transporter		
OATP2 (GenBank accession number AF205071)		

Table 1. continued.

mRNA	Sequence	Position
Forward primer	TGGAATAACTTACATCTCACCCTGT	1431–1455
Reverse primer	TTCTGGAGACCAGTTACTTCCAA	1541–1519
Probe	AGCAGGTTGCAAATCTTCAAGTGGCAATA	1458–1486

All sequences are shown from the 5′ to 3′ end.

6. Statistical Analysis Samples were deemed positive at any given cycle when the value of the emitted fluorescence was greater than the threshold value calculated by the instrument's software (Sequence Detector Ver. 1.6.3). The threshold cycle (Ct), which is defined as the cycle at which PCR amplification reaches a significant value (i.e., usually 15 times the standard deviation of the baseline), is given as a mean value. The source of total RNA for the measurement of calibration data was the human adult liver.

The data are expressed as the ratio of target mRNA to GAPDH mRNA, and the data are then shown as a percentage relative to control at each time point. Experiments involving human hepatocyte cultures were performed in triplicate, and results for the ratio of target mRNA to GAPDH mRNA and percentage relative to control at each time point are shown as mean ±SD of three runs.

RESULTS

1. Detection of mRNAs Using Quantitative, High-Throughput, Real-Time RT–PCR Pairs of primers and TaqMan probes specific to each phase I enzyme, phase II enzyme, and transporter were generated (Table 1). The sequence of each was obtained from GenBank, and GenBank accession numbers are shown in the table. The positions of the primers and probes were counted from the position of the initiation codon, except for MGST1, ABCA2, and SLC22A3, for which the position of the initiation codon is not known. Analysis was performed in the presence of the TaqMan probes by real-time fluorescence monitoring on an ABI PRISM 7700 Sequence Detector. Table 2 shows the calibration data and limit of quantification for each mRNA in the total RNA studied. The source of total RNA was the human adult liver pool. The lower limit of quantification of each of the mRNAs ranged from 6.4 to 4000 pg of total RNA per 50 μl of reaction mixture.

2. Effect of Inducers on mRNA Expression In the present study, the levels of gene expression for phase I enzymes, phase II enzymes, and transporters were evaluated in cultures of human hepatocytes after 48–h exposure to known typical enzyme inducers such as rifampicin and omeprazole. All experiments were performed in the presence of 0.1% DMSO. Expression data are shown in Table 3. None of the treatments showed any identifiable toxic effects such as cell death or loss of adhesion of hepatocytes at the study concentrations employed, as assessed by microscopic examination. The levels of CYP1A1, CYP2B6, CYP2C8, CYP3A4, CYP3A5, ADH3, and ABCG1 mRNA in human hepatocytes after exposure to rifampicin were two to five times higher than those in controls. The levels of CYP1A1, CYP1A2, CYP1B1, ALDH3, and ALDH6 mRNA after exposure to omeprazole were 2.5 to 224 times higher than those in controls. On the other hand, the levels of ADH3 and ABCB4 mRNA after exposure to omeprazole were 0.27 to 0.5 times lower than those in controls.

The time course and effects of these doses (2, 10, and 50 μM) on expression of the mRNAs (CYP1A1, CYP1A2, CYP1B1, CYP2B6, CYP2C8, CYP3A4, CYP3A5, ADH3, ALDH3, ALDH6, ABCB4, and ABCG1) were evaluated, and mRNA levels were two times higher or lower than in controls after exposure to rifampicin or omeprazole, as shown in Table 3. None of the treatments showed any identifiable toxic effects such as cell death or loss of adhesion of hepatocytes at the study concentrations employed, as assessed by microscopic examination. Figures 1 and 2 show the levels of mRNA expression after rifampicin and omeprazole exposure, respectively. The human hepatocytes were treated with rifampicin or omeprazole for 24 and 48 h. The medium with rifampicin or omeprazole were then replaced with fresh medium, and the hepatocytes were cultured for an additional 24 and 48 h (72 and 96 h, respectively, in Figs. 1 and 2). Exposure to rifampicin increased the levels of CYP1A1, CYP2B6, CYP2C8, CYP3A4, CYP3A5,

Table 2. Calibration Data and limit of quantification for each mRNA in the Total RNA Studied

mRNA	No. of data points	Calibration curve Slope	Intercept	Correlation coefficient r	Limit of quantification (pg total RNA/50 μl reaction mixture)
Phase I enzymes					
CYP1A1	5	−3.42	42.14	1.00	160
CYP1A2	7	−3.35	36.16	1.00	6.4
CYP1B1	4	−3.36	44.65	0.99	800
CYP2A6	7	−3.46	34.56	1.00	6.4
CYP2B6	7	−3.39	37.30	1.00	6.4
CYP2C8	7	−3.14	32.95	1.00	6.4
CYP2C9	7	−3.49	36.57	1.00	6.4
CYP2C18	5	−3.46	38.09	1.00	160
CYP2C19	6	−3.59	42.07	1.00	32
CYP2D6	4	−3.05	39.24	1.00	800
CYP2E1	7	−3.30	31.83	1.00	6.4
CYP2J2	5	−3.36	39.55	1.00	160
CYP3A4	7	−3.50	37.14	1.00	6.4
CYP3A5	4	−3.70	42.74	1.00	800
CYP4A11	7	−3.29	35.04	1.00	6.4
CYP27	6	−3.53	38.42	1.00	32
EPHX1	7	−3.30	34.52	1.00	6.4
EPHX2	7	−3.26	37.97	1.00	6.4
PIG3	5	−3.35	44.88	1.00	160
NMOR2	7	−3.26	37.97	1.00	6.4
MAOA	7	−3.23	37.86	1.00	6.4
MAOB	7	−3.40	38.72	1.00	6.4
PTGS1	3	−3.25	41.58	1.00	4000
PTGS2	4	−3.31	44.81	1.00	800
DPYD	6	−3.38	38.67	1.00	32
AOX1	7	−3.43	36.52	1.00	6.4
XDH	6	−3.28	38.87	1.00	32
CES1	7	−3.39	36.29	1.00	6.4
CES2	7	−3.43	37.52	1.00	6.4
AADAC	6	−3.27	37.61	1.00	32
LIPA	7	−3.31	39.54	1.00	6.4
NTE	7	−3.24	40.41	0.99	6.4
UCHL1	6	−3.33	43.03	1.00	32
UCHL3	7	−3.11	37.83	1.00	6.4
ESD	7	−3.25	37.59	1.00	6.4
FMO3	7	−3.68	41.19	1.00	6.4
FMO4	6	−3.07	39.54	0.99	32
FMO5	6	−3.45	38.40	1.00	32
ADH1	5	−3.57	36.32	1.00	160
ADH2	4	−3.14	43.01	1.00	800
ADH3	7	−3.33	35.57	1.00	6.4
ADH4	7	−3.36	34.27	1.00	6.4
ADH5	7	−3.38	38.07	1.00	6.4
ADH6	7	−3.41	37.81	1.00	6.4
ADH7	3	−3.01	44.19	1.00	4000
HADH2	7	−3.25	38.29	1.00	6.4
HEP27	5	−3.07	41.29	0.99	160
ALDH1	7	−3.19	37.03	1.00	6.4
ALDH2	7	−3.35	34.99	1.00	6.4

Table 2. continued.

mRNA	No. of data points	Calibration curve Slope	Intercept	Correlation coefficient r	Limit of quantification (pg total RNA/50 μl reaction mixture)
ALDH3	4	−4.48	51.97	0.99	800
ALDH4	7	−3.35	40.14	1.00	6.4
ALDH5	7	−3.43	37.04	1.00	6.4
ALDH6	4	−3.48	44.13	0.99	800
ALDH9	7	−3.53	38.23	1.00	6.4
ALDH10	7	−3.21	38.25	1.00	6.4
Phase II enzymes					
TPMT	4	−3.57	40.56	1.00	800
HNMT	5	−3.58	39.71	1.00	160
COMT	5	−3.56	43.10	0.99	160
ASMT	7	−3.46	34.92	1.00	6.4
GAMT	7	−3.68	40.34	1.00	6.4
NNMT	7	−3.32	37.75	0.99	6.4
PEMT	5	−3.73	42.60	0.99	160
PNMT	4	−3.58	48.19	0.99	800
NAT1	3	−3.65	45.37	1.00	4000
NAT2	6	−3.46	41.73	1.00	32
ARD1	7	−3.24	39.07	1.00	6.4
TST	7	−3.34	37.07	1.00	6.4
BAAT	7	−3.55	37.25	1.00	6.4
GSTP1	6	−3.26	40.02	1.00	32
GSTT1	4	−3.30	37.64	1.00	800
GSTM3	4	−3.27	42.77	1.00	800
GSTM4	4	−3.53	40.87	1.00	800
GSTA2	5	−3.77	42.20	1.00	160
GSTA4	5	−3.75	42.99	1.00	160
MGST1	7	−3.31	34.31	1.00	6.4
MGST2	7	−3.38	39.30	1.00	6.4
MGST3	6	−3.32	37.99	1.00	32
GSTZ1	4	−3.52	39.20	1.00	800
CHST1	4	−3.10	42.10	1.00	800
CHST3	5	−3.82	43.58	0.99	160
CHST4	5	−3.29	42.65	1.00	160
CST	5	−3.52	44.83	1.00	160
SULT2A1	5	−3.29	39.91	1.00	160
TPST2	5	−3.63	39.30	1.00	160
UGT1	7	−3.29	39.26	1.00	6.4
UGT1A9	5	−3.72	45.17	1.00	160
UGT2B10	7	−3.46	36.85	1.00	6.4
UGT2B11	4	−3.89	49.64	0.99	800
UGT2B17	6	−3.41	39.20	1.00	32
Transporters					
ABCA1	7	−3.31	39.37	1.00	6.4
ABCA2	6	−3.27	41.95	1.00	32
ABCA4	4	−2.89	45.85	0.99	800
ABCA8	5	−3.96	43.85	1.00	160
ABCB1	5	−3.40	43.08	1.00	160
ABCB2	5	−3.49	43.10	1.00	160
ABCB3	5	−3.29	43.54	1.00	160
ABCB4	7	−3.44	40.41	1.00	6.4

Table 2. continued.

mRNA	No. of data points	Calibration curve Slope	Intercept	Correlation coefficient r	Limit of quantification (pg total RNA/50 μl reaction mixture)
ABCB6	4	−3.62	44.77	1.00	800
ABCB7	5	−3.23	42.87	1.00	160
ABCB8	5	−3.39	42.21	1.00	160
ABCB9	6	−3.30	40.57	1.00	32
ABCB11	6	−3.17	38.60	1.00	32
ABCC1	3	−3.77	44.63	1.00	4000
ABCC2	7	−3.26	38.28	0.99	6.4
ABCC3	6	−3.38	39.74	1.00	32
ABCC5	3	−3.25	41.54	1.00	4000
ABCC6	5	−3.70	43.15	1.00	160
ABCC9	6	−3.51	41.28	1.00	32
ABCD1	4	−3.10	40.50	1.00	800
ABCD3	6	−3.22	37.99	1.00	32
ABCE1	6	−3.41	41.68	1.00	32
ABCF1	5	−3.08	39.50	1.00	160
ABCF2	5	−3.24	40.97	1.00	160
ABCG1	7	−3.53	43.03	1.00	6.4
ABCG2	4	−3.31	40.93	1.00	800
SLC22A1	7	−3.34	35.79	1.00	6.4
SLC22A3	7	−3.26	38.95	1.00	6.4
SLC22A5	6	−3.56	44.54	1.00	32
OATP2	4	−3.60	37.60	0.99	800

The calibration curves show the threshold cycle (Ct) for analysis of total RNA per 50 μl of reaction mixture. The source of total RNA was the human adult liver.

ADH3, and ABCG1 mRNA in a concentration-dependent manner (Fig. 1A, D, E, F, G, H, L). The levels of CYP1A1, CYP2B6, CYP3A4, CYP3A5, and ABCG1 mRNA decreased after a change to media without rifampicin, and levels 48 h after a change to media without rifampicin (96 h in the figure) were comparable to those in controls (untreated cells) (Fig. 1A, D, F, G, L). The levels of CYP2C8 and ADH3 mRNA decreased slightly 48 h after a change to media without rifampicin (Fig. 1E, H). The levels of CYP1A2, CYP1B1, ALDH3, ALDH6, and ABCB4 mRNA after rifampicin exposure did not change to the levels in controls (Fig. 1B, C, I, J, K). Exposure to omeprazole increased the levels of CYP1A1, CYP1A2, CYP1B1, ALDH3, and ALDH6 mRNA in a concentration-dependent manner, and the levels of these mRNAs decreased after a change to media without omeprazole (Fig. 2A, B, C, I, J). Exposure to omeprazole increased the levels of CYP2B6 and CYP3A4 mRNA, and the levels of these mRNAs decreased after a change to media without omeprazole (Fig. 2D, F). Exposure to omeprazole slightly increased the levels of CYP2C8 and CYP3A5 mRNA (Fig. 2E, G). On the other hand, exposure to omeprazole decreased the levels of ADH3 and ABCB4 mRNA, and the levels of these mRNAs recovered after a change to media without omeprazole (Fig. 2H, K). Exposure to omeprazole for an additional 24 h decreased the level of ABCG1 mRNA in a concentration-dependent manner, but the level of this mRNA recovered after an additional 48 h of omeprazole exposure (Fig. 2L).

DISCUSSION

A new analytic method for quantifying mRNA levels in small numbers of human hepatocytes has been developed for phase I enzymes, phase II enzymes, and transporters. This method employs sets of forward and reverse primers and TaqMan probes to hybridize to each target cDNA of phase I enzymes, phase II enzymes, and transporters. Both the primers and/or probes were homology searched by an NCBI BLAST search to ensure that they are specific for the target mRNA transcript. To our knowledge, this is the first

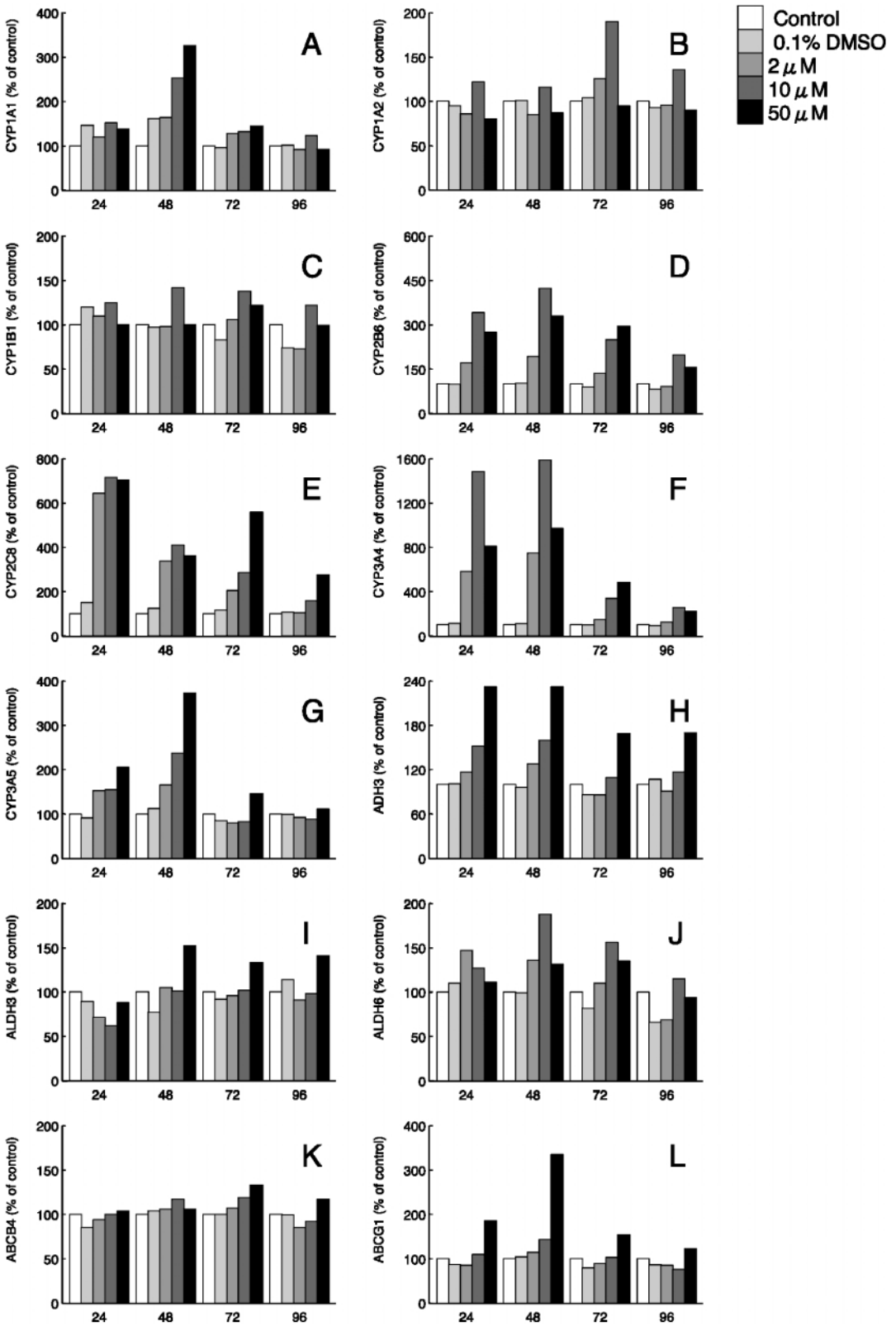

Fig. 1. Time Course and Effects on Levels of mRNA Expression after Rifampicin Exposure

Human hepatocytes were treated with rifampicin for 24 and 48 h. The media with rifampicin were then replaced with fresh medium, and the hepatocytes were cultured for an additional 24 and 48 h (72 and 96 h, respectively, in the figure). The medium was changed daily. Data are expressed as the ratio of target mRNA to GAPDH mRNA, and data are then shown as a percentage relative to control at each time point. Experiments (cultured human hepatocytes) were performed in triplicate, and data are shown as the mean value for three runs. A: CYP1A1; B: CYP1A2; C: CYP1B1; D: CYP2B6; E: CYP2C8; F: CYP3A4; G: CYP3A5; H: ADH3; I: ALDH3; J: ALDH6; K: ABCB4; L: ABCG1.

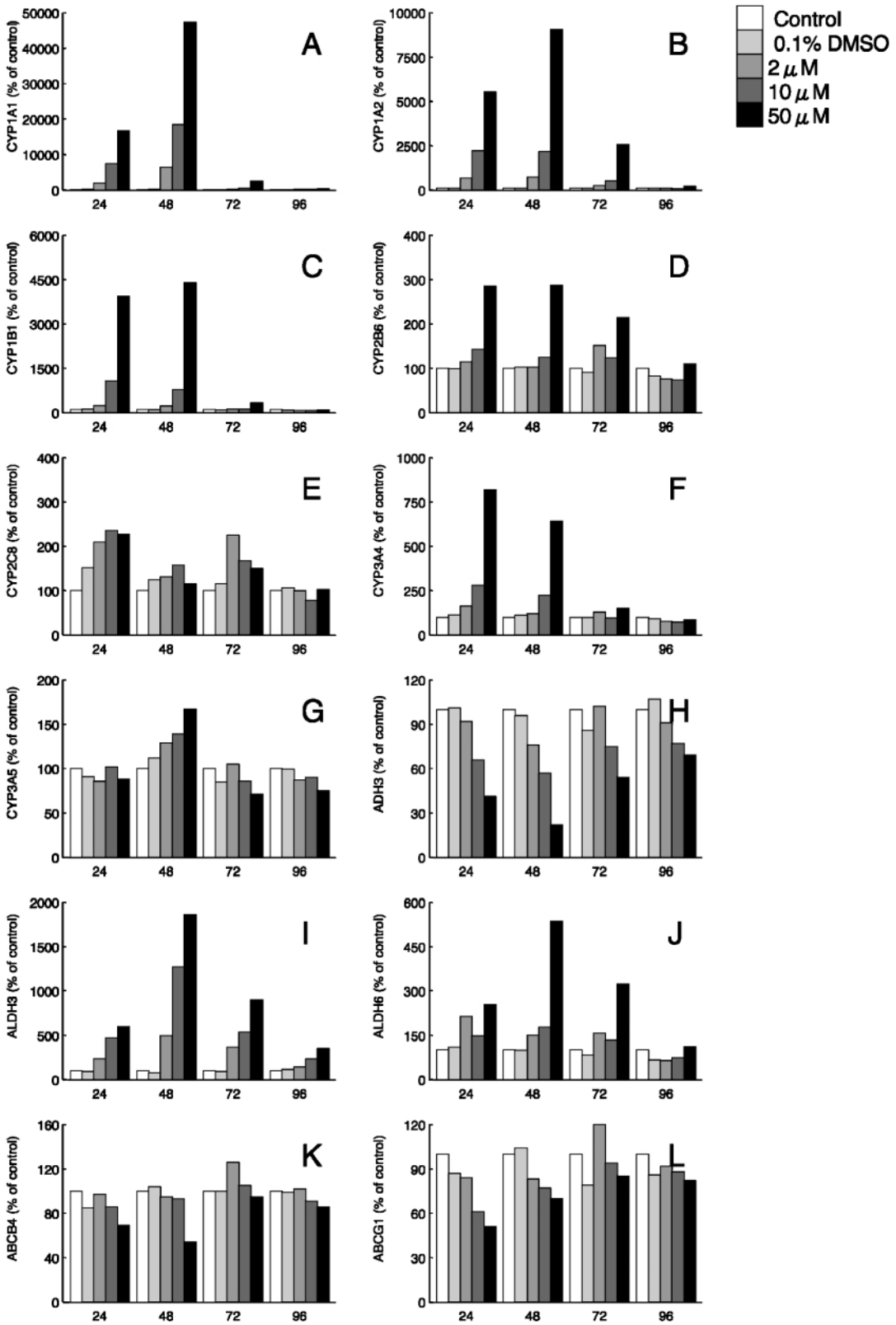

Fig. 2. Time Course and Effects on Levels of mRNA Expression after Omeprazole Exposure

Human hepatocytes were treated with omeprazole for 24 and 48 h. The media with omeprazole were then replaced with fresh medium, and the hepatocytes were cultured for an additional 24 and 48 h (72 and 96 h, respectively, in the figure). The medium was changed daily. Data are expressed as the ratio of target mRNA to GAPDH mRNA, and data are then shown as a percentage relative to control at each time point. Experiments (cultured human hepatocytes) were performed in triplicate, and data are shown as the mean value for three runs. A: CYP1A1; B: CYP1A2; C: CYP1B1; D: CYP2B6; E: CYP2C8; F: CYP3A4; G: CYP3A5; H: ADH3; I: ALDH3; J: ALDH6; K: ABCB4; L: ABCG1.

application of the real-time one-step RT–PCR TaqMan method to the study of phase I enzymes, phase II enzymes, and transporters. In the present study, the TaqMan method was found to be exquisitely sensitive and to permit quantitative evaluation, and it was found that the quantification limit was two times higher or lower than in control. This is in contrast to existing methods such as Northern blotting, which often require micrograms of total RNA. This method could therefore prove to be particularly useful in studies where the amount of target mRNA may be very low, as in cultured cells. In addition, this method does not require electrophoretic gels to be run, and the assay can be performed in a high-throughput manner using 96–well or 384–well plates.

The hybridization efficiency to target mRNA differed according to the design position of the reverse primer because the RT reaction carried out at 48℃ preserved the higher-order structure of the mRNA. Therefore the position of the reverse primer set the upper limit of the hybridization efficiency to the target mRNA. This being the case, the design positions of sequences that can select an enzyme family are limited. It is thus more difficult to design both primers and probes for use in one-step RT–PCR compared with two-step RT–PCR. This method avoids complicated operations such as the need to perform separate reactions for RT and PCR, assuming that the set of primers and probes is designed to target selectively mRNA in which the high-order structure is preserved. To standardize the reaction efficiency, the concentrations of the forward primer, the reverse primer, and the TaqMan probe were 300 nM, 900 nM, and 200 nM, respectively. With regard to TaqMan RT–PCR conditions, the concentration of the reverse primer was three times higher than that of the forward primer due to an increase in hybridization efficiency to mRNA in the RT reaction. The calculated standard values using the human total RNA were affected by the quantity of the target mRNA in the human total RNA. Therefore the same human total RNA was used in this study.

In standard induction studies, drug-metabolizing enzyme activities are evaluated in liver samples after repeated administration to female rats. However, species differences in the induction of drug-metabolizing enzymes and transporters are frequently observed in the metabolism and transport of xenobiotics. Studies employing human hepatocytes are useful in overcoming this problem. The quantification of enzyme and transporter induction has been achieved primarily by measuring changes in the metabolism and transport of enzyme- and transporter-selective substrates or by direct quantification of enzyme and transporter proteins using specific antibodies and Western blotting. However, using these analytic methods for quantifying enzyme activities and protein levels, it is difficult to measure phase I enzymes, phase II enzymes, and transporters in the same sample because a large amount of sample is required for measurement. In the present study, a new analytic method for quantifying mRNA levels was used to measure phase I enzymes, phase II enzymes, and transporters in the same sample obtained from small numbers of human hepatocytes. Recently, direct studies of enzyme mRNA expression have been used because up-regulation of enzyme gene transcription and the subsequent increase in protein levels are thought to be primarily responsible for the enhanced metabolic function of enzymes after exposure to inducers.[15—17] It has been suggested that the induction levels show a correlation between enzyme activities or protein levels and mRNA levels after exposure to inducers.[18,19] One possible exception is the induction of CYP2E1 by ethanol and isoniazid, which is thought to result either from protein stabilization[20] or increased protein translation.[21] Therefore we considered that rapid estimation should be possible by evaluating the changes in the mRNA levels of target enzymes and transporters and then evaluating the changes in corresponding protein and activity levels. It is also possible that mRNA induction occurs to counter a reduction in activity due to inhibition of the target enzyme or transporter. Therefore we carefully considered the targets for evaluating changes in mRNA levels when mRNA levels were not correlated with protein or activity levels.

The results shown in Fig. 1 are similar to those shown in Table 3 after exposure to rifampicin. However, the exposure to omeprazole 50 μM for 48 h had no remarkable effect on CYP2B6 or CYP3A4 mRNA expression (Table 3). On the other hand, the levels of CYP2B6 and CYP3A4 mRNA after exposure to omeprazole 50 μM for 48 h, in the same hepatocytes increased to levels approximately three and seven times higher than those in controls, respectively (Fig. 2). The levels of CYP1A1, CYP1A2, CYP1B1, ALDH3, and ALDH6 mRNA shown in

Table 3. Effect on mRNA Expression after Drug exposure for 48 h

	Control	DMSO 0.1%	Rifampicin 50 μM	Omeprazole 50 μM
β-actin	3.01 ± 0.25 (100)	2.73 ± 0.20 (91)	2.67 ± 0.20 (88)	3.48 ± 0.20 (115)
Phase I enzymes				
CYP1A1	0.0243 ± 0.021 (100)	0.0299 ± 0.0032 (123)	0.0684 ± 0.0098 (281)	5.45 ± 0.07 (22426)
CYP1A2	0.00059 ± 0.00010 (100)	0.00059 ± 0.00014 (99)	0.00060 ± 0.00015 (102)	0.0275 ± 0.0052 (4652)
CYP1B1	0.138 ± 0.019 (100)	0.149 ± 0.047 (108)	0.120 ± 0.013 (87)	3.85 ± 1.67 (2799)
CYP2A6	0.00022 ± 0.00002 (100)	0.00023 ± 0.00003 (103)	0.00037 ± 0.00007 (169)	0.00024 ± 0.00009 (106)
CYP2B6	0.00254 ± 0.00060 (100)	0.00255 ± 0.00031 (100)	0.00700 ± 0.00103 (276)	0.00296 ± 0.00109 (116)
CYP2C8	0.00025 ± 0.00015 (100)	0.00017 ± 0.00007 (69)	0.00085 ± 0.00037 (340)	0.00019 ± 0.00012 (77)
CYP2C9	0.00984 ± 0.00169 (100)	0.0106 ± 0.0013 (107)	0.0108 ± 0.0004 (110)	0.00710 ± 0.00259 (72)
CYP2C18	0.247 ± 0.021 (100)	0.242 ± 0.021 (98)	0.267 ± 0.027 (108)	0.212 ± 0.053 (86)
CYP2C19	0.0374 ± 0.0012 (100)	0.0299 ± 0.0050 (80)	0.0261 ± 0.0036 (70)	0.0235 ± 0.0080 (63)
CYP2D6	0.00458 ± 0.00074 (100)	0.00514 ± 0.00039 (112)	0.00362 ± 0.00064 (79)	0.00464 ± 0.00036 (101)
CYP2E1	0.00058 ± 0.00003 (100)	0.00070 ± 0.00002 (121)	0.00100 ± 0.00012 (172)	0.00092 ± 0.00008 (158)
CYP2J2	0.0685 ± 0.0088 (100)	0.0659 ± 0.0036 (96)	0.0720 ± 0.0034 (105)	0.0579 ± 0.0160 (84)
CYP3A4	0.00102 ± 0.00001 (100)	0.00099 ± 0.00011 (97)	0.00505 ± 0.00049 (496)	0.00149 ± 0.00027 (146)
CYP3A5	0.0473 ± 0.0134 (100)	0.0534 ± 0.0058 (113)	0.132 ± 0.022 (278)	0.0626 ± 0.0032 (132)
CYP4A11	0.00051 ± 0.00011 (100)	0.00045 ± 0.00004 (89)	0.00031 ± 0.00005 (61)	0.00027 ± 0.00008 (52)
CYP27	0.0203 ± 0.0025 (100)	0.0191 ± 0.0018 (94)	0.0178 ± 0.0018 (88)	0.0190 ± 0.0054 (94)
EPHX1	0.0414 ± 0.0036 (100)	0.0374 ± 0.0027 (90)	0.0490 ± 0.0049 (118)	0.0390 ± 0.0106 (94)
EPHX2	0.0165 ± 0.0018 (100)	0.0146 ± 0.0011 (89)	0.0149 ± 0.0002 (91)	0.0140 ± 0.0028 (85)
PIG3	6.98 ± 1.10 (100)	6.34 ± 0.65 (91)	7.51 ± 0.67 (108)	6.54 ± 0.83 (94)
NMOR2	0.0818 ± 0.0068 (100)	0.0711 ± 0.0045 (87)	0.0929 ± 0.0089 (114)	0.0910 ± 0.0057 (111)
MAOA	0.212 ± 0.043 (100)	0.180 ± 0.009 (85)	0.169 ± 0.012 (79)	0.136 ± 0.026 (64)
MAOB	0.0597 ± 0.0072 (100)	0.0579 ± 0.0092 (97)	0.0622 ± 0.0044 (104)	0.0601 ± 0.0122 (101)
PTGS1	0.218 ± 0.046 (100)	0.225 ± 0.059 (103)	0.136 ± 0.038 (63)	0.166 ± 0.110 (76)
PTGS2	0.697 ± 0.154 (100)	0.690 ± 0.153 (99)	0.474 ± 0.099 (68)	0.930 ± 0.389 (133)
DPYD	0.0291 ± 0.0018 (100)	0.0281 ± 0.0004 (97)	0.0310 ± 0.0024 (106)	0.0237 ± 0.0079 (81)
AOX1	0.127 ± 0.031 (100)	0.125 ± 0.011 (99)	0.107 ± 0.019 (84)	0.107 ± 0.041 (84)
XDH	0.0109 ± 0.0024 (100)	0.0107 ± 0.0004 (99)	0.0172 ± 0.0014 (158)	0.0149 ± 0.0071 (137)
CES1	0.109 ± 0.037 (100)	0.135 ± 0.012 (124)	0.204 ± 0.038 (187)	0.141 ± 0.061 (130)
CES2	0.136 ± 0.019 (100)	0.144 ± 0.016 (106)	0.119 ± 0.009 (88)	0.203 ± 0.077 (150)
AADAC	0.239 ± 0.053 (100)	0.222 ± 0.030 (93)	0.196 ± 0.044 (82)	0.193 ± 0.079 (81)
LIPA	0.164 ± 0.037 (100)	0.140 ± 0.011 (85)	0.154 ± 0.024 (94)	0.151 ± 0.072 (92)
NTE	0.448 ± 0.104 (100)	0.494 ± 0.029 (110)	0.601 ± 0.065 (134)	0.576 ± 0.253 (129)
UCHL1	5.56 ± 0.49 (100)	5.79 ± 0.53 (104)	6.82 ± 0.37 (123)	9.61 ± 2.60 (173)
UCHL3	0.852 ± 0.139 (100)	0.843 ± 0.004 (99)	0.879 ± 0.168 (103)	0.892 ± 0.423 (105)
ESD	0.250 ± 0.041 (100)	0.255 ± 0.018 (102)	0.225 ± 0.040 (90)	0.324 ± 0.139 (130)
FMO3	0.00435 ± 0.00175 (100)	0.00521 ± 0.00098 (120)	0.00421 ± 0.00077 (97)	0.00338 ± 0.00168 (78)
FMO4	0.0130 ± 0.0024 (100)	0.0139 ± 0.0020 (107)	0.0134 ± 0.0011 (103)	0.0110 ± 0.0042 (85)
FMO5	0.00486 ± 0.00094 (100)	0.00568 ± 0.00053 (117)	0.00736 ± 0.00058 (151)	0.00531 ± 0.00198 (109)
ADH1	ND (—)	ND (—)	ND (—)	ND (—)
ADH2	0.00066 ± 0.00044 (100)	0.00094 ± 0.00034 (141)	0.00060 ± 0.00015 (90)	0.00060 ± 0.00037 (91)
ADH3	0.00062 ± 0.00015 (100)	0.00063 ± 0.00014 (101)	0.00124 ± 0.00028 (200)	0.00017 ± 0.00003 (27)
ADH4	0.00032 ± 0.00007 (100)	0.00031 ± 0.00005 (95)	0.00024 ± 0.00006 (73)	0.00023 ± 0.00009 (69)
ADH5	0.131 ± 0.032 (100)	0.131 ± 0.013 (100)	0.168 ± 0.062 (128)	0.196 ± 0.068 (149)
ADH6	0.00118 ± 0.00020 (100)	0.00131 ± 0.00022 (111)	0.00118 ± 0.00047 (100)	0.00087 ± 0.00027 (73)
ADH7	0.387 ± 0.101 (100)	0.338 ± 0.090 (87)	0.312 ± 0.103 (81)	0.337 ± 0.203 (87)
HADH2	0.135 ± 0.053 (100)	0.136 ± 0.018 (101)	0.130 ± 0.033 (96)	0.154 ± 0.071 (114)
HEP27	0.00737 ± 0.00146 (100)	0.00633 ± 0.00136 (86)	0.00956 ± 0.00163 (130)	0.0136 ± 0.0029 (185)

Table 3. continued.

	Control		DMSO 0.1%		Rifampicin 50 μM		Omeprazole 50 μM	
ALDH1	0.401 ± 0.184	(100)	0.425 ± 0.028	(106)	0.390 ± 0.153	(97)	0.555 ± 0.199	(139)
ALDH2	0.0149 ± 0.0056	(100)	0.0164 ± 0.0018	(110)	0.0229 ± 0.0089	(153)	0.0293 ± 0.0153	(196)
ALDH3	1.51 ± 0.32	(100)	1.83 ± 0.07	(121)	2.16 ± 0.76	(143)	12.9 ± 7.0	(853)
ALDH4	0.105 ± 0.074	(100)	0.130 ± 0.043	(124)	0.133 ± 0.074	(127)	0.171 ± 0.114	(163)
ALDH5	0.231 ± 0.046	(100)	0.208 ± 0.027	(90)	0.215 ± 0.034	(93)	0.301 ± 0.104	(130)
ALDH6	0.138 ± 0.025	(100)	0.132 ± 0.028	(96)	0.199 ± 0.039	(144)	0.339 ± 0.120	(246)
ALDH9	0.175 ± 0.040	(100)	0.177 ± 0.006	(101)	0.149 ± 0.049	(85)	0.179 ± 0.070	(102)
ALDH10	0.147 ± 0.007	(100)	0.134 ± 0.004	(91)	0.176 ± 0.024	(120)	0.171 ± 0.021	(117)
Phase II enzymes								
TPMT	0.204 ± 0.031	(100)	0.216 ± 0.028	(106)	0.192 ± 0.011	(94)	0.196 ± 0.094	(96)
HNMT	0.123 ± 0.019	(100)	0.114 ± 0.019	(93)	0.140 ± 0.016	(114)	0.076 ± 0.026	(61)
COMT	0.160 ± 0.023	(100)	0.168 ± 0.022	(105)	0.149 ± 0.015	(94)	0.135 ± 0.057	(84)
ASMT	0.237 ± 0.029	(100)	0.248 ± 0.054	(105)	0.262 ± 0.042	(111)	0.248 ± 0.088	(105)
GAMT	0.0371 ± 0.0045	(100)	0.0407 ± 0.0044	(109)	0.0343 ± 0.0050	(92)	0.0418 ± 0.0179	(113)
NNMT	1.10 ± 0.08	(100)	1.15 ± 0.09	(105)	0.794 ± 0.106	(72)	0.873 ± 0.042	(80)
PEMT	0.0495 ± 0.0111	(100)	0.0539 ± 0.0057	(109)	0.0487 ± 0.0109	(98)	0.0535 ± 0.0287	(108)
PNMT	0.460 ± 0.095	(100)	0.446 ± 0.148	(97)	0.473 ± 0.068	(103)	0.396 ± 0.155	(86)
NAT1	0.206 ± 0.050	(100)	0.207 ± 0.039	(100)	0.160 ± 0.038	(78)	0.133 ± 0.066	(64)
NAT2	0.0513 ± 0.0061	(100)	0.0559 ± 0.0072	(109)	0.0541 ± 0.0055	(105)	0.0459 ± 0.0097	(89)
ARD1	0.448 ± 0.040	(100)	0.452 ± 0.033	(101)	0.442 ± 0.071	(99)	0.517 ± 0.174	(116)
TST	0.101 ± 0.024	(100)	0.113 ± 0.017	(112)	0.135 ± 0.022	(134)	0.123 ± 0.051	(123)
BAAT	0.0745 ± 0.0089	(100)	0.0688 ± 0.0044	(92)	0.0637 ± 0.0055	(85)	0.0822 ± 0.0327	(110)
GSTP1	0.206 ± 0.028	(100)	0.214 ± 0.007	(104)	0.223 ± 0.018	(108)	0.173 ± 0.041	(84)
GSTT1	0.160 ± 0.022	(100)	0.141 ± 0.014	(88)	0.169 ± 0.021	(105)	0.141 ± 0.033	(88)
GSTM3	0.343 ± 0.067	(100)	0.324 ± 0.018	(94)	0.429 ± 0.094	(125)	0.362 ± 0.089	(106)
GSTM4	0.532 ± 0.071	(100)	0.464 ± 0.051	(87)	0.499 ± 0.086	(94)	0.449 ± 0.118	(84)
GSTA2	0.0642 ± 0.0114	(100)	0.0591 ± 0.0059	(92)	0.0736 ± 0.0011	(115)	0.101 ± 0.017	(158)
GSTA4	0.0502 ± 0.0058	(100)	0.0472 ± 0.0023	(94)	0.0615 ± 0.0065	(122)	0.0689 ± 0.0236	(137)
MGST1	0.506 ± 0.065	(100)	0.469 ± 0.062	(93)	0.493 ± 0.035	(97)	0.482 ± 0.137	(95)
MGST2	0.121 ± 0.017	(100)	0.114 ± 0.014	(94)	0.107 ± 0.002	(88)	0.0928 ± 0.0345	(76)
MGST3	0.660 ± 0.065	(100)	0.661 ± 0.005	(100)	0.724 ± 0.027	(110)	0.692 ± 0.013	(105)
GSTZ1	0.115 ± 0.009	(100)	0.112 ± 0.014	(98)	0.138 ± 0.016	(120)	0.119 ± 0.042	(103)
CHST1	0.230 ± 0.033	(100)	0.0987 ± 0.0135	(76)	0.182 ± 0.025	(140)	0.144 ± 0.008	(111)
CHST3	0.644 ± 0.113	(100)	0.547 ± 0.0656	(85)	0.564 ± 0.0539	(88)	0.484 ± 0.154	(75)
CHST4	0.372 ± 0.045	(100)	0.417 ± 0.089	(112)	0.358 ± 0.009	(96)	0.270 ± 0.124	(73)
CST	0.194 ± 0.064	(100)	0.198 ± 0.030	(102)	0.194 ± 0.036	(100)	0.215 ± 0.135	(111)
SULT2A1	0.0232 ± 0.0055	(100)	0.0277 ± 0.0027	(119)	0.0236 ± 0.0060	(102)	0.0458 ± 0.0200	(197)
TPST2	0.193 ± 0.016	(100)	0.201 ± 0.018	(104)	0.176 ± 0.022	(91)	0.166 ± 0.071	(86)
UGT1	0.628 ± 0.109	(100)	0.636 ± 0.058	(101)	0.514 ± 0.018	(82)	0.602 ± 0.190	(96)
UGT1A9	0.181 ± 0.058	(100)	0.226 ± 0.039	(125)	0.246 ± 0.023	(136)	0.284 ± 0.117	(156)
UGT2B10	0.00096 ± 0.00012	(100)	0.00101 ± 0.00013	(106)	0.00100 ± 0.00012	(105)	0.00091 ± 0.00049	(95)
UGT2B11	0.237 ± 0.048	(100)	0.260 ± 0.086	(110)	0.127 ± 0.021	(53)	0.131 ± 0.095	(55)
UGT2B17	0.00268 ± 0.00024	(100)	0.00270 ± 0.00049	(101)	0.00263 ± 0.00013	(98)	0.00186 ± 0.00117	(69)
Transporters								
ABCA1	0.0561 ± 0.0059	(100)	0.0519 ± 0.0034	(93)	0.0716 ± 0.0110	(128)	0.0473 ± 0.0098	(84)
ABCA2	0.253 ± 0.008	(100)	0.223 ± 0.009	(88)	0.221 ± 0.009	(87)	0.308 ± 0.062	(122)
ABCA4	0.373 ± 0.120	(100)	0.367 ± 0.128	(98)	0.406 ± 0.094	(109)	0.321 ± 0.079	(86)
ABCA8	0.0332 ± 0.0066	(100)	0.0317 ± 0.0026	(95)	0.0161 ± 0.0011	(49)	0.0176 ± 0.0055	(53)
ABCB1	0.639 ± 0.126	(100)	0.624 ± 0.047	(98)	1.01 ± 0.05	(158)	0.653 ± 0.109	(102)

Table 3. continued.

	Control	DMSO 0.1%	Rifampicin 50 μM	Omeprazole 50 μM
ABCB2	1.05 ± 0.18 (100)	1.01 ± 0.07 (96)	1.20 ± 0.09 (114)	1.20 ± 0.25 (114)
ABCB3	0.686 ± 0.099 (100)	0.640 ± 0.015 (93)	0.661 ± 0.072 (96)	0.547 ± 0.113 (80)
ABCB4	0.0373 ± 0.0050 (100)	0.0364 ± 0.0013 (98)	0.0298 ± 0.0013 (80)	0.0187 ± 0.0022 (50)
ABCB6	0.775 ± 0.151 (100)	0.670 ± 0.090 (86)	0.752 ± 0.081 (97)	0.843 ± 0.274 (109)
ABCB7	0.280 ± 0.031 (100)	0.252 ± 0.026 (90)	0.243 ± 0.024 (87)	0.237 ± 0.068 (85)
ABCB8	0.694 ± 0.096 (100)	0.644 ± 0.056 (93)	0.643 ± 0.040 (93)	0.625 ± 0.141 (90)
ABCB9	0.262 ± 0.045 (100)	0.247 ± 0.024 (94)	0.284 ± 0.035 (108)	0.305 ± 0.086 (116)
ABCB11	0.00827 ± 0.00105 (100)	0.00741 ± 0.00074 (90)	0.00603 ± 0.00037 (73)	0.00726 ± 0.00170 (88)
ABCC1	0.610 ± 0.093 (100)	0.650 ± 0.056 (107)	0.672 ± 0.104 (110)	0.674 ± 0.094 (111)
ABCC2	0.373 ± 0.048 (100)	0.332 ± 0.028 (89)	0.474 ± 0.052 (127)	0.472 ± 0.113 (126)
ABCC3	0.359 ± 0.044 (100)	0.346 ± 0.047 (96)	0.338 ± 0.024 (94)	0.345 ± 0.086 (96)
ABCC5	0.381 ± 0.041 (100)	0.361 ± 0.013 (95)	0.364 ± 0.051 (95)	0.349 ± 0.080 (91)
ABCC6	0.0323 ± 0.0035 (100)	0.0287 ± 0.0050 (89)	0.0196 ± 0.0015 (61)	0.0190 ± 0.0018 (59)
ABCC9	0.0207 ± 0.0024 (100)	0.0193 ± 0.0007 (93)	0.0153 ± 0.0020 (74)	0.0167 ± 0.0058 (81)
ABCD1	0.242 ± 0.029 (100)	0.254 ± 0.016 (105)	0.314 ± 0.015 (129)	0.338 ± 0.070 (139)
ABCD3	0.146 ± 0.014 (100)	0.139 ± 0.003 (95)	0.142 ± 0.014 (97)	0.134 ± 0.026 (91)
ABCE1	0.924 ± 0.061 (100)	0.857 ± 0.062 (93)	0.996 ± 0.109 (108)	1.14 ± 0.178 (123)
ABCF1	0.651 ± 0.064 (100)	0.553 ± 0.035 (85)	0.554 ± 0.030 (85)	0.606 ± 0.082 (93)
ABCF2	1.27 ± 0.22 (100)	1.12 ± 0.16 (89)	1.09 ± 0.09 (86)	1.19 ± 0.22 (94)
ABCG1	0.0105 ± 0.0011 (100)	0.0113 ± 0.0009 (108)	0.0272 ± 0.0019 (259)	0.0074 ± 0.0018 (70)
ABCG2	0.237 ± 0.018 (100)	0.222 ± 0.027 (94)	0.262 ± 0.031 (110)	0.319 ± 0.067 (134)
SLC22A1	0.00046 ± 0.00006 (100)	0.00054 ± 0.00002 (117)	0.00044 ± 0.00002 (95)	0.00085 ± 0.00023 (185)
SLC22A3	0.113 ± 0.022 (100)	0.104 ± 0.008 (92)	0.111 ± 0.005 (98)	0.192 ± 0.063 (170)
SLC22A5	1.18 ± 0.20 (100)	1.11 ± 0.04 (94)	1.43 ± 0.16 (121)	1.25 ± 0.03 (106)
OATP2	0.0229 ± 0.0024 (100)	0.0234 ± 0.0024 (102)	0.0260 ± 0.0260 (114)	0.0236 ± 0.0204 (103)

The effects of rifampicin and omeprazole were studied at doses of 50 μM. Data are expressed as the ratio of target mRNA to GAPDH mRNA. Experiments (cultured human hepatocytes) were performed in triplicate, and data are shown as mean ± SD of three runs. Values in parentheses indicate percentages relative to control.

Fig. 2 after exposure to omeprazole 50 μM for 48 h were about two times higher than those shown in Table 3. It is considered that the sensitivity of hepatocytes after drug exposure was due to experimental variation. However, the biochemical cause of this difference is unknown. Therefore we feel that it is important to obtain measurements using positive controls such as rifampicin for CYP3A4 mRNA and omeprazole for CYP1A1 and CYP1A2 mRNA. We are planning to perform additional studies to identify appropriate positive controls for each enzyme and transporter.

As shown in Fig. 2, the level of ABCG1 mRNA decreased to 50% after 24-h exposure to omeprazole 50 μM, whereas the level of ABCG1 mRNA was 70% after 48-h exposure to omeprazole 50 μM, as shown in Table 3. Exposure to a drug at a dose of 50 μM for 48 h is not the most suitable method for identifying mRNAs that are regulated by drug exposure. However, at the present time, even using high-throughput analysis, it is difficult to measure mRNA levels for more than 100 enzymes and transporters at various concentrations and at multiple time points. We therefore investigated exposure to compounds at the dose of 50 μM for 48 h for initial screening in the present study.

In conclusion, the results of the present study demonstrate the applicability of quantitative real-time RT-PCR to the evaluation of gene induction and recovery of drug-metabolizing enzymes and transporters after exposure to drugs in human hepatocytes. This method has the advantages of high sensitivity, simplicity, and linearity of quantification over a wide range of mRNA concentrations, making it particularly suitable for evaluating large numbers of samples, as required in expression profile determinations. We

will present further results demonstrating the usefulness of this system employing human hepatocytes as *in vitro* induction studies.

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