Decreased Expression of Cytochromes P450 1A2, 2E1, and 3A4 and Drug Transporters Na\textsuperscript{+}-Taurocholate-Cotransporting Polypeptide, Organic Cation Transporter 1, and Organic Anion-Transporting Peptide-C Correlates with the Progression of Liver Fibrosis in Chronic Hepatitis C Patients


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ABSTRACT:

Patients with chronic hepatitis C viral infection underwent liver biopsies and laboratory studies for evaluation and to determine subsequent treatment. Changes in status of drug metabolism and disposition may vary with chronic hepatitis C stage and should be assessed. Total RNA was extracted from liver biopsy specimens \((n = 63)\) and reverse transcribed to yield cDNA. Relative mRNA levels of drug-metabolizing enzymes, transporters, nuclear receptors, and proinflammatory cytokines were analyzed with normalization to glyceraldehyde 3-phosphate dehydrogenase expression. mRNAs encoding cytochromes P450 1A2, 2E1, and 3A4, and drug transporters, Na\textsuperscript{+}-taurocholate-cotransporting polypeptide, organic anion-transporting peptide-C, and organic cation transporter 1 showed remarkable decreases, and tumor necrosis factor-\(\alpha\) showed an increase according to fibrosis stage progression. HepG2 cells and primary hepatocytes of two human individuals were treated with interleukin 1\(\beta\), interleukin 6, or tumor necrosis factor-\(\alpha\). CYP1A2 and Na\textsuperscript{+}-taurocholate-cotransporting polypeptide mRNA levels significantly decreased in HepG2 cells with interleukin 1\(\beta\) and interleukin 6 treatments. CYP2E1 and organic cation transporter 1 mRNA levels significantly decreased with tumor necrosis factor-\(\alpha\) treatment only in HepG2. These results suggested that down-regulation of CYP1A2, 2E1, and 3A4, and drug transporters, Na\textsuperscript{+}-taurocholate-cotransporting polypeptide, organic anion-transporting peptide-C, and organic cation transporter 1, manifested in livers of patients with chronic hepatitis C viral infection, was associated, at least in part, with the elevated production of proinflammatory cytokines, including tumor necrosis factor-\(\alpha\).

Infection and inflammation generally cause a decrease in hepatic capacity for drug metabolism and disposition. Using lipopolysaccharide models of hepatic inflammation, a number of investigators have demonstrated decreases in the expression of various drug-metabolizing enzymes (Iber et al., 1999; Renton, 2004). Bacterial and viral infections are associated with the induction of various cytokines, including interleukins (ILs) 1\(\beta\) and 6, tumor necrosis factor (TNF)-\(\alpha\), and interferon-\(\gamma\), which decrease the level of hepatic drug-metabolizing enzymes (Iber et al., 1999; Renton, 2004). Hepatitis C virus (HCV) infection was reported to cause changes in levels of drug-metabolizing enzymes cytochrome P450 2E1 (Gochee et al., 2003), an oxidative stress-related enzyme, and glutathione peroxidase (Levent et al., 2006); and nuclear receptors, including peroxisome proliferator-

ABBREVIATIONS: IL, interleukin; TNF, tumor necrosis factor; HCV, hepatitis C virus; ROS, reactive oxygen species; PCR, polymerase chain reaction; NTCP, Na\textsuperscript{+}-taurocholate-cotransporting peptide; OATP-C, organic anion-transporting peptide-C; OCT1, organic cation transporter 1; MRP, multidrug resistance-associated protein; MDR, multidrug resistance protein; CAR, constitutive androstane receptor; SULT, sulfotransferase; HNF, hepatocyte nuclear factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ANOVA, analysis of variance; INR, international normalized ratio.
expansion; F2, portoportal septa; and F3, portocentral linkage or bridging.

No necroinflammatory reaction; A1, mild necroinflammatory reaction; and A2, fibrosis (Desmet et al., 1994). No patients with liver cirrhosis (F4) were included in this study. Fibrosis staging was divided into four classes: F0, no fibrosis; F1, periportal fibrosis; F2, pericentral fibrosis; and F3, bridging or septal fibrosis.

63 patients with chronic hepatitis C at Toranomon Hospital, Tokyo, Japan. Using quantitative real-time polymerase chain reaction (PCR) analysis, liver biopsy samples from 63 Japanese patients were examined. We observed clear correlations between fibrosis stage and mRNA levels of hepatic CYP1A2, IL-1 and IL-6 resulted in a decrease in CYP1A2 and NTCP mRNA activity and OCT1 mRNA levels. Exposure of HepG2 cells to TNF-α and interferon (IFN)-α partly resulted in a decrease in CYP2E1 mRNA level was observed in our study.

Fibrosis staging (n) 0, F1, 35; F2, 11; F3, 17; F4, 0
Inflammation grading (n) A0, 0; A1, 38; A2, 25; A3, 0

Materials and Methods

Subjects. Ultrasound-guided or laparoscopic liver biopsy was performed on 63 patients with chronic hepatitis C at Toranomon Hospital, Tokyo, Japan. Fibrosis staging was divided into four classes: F0, no fibrosis; F1, 2100 Bioanalyzer. Total RNA extracted from 63 Japanese patients was subjected to electrophoretic analysis. As shown in sample A, 28S and 18S rRNAs were detected as sharp peaks, whereas in sample B, both rRNAs sizes were completely degraded. Of the 63 samples, eight samples showed a high degree of RNA degradation and therefore were excluded from subsequent analyses.

Clinical Laboratory Data Number or Value (Mean ± S.D.; Range)

Viral load (KIU/ml) 777 ± 596
HCV genotype
1a 1
1b 42
2a 13
2b 7
ALT (IU/l) 139 ± 93; 36–414
AST (IU/l) 88 ± 53; 23–276
γ-GTP (IU/l) 89 ± 73; 17–264
ALP (IU/l) 218 ± 78; 81–430
LDH (IU/l) 159 ± 27; 104–256
Total bilirubin (mg/dl) 0.9 ± 0.3; 0.4–2.2
Total cholesterol (mg/dl) 165 ± 27; 109–222
Serum triglycerides (mg/dl) 99 ± 34; 38–183
HDL (mg/dl) 44 ± 11; 26–77
Cholesterol (mg/dl) 7.7 ± 0.7; 6.5–9.4
Prothrombin time (INR) 1.0 ± 0.09; 0.86–1.34
AFP (ng/ml) 16.2 ± 36.3; 2–256
Fe (μg/dl) 168.1 ± 58.4; 20–256
UIBC (μg/dl) 184.3 ± 84.2; 18–449
Ferritin (μg/ml) 240 ± 286; 12–1378
Hyaluronic acid (μg/ml) 104.6 ± 117; 15–589

ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyl transferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; HDL, high density lipoprotein; AFP, α-fetoprotein; UIBC, unsaturated iron binding capacity.

The analyzed liver tissues of chronic hepatitis C patients weighed 1 to 25 mg (mean ± S.D. = 5.9 ± 5.4 mg), weight of nonhepatitis C patients ranged from 50 to 750 mg, and samples were stored at −70°C until used. The liver specimens were homogenized three times for 15 s in Lysis/Binding Solution from an RNA isolation kit (RNAqueous; Ambion, Austin, TX), using a Kinematica Polytron homogenizer (PT10–35; Kinematica, Lucerne, Switzerland). Total RNA (200 ng) was reverse transcribed to yield cDNA using TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA). Real-time PCR was performed with probe and primer sets (TaqMan Gene Expression Assays) available from Applied Biosystems using an ABI PRISM 7500 Fast Real-Time PCR System. The probes and primer sets are specific for human CYP1A2, IL-1, IL-6, CYP2E1, NTCP, OCT1, CYP3A4, CYP3A5, CYP2C9, and CYP2C19. The fold change in gene expression was calculated using the ΔΔCT method. The fold change in gene expression was calculated using the ΔΔCT method. The fold change in gene expression was calculated using the ΔΔCT method.
7700 Sequence Detection System (Applied Biosystems) for the quantitative analysis of mRNA encoding the following drug metabolism enzymes, drug transporters, nuclear receptors, and proinflammatory cytokines: CYP1A2, 2A6, 2B6, 2C8, 2C9, 2D6, 3A4, and 3A5; multidrug resistance-associated proteins (MRPs) MRP1, MRP2, and MRP3; multidrug resistance proteins (MDRs) MDR1 and MDR3; NTCP; OATP-A, OATP-B, and OATP-C; bile salt export pump; breast cancer resistance protein; OCT1; organic anion transporter; constitutive androstane receptor; pregnane X receptor; vitamin D receptor; UDP-glycuronosyltransferase 1A1; peroxisome proliferator-activated receptor-α; sulfotransferases (SULTs) SULT2A1 and SULT2B1; and hepatocyte nuclear factor (HNF) HNF4α and HNF1α dimerization cofactor; and IL-1, IL-6, and TNF-α (Table 3). mRNA levels were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

The ABI PRISM 7700 Sequence Detection System automatically created a standard curve by plotting the threshold cycle values against each standard dilution of a known standard human liver poly (A) RNA. Therefore, comparison of A/B mRNA levels well correlated (r = 0.7975, p < 0.001), indicating that using GAPDH and β-actin gave quite similar results. We decided to use GAPDH for normalization, although justification of GAPDH usage has not fully been verified. All amplification reactions were carried out in duplicate. All mRNA level measurements were performed in at least two separate experiments, which had deviations within 7% of the mean values.

### mRNA Levels for Genes Involved in Drug Metabolism and Disposition in HepG2 Cells and Primary Human Hepatocytes Treated with IL-1β, IL-6, or TNF-α

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* Dimerization cofactor of HNF1α (TCF1) 2.
Figure 2. Relationships between disease progression (fibrosis) and prothrombin time (INR) (A) and hyaluronic acid level (B). Clinical laboratory data were plotted in relationship to fibrosis stage. The Kruskal-Wallis test and shown as p-values.

Results

Patient Characteristics. Table 1 describes the histopathological features of the liver samples, together with the age, sex, and smoking, drinking habits, and major medications of the chronic hepatitis C patients recruited in the present study. Liver biopsy was performed to decide subsequent therapy. Therefore, no patient received interferon before liver biopsy. There were 34 males, 29 females, and 20 smokers. There were no heavy drinkers, although 21 reported alcohol consumption before liver biopsy. There were 34 males, 29 females, and 20 smokers.

Measurement of CYP2E1 Activity. CYP2E1 activity in human primary hepatocytes was determined according to the method by Ito et al. (2007). In brief, cultured primary hepatocytes were incubated in a fresh media with 0.5 mM p-nitrophenol at 37°C for 60 min. The supernatant was assayed for 4-nitrophenol by the addition of 10 M NaOH (1:10) and immediate determination of the absorbance at 454 nm. Activity after cytokine exposure was compared with that of control.

Determination of Viral Load. HCV RNA viral load in serum samples was calculated by the Amplicor quantification method (Amplicor HCV Monitor assay version 2.0; Roche Diagnostics, Tokyo, Japan).

Statistical Analysis. Statistical analysis was performed using the Kruskal-Wallis test, one-way analysis of variance (ANOVA) with a post hoc test (Tukey’s multiple comparison test), the Mann-Whitney test, χ² test, and the Spearman rank correlation using GraphPad Prism version 4.02 (GraphPad Software Inc., San Diego, CA). In each test, p < 0.05 was considered statistically significant.

Expression of CYP1A2, CYP2E1, CYP3A4, NTCP, OATP-C, and OCT1 mRNAs in HepG2 Cells and Primary Human Hepatocytes Treated with Proinflammatory Cytokines. HepG2 cells were exposed to 4 ng/ml recombinant IL-1β, 50 ng/ml recombinant IL-6, or 0.25 ng/ml recombinant TNF-α at 37°C for 24 h, all of which did not show any appreciable cytotoxicity. mRNA levels of CYP1A2, CYP2E1, CYP3A4, NTCP, OATP-C, and OCT1 decreased as the fibrosis stage progressed and were measured after 24 h of cytokine exposure using real-time PCR as described under Materials and Methods. As shown in Fig. 5, CYP1A2 and 89.6 and 96.4% for NTCP, respectively). CYP2E1 and NTCP (Fig. 3D), OATP-C (Fig. 3E), and OCT1 (Fig. 3F), showed remarkable decreases as the fibrosis stage progressed. These parameters failed to correlate with increased inflammation, except for CYP1A2. The relative CYP1A2 mRNA levels showed marginal statistical significance with increased inflammation (plot not shown, p = 0.0369 by nonparametric Mann-Whitney test). No fibrosis stage-dependent differences were observed for all other genes studied (see Materials and Methods). For example, mRNA levels for CYP2D6 and MDR1 did not correlate (plots not shown, p = 0.7059 and p = 0.9932, respectively). For reference, mRNA levels for CYP1A2, CYP2E1, CYP3A4, NTCP, OATP-C, and OCT1 in the noncancerous and non-hepatitis C liver tissues from 24 human individuals, who suffered from colon cancer and underwent resection of liver metastasis, were used. In the case of CYP3A4 and OATP-C, mean mRNA levels of the nonhepatitis C livers were higher than those of F1 patients, but not in regard to the other four mRNAs. Although determinations of mRNA levels of interest were performed in the same way after checking the quality of RNAs as in hepatitis C cases, the nonhepatitis C livers were derived from tissues surrounding tumors, not from healthy subjects. Therefore, some mRNAs may be down-regulated in the liver tissues surrounding cancerous tissues as in the case of hepatitis C patients. Alternatively, CYP1A2, CYP2E1, NTCP, and OCT1 might be once up-regulated in the initial F1 stage after hepatitis C viral infection; thereafter, their expression levels gradually decrease to those in the non-HCV patients. One-way ANOVA analyses of these gene expressions indeed revealed statistically significant elevation of CYP1A2 (p < 0.01), CYP2E1 (p < 0.01), NTCP (p < 0.01), and OCT1 (p < 0.05) mRNA levels in the patients of the F1 stage as compared with those in the non-HCV patients. Those in the F3 patients were not statistically different from those in the non-HCV patients. This hypothesis should be clarified by further studies performed using liver samples obtained in one hospital. mRNA levels of TNF-α, but not those of IL-1β, increased with statistical significance as the fibrosis stage progressed. As illustrated in Fig. 4, TNF-α mRNA levels in F3 patients were significantly higher than those of F1 patients (one-way ANOVA, p < 0.05). IL-6 mRNA levels were too low to evaluate in relation to fibrosis stage: IL-6 was detected in two of 31 subjects with F1 stage, one in 10 (F2), and four in 14 (F3) (χ² test, p < 0.05).

Relationships among Relative mRNA Expression for Cytochromes P450, Drug Transporters, and Nuclear Receptors and Fibrosis Staging and Inflammation Grading in Chronic Hepatitis C Patients. We analyzed trends in mRNA expression of drug-metabolizing enzymes, drug transporters, nuclear receptors, and proinflammatory cytokines according to the progression of chronic hepatitis C measured by histological staging and grading. Samples of 55 of 63 chronic hepatitis C patients (eight samples were excluded because of RNA degradation, Fig. 1) were evaluated by a pathologist from F1 to F3: F1, 31; F2, 10; and F3, 14; and from A1 to A2, A3, 34; and A2, 21. mRNA levels of drug-metabolizing enzymes [CYP1A2 (Fig. 3A), CYP2E1 (Fig. 3B), and CYP3A4 (Fig. 3C)], and drug transporters, NTCP (Fig. 3D), OATP-C (Fig. 3E), and OCT1 (Fig. 3F), showed statistically significant elevation of CYP1A2 (p < 0.01), CYP2E1 (p < 0.01), NTCP (p < 0.01), and OCT1 (p < 0.05) mRNA levels in the patients of the F1 stage as compared with those in the non-HCV patients. Those in the F3 patients were not statistically different from those in the non-HCV patients. This hypothesis should be clarified by further studies performed using liver samples obtained in one hospital. mRNA levels of TNF-α, but not those of IL-1β, increased with statistical significance as the fibrosis stage progressed. As illustrated in Fig. 4, TNF-α mRNA levels in F3 patients were significantly higher than those of F1 patients (one-way ANOVA, p < 0.05). IL-6 mRNA levels were too low to evaluate in relation to fibrosis stage: IL-6 was detected in two of 31 subjects with F1 stage, one in 10 (F2), and four in 14 (F3) (χ² test, p < 0.05).
with 4 ng/ml recombinant IL-1β, 50 ng/ml recombinant IL-6, or 0.25 ng/ml recombinant TNF-α at 37°C for 24 h exactly in the same way as HepG2 cells. Our preliminary experiments on p-nitrophenol oxidation using human hepatocyte no. 2 showed that IL-1β and TNF-α almost completely suppressed the activity, whereas IL-6 showed only 44% inhibition despite no appreciable change in mRNA expression. Apparently, regulatory mechanisms of CYP2E1 seemed quite different between HepG2 and human primary hepatocytes. Expressed levels of drug transporters, NTCP, OATP-C, and OCT-1, after normalization by GAPDH, were roughly 100-, 1000-, and 10-fold higher in primary hepatocytes than HepG2, respectively. Our preliminary data suggested IL-6 down-regulation by larger than 45% of CYP1A2, CYP3A4, NTCP, OATP-C, and OCT1 in the hepatocytes of the two human individuals. Our present in vitro study using HepG2 and primary hepatocytes may indicate both systems should be used complementarily to clarify mechanisms for cytokine-mediated down-regulation of drug metabolism and disposition genes.

Discussion

There has been little information published on which genes involved in drug metabolism and disposition undergo alteration of their expression during chronic hepatitis C viral infection. Because data
have been accumulating on substrate specificities and function for a number of cytochromes P450 and drug transporters, it is valuable to elucidate the changes in gene expression for those cytochromes P450 and transporters for which information is available. We have examined chronic hepatitis C-related changes in the expression of drug metabolism and disposition genes using total RNA extracted from liver biopsy samples from 63 Japanese patients who were diagnosed with chronic hepatitis C at Toranomon Hospital, Tokyo, Japan. The patients recruited in this study exhibited a tendency toward higher values of prothrombin times (INR) and hyaluronic acid levels with HCV-related liver fibrosis progression (Fig. 2).

In agreement with these clinical laboratory observations, a remarkable decrease in CYP1A2, CYP2E1, CYP3A4, NTCP, OATP-C, and OCT1 mRNA levels was observed in relationship to liver fibrosis progression (Fig. 3). Hepatic expressions of CYP1A2, CYP2E1, NTCP, and OCT1 in the initial fibrosis stage (F1) of hepatitis C infection seemed to be once up-regulated as compared with non-HCV patients according to the statistical analyses throughout non-HCV and F1 to F3 HCV patients (Fig. 3). This hypothesis should be evaluated by further studies using liver specimens obtained in one hospital. These mRNA levels overall showed negative correlations with clinical laboratory results of aspartate amino transferase, prothrombin time (INR), and hyaluronic acid levels (K. Nakai, unpublished data). However, no fibrosis stage-dependent decrease was observed for any of the other genes studied, including CYP2D6 and MDR1 (plots not shown). Only some of the drug metabolism and disposition gene expressions showed fibrosis-stage dependent decrease. During fibrosis progression, fibrotic hepatocytes would be replaced by fibroblasts. This might lead an assumption that a few hepatic genes would not be simultaneously down-regulated. The present study excludes this most trivial explanation on mechanisms of down-regulation of hepatic genes. In conjunction with the inflammatory state of hepatitis C infection, an increase in the TNF-α mRNA level was manifested with the progress of liver fibrosis staging (Fig. 4).

We found a clear relationship between the decrease in hepatic CYP1A2 expression and fibrosis stage progression (Fig. 3A) and inflammation grading (plot not shown) in patients with chronic HCV infection, which is generally consistent with observations by Congiu et al. (2002). CYP1A2, but not CYP4A, decreased at both the mRNA and protein levels during sepsis progression in the rat. Cytochrome P450 blockade by pretreatment with 1-aminobenzotriazole exacerbated the inflammatory response in sepsis (Crawford et al., 2004). These results, together with the finding that CYP1A2 has a protective role against ROS production (Shertzer et al., 2004), indicate that reduction in CYP1A2 expression during HCV infection state might be deleterious. A number of reports in the literature observed that there is a reduction of CYP2E1 expression in chronic HCV infection (Gochee et al., 2003; Asselah et al., 2005; Bieche et al., 2005). The results of this study are consistent with those reports. CYP2E1 has been documented to have a role in ROS generation during sepsis and ethanol metabolism (Navasumrit et al., 2000). Singlet oxygen (1O2) has been suggested to be involved with CYP2E1 function (Hayashi et al., 2005). Oxidative stress by ethanol has been associated with ethanol-induced and -metabolizing CYP2E1, converting it to more reactive intermediates (Kessova and Cederbaum, 2003). The HCV core protein, in coordination with ethanol, was reported to increase oxidative stress (Wen et al., 2004). The findings using CYP2E1-overexpressing cells have been reviewed
regarding the toxicological properties of CYP2E1 in conjunction with alcohol (Caro and Cederbaum, 2004). Mitochondrial ROS species production is synergistically induced by HCV core protein and CYP2E1, resulting in a reduction of mitochondrial antioxidant capacity and sensitivity to oxidants and TNF-α (Otani et al., 2005). CYP2E1-mediated oxidative stress reportedly down-regulates glucose-regulated proteins 78 and 94 that reside in endoplasmic reticulum and protect endoplasmic reticulum from CYP2E1-dependent oxidative stress (Dey et al., 2006). Taken together, CYP2E1 down-regulation in the chronic hepatitis C-infected liver may be a protective response of liver cells against oxidative stress. The CYP3A4 mRNA titer in blood showed a tendency to decrease with the progression of viral liver disease, which is consistent with our present study (Horiike et al., 2005). Pregnen X receptor, controlling CYP3A4 expression, failed to change in accordance with fibrosis stage progression (K. Nakai, unpublished data). The biological significance of decreases in liver CYP3A4 levels and drug transporters is, at present, difficult to interpret, due to little knowledge of biological function of CYP3A4 and drug transporters and the mechanisms and pathophysiology of chronic hepatitis C.

Genome-wide analyses of gene expression in liver biopsy specimens from patients with mild or early stage fibrosis caused by chronic hepatitis C have been reported. Up-regulation in comparison with normal liver patients of IL-6 and TNF and down-regulation of CYP2E1 was observed as compared with normal liver patients (Asselah et al., 2005; Bièche et al., 2005). It was also shown that IL-6 and TNF levels in patients with liver fibrosis stages 2 to 4 were significantly higher than those in patients with stage 1 fibrosis (Asselah et al., 2005). In our study population, up-regulation of TNF-α was consistent overall with the studies by Asselah et al. (2005), but IL-6 mRNAs were below the limit of detection in many subjects. IL-6 has been shown to down-regulate CYP1A1, CYP1A2, and CYP3A4 in human hepatoma cells (Fukuda et al., 1992) and to down-regulate CYP2E1 (Hakkola et al., 2003) and CYP3A4 (Jover et al., 2002). Abdel-Razzak et al. (1993) showed that IL-6, IL-1β, and TNF-α down-regulated expression of human CYP1A1, 1A2, and 3A in adult human hepatocytes in primary culture. These results were consistent overall with our experiments using HepG2 cells and human primary hepatocytes (Fig. 5). Interferon-γ also suppressed CYP1A2 and CYP2E1 in the human primary hepatocyte system (Abdel-Razzak et al., 1993). Similarly, in human primary hepatocyte cultures, cytokines negatively affected inducible expression of CYP1A1, CYP1A2, and CYP3A4 (Muntané-Relat et al., 1995). With respect to drug transporters, murine Ntcp was shown to be down-regulated by IL-1β in vivo (Geier et al., 2005). Very recently, Le Vee et al. (2008) reported repression of NTCP mRNA and protein expression by IL-1β in human primary hepatocytes. IL-1β was reported to inhibit CAR-induced expression of hepatic CYP3A4 (Assenat et al., 2004). A decrease in CYP3A4 is consistent with the present results, but a decrease in CAR mRNA levels has not been related to fibrosis stage progression (K. Nakai, unpublished data). Gene promoter analysis of human OATP-C expression revealed that HNF1α stimulated OATP-C expression (Jung et al., 2001). HNF1α transcriptional activator did not strikingly correlate with fibrosis staging (K. Nakai, unpublished data). Expression of human OCT1 was activated by HNF4α, according to a study that used a luciferase reporter assay (Saborowski et al., 2006). Expression of HNF4α was also not correlated with fibrosis staging (K. Nakai, unpublished data), which suggests that HNF4α by itself does not fully explain the mechanisms involved in the decrease in OCT1 expression in chronic hepatitis C patients. TNF-α repressed CYP1A2, CYP3A4, and OCT1 expression in HepG2 cells. Our preliminary results showed IL-6 down-regulation of CYP1A2, CYP3A4, NTCP, and OCT1 expression in the human hepatocytes of both two individuals. Our present results on fibrosis stage-dependent decreases in the expression of CYP1A2, CYP2E1, CYP3A4, NTCP, OATP-C, and OCT1 suggest that these decreases were likely correlated, at least in part, by mechanisms associated with the elevated cytokine production of TNF-α. CYP1A2 mRNA levels significantly correlated with CYP2E1 (r = 0.705, p < 0.0001) and CYP3A4 (r = 0.535, p < 0.0001), but not with CYP2D6 (r = 0.231, p > 0.05). In addition, OCT1 mRNA levels significantly correlated with NTCP (r = 0.6341, p < 0.0001), but not with MDR1 (r = 0.231, p > 0.05). These results are consistent with our hypothesis that inflammatory cytokines are involved in the down-regulation of the expression of these genes. The precise molecular mechanisms of fibrosis stage-dependent decreases in the expression of the CYP1A2, CYP2E1, CYP3A4, NTCP, OATP-C, and OCT1 genes governing drug metabolism and transport should be further clarified. The data, providing information on fibrosis stage-associated changes in the gene expression related to hepatic drug metabolism and disposition, are useful for drug therapy for patients with chronic hepatitis C infection. In fact, NTCP reportedly has an important role in hepatic uptake of ursodeoxycholic acid (Mita et al., 2006). Recent report indicated INR of prothrombin time negatively correlated with antipyrene clearance, informative indices for hepatic impairment in hepatitis C patients (Mahmoud et al., 2007). The results were quite consistent with our results because antipyrene was shown to be a substrate for CYP1A2 and CYP3A4 (Rendic, 2002). In conclusion, our results indicate that in the relatively early stages of chronic hepatitis C infection without cirrhosis, factors such as cytokines are likely to affect the expression of drug metabolism enzymes and drug transporters, such as CYP1A2, CYP2E1, CYP3A4, NTCP, OATP-C, and OCT1.

References


