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Effect of Nuclear Receptor Downregulation on Hepatic Expression of Cytochrome P450 and Transporters in Chronic Hepatitis C in Association with Fibrosis Development

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Summary: Analysis of mRNAs from liver biopsy samples of patients with chronic hepatitis C revealed that the levels of nuclear receptor expression were correlated with those of drug-metabolizing enzymes and transporters in relation to the development of fibrosis. Overall, the median mRNA level was largely dependent on fibrosis stage (F), and that for stage 3 patients (F3) was about 50% less than that for F1 patients. Levels of expression of AhR, together with CAR and PXR, were lowest in livers of F3 patients. Multivariate linear regression analysis revealed that AhR expression appeared to be involved in the regulation of CYP1A2, 2E1, 2D6, UGT1A, MDR1/3, MRP2/3, NTCP and OCT1 in the livers of patients with chronic hepatitis C. These results suggest that downregulation of AhR during the progression of liver fibrosis is associated with decreased expression levels of these phase I and II enzymes and drug transporters during inflammation-related signal transduction between AhR and other nuclear receptors.

Keywords: mRNA downregulation; cytochrome P450 and transporters; nuclear receptors; hepatitis C; liver fibrosis

Introduction

Hepatic expression of drug-metabolizing enzymes and transporters, which are heterogeneous in nature and influenced by many factors including genetics, disease and concomitant drug intake, is one of the major determinants of drug elimination capacity. Administration of individual probe drugs that are metabolized by specific cytochromes P450 (CYPs), such as the CYP3A-specific substrate midazolam, make it possible to relate hepatic drug metabolic capacity to liver function parameters using systems such as the Child-Pugh score. Frye *et al.* recently administered probe drugs specific for the drug-metabolizing enzymes CYP1A2, -2C19, -2D6, and -2E1 to patients with various liver diseases,[1)] and showed that liver disease severity had differential effects on their metabolic activity.

The levels of mRNA for several CYPs, such as CYP1A2, -2C, -2D6, and -3A4, but not CYP2E1, are well correlated with their protein levels and activities in patients with liver cirrhosis.[2,3)] Recently, we have reported CYPs and drug transporter mRNA levels in patients with chronic hepatitis C.[4)] As liver fibrosis progressed, the levels of the mRNAs encoding CYP1A2, -2E1, and -3A4, and Na^+-taurocholate cotransporting polypeptide (NTCP), organic anion transporting peptide-C (OATP-C), and organic cation transporter 1 (OCT1) show marked decreases, whereas that of the mRNA encoding tumor necrosis factor-α (TNF-α) increased.[4)]

Long-term hepatitis C virus (HCV) infection induces cellular oxidative stress including an increase of reactive oxygen species (ROS).[5)] Waris *et al.* have reported that HCV infection induces endoplasmic reticulum (ER) stress

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through ER-nucleus signal transduction and activation of STAT-3[6] and nuclear factor kappa B (NFκB), associated with production of proinflammatory cytokines such as TNF-α, after infection with human immunodeficiency virus.[7] It has been proposed that the association of NFκB (RelA) and activated aryl hydrocarbon receptor (AhR) proteins plays a role in the toxicity of dioxin. Furthermore, activation of AhR leads to repression of NFκB-mediated transcription,[8] and AhR-mediated gene expression has been extensively investigated to clarify its biochemical and pathological roles.[9] Induction of CYP1A1 through AhR signaling upon treatment with dioxin is impaired in human hepatocarcinoma cells overexpressing non-structural protein (NS) 5A of HCV.[10,11] Thus, HCV infection and progression of the disease to a severer state probably affects drug metabolism and disposition capacity *via* alterations of nuclear receptor signaling, including AhR. In the present study, we used quantitative real-time PCR analysis to focus on the expression of nuclear factors such as the constitutive androstane receptor (CAR), pregnane X receptor (PXR), hepatocyte nuclear factor 4 α (HNF4α), peroxisomal proliferator-activated receptor α (PPARα), nuclear factor-erythroid 2 p45-related factor 2 (NRF2), glucocorticoid receptor (GR), AhR, aryl hydrocarbon receptor nuclear translocator (ARNT) and NFκB, which were not investigated extensively in our previous study,[4] to further establish their role in the regulation of CYPs and drug transporters in patients with chronic hepatitis C.

Table 1. Patient backgrounds and laboratory data ($n = 49$)

	Mean ± SD	Range
Age (years)	53 ± 10	23–69
Sex (M/F)	24/25	
Smoking	14	
Drinking	14	
HCV(KIU/mL)	725 ± 609	1.2–2,300
HCV genotype (*n*)	1a(1), 1b(32), 2a(10), 2b(6)	
Fibrosis staging (*n*)	F1(27), F2(9), F3(13), F4(0)	
Activity grading (*n*)	A1(28), A2(21), A3(0)	
ALT (IU/L)	130 ± 81	36–414
AST (IU/L)	87 ± 53	23–276
γ-GTP (IU/L)	78 ± 57	81–430
ALP (IU/L)	221 ± 79	81–430
D-Bil (mg/dL)	0.5 ± 0.2	0.2–1.1
Platelets ($\times 10^3$ μL)	165 ± 44	92–267
Albumin (g/dL)	3.9 ± 0.3	3.1–4.6
Cholinesterase ΔpH	1.10 ± 0.28	0.6–2.1
ZTT (KU)	9.5 ± 3.1	3.1–18.3
TTT (KU)	8.3 ± 6.5	0.7–26.9
PT(s)	12.6 ± 1.0	10.2–15.2
AFP (μg/L)	18 ± 41	2–256
Fe (μg/dL)	160 ± 53	20–256
Ferritin (μg/L)	206 ± 254	12–1,378
HA (μg/L)	110 ± 127	15–589

ALT, alanine amino transferase; AST, aspartate amino transferase; γ-GTP, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; D-Bil, direct bilirubin; ZTT, zinc sulfate turbidity test; TTT, thymol turbidity test; PT, prothrombin time; AFP, alpha fetoprotein; HA, hyaluronic acid

Materials and Methods

Subjects: Liver biopsy was performed in 63 patients with chronic hepatitis C at Toranomon Hospital, Tokyo, as reported previously.[4] Fibrosis staging and inflammation grading in these patients were determined according to the New Inuyama Classification by an independent pathologist. The clinicopathological characteristics of the patients are shown in **Table 1**. We also collected clinical laboratory data on the day of liver biopsy, or on the preceding day. Some of the patients were taking prescribed drugs, but we considered these medications unlikely to influence the production of the enzymes under investigation. Eight of the liver biopsy samples were excluded because the RNA had been degraded through use of the Agilent 2100 Bioanalyzer (Agilent Biotechnologies, Japan), and a further 6 samples were excluded because of insufficient volume. The study was conducted in accordance with the Helsinki Declaration, and was approved by the ethics committee of Toranomon Hospital. Informed consent was obtained from all patients.

RNA extraction, reverse transcription, and real-time PCR: For the present study, we measured the levels of mRNA encoding CYP1A1, -2C8, -2C9, -2C19, -2D6, -3A4 and -3A5, and OATP-A, OATP-B, OATP-C, MDR1, MDR3, MRP1 and MRP3, and the newly determined nuclear receptors, CAR, PXR, HNF4α, PPARα, NRF2, GR, AhR, ARNT and NFκB. cDNA from patients was prepared as reported previously.[4] cDNA amplification was performed with an ABI PRISM 7700 Sequence Detection System or 7300 Real Time PCR System (Applied Biosystems, Japan). We used real-time PCR to analyze the levels of mRNAs encoding drug-metabolizing enzymes, transporters, and nuclear-related proteins (**Supplementary Table A**). Messenger RNA levels were normalized relative to those of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and β-actin. Human liver poly (A)+RNA (1 μg/μL, Lot#2070753, Becton Dickinson, Franklin Lakes, NJ, USA) was used as a standard for quantification of each mRNA. cDNA was prepared in the same way as described above, after the poly (A)+RNA had been diluted to 10,000, 2,000, 400, 80, 16, or 3.2 pg/μL. In the real-time PCR reaction, we used 2× TaqMan Universal PCR Master Mix (Applied Biosystems), which is optimized for TaqMan reactions. The primers and probe were designed and manufactured by Applied Biosystems (**Supplementary Table A**). The conditions for the thermal cycler were 50°C for 2 min and 90°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. All amplification reactions were performed in duplicate. If the deviation between the duplicated data was greater than 50%, re-analysis was performed.

Statistical analysis: Statistical analysis was performed using the SPSS program (SPSS, Chicago, IL, USA). The