Changes in the expression of cholesterol metabolism-associated genes in HCV-infected liver: A novel target for therapy?

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Received July 13, 2009; Accepted August 20, 2009

DOI: 10.3892/ijmm_00000299

Abstract. Recent investigations indicate that hepatitis C virus (HCV) infection is closely associated with hepaticocytic lipid metabolism and induces hepatic steatosis. However, the actual lipid metabolism in HCV-infected liver has not been extensively investigated in humans. In this study, we evaluated the expression of lipid metabolism-associated genes in patients with HCV infection by real-time PCR. Sterol regulatory element-binding protein (SREBP)-2 expression was unchanged and low density lipoprotein receptor expression was markedly reduced by 90% in HCV-infected liver. The expression of apolipoprotein B100, microsomal triglyceride transfer protein and ATP-binding cassette G5 was significantly increased. Up-regulation of cholesterol synthesis-associated genes, including HMG-CoA reductase, HMG-CoA synthase, farnesyl-diphosphate synthase and squalene synthase, confirmed enhanced de novo cholesterol synthesis. The expression of cholesterol 7α-hydroxylase and farnesoid X receptor was enhanced, while bile salt export pump expression was unchanged. Fatty acid synthase expression was increased which was accompanied by increased expression of liver X receptor α and SREBP-1c. In summary, the regulation of lipid metabolism was impaired and cholesterol and fatty acid synthesis continued to increase without negative feedback in HCV-infected liver. These changes may be beneficial for HCV replication.

Introduction

A close association between hepatitis C virus (HCV) infection and lipid metabolism was previously reported. For example, the low density lipoprotein receptor (LDLR) is a target for HCV entry into hepatocytes (1,2), therefore, β-lipoproteins influence HCV proliferation. Serum HCV-Ag levels are negatively correlated with serum β-lipoproteins (3) and LDL-cholesterol levels are correlated with the outcome of HCV treatment with interferon (IFN) (4,5). HCV core protein induces hepatic lipid accumulation by activating sterol regulatory element-binding protein (SREBP)-1c (6,7). In addition, liver microsomal triglyceride transfer protein (MTP), a key enzyme for the assembly of very low density lipoprotein (VLDL), may be involved in HCV-related steatosis, and hepatic MTP expression and steatosis showed significant negative correlation in patients with chronic hepatitis C (8-11). Approximately 50% of patients with chronic hepatitis C have hepatic steatosis which enhances disease progression (12-14). Host metabolic factors as well as viral factors should be involved in the pathogenesis of hepatic steatosis. However, the actual lipid metabolism in HCV-infected liver has not been extensively

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Abbreviations: ABC, ATP binding cassette; BSEP, bile salt export pump; CYP7A1, cholesterol 7α-hydroxylase; EPA, eicosapentaenoic acid; FAS, fatty acid synthase; FXR, farnesoid X receptor; HMGR, HMG-CoA reductase; IFN, interferon; LDLR, low density lipoprotein receptor; LXR, liver X receptor; MTP, microsomal triglyceride transfer protein; SREBP, sterol regulatory element-binding protein; VLDL, very low density lipoprotein

Key words: hepatitis C virus, cholesterol, lipid-metabolism, chronic hepatitis, fatty acid
investigated in humans. Therefore, we evaluated the expression of lipid metabolism-associated genes in HCV-infected liver.

**Materials and methods**

Tissue samples were obtained by liver biopsy from 70 patients (males/females, 29/41; age, 56.1±11.5 years old) with chronic hepatitis C (genotype 1b, n=45; 2a/2b, n=25), who were admitted to the Kyushu Medical Center in 2007-2008. For a control, normal liver tissue was obtained from 10 living donors together with VLDL together with triglyceride and apoB100 by MTP. Cholesterol is also secreted into bile via ATP binding cassette (ABC) G5/8. Microsomal triglyceride transfer protein; HMG-CoA reductase; HMG-CoA synthase; FDPS, farnesyl-diphosphate synthase; SS, squalene synthase, NPC1L1, Niemann-Pick C1 like 1; FAS, fatty acid synthase.

**Results**

Expression levels of cholesterol metabolism-associated genes in HCV-infected liver were compared with those in normal controls. The results of real-time PCR are shown in Figs. 1 and 2. Serum LDL-cholesterol is taken into hepatocytes via the LDLR. For secretion, cholesterol is packed into VLDL together with triglyceride and apoB100 by MTP. Cholesterol accumulation in hepatocytes down-regulates SREBP-2 activity, thus decreasing cholesterol synthesis/uptake. Surprisingly, in HCV-infected liver, HMGR expression was increased by >5-fold, while SREBP-2 expression was unchanged (Figs. 1 and 2). In contrast, LDLR expression was markedly reduced by 90% (Fig. 1). The expression of apoB100 and MTP was increased by >3-fold and ABCG5 expression was also increased (Fig. 1). Up-regulation of other cholesterol synthesis-associated genes, including HMG-CoA synthase, farnesyl-diphosphate synthase and squalene synthase, confirmed enhanced de novo cholesterol synthesis (Fig. 2). Cholesterol 7α-hydroxylase (CYP7A1) is a key enzyme involved in bile acid synthesis and its expression is negatively regulated by farnesoid X receptor (FXR). Bile acid is transported into bile by the bile salt export pump (BSEP), whose expression is positively regulated by farnesoid X receptor (FXR). Bile acid is transported into bile by the bile salt export pump (BSEP), whose expression is positively regulated by farnesoid X receptor (FXR). In the HCV-infected liver, CYP7A1 expression was increased by >5-fold, while SREBP-2 expression was unchanged (Fig. 1). The expression of apoB100 and MTP was increased by >3-fold and ABCG5 expression was also increased (Fig. 1). Up-regulation of other cholesterol synthesis-associated genes, including HMG-CoA synthase, farnesyl-diphosphate synthase and squalene synthase, confirmed enhanced de novo cholesterol synthesis (Fig. 2). Cholesterol 7α-hydroxylase (CYP7A1) is a key enzyme involved in bile acid synthesis and its expression is negatively regulated by farnesoid X receptor (FXR). Bile acid is transported into bile by the bile salt export pump (BSEP), whose expression is positively regulated by farnesoid X receptor (FXR). In the HCV-infected liver, CYP7A1 expression was increased by >5-fold, while SREBP-2 expression was unchanged (Fig. 1). The expression of apoB100 and MTP was increased by >3-fold and ABCG5 expression was also increased (Fig. 1). Up-regulation of other cholesterol synthesis-associated genes, including HMG-CoA synthase, farnesyl-diphosphate synthase and squalene synthase, confirmed enhanced de novo cholesterol synthesis (Fig. 2). Cholesterol 7α-hydroxylase (CYP7A1) is a key enzyme involved in bile acid synthesis and its expression is negatively regulated by farnesoid X receptor (FXR). Bile acid is transported into bile by the bile salt export pump (BSEP), whose expression is positively regulated by farnesoid X receptor (FXR).
was enhanced by 4-fold, which was accompanied by increased expression of FXR, while BSEP expression was unchanged (Fig. 1). FAS expression was increased by ~3-fold and was accompanied by increased expression of LXR\textsuperscript{·} and SREBP-1c (Fig. 1).

**Discussion**

Expression pattern of examined lipid metabolism-associated genes in the liver of chronic hepatitis C is summarized in Fig. 3. In our investigation, the regulation of lipid metabolism was impaired in HCV-infected liver. It is probable that HCV infection induces intra-hepatic accumulation of cholesterol, which results in decreased LDL-cholesterol uptake and increased lipoprotein and cholesterol output. Nevertheless, \textit{de novo} cholesterol synthesis and fatty acid synthesis continued to increase without negative feedback (Fig. 2). We cannot explain the phenomena clearly but the same discrepancy was found in nonalcoholic fatty liver disease (15). The expression patterns of the tested genes were also apparent in a preliminary evaluation in an HCV replicon system (data not shown).

These changes seem to be needed or are beneficial for HCV replication. Considering the enhanced cholesterol synthesis in HCV-infected liver, it is plausible that HMG\textsubscript{R} inhibitors (statins) elicit inhibitory effects on viral replication. Statins were recently reported to suppress HCV replication in a clinical trial on peg-IFN plus ribavirin combination therapy, fluvastatin showed synergistic antiviral effects (16,17). In addition, geranylgeranyl-diphosphate and farnesyl-diphosphate, which are produced through the \textit{de novo} cholesterol synthesis pathway, are reported to be essential for viral replication (18). They are needed to activate small GTPases such as Rho and Ras, therefore, HCV may need lipids not only for components of virus particles but also for the modulation of cell signaling pathways. It is also expected that bisphosphonate has antiviral effects because bisphosphonate inhibits farnesyl-diphosphate synthase, the expression of which was enhanced in HCV-infected liver. Therefore, EPA might elicit antiviral effects via the inhibition of SREBP-1c. We are now performing a clinical trial using the lipid modulators, statins, bisphosphonate and/or EPA, in combination with peg-IFN plus ribavirin therapy.
References


