Cell surface expression of LDL receptor in chronic hepatitis C: correlation with viral load

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THE MECHANISM BY WHICH HEPATITIS C VIRUS (HCV) binds to and enters cells appears to be complex (9). Several cellular receptors have been proposed as mediating the entry of HCV into cells, namely the CD81 receptor (19), the scavenger receptor class B type I receptor (23), and the low-density lipoprotein (LDL) receptor (15). Several studies have reported interaction between lipoprotein metabolism and the HCV life cycle (1, 2, 9, 15, 17, 18). It has been suggested that HCV particles associated with lipoproteins represent the species with the highest infectiousness, whereas lipoprotein-free virions are poorly infectious (5, 11). On the basis of in vitro studies, the hypothesis can be made that one of the crucial parameters for viral entry is therefore the LDL receptor at the cell surface of hepatocytes (1, 9, 14).

However, these studies were carried out using cell lines or virus particles that do not mimic natural infection. To our knowledge, there are no data based on human studies about the implication of the LDL receptor in HCV viral replication.

Some studies have reported a higher prevalence of hypocholesterolemia and hypobetalipoproteinaemia in HCV-infected patients compared with control groups (8, 14, 24). Permuter et al. (17) demonstrate that hepatic overexpression of HCV core protein interferes with the hepatic assembly and secretion of VLDL and inhibits microsomal triglyceride transfer protein (MTP) activity. These data show that the effect of HCV core protein on liver very low-density lipoprotein (VLDL) assembly and secretion is the result of marked reduction of MTP function (17). The hypocholesterolemia observed in these subjects may well modify the regulation of LDL-R expression. Indeed, expression of LDL-Rs on the cell surface of hepatocytes is inversely related to the concentration of LDL cholesterol (6).

The purpose of the current study was to determine the cell surface expression of LDL receptors in patients with HCV chronic infection, according to HCV viral load. An association between LDL-R expression and HCV viral load could suggest in human subjects an implication of the LDL-R in the HCV replication cycle.

MATERIALS AND METHODS

Patients. Sixty-eight untreated chronic hepatitis C patients were recruited for this study. Collected data included age, gender, and alcohol use. Body mass index (BMI) was calculated as body weight in kilograms divided by height in meters squared (kg/m²). The study protocol was approved by the Ethics Committees of the University of Dijon, and subjects gave written consent to participate in the present study.

Laboratory determinations. Venous blood samples were taken in the morning after 12 h of overnight fasting, before the liver biopsy or the fibrotest was performed. Plasma glucose concentration was measured by a glucose oxidase method using a Vitros 750 analyzer (Johnson & Johnson Clinical Diagnostics, Rochester, NY). Total cholesterol, triglycerides, HDL-cholesterol, apolipoprotein A1 (apoA1), and apoB were determined in total serum samples using a Cobas Integra 700 analyzer with commercially available kits (Roche Diagnostics Systems, Basel, Switzerland). HDL triglycerides were measured by an enzymatic assay (Triglycerides 25 reagent ABX Diagnostics) after selective precipitation of plasma apoB-containing lipoproteins with phosphotungstic acid and MgCl₂ (Roche Diagnostics). LDL cholesterol was calculated using the Friedewald formula when the triglyceride level was < 4 g/l.

LDL-receptor measurement. LDL receptors were quantified at the surface of mononuclear cells in fresh blood samples taken at 8 AM after fasting. At the same time, blood was taken for the measurement of lipid parameters, glycemia, and HCV viral load. LDL-R expression was quantified on peripheral mononuclear cells isolated from blood.
samples immediately after blood drawing. Erythrocytes were lysed by mixing 1 ml blood with 4 ml of a hypotonic solution containing 0.83% NaCl, 0.004% EDTA, and 0.1% KHCO₃. After a 30-min incubation at 4°C, samples were centrifuged at 300 g for 5 min, and the pellet was washed twice with the lysis solution. The pellet was then suspended in ice-cold PBS with 1% BSA and 0.1% NaN₃. A volume containing 10⁵ cells was then centrifuged, supernatant was removed, and the cells incubated at 4°C for 30 min in the dark with 50 µl FITC-labeled goat anti-mouse IgG diluted 1:50 (QIFIKIT; Dako, Glostrup, Denmark). Again, cells were washed twice with PBS/1% BSA/0.1% NaN₃ and re-suspended in 1 ml of the same solution. Blanks were processed in parallel with the samples and in the same way, except that the anti-LDL receptor antibody was replaced with PBS/1% BSA/0.1% NaN₃. A calibration curve linking the intensity of fluorescence and the number of antigenic sites was established using QIFIKIT. Setup and calibration beads (50 µl each) were washed, as recommended by the manufacturer, and incubated at 4°C for 30 min in the dark with 50 µl goat FITC-labeled anti-mouse IgG diluted 1:50. Then, they were washed and suspended in PBS before flow cytometer analysis.

Measurements were performed on a GALAXY flow cytometer (Dako). The monocyte population was selected in a forward vs. side-scatter window. For each sample, F1 channel fluorescence (FITC) was recorded. Data were analyzed using FluoMax software (Dako). The fluorescence related to specific binding to LDL-Rs was obtained by subtracting the mean fluorescence of the blank from the mean fluorescence of the sample. The number of antigenic sites per cell was calculated from the calibration curve derived from QIFIKIT beads. The coefficient of variation evaluating the repeatability of the assay was 5%.

**Virology.** Serological testing for anti-HCV was carried out using a commercial microparticle enzyme immunoassay (AxSYM HCV version 3.0, Abbott Laboratories, Chicago, IL) according to the manufacturer’s instructions. Patients were tested for viral load by RT-PCR (Amplicor HCV, Roche) followed by reverse hybridization for genotyping (Inno-lipa HCVII; Innogenetics, Swigdrecht, Belgium). Serum samples were diluted for measurement when values were outside the linear range of the method.

**Table 1. Clinical and epidemiological characteristics of HCV patients.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Means</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>68</td>
<td>19</td>
<td>72</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.6±12.4</td>
<td>19</td>
<td>72</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.2±5.0</td>
<td>15.6</td>
<td>38.9</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>48</td>
<td>9</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>3</td>
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<td>2</td>
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<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.77±0.82</td>
<td>3.13</td>
<td>6.66</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>3.00±0.81</td>
<td>1.36</td>
<td>5.32</td>
</tr>
<tr>
<td>Glocemia</td>
<td>5.38±0.89</td>
<td>3.72</td>
<td>8.91</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.30±0.43</td>
<td>0.41</td>
<td>2.34</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.04±0.51</td>
<td>0.35</td>
<td>2.64</td>
</tr>
<tr>
<td>ApoB, g/l</td>
<td>0.83±0.18</td>
<td>0.49</td>
<td>1.44</td>
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<tr>
<td>ApoAI, g/l</td>
<td>1.45±0.34</td>
<td>0.86</td>
<td>2.45</td>
</tr>
<tr>
<td>ASAT</td>
<td>58±34</td>
<td>18</td>
<td>216</td>
</tr>
<tr>
<td>LDL receptor per monocyte</td>
<td>7.246±3.160</td>
<td>1,007</td>
<td>15,377</td>
</tr>
<tr>
<td>Log viral load IU/ml, median</td>
<td>6.21±0.67</td>
<td>4.66</td>
<td>7.67</td>
</tr>
</tbody>
</table>

BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ApoB and ApoA1, apolipoprotein B, A1, respectively; ASAT, aspartate aminotransferase.

**Results.** Statistics. Results were expressed as means ± SD. Comparisons between groups were made using the Student’s t-test or the Mann-Whitney U-test for continuous variables. Comparisons between different groups were performed using ANOVA. Statistical correlations were determined by the nonparametric Spearman test. Probability levels lower than 0.05 were considered significant. Normality of continuous variables was assessed. LDL-R expression was distributed normally and so was HCV viral load after log transformation. Normality having been presumed as established, a multiple linear regression was performed on LDL-R expression per monocyte.

**RESULTS**

**Characteristics of patients.** The main clinical and laboratory data are summarized in Table 1: 32 males (mean age: 42.2 ± 10.3) and 36 females (mean age: 48.6 ± 13.5) were studied. The mean BMI was 25.2 ± 5.0 (range: 15.6 ± 38.9 kg/m²): 25.6 ± 5.4 in males and 24.9 ± 4.6 in females. Fibrosis was determined by liver biopsy for 49 patients, and by fibrotest for 19 patients. Fibrosis grade 0–1 was found in 37 patients (54.4%), grade 2 in 18 patients (26.4%), grade 3 in 6 patients (8.8%), and grade 4 in 7 patients (10.2%). Forty-eight patients (70.5%) were infected by HCV genotype 1, nine patients (13.2%) by genotype 2, nine patients (13.2%) by genotype 3, and two patients (2.9%) by HCV genotype 4.

**Monocytes LDL-receptor expression.** LDL-R expression was negatively correlated with total cholesterol (r = −0.28, P = 0.01), with LDL cholesterol (r = −0.25; P = 0.03) (Fig. 1), and with apolipoprotein B (apoB) level (r = −0.25; P = 0.04). LDL-R expression was not correlated with age (r = 0.14, P = 0.24), BMI (r = 0.22, P = 0.06), HDL cholesterol (−0.07, P = 0.51), triglyceride level (r = 0.09, P = 0.42), apoA1 level (r = 0.11, P = 0.36), or glycemia (r = 0.14, P = 0.23). LDL-R expression was positively correlated with HCV viral load (r = 0.37, P = 0.002), (Fig. 2). The median of HCV viral load was 1967,300 IU/ml. Patients with HCV viral load under the median had a lower monocyte LDL-R expression than patients with HCV viral load over the median (5,854 ± 2,932 vs. 8,639 ± 2,772, P = 0.0005). HCV viral load was not correlated with HDL cholesterol (r = −0.16, P = 0.18), LDL cholesterol (r = −0.04, P = 0.71) or triglyceride level (r = 0.07, P = 0.55). LDL-R expression was not significantly correlated with age (r = 0.24, P = 0.02), BMI (r = 0.29, P = 0.005), or HCV genotype (r = −0.23, P = 0.03).
Fig. 2. Correlation between HCV-viral load [log (IU/ml)] and LDL-receptors per monocyte ($r = 0.37; P = 0.002$).

different in patients with fibrosis scores 0 and 1 than in patients with score 2 (6,740 ± 2,972 vs. 7,945 ± 3,432; $P = 0.27$) or with scores 3/4 (6,740 ± 2,972 vs. 7,720 ± 3,286; $P = 0.31$). LDL-R expression was not significantly different in patients with necroinflammatory activity scores 0 and 1 than in patients with scores 2 and 3 (7,139 ± 3,001 vs. 7,252 ± 3,550; $P = 0.95$). The correlation between LDL-R expression and aspartate aminotransferase (ASAT) level was border-significant in all the subjects ($r = 0.21$, $P = 0.08$) and was significant in the group of patients without cirrhosis ($n = 61$), ($r = 0.29$, $P = 0.02$). In the group of patients without cirrhosis, LDL-R expression was significantly different in patients with fibrosis score 0 compared with patients with scores 2/3 (5,646 ± 3,213 vs. 8,189 ± 3,406; $P = 0.02$) and marginally with patients score 1 (5,646 ± 3,213 vs. 7,146 ± 2,833; $P = 0.10$).

Factors associated with monocyte LDL-R expression. In multivariate analysis, the predictive variables for monocyte LDL-R expression were HCV viral load and LDL cholesterol whereas gender, BMI, and age were not (Table 2).

**DISCUSSION**

In this study, we demonstrated that HCV viral load was associated with LDL-R expression in mononuclear cells. These data strongly suggest that one of the crucial parameters for HCV cell entry could be the LDL receptor. In vitro, the importance of the LDL receptor in HCV binding was demonstrated by increasing viral binding and entry, under conditions that led to upregulation of the LDL-R (26). Consistent with these findings, we found in our in vivo study that HCV-infected patients with an HCV viral load over the median had a higher LDL-R expression per monocyte compared with patients with a viral load under the median.

Liver, but not mononuclear cell, LDL receptors play a key role in LDL-cholesterol catabolism in vivo. However, several studies have shown that LDL-R measurement on mononuclear cells is physiologically relevant for the determination of LDL-receptor status. Indeed, in human mononuclear cells, LDL-receptor gene expression has been demonstrated to parallel and be coordinately regulated to gene expression in human and rabbit livers (20, 22). Moreover, the number of LDL receptors on mononuclear cells has been shown to be susceptible to the effect of well-recognized modulators of LDL receptor numbers such as saturated fatty acids and dietary cholesterol (16, 22). It is noteworthy that we performed LDL-R measurements on freshly isolated mononuclear cells; therefore, our results are likely to reflect an in vivo situation.

There are several reports indicating that infectious HCV particles in plasma are associated with lipoproteins and that binding of HCV to the LDL-receptor may be mediated by LDL (2, 15, 26). Andre et al. (2) showed that the upregulation of LDL-receptor expression significantly increased the internalization of purified HCV RNA-containing particles. Conflicting results were reported about the role of the LDL-R on cellular entry of HCV (3, 7, 14). The LDL receptor is a plausible candidate receptor for hepatitis C virus in certain in vitro studies according to several authors (1, 15). Whereas, others provide evidence that the LDL receptor is not sufficient to mediate HCV cell entry (4). Recently, results obtained using human hepatocytes inoculated with serum-derived HCV virions, support the notion that the LDL receptor plays a role at an early stage of virus entry (14). The discrepancies between these studies are probably due to the fact that all these studies were carried out using cell lines or virus pseudoparticles that do not mimic natural infection. Here, we provide new data from a human study, suggesting that LDL-R expression in HCV-infected patients is associated with the level of HCV viral replication. Recently, a study has indicated that functional apolipoprotein E gene polymorphism may be a determinant of outcome in HCV infection (21). In this study, apolipoprotein E2 allele is associated with an increased likelihood of viral clearance. Interestingly, the E2 allele is associated with defective binding to the LDL receptor; hence, these results could suggest that such defective binding may result in poor uptake of HCV lipoviral particles by the LDL receptor into hepatocytes (21). This study shows that the LDL receptor plays an important role in HCV entry, as suggested by our data. However, in our study, univariate analysis shows that HCV viral load variations only explain 13% of LDL-R expression variability. This means that other factors in addition to HCV receptors play an important role in HCV viral replication.

The potential involvement of the LDL receptor in HCV infection has implications for the therapy currently used for HCV infection. It has been suggested that the antiviral effect of interferon alpha may be mediated in part by the down-regulation of LDL receptors (1). Recently, it has been shown that the lipoprotein level could be a prognostic factor regarding response to treatment by interferon and ribavirin.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient</th>
<th>95% CI</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI &gt;27</td>
<td>1.30</td>
<td>-0.45</td>
<td>3.06</td>
</tr>
<tr>
<td>Age &gt;45</td>
<td>0.03</td>
<td>-0.03</td>
<td>0.09</td>
</tr>
<tr>
<td>Female gender</td>
<td>0.83</td>
<td>-0.66</td>
<td>2.33</td>
</tr>
<tr>
<td>Genotype 1</td>
<td>-0.34</td>
<td>-1.88</td>
<td>1.20</td>
</tr>
<tr>
<td>Log HCV viral load</td>
<td>0.79</td>
<td>0.32</td>
<td>1.26</td>
</tr>
<tr>
<td>Fibrosis &gt;1</td>
<td>0.72</td>
<td>-0.88</td>
<td>2.34</td>
</tr>
<tr>
<td>LDL-cholesterol, g/l</td>
<td>-3.04</td>
<td>-5.37</td>
<td>-0.72</td>
</tr>
</tbody>
</table>

HCV, hepatitis C virus; CI, confidence interval.
Indeed, LDL and cholesterol levels before treatment were found to be higher in patients with positive early viral response, end-of-treatment response, and sustained viral response. The LDL-receptor is responsible for the binding and subsequent cellular uptake of apolipoprotein B- and E-containing lipoproteins. By controlling LDL catabolism, the number of hepatic LDL receptors directly governs plasma LDL cholesterol concentrations. Expression of LDL receptors on the cell surface of hepatocytes is inversely related to the concentration of LDL cholesterol (6). Our results presenting a negative correlation between LDL-R expression and LDL cholesterol are consistent with this concept. In the multivariate regression analysis, we found that LDL-R expression was associated with HCV viral load whatever the LDL cholesterol level. These results suggest that the relationship between LDL-R expression and viral load is not a simple consequence of the hypolipoproteinemia secondary to the severity of HCV infection. We can argue that the good prognostic factor regarding viral treatment response of high LDL cholesterol concentration, observed by Gopal et al. (10), could be the consequence of low LDL-R expression. Thus, a prospective study is needed to investigate the association between serum lipid level, HCV viral load, LDL-R expression, and response to pegylated interferon and ribavirin treatment in patients with chronic hepatitis C.

An in vitro study has demonstrated that the combination of interferon and statins exhibit strong inhibitory effects on HCV replication (12). These authors propose that therapy combining statins with interferon may be effective for the treatment of patients with chronic hepatitis C. However, the complexity of the interaction between HCV and lipoprotein metabolism should discourage immediate extrapolation of these findings to clinical practice (13). Indeed, the use of HMG-CoA reductase inhibitors is associated with an up-regulation in LDL receptors, and when examining our findings for a correlation between HCV viral load and LDL-receptor expression, we cannot exclude that the use of statins could lead to an upregulation of HCV replication. Our results, suggesting the important role of the LDL receptor in HCV replication, do not recommend immediate use of statins in patients with HCV infection without careful prospective study.

In conclusion, our data provided by a human study suggest that the LDL receptor may be one of the receptors implicated in HCV replication. Prospective studies are needed to investigate the influence of LDL-R expression on the response to antiviral therapy in chronic hepatitis C patients treated with peginterferon plus ribavirin.

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GRANTS

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REFERENCES


