Significance of Hepatic Insulin Clearance in Patients with Chronic Hepatitis C and Non-alcoholic Fatty Liver Disease

Hisamitsu Miyaaki, Tatsuki Ichikawa, Naota Taura, Satoshi Miuma, Takuya Honda, Hidetaka Shibata, Kan Toriyama and Kazuhiko Nakao

Abstract

Objective  Hyperinsulinemia plays an important role in the pathophysiological processes of chronic hepatitis C (CHC) and non-alcoholic fatty liver disease (NAFLD). However, there are few reports on hepatic insulin clearance in patients with these diseases.

Methods  A total of 74 CHC patients and 37 NAFLD patients were enrolled in this study. We evaluated their hepatic insulin clearance, insulin sensitivity and β-cell function with an oral glucose tolerance test.

Results  Hepatic insulin clearance in the patients with CHC was significantly correlated with platelets (r=0.271, p=0.020) and liver fibrosis (r=-0.234, p=0.045) and was significantly affected by both steatosis (mild: 0.157±0.078, severe: 0.114±0.053, p=0.024) and fibrosis (mild: 0.167±0.0857, severe: 0.125±0.052, p=0.010). There were no significant differences in (homeostasis model assessment) HOMA-β among steatosis and fibrosis stages. In the NAFLD patients, those with severe fibrosis had significantly reduced hepatic insulin clearance (mild: 0.135±0.045, severe: 0.098±0.031, p=0.013) and significantly increased HOMA-β (mild: 115.6±67.1, severe: 172.8±65.7, p=0.018) compared with the patients with mild fibrosis.

Conclusion  Liver fibrosis development is associated with hepatic insulin clearance in both the CHC and NAFLD patients.

Key words: insulin clearance, NAFLD, chronic hepatitis C

(Intern Med 55: 1049-1054, 2016)  
(DOI: 10.2169/internalmedicine.55.5288)

Introduction

The liver is one of the most important organs for metabolizing glucose and lipids. Therefore, liver disease is associated with metabolic factors, including glucose metabolism and lipid metabolism. In particular, non-alcoholic fatty liver disease (NAFLD) and hepatitis C virus (HCV) infection have common features, such as insulin resistance and hepatic steatosis (1-5). Insulin resistance results from a dysregulation of insulin secretion and clearance. Regarding insulin secretion, in some previous reports, HCV impaired β-cell function, which resulted in hyperinsulinemia (6, 7).

The liver is the primary site of insulin clearance. In advanced fibrotic liver disease, insulin clearance decreases (8), indicating that hepatic damage may affect insulin clearance in the liver. However, decreased insulin clearance is also associated with obesity (9) and type 2 diabetes (10). Previous data have shown that liver fat deposition and free fatty acid impair insulin clearance (10-12). However, there are few published studies that have examined hepatic insulin clearance in patients with HCV. In this study, we examined the influence of liver disease extent on hepatic insulin clearance and secretion in patients with HCV or NAFLD.

Materials and Methods

Patients

Our retrospective study included 74 patients with chronic hepatitis C (CHC) who were admitted to our hospital between January 2008 and December 2011 and fulfilled the
criteria specified below. All subjects were interferon treatment-naïve, exhibited positive HCV viremia and were diagnosed with chronic hepatitis based on histological findings. Additionally, 37 consecutive patients with biopsy-confirmed NAFLD were also included. Liver ultrasonography revealed increased echogenicity, suggestive of steatosis, and a liver biopsy confirmed NAFLD as the only explanation for the elevated aminotransferase levels. The diagnosis of NAFLD was based on the presence of steatosis (>10%), an ethanol intake of less than 20 g per week (as confirmed by the physician and family members) and the appropriate exclusion of other liver diseases, such as alcoholic liver disease, viral hepatitis and autoimmune hepatitis. The patients with type 2 diabetes had to be on a stable dose of their antidiabetic medications. To avoid the confounding effect of end-stage liver disease on the interpretation of the insulin clearance, we excluded the patients with collateral formation.

Written informed consent was obtained from each patient and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki (1975).

**Oral glucose tolerance test and insulin clearance**

All subjects with CHC underwent an oral glucose tolerance test (OGTT) with 75 g of glucose according to the recommendations of the National Diabetes Data Group of the National Institutes of Health. At 8:00 AM, after a 10- to 12-h overnight fasting period, subjects received 75 g of glucose orally. Blood samples were taken 0, 30, 60, 90 and 120 minutes after administration to measure plasma glucose, insulin and C-peptide concentrations. For estimating hepatic insulin clearance, we used the C-peptide: insulin area under the curve ratio calculated by the trapezoidal rule during OGTT, as previously described (13-15).

**β-Cell function**

The homeostasis model assessment (HOMA-β) was developed to validate a hyperglycemic clamp, and the early insulin response (Δ-insulin/Δ-glucose 30) after a glucose load was used as a marker of early insulin secretion.

**Definition of obesity, hypertension and dyslipidemia**

Obesity, hypertension (HT) and dyslipidemia were diagnosed based on the criteria of the appropriate Japanese societies (Japan Society for the study of Obesity, The Japanese Society of Hypertension and Japan Atherosclerosis Society). Obesity was defined as a body mass index (BMI) of more than 25 kg/m². Hypertension was defined as a blood pressure of 140/90 mmHg or higher, or if any drug was being taken to treat HT. Dyslipidemia was defined as a low-density lipoprotein level of 140 mg/dL or higher, triglyceride level of 150 mg/dL or higher, or if any drug was being taken to treat dyslipidemia.

**Histopathological examination of the liver**

Ultrasound-guided liver biopsy specimens were fixed in 10% formalin, embedded in paraffin, cut to a thickness of 4 μm and stained with hematoxylin-eosin and Azan. All liver tissue specimens were evaluated by two pathologists who were unaware of the clinical condition of the patients.

The liver histology in the CHC patients was evaluated according to the degree of necroinflammatory activity and steatosis. The necroinflammatory activity (grading) was classified as follows: A1 (mild), A2 (moderate) and A3 (severe).

The liver histology in the NAFLD patients was evaluated according to the degree of fibrosis, necroinflammatory activity and steatosis. Steatosis was graded on a scale of 1 to 3. Grade 1 was defined as mild steatosis (11-33% of the hepatocytes affected), grade 2 was moderate (34-66% of the hepatocytes affected) and grade 3 was severe (>66% of the hepatocytes affected). Necroinflammation was graded on a scale of 0 to 3. Grade 0 was defined as the absence of intracanalicular inflammation or ballooning, grade 1 was mild inflammation and minimal ballooning, grade 2 was moderate inflammation and ballooning in zone 3 and grade 3 was severe inflammation and marked ballooning in zone 3 (16).

The extent of fibrosis (staging) was classified as follows: F1 (periportal expansion), F2 (portalportal septa), F3 (portal-central linkage or bridging fibrosis) and F4 (cirrhosis).

**Statistical analysis**

The data were analyzed using the Student’s t test. Spearman’s rank correlation coefficient was used to analyze the correlation. A multivariate analysis was performed using multiple linear analyses. Analyses were performed using the SPSS statistics software program, version 20 (SPSS Inc., Chicago, USA).

**Results**

**Clinical characteristics of the patients with HCV and NAFLD**

Table 1 summarizes the clinical characteristics of the patients with HCV and NAFLD.

**Factors correlated with hepatic insulin clearance in the patients with CHC and NAFLD** (Table 2)

There were no significant differences in hepatic insulin clearance in the CHC patients with or without HT (HT: non-HT=0.149±0.050:0.137±0.046, p=0.408), or obesity (obesity: non-obesity=0.150±0.124:0.151±0.053, p=0.985) or dyslipidemia (DL: non-DL=0.155±0.066:0.149±0.075, p=0.775).

There were also no significant differences in hepatic insulin clearance in the CHC patients regardless of HCV genotype (genotype 1:2=0.143±0.054:0.171±0.109, p=0.299).

Hepatic insulin clearance in the patients with CHC showed significant correlations with platelets (r=0.271, p=
Table 1. Clinical Characteristics of the Patients with the Hepatitis C Virus (n=74).

<table>
<thead>
<tr>
<th></th>
<th>HCV n=74</th>
<th>NAFLD n=37</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.8±10.3</td>
<td>55.4±15.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.6±4.2</td>
<td>26.5±4.3</td>
</tr>
<tr>
<td>Obesity</td>
<td>16 cases</td>
<td>21 cases</td>
</tr>
<tr>
<td>Hypertension</td>
<td>17 cases</td>
<td>14 cases</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>17 cases</td>
<td>16 cases</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>46.2±27.2</td>
<td>95.6±72.6</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>55.5±34.7</td>
<td>67.1±45.1</td>
</tr>
<tr>
<td>Platelets (10^4/μL)</td>
<td>16.7±6.3</td>
<td>20.2±5.5</td>
</tr>
<tr>
<td>HCV viral load (log IU/mL)</td>
<td>6.00±0.98</td>
<td></td>
</tr>
<tr>
<td>Steatosis (%)</td>
<td>5.15±9.8</td>
<td>1.95±0.88</td>
</tr>
<tr>
<td>Necroinflammatory grade</td>
<td>1.15±0.488</td>
<td>1.35±0.78</td>
</tr>
<tr>
<td>Fibrosis stage (0-4)</td>
<td>1.66±1.44</td>
<td>1.78±1.35</td>
</tr>
</tbody>
</table>

NAFLD: non-alcoholic fatty liver disease, BMI: body mass index, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HCV: hepatitis C virus. Data are presented as the mean±SD.

0.020) and liver fibrosis (r=0.234, p=0.045), but not with age, BMI, aspartate aminotransferase (AST), alanine aminotransferase (ALT), steatosis or HCV viral load.

There were no significant differences in hepatic insulin clearance in the NAFLD patients with or without HT (HT: non-HT=0.115±0.033:0.117±0.038, p=0.886), or obesity (obesity:non-obesity=0.118±0.041:0.114±0.029, p=0.715) or dyslipidemia (DL: non-DL=0.120±0.047:0.114±0.024, p=0.641).

Hepatic insulin clearance in the patients with NAFLD showed significant correlations with liver fibrosis (r=0.337, p=0.042), but not with age, BMI, AST, ALT, platelet or steatosis.

**Hepatic insulin clearance by histology group in the patients with CHC (Table 2), (Fig. 1)**

The patients with ≥10% steatosis had a significant reduction in hepatic insulin clearance when compared with the patients with less than 10% steatosis (0.114±0.053 vs. 0.157±0.078, p=0.024).

There was no significant difference in hepatic insulin clearance by necroinflammation group (mild A0, 1, 0.155±0.081; severe A2, 3, 0.125±0.039; p=0.184).

The patients with severe fibrosis had significantly reduced hepatic insulin clearance compared with the patients with mild fibrosis (F0/1, 0.167±0.0857; F2-3, 0.125±0.052, p=0.010).

According to a multivariate analysis, fibrosis was the only independent factor for hepatic insulin clearance (β=-0.256, p=0.026).

**Hepatic insulin clearance by histology group in the patients with NAFLD (Fig. 2)**

There were no significant differences in hepatic insulin clearance by steatotic group (<33%, 0.118±0.030; ≥33%, 0.125±0.052, p=0.661) or by necroinflammation grade (G0/1, 0.127±0.397; G2/3, 0.111±0.052; p=0.288).

The patients with severe fibrosis had a significant reduction in hepatic insulin clearance, when compared with the patients with mild fibrosis. (F0/1, 0.135±0.045; F2-3, 0.098±0.031; p=0.013).

According to a multivariate analysis, fibrosis was the only independent factor for hepatic insulin clearance (β=-0.424, p=0.015).

**Correlations with HOMA-β in the patients with CHC (Table 3)**

There were no significant differences in HOMA-β in the CHC patients with or without HT (HT: non-HT=113.8±88.9:143.5±87.7, p=0.216), or obesity (obesity:non-obesity=153.4±66.2:131.7±93.3, p=0.300) or dyslipidemia (DL: non-DL=117.2±58.2:141.2±94.1, p=0.222).

There were no significant differences in HOMA-β in the CHC patients regardless of HCV genotype (genotype 1: 2=137.3±86.6:133.8±86.6, p=0.890).

HOMA-β in the patients with CHC showed a significant correlation with age (r=0.271, p=0.0079), but not with BMI, AST, ALT, platelet (PLT), steatosis or HCV viral load.

**HOMA-β by histology group in the patients with CHC**

There were no significant differences in HOMA-β by steatotic group (<10%, 132.6±91.2; >10%, 150.9±73.6; p=0.449), by grade (G0/1: 145.4±92.4; G2/3, 94.7±51.4; p=0.062) or by stage (F0-2, 127.4±84.5; F2/3, 147.4±93.2, p=0.348).
Correlations with HOMA-\(\beta\) in the patients with NAFLD (Table 3)

There were no significant differences in HOMA-\(\beta\) in the NAFLD patients with or without HT (HT: non-HT=119.5±73.9:150.1±68.9, \(p=0.228\)), or obesity (obesity:non-obesity=149.4±64.9:120.6±79.8, \(p=0.273\)) or dyslipidemia (DL: non-DL=142.2±70.9:134.6±73.6, \(p=0.760\)).

HOMA-\(\beta\) in the patients with NAFLD did not show significant correlations with any clinical factors.

HOMA-\(\beta\) by histology group in the patients with NAFLD

There were no significant differences in hepatic HOMA-\(\beta\) by steatotic group (<33%, 120.3±51.9; ≥33%, 146.2±81.4; \(p=0.629\)). The patients with severe necroinflammatory activity had significantly increased HOMA-\(\beta\) compared with the patients with mild activity (A0/1, 114.28±56.9; A2/3, 180.42±79.5; \(p=0.020\)). The patients with severe fibrosis had a significantly increased HOMA-\(\beta\) level compared with the patients with mild fibrosis (F0-2: 115.6±67.1; F3/4, 172.8±
Table 3. Correlations with HOMA-β in the Patients with the Hepatitis C Virus (n=74) and NAFLD (n=37).

<table>
<thead>
<tr>
<th></th>
<th>Chronic hepatitis C</th>
<th>NAFLD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correlation coefficient</td>
<td>p value</td>
</tr>
<tr>
<td>Age</td>
<td>-0.309</td>
<td>0.007</td>
</tr>
<tr>
<td>BMI</td>
<td>0.075</td>
<td>0.525</td>
</tr>
<tr>
<td>AST</td>
<td>0.106</td>
<td>0.367</td>
</tr>
<tr>
<td>ALT</td>
<td>0.192</td>
<td>0.104</td>
</tr>
<tr>
<td>Platelet count</td>
<td>-0.077</td>
<td>0.514</td>
</tr>
<tr>
<td>HCV viral load</td>
<td>0.058</td>
<td>0.624</td>
</tr>
<tr>
<td>Steatosis</td>
<td>-0.018</td>
<td>0.876</td>
</tr>
<tr>
<td>Fibrosis stage</td>
<td>0.003</td>
<td>0.979</td>
</tr>
</tbody>
</table>

NAFLD: non-alcoholic fatty liver disease; BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; HCV: hepatitis C virus

65.7; p=0.018).

Discussion

Although insulin resistance is a major issue for HCV and NAFLD patients with glucose intolerance, previous studies have not demonstrated a relationship between the histopathological factors and hepatic insulin clearance. The plasma insulin level is regulated by insulin secretion and its clearance. Hyperinsulinemia is an important pathophysiological factor and previous studies have reported that it was associated with cardiovascular disease (17) and hepatocellular carcinoma (HCC) (18). Moreover, our previous study showed that hyperinsulinemia affected the clinical course of early stage HCC (19).

A recent study in patients with NAFLD suggested that even mild hepatic steatosis impaired hepatic insulin clearance (15). In the CHC patients, our data also suggested that the patients with more than 10% steatosis had reduced hepatic insulin clearance. The patients with HCV may have two main types of liver steatosis. One type is associated with metabolic syndrome. These patients usually have comorbid high BMI, hyperlipidemia and type 2 diabetes. The other type is HCV-induced steatosis that develops in the absence of obesity. The replication of HCV correlates with the severity of steatosis (20, 21). However, in our study, the extent of steatosis was not significantly correlated with BMI (data not shown). Therefore, in our patients, HCV may have induced mild liver steatosis and impaired insulin clearance.

However, for the NAFLD patients, insulin clearance did not show any significant differences in the steatosis group. The diagnosis of NAFLD was based on the presence of steatosis (>10%). As noted in a previous study, such mild hepatic steatosis (>10%) reduced insulin clearance (15).

To avoid the confounding effects of end-stage liver disease on the interpretation of the insulin and insulin resistance data, we excluded the patients with Child-Pugh classifications of B and C. However, in the HCV patients, hepatic insulin clearance was inversely associated with fibrosis stage. Moreover, hepatic insulin clearance significantly decreased in the severe fibrosis group. Conversely, HOMA-β, which indicated the cell function, showed no significant differences in the fibrosis group. We previously showed that the development of liver fibrosis in patients with HCV was associated with insulin resistance (22). Taken together, these results suggest that hyperinsulinemia and insulin resistance in severe fibrosis patients with CHC were caused by insulin clearance impairment. In the present study, the NAFLD patients with severe fibrosis also showed significantly reduced insulin clearance compared with those with mild fibrosis. Moreover, HOMA-β in the NAFLD patients was significantly increased in the patients with severe fibrosis when compared with those with mild fibrosis. Therefore, insulin resistance in the NAFLD patients appears to have been caused not only by insulin clearance impairment but also by the hypersecretion of insulin in β-cells.

Insulin clearance in the NAFLD patients with severe fibrosis was significantly increased compared to that in the HCV patients with severe fibrosis. Insulin clearance was impaired by adipocytokines secreted in adipocytes (10). In our study, the NAFLD patients showed more severe steatosis than did the CHC patients, thus suggesting that the differences in steatosis might affect hepatic insulin clearance.

In conclusion, we demonstrated that the development of liver fibrosis is associated with hepatic insulin clearance in the patients with CHC or NAFLD. Further studies are needed to determine the underlying cause of this association.

The authors state that they have no Conflict of Interest (COI).

References

2006.

© 2016 The Japanese Society of Internal Medicine
http://www.naika.or.jp/imonline/index.html