ELSEVIER

Journal of Hepatology 49 (2008) 702–710

Journal of Hepatology

www.elsevier.com/locate/jhep

Patients with chronic hepatitis C achieving a sustained virological response to peginterferon and ribavirin therapy recover from impaired hepcidin secretion☆

Naoki Fujita[1,*], Ryosuke Sugimoto[1], Satoshi Motonishi[2,3], Naohisa Tomosugi[2], Hideaki Tanaka[1], Masaki Takeo[1], Motoh Iwasa[1], Yoshinao Kobayashi[1], Hisao Hayashi[3], Masahiko Kaito[1], Yoshiyuki Takei[1]

[1]*Department of Gastroenterology and Hepatology, Division of Clinical Medicine and Biomedical Science, Institute of Medical Sciences, Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu, Mie 514-8507, Japan*

[2]*Division of Clinical Science, Medical Research Institute, Kanazawa Medical University, Kanzawa, Japan*

[3]*School of Pharmacy, Aichi Gakuin University, Aichi, Japan*

See Editorial, pages 680–685

***Background/Aims*: The aim of this study is to determine the clinical relevance of hepatic producing iron regulatory hormone-hepcidin, on iron overload in patients with chronic hepatitis C (CHC).**

***Methods*: Serum hepcidin was measured in 73 CHC patients by surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF-MS), and compared to those of healthy controls and anemia of inflammation patients, and analyzed their relationship to hepatic hepcidin mRNA expression levels and clinical, hematological, and histological findings. The sequential changes of hepcidin were investigated in 27 CHC patients treated with a 48 week-course of pegylated-interferon (PEG-IFN) plus ribavirin therapy.**

***Results*: Serum hepcidin was positively correlated with hepatic hepcidin mRNA levels, serum ferritin and the degree of hepatic iron deposition in CHC. Serum hepcidin-to-ferritin ratios were significantly lower in HCV positive patients than in HCV negative controls in both hyper- and normal-ferritinemic conditions. This relative impairment of hepcidin production was fully reversible after successful HCV eradication by PEG-IFN plus ribavirin, concomitantly with the improvement of the iron overload condition.**

***Conclusions*: The impairment of hepatic hepcidin production occurring with chronic HCV infection may enhance iron toxicity and lead to disease progression, and modulation or supplementation of hepcidin may be beneficial for these conditions in CHC.**

***Keywords*: Chronic hepatitis C; Iron; Iron-regulated genes; Interferon; Ribavirin; Surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF-MS); Real-time PCR**

Received 12 January 2008; received in revised form 30 April 2008; accepted 1 May 2008; available online 6 June 2008

Associate Editor: Y.M. Deugnier

☆ The authors declare that they do not have anything to disclose regarding funding from industries or conflict of interest with respect to this manuscript.

* Corresponding author. Tel.: +81 59 231 5017; fax: +81 59 231 5223.

E-mail address: nfujita@clin.medic.mie-u.ac.jp (N. Fujita).

Abbreviations: CHC, chronic hepatitis C; SELDI-TOF-MS, surface-enhanced laser desorption/ionization time of flight mass spectrometry; PEG, pegylated; IFN, interferon; HCV, hepatitis C virus; AI, anemia of inflammation; SD, standard deviation; CHCA, α-cyano-4-hydroxy-cinnamic acid; AU, arbitrary units; PCR, polymerase chain reaction; TIS, total iron score; SVR, sustained virological responders; IL, interleukin; LPS, lipopolysaccharide.

0168-8278/$34.00
doi:10.1016/j.jhep.2008.05.014

1. Introduction

Hepatitis C virus (HCV) infection is a major global health problem, affecting more than 170 million people worldwide [1]. Although the causative factor responsible for the initiation of hepatic disease processes in patients with chronic hepatitis C (CHC) is a single insult, i.e. HCV infection, it has become increasingly evident that the involvement of cofactors is critical in determining disease progression. Importantly, CHC often appears to be associated with disturbances in iron homeostasis, with serum ferritin and hepatic iron stores being elevated in approximately 50% of patients [2–4]. Because iron is a redox-active metal, catalyzing free radical reactions via the Fenton reaction, and because there is substantial evidence to implicate redox active mechanisms in the pathogenesis of CHC [5], iron has been highlighted as an important element affecting the natural history of CHC. However, little is known about the mechanism of excess iron accumulation during chronic HCV infection.

Recently, hepcidin, a 25 amino acid antimicrobial peptide hormone [6,7], has been shown to play a central role in the regulation of iron homeostasis [8]. Hepcidin is mainly secreted by the liver [6,7] and induces internalization and degradation of the iron exporter ferroportin in absorptive enterocytes and reticuloendothelial cells, thereby inhibiting iron absorption from the intestine and iron release from reticuloendothelial cell stores [9]. Hepcidin expression is induced by iron loading, thus providing a feedback mechanism to limit further iron absorption [6,8]. Hepcidin is also recognized as a key mediator of anemia of inflammation (AI) [10]; characterized by impaired intestinal iron absorption, reticuloendothelial cell iron sequestration, and hypo-ferremia and hyper-ferritinemia [11].

Previously, using liver biopsy samples, we demonstrated that hepatic hepcidin mRNA expression levels were relatively low in CHC, compared to chronic hepatitis B [12], suggesting that hepcidin may be involved in the iron overload in chronic HCV infection. But liver biopsy cannot be performed ethically on patients without liver disease, and cannot be carried out repeatedly, even on CHC patients. Thus, comparison of hepatic mRNA expression levels of hepcidin between HCV positive and negative cases, and evaluation of sequential changes after HCV eradication by interferon (IFN)-based therapy, cannot be determined. Recently two reports described the use of surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF-MS) for the semiquantitative determination of hepcidin in human serum [13,14]. Therefore, using this assay, we measured the serum hepcidin levels in patients with CHC and compared them to normal subjects and patients with AI, as the normal and positive controls for serum hepcidin. The sequential changes of serum hepcidin also were investigated in patients who cleared HCV infection after a 48-week course of pegylated (PEG)-IFN plus ribavirin combination therapy.

2. Patients and methods

2.1. Patients

A total of 73 consecutive patients with CHC, who were hospitalized at Mie University Hospital between June 2005 and June 2006, were enrolled in this study (Table 1). The diagnosis of chronic HCV infection was based on the consistent detection of serum anti-HCV antibody (the third-generation EIA; Ortho Diagnostic Systems, Raritan, NJ, USA) and HCV RNA (Amplicor-HCV assay; Roche Molecular Diag. Co., Tokyo, Japan). None of the patients complicated with acute inflammatory disorders and received phlebotomy in the year preceding the study. All patients showed absence of the HFE mutations C282Y or H63D. Serum HCV RNA titer was quantified by the Amplicor monitor assay (Roche Molecular Diag. Co.). We also recruited patients with AI, who were hospitalized between August 2005 and June 2006, as a control for high serum hepcidin levels. Ten AI patients attributed to various pathologies (five suffered from rheumatoid arthritis, two from malignant lymphoma, and one from systemic lupus erythematosus, pyelonephritis, and pulmonary tuberculosis, respectively) participated in the study. We also studied a group of age-matched healthy volunteers ($n = 10$) with normal serum iron status, no signs of anemia, liver disease, or inflammation, and without HCV infection as controls for normal serum hepcidin levels between April 2006 and June 2006. Serum ferritin levels were measured by RPHA method (Ohthuka assay Co. Ltd., Tokushima, Japan) in this study. The upper limit of normal-ferritin was defined as 170 ng/mL for males and 120 ng/mL for females by the value of mean ±3 standard deviations (SD) obtained using the screening serum samples of Japanese volunteers ($n = 1562$, 823 males and 739 females) (unpublished data). This normal range of ferritin deviates from the more universally used upper reference values of 300 ng/mL for males and 200 ng/mL for females, and may be a distinctive feature of Japanese population. Blood samples of all these subjects were collected on the morning under fasting conditions, and sera were separated immediately after blood collection and stored at −80 °C until use. Informed consent was obtained from each patient included in the study and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a *priori* approval by the Ethical Committee of Mie University.

2.2. Semi-quantitative assay for serum hepcidin using the SELDI-TOF MS system

We performed serum hepcidin measurement by SELDI-TOF MS system between October 2006 and February 2007 (storage period of serum samples was 5–19 months), as reported previously, with some modification [13]. In brief, 40 μL of the diluted serum samples was applied onto an IMAC 30 chip array (Ciphergen Biosystems, Freemont, CA, USA) and incubated at room temperature for 30 min. The chip arrays were washed and air-dried, and 0.5 μL α-cyano-4-hydroxy-cinnamic acid (CHCA) was added twice to the array surface, and arrays were analyzed with the Chiphergen ProteinChip Reader (model PBS II; Chiphergen Biosystems). The mass–charge ratio (m/z) of each of the proteins/peptides captured on the array surface was determined according to the externally calibrated standards: Arg8-vasopressin (1084.25 Da), somatostatin (1637.9 Da), dynorphin (2147.5 Da), bovine insulin β-chain (3495.95 Da), human insulin (5807.65 Da), bovine ubiquitin (8564.8 Da), and bovine cytochrome *C* (12,230.9 Da). Serially diluted synthetic hepcidin-25 (Peptide Institute, Osaka, Japan) was used as a quantitative control. The data from the peak intensities were normalized with total ion current using Biomarker Wizard (Ciphergen ProteinChip Software 3.1.1; Chiphergen Biosystems) to compensate for the variations in sample concentrations loaded onto a spot; the data were represented as arbitrary units (AU) calculated using this software.