derived ROS may act as common intracellular signaling molecules for hepatic fibrogenesis of HSCs in human chronic liver diseases.

In the present study, we used DPI as an inhibitor of NAD(P)H oxidase. However, a high concentration of DPI has known to inhibit flavoprotein enzymes including NAD(P)H oxidase, mitochondrial oxidases, xanthine oxidase, and cyclooxygenase.[26] To ensure that the effect of DPI (25 μmol/L) actually reflected NAD(P)H oxidase inhibition, we examined whether the observed chemiluminescence was sensitive to enzyme inhibitors of mitochondrial oxidases, xanthine oxidase, and cyclooxygenase. In intact HSCs, we found that PDGF-BB–induced ROS production was insensitive to KCN, an inhibitor of mitochondria oxidases, allopurinol, an inhibitor of xanthine oxidase, and indomethacin, an inhibitor of cyclooxygenase. PDGF-BB–induced HSCs proliferation was also inhibited by DPI but not by allopurinol and indomethacin. Moreover, apocynin, another type of NAD(P)H oxidase inhibitor, inhibited PDGF-BB–mediated ROS production and HSC proliferation. Therefore, the experimental results obtained from DPI at a concentration of 25 μmol/L reflected the inhibition of NAD(P)H oxidase.

Previous studies have shown that PDGF positively regulates HSC proliferation by indirectly activating ERK via the activation of Ras in culture systems.[27,28] Our results show that ERK is involved in PDGF-mediated HSC proliferation, independently of its association with oxidative stress. However, activation of ERK alone did not sufficiently induce HSC proliferation. Our results showed that p38 MAPK, a redox-sensitive MAPK, was more closely linked to PDGF-induced HSC proliferation than was ERK. These results suggest strongly that in relation to PDGF-NAD(P)H oxidase–mediated HSC proliferation, p38 MAPK is a growth stimulator. Recently, it was proposed that p38 MAPK plays functional roles in HSC activation and proliferation.[29,30] Our results that p38 MAPK plays an important role in HSC proliferation is inconsistent with these reports.

Phosphatidylinositol 3-kinase or Na^+/K^+ exchanger activation have also been reported to be involved in PDGF-induced HSC proliferation.[31,32] Phosphatidylinositol 3-kinase associates with phosphotyrosine residue of PDGF receptor (src homology 2 domains) and is considered to be relevant to PDGF-mediated HSC proliferation.[31] Although we did not show the data, we found that LY294002, an inhibitor of phosphatidylinositol 3-kinase, suppressed NAD(P)H oxidase activity. We therefore consider that phosphatidylinositol 3-kinase is located upstream of NAD(P)H oxidase. Oxidative stress is known to increase Na^+/K^+ exchanger activation, and thus NAD(P)H oxidase–derived ROS may induce HSC proliferation through Na^+/K^+ exchanger activation. Nuclear receptors such as peroxisome proliferator–activated receptors are also thought to be involved in PDGF-induced HSC proliferation.[27,30] The expression of peroxisome proliferator–activated receptor β was reported to be stimulated by p38 MAPK. The results of the present study reveal that the PDGF/NAD(P)H oxidase/ROS/p38 MAPK activation pathway may mediate HSC proliferation and may form a network linking the already well-established pathways that mediate PDGF-induced HSC proliferation.

Our *in vitro* results revealed that NAD(P)H oxidase–derived ROS play an important role in PDGF-mediated HSC proliferation. NAD(P)H oxidase–derived ROS are also thought to be involved in angiotensin II–mediated liver fibrosis.[25] Furthermore, it has been reported that NAD(P)H oxidase is involved in the transforming growth factor β1 signaling pathway in various types of cells.[33] To explore the possibility that the findings for HSCs can also be adapted to an *in vivo* model of hepatic fibrosis, we performed the *in vivo* experiments using mice with chronic liver injury. An experiment involving an *in vivo* animal experiment of hepatic fibrosis induced by DMN showed that Mn-TBAP, an intracellular ROS scavenger, and DPI, an inhibitor of NAD(P)H oxidase, significantly suppressed the activation and proliferation of HSCs. Although further study using more selective and specific drugs will be required, our preliminary *in vivo* findings suggest that NAD(P)H oxidase and NAD(P)H oxidase–derived ROS might constitute a pathway for activation and proliferation of HSCs, consistent with the *in vitro* findings. Recently, carriers for the targeting of specific drugs to activated HSCs have been under intense investigation.[34] By controlling the delivery and release of drugs within activated HSCs *in vivo*, drugs such as antioxidants, NAD(P)H oxidase inhibitors, or small interfering RNA for enzymatic components of NAD(P)H oxidase may be successfully targeted to activated HSCs, providing a novel therapy for chronic liver diseases.

In conclusion, we have investigated the mechanisms underlying PDGF-BB–mediated HSC proliferation using LI-90 cells and primary cultured HSCs. In this study, PDGF-BB activated NAD(P)H oxidase, resulting in the generation of ROS. NAD(P)H oxidase–derived ROS then activated p38 MAPK and induced the proliferation of HSCs. An *in vivo* hepatic fibrosis model also supported the critical role of NAD(P)H oxidase in the activation and proliferation of HSCs. We have clarified a pathway that mediates the proliferation of HSCs: PDGF/NAD(P)H oxidase/ROS/p38 MAPK.

Acknowledgment: The authors thank Dr. S. Imajoh-Ohmi (The Institute of Medical Science, University of Tokyo) for providing anti-human $p21^{phox}$, $gp91^{phox}$, $p47^{phox}$, and $p67^{phox}$ antibodies and the Human Science Cell Bank (Saitama, Japan) for providing the human hepatic stellate cell line LI-90. The authors also thank the editors and reviewers for their helpful comments and suggestions.

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In October 2002, an extended left hepatic lobectomy was carried out. The cut surface of the resected specimen showed a green-brown mass measuring 5.0 cm in diameter, with a well-encapsulated margin that had several small daughter nodules in the periphery in the S5. In addition, there was an irregularly bordered white mass in S4, measuring 1.0 cm in diameter. We carried out an intraoperative pathologic examination of a lymph node at the hepatic portal region in order to discern the indications for extensive lymphadenectomy; intrahepatic cholangiocarcinoma (ICC) could not be completely excluded preoperatively. Because tumor cells were not recognized in the presented lymph node, only hepatoduodenal ligament lymphadenectomy was added to the hepatectomy. Microscopically, the S5-tumor was moderately differentiated HCC, whereas the S4-tumor was moderately differentiated ICC. Neither continuity nor transitional images were recognized between the two tumors, and a diagnosis of double primary cancer was made. The non-cancerous portion of the liver showed liver fibrosis.

HCC and ICC can be recognized in the same liver as primary hepatic cancer; this is defined as combined hepatocellular and cholangiocarcinoma.[1,2] Allen and Lisa classed these tumors in three categories: (i) separate nodules of hepatocellular and bile duct carcinoma; (ii) contiguity with intermingling; and (iii) intimate association as a result of origininating from the same focus.[3] The incidence of combined hepatocellular carcinoma and cholangiocarcinoma (HCC–CC) is only 0.54% among histologically verified primary liver cancers.[4] Combined, or mixed, HCC and ICC are sometimes encountered in resected livers, but cases of double cancer of HCC and ICC are extremely rare. Previous reports produced only 17 cases of double primary liver cancer that were synchronously resected.

We analyzed 18 reported cases, including the present case, of double primary liver cancer resected synchronously (Table 1). The average age of the patients was 63.6 ± 7.8 (mean ± standard deviation) years old, and all patients were male. In hepatitis virus serology, the percentages of HBsAg-positive and HCV-positive patients were 11.8% and 73.3%, respectively. Analysis of the reported cases showed that 83.3% of patients had chronic hepatitis, liver fibrosis or cirrhosis in the tissue of the non-cancerous portion of the liver. It was found that 66.7% of patients had a high level of serum AFP, and the serum CA 19–9 level and CEA level was high in 50.0% and 25.0% of patients, respectively. Analysis of the reported cases found that 88.9% and 11.6% had HCC in the right and the left lobe, respectively, whereas 61.1% and 38.9% had ICC in the right and the left lobe, respectively. Furthermore, five cases (27.7%) had separate masses of double cancer in different lobes. We examined the preoperative diagnoses in these 18 cases. Including intrahepatic metastasis, 83.1% of the patients were diagnosed as HCC preoperatively, and 5.1% were diagnosed as ICC. In only two cases, one using a needle biopsy and one using images of several examinations, were separate tumors correctly diagnosed preoperatively as double cancer of HCC and ICC. Thus, the findings of the present study suggest that in cases where HCC might be clinically doubtful because serum CA 19–9 level is high and serum AFP level is comparatively low, the possibility of double liver cancer should be taken into consideration.

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DIRECT CYTOPATHIC LIVER INJURY AND ACUTE RESPIRATORY DISTRESS SYNDROME ASSOCIATED WITH GILLIAM-TYPE TSUTSUGAMUSHI DISEASE

To the Editor,

Tsutsugamushi disease is an infectious disease caused by *Rickettsia tsutsugamushi*, which is transmitted by the bite of the tsutsugamushi, a type of tick. There are two types of this disease in Japan; the 'new type' Gilliam strain, which has a good natural course and occurs in autumn to spring, and the 'classical type' Kato-strain, which varies from mild to severe and occurs in summer.[1] Clinical features of high fever, exanthema, severe malaise, myalgia and an eschar with a black crust make this disease relatively easy to identify. In most cases, the disease is not problematic if treated early with the correct antibiotics. However, some cases can become serious with complications including acute respiratory distress syndrome (ARDS), meningitis, myocarditis and pericarditis.[1]

A 73-year-old man suffering from high fever, nausea and right upper-abdominal pain for 10 days visited a local clinic. He was prescribed antibiotics and non-steroidal anti-inflammatory drugs. As the high fever continued after medication, he was transferred to our university hospital for further examination and treatment. On admission, his body temperature was 39.0°C. There were some small eruptions on his left upper-arm.

The patient's abdomen was tender over the right upper-quadrant with Murphy's sign. Emergency laboratory tests showed white blood cell count 12,700/μL, platelet count 9.7×10^4/μL, albumin 2.2 g/dL (normal range, 3.8–4.9 g/dL), total bilirubin 1.7 mg/dL (normal range, 0.3–1.3 mg/dL), alanine aminotransferase (ALT) 301 IU/L (normal range, 4–30 IU/L), alkaline phosphatase (ALP) 942 IU/L (normal range, 39–125 IU/L), γ-glutamyltranspeptidase (γ-GTP) 1243 IU/L (<45), lactate dehydrogenase 1046 IU/L (normal range, 202–357 IU/L), and C-reactive protein 12.9 mg/dL. Both hepatitis B virus surface antigen and hepatitis C virus antibody were negative. Sustained high fever, right hypochondralgia and high serum levels of ALP and γ-GTP mimicked the clinical features of acute obstructive suppurative cholangitis (AOSC). However, computed tomography and endoscopic retrograde cholangiopancreatography results were normal. When it was learned that he had visited a mountain area a few weeks before developing the fever, we re-examined his extremities and found an eschar with a black crust, about 15 mm diameter, over the left internal femoral region. *Rickettsia tsutsugamushi* disease was highly suspected and minocycline 200 mg was given immediately. The indirect immunofluorescent antibody test for *Rickettsia tsutsugamushi* showed an IgM titer of 1:1280 to the Gilliam strain, 1:80 to the Karp strain and 1:20 to the Kato strain. The Weil–Felix test for Proteus OXK was also positive. These results confirmed the diagnosis of Gilliam-type tsutsugamushi disease.

Two days after admission, the patient suddenly complained of dyspnea and wheezing. His arterial oxygen saturation was 35 mmHg on room air, and a chest X-ray film showed bilateral diffuse infiltration suggesting ARDS. Because his respiratory state was worsening, he was intubated and placed on ventilator support with positive end expiratory pressure. Bronchoalveolar lavage fluid had a cell count of 56×10^4/mL, which included 16% macrophages, 4% neutrophils, 80% lymphocytes, and no eosinophils or basophils. We diagnosed lymph-alveolitis and administered a high dose of methylprednisolone, he subsequently recovered from his respiratory distress.

After recovery, an echo-guided liver biopsy was carried out on the 30th hospital day. This showed no aggregation of lymphocytes and fibrotic changes in the portal area, but degeneration of the bile duct epithelium (Fig. 1A). Only focal necrosis of hepatocytes was evident; we did not observe inflammatory changes in the hepatic lobules (Fig. 1B). There was no evidence of granuloma or vasculitis. *Rickettsia tsutsugamushi* could not be detected in the liver by microscopic observation with Giemsa staining and immunofluorescence staining.

Severe hepatic dysfunction related to this disease has been reported only rarely.[2,3] Elevation of serum ALT level was observed in 77% of patients,[2] few reports have described high elevation of ALP or γ-GTP levels. Microscopic findings of the liver have varied, and reports of degeneration of the bile duct epithelium are rare.[4] Previous reports of the human liver histology of this disease have included granulomatous hepatitis,[3] acute viral hepatitis-like changes, and non-specific reactive hepatitis.[5] However, as the day on which the biopsy samples were obtained differed in these reports, and each report described only a single patient, the characteristic *Rickettsia tsutsugamushi*-related liver histology remains unclear. According to Pongponratn *et al.*,[4] *Rickettsia tsutsugamushi* in the sinusoid first makes direct contact with the cell membrane, then after endocytosis, penetrates to the cytoplasm, leading to damage to the organelles. Finally, cytoplasmic vacuolation occurs. In the patient in the present study, only liver cell necrosis in the lobules and degeneration of the bile duct were seen by biopsy on the 30th hospital day; inflammatory changes were not observed. The liver function parameters at that time were still not improved: aspartate aminotransferase 35 IU/l (normal range, 13–33 IU/l), ALT 71 IU/l, γ-GTP 74 IU/l, and did not become normalized until two months later. We believe that the liver histology obtained from the patient in the present study partly supported the mechanism of liver injury in this disease. These findings suggest that, especially in the case of marked elevation of ALP and γ-GTP levels, liver dysfunction associated with *Rickettsia tsutsugamushi*

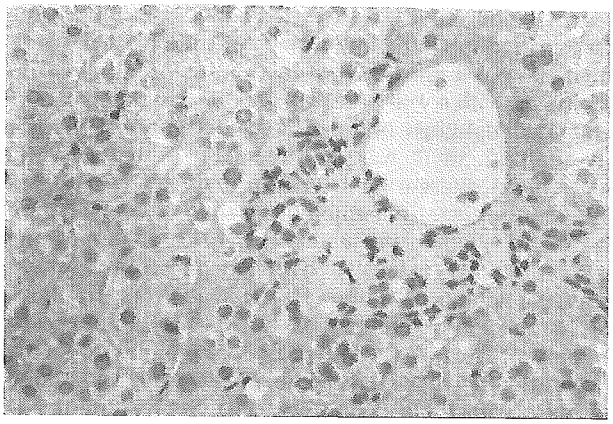

Figure 1 In the portal area, some eosinophilic infiltration and degeneration of the bile duct epithelium were observed, but there was no apparent aggregation of lymphocytes or fibrotic change (HE; original magnification, ×200).

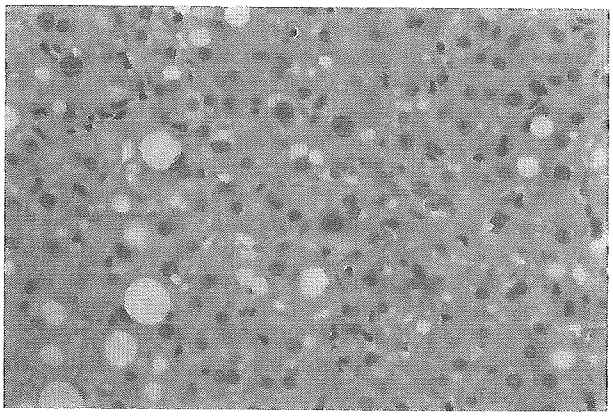

Figure 2 Only liver cell necrosis was seen in the hepatic lobules. (HE; original magnification, ×200)

infection is not a result of immunoreaction, but of direct hepatocyte injury.

Immunity to *Rickettsia tsutsugamushi* is believed to be serotype specific,[1] therefore, it is important to know the serotype of the organism. The Gilliam strain appears not to be lethal, in contrast to the Kato strain, which can easily progress to a serious state. The patient in the present study had a life-threatening Gilliam strain infection, which usually has a good clinical course. By bronchoalveolar lavage examination, it was shown that lymph-alveolitis, not intestinal pneumonia, was the major feature in this case of *Rickettsia tsutsugamushi*-related pulmonary disease, and a high dose of methylprednisolone proved to be effective. Respiratory failure caused by ARDS and requiring supportive mechanical ventilation has rarely been described in previous studies. Therefore, if it is diagnosed and treated early, respiratory distress might not develop in this disease. Previously reported pathological findings in the lung, heart, and brain are focal vasculitis and perivasculitis of the small blood vessels.[1] To our knowledge, no previously reported study of *Rickettsia tsutsugamushi* disease has evaluated both liver and lung pathology in the same patient. In the present case, we were unable to obtain more information to indicate whether injuries to both organs were based on the same mechanism. This is because we could not carry out a lung biopsy for ethical reasons. The possibility remains that the target epitopes of *Rickettsia tsutsugamushi* differ between the liver and the lung, so both humoral and cellular immunological responses might differ among organs. We had no choice but to give the patient a high dose of methylprednisolone for relief of severe respiratory distress, and this might have changed the pathological features of the liver biopsy sample obtained. Further histological and immunological studies of large numbers of patients infected with *Rickettsia tsutsugamushi* are needed.

In conclusion, the present case indicates that serum ALP or γ-GTP levels are markedly dominant, resembling AOSC or drug-induced hepatitis. The mechanism of this liver dysfunction might be a result of a direct cytopathic effect on hepatocytes rather than immunoreaction as in infections caused by other hepatitis viruses. Prompt diagnosis, timely antimicrobial therapy and intensive supportive care are important in order to avoid ARDS and other life-threatening complications. The present case provides useful information for understanding the pathogenesis of tsutsugamushi disease.

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HEPATOLOGY RESEARCH
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Editorial

Expression of angiotensin II type I receptor in human cirrhotic livers: Its relation to fibrosis and portal hypertension

Recent evidence indicates that angiotensin II (ANG II), a potent vasoconstrictor peptide, is involved in the key events of the inflammation process [1]. A large number of clinical trials and experimental studies have demonstrated the benefits of ANG II blockade for the inflammatory disease of several organs, such as heart, kidney, lung, and liver. In particular, the regulation of liver fibrosis has gained attention recently, and the understanding of its mechanism is desired from the point of view of its ultimate results: liver cirrhosis and/or hepatocellular carcinoma. In this issue of the journal, Ikura et al. [2] reported the precisely investigated expression pattern of ANG II type I receptor (AT1) in liver specimen and discussed the mechanisms of liver fibrosis and portal hypertension in human cirrhotic liver.

Considerable progress has been made toward the understanding of how ANG II regulates the tissue fibrogenesis and also local blood flow. ANG II, the major effecter peptide of the renin–angiotensin system (RAS), is generated mainly by cleavage of its precursor ANG I by angiotensin converting enzyme (ACE). RAS is well known as an adaptive mechanism to regulate systemic circulatory homeostasis. It is also evidently demonstrated that the local RAS is involved in various biological phenomena, such as alteration of hemodynamics and vascular permeability, initiation of inflammation, and tissue repair and remodeling [1]. Thus, ANG II is supposed to be recognized as the key mediator of inflammation, and a substantial amount of information on its physiological effects in the inflammation process is available from previous investigations both in vitro and in vivo. Concerning fibrosis, which is the final step of the inflammatory cascade process, available evidences indicate that ANG II participates intensively in cell growth, angiogenesis, and matrix synthesis thorough the regulation of transforming growth factor β (TGF β), platelet-derived growth factor (PDGF), plasminogen activator-1, and other cytokines [1,3].

In the liver, hepatic stellate cells (HSCs) play an important role in the regulation of intrahepatic vascular resistance and liver fibrosis. The prevalence of the two types of ANG II receptor, AT1 and AT2, are known to depend on cell type. Binding studies showed that the receptor of ANG II in human HSCs was predominantly of the AT1 type [4]. It was also demonstrated that ANG II acts as a mitogenic factor for HSCs through AT1, resulting in HSC contractility and proliferation. Quiescent stellate cells are activated after liver injury mainly through the effect of TGF β, PDGF, and endothelin-1 (ET1) [3]. Then, activated HSCs would be stimulated to increase the number of TGF β-1 receptors on the cell surface, and be induced to generate TGF β-1 by themselves in autocrine manner. TGF β-1 is, as is well known, one of the most important stimulators for matrix synthesis in the cascade of ANG II mediated fibrosis. Thus, ANG II not only initiates the inflammatory process through the increasing of vascular permeability and the activating of inflammatory cells, but also induces matrix synthesis directly through HSC activation. On the other hand, activated HSCs also express the receptor of several vasoactive factors, such as ET1 and vasopression, that increase intrahepatic resistance to blood flow, resulting in portal hypertension [5,6]. Taken together, HSCs are supposed to be one of the most important cell targets of the pathogenetic action of ANG II for liver fibrosis and portal hypertension.

In one of the most outstanding studies regarding regulation of liver fibrosis by ANG II blockade, Terui et al. reported a clinical trial using an AT1 antagonist in patients with early stages of hepatic fibrosis of chronic hepatitis C [7]. The results suggest that AT1 antagonist may have a beneficial role in the early stage of hepatic fibrosis. On the other hand, in the case of advanced hepatic fibrosis, the effect of ANG II seems to be more complicated and the benefit of ANG II blockade for portal hypertension is still controversial [8,9]. More precise information on the ANG II effecting reactions in cirrhotic liver is necessary to understand the mechanism of ANG II mediated regulation of portal hypertension in human cirrhotic livers.

Ikura et al., in this issue of the journal, reported the following precisely investigated features of cirrhotic liver specimens: (1) AT1-positive vessels distinctly increased in fibrous septa; (2) both SMA and AT1-positive mesenchymal cells, which were presumably activated HSCs and myofibroblasts, especially located at the marginal portion of fibrous septa; (3) the AT1 positivity on hepatocytes was weakened and less

1386-6346/$ – see front matter
doi:10.1016/j.hepres.2005.03.006

frequent than in normal liver. These results are coincident with the previous studies [10,11]. Vollmar et al. reported that the spatial distribution of Ito cells (HSCs) changes from a homogeneous distribution to a zonal redistribution, mainly to fibrotic septa, during the development of fibrosis in experimental rat liver [10]. Moreover, similar results of the receptor expression pattern were observed in the case of PDGF, which is one of the most potent mitogen for HSCs. Immunostaining assay for PDGF receptor subunits showed that the expression of PDGF receptor subunits were increased in mesenchymal cells and stromal cells within fibrous septa in cirrhotic human liver [11]. These results are useful information to understand the regulation of liver fibrosis, but also it would help greatly to understand the detail of the regulation of local blood flow and pressure. Authors tried to analyze the relationship between AT1 expression and portal hypertension in this paper. Although the pressure of portal vein was not measured directly in the study, the amount of AT1-positive vessels was significantly greater in cirrhotic patients with esophageal varices than those without varices, suggesting that ANG II is involved in the key event of regulating blood flow through AT1 in liver cirrhosis.

Another interesting finding in this article is the redistribution of chymase-positive mast cells in the liver. The number of chymase-positive mast cells in cirrhotic livers significantly increased than that in normal livers. Furthermore, chymase-positive cells were localized in fibrous septa, which are supposed to be the key area of local RAS action. Chymase mimics an action of ACE converting ANG I to ANG II. A possible interpretation is that redundant, and also active, pathways of generating ANG II exist in fibrotic septa in human cirrhotic liver.

Despite the previous randomized trial of long-term administration of an AT1 antagonist [9], it did not significantly decrease portal pressure in patients with liver cirrhosis. Accumulated evidence, including this study in this issue, indicates that ANG II certainly involves in the key events of regulating blood flow in cirrhotic liver. It will be interesting to know why AT1 antagonist administration does not decrease portal hypertension. Currently, the beneficial effects of AT1 antagonist administration on liver fibrosis have gained more attention. Does AT1 antagonist really reduce the risk of progression to liver cirrhosis and eventually prevent the occurrence of hepatocellular carcinoma? Hopefully, we can foresee the answer of above question in the next few years.

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4 March 2005

Available online 28 April 2005

Available online at www.sciencedirect.com
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Hepatology Research 33 (2005) 14–18
HEPATOLOGY RESEARCH
http://www.elsevier.com/locate/hepres

Reduction of serum ghrelin concentration during interferon-α therapy in patients with chronic hepatitis C

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Received 10 March 2005; received in revised form 16 June 2005; accepted 29 June 2005
Available online 19 August 2005

Abstract

The efficacy of interferon (IFN) therapy for chronic hepatitis C is dependent on compliance. Anorexia is an important adverse effect in determining compliance. To clarify the mechanisms underlying anorexia, the level of ghrelin was determined during therapy. Fourteen patients with chronic hepatitis C received IFN-α2b with or without ribavirin (Rib+ or Rib− group; $n=7$ in each group) for 24 weeks. Serum ghrelin concentrations and body weight were determined before, 2 and 24 weeks after initiation of therapy. Serum ghrelin concentrations and body weight significantly decreased 2 weeks after initiation of therapy ($P=0.0008$ and 0.0062, respectively), and then returned to the level before therapy. The Δghrelin concentration correlated with Δbody weight after 2 weeks ($r=0.726$, $P=0.023$). Percentage reduction of serum ghrelin was significantly higher in the Rib+ group than in the Rib− group ($P=0.046$). Percentage reduction in body weight tended to be higher in the Rib+ group ($P=0.057$). IFN-α2b therapy causes short-term reduction of serum ghrelin and body weight, and this may occur to a greater extent with combination therapy. Reduction of serum ghrelin might contribute partly to anorexia, leading to weight loss.

Keywords: Ribavirin; Hormone; Anorexia; Body weight; Weight loss; Adverse effect

1. Introduction

Treatment using interferon (IFN)-α forms the basis of all effective regimens against HCV [1,2], and combination therapy with ribavirin is more effective against HCV than IFN-α alone [3,4]. However, patients receiving IFN-α therapy often experience such upper abdominal side effects as anorexia, discomfort, nausea, and vomiting. Hence, to enhance the efficacy of IFN-α therapy, improvement of compliance is very important.

Ghrelin is a circulating hormone synthesized in the stomach [5]. It is the endogenous ligand for the growth hormone secretagogue receptor, which is expressed in arcuate nuclei and in other hypothalamic and brain stem nuclei [6,7]. Ghrelin levels are highest in the fasting state, rising sharply prior to eating and then falling within one hour of a meal [8]. Ghrelin peaks are of similar magnitude before each meal of the day, and it is thought that ghrelin might be involved in initiation of eating [9].

Ghrelin potently stimulates food intake following peripheral administration in humans [10] and rats [11–13]. The injected ghrelin potently stimulates feeding in rodents, with the maximum effect being seen within one hour of administration [13]. Moreover, the dose that stimulates feeding results in similar circulating ghrelin levels to those seen after a 24-h fasting period [14], suggesting that ghrelin regulates day-to-day food intake. Chronic peripheral ghrelin administration leads to a significant increase in cumulative food intake, and to body weight gain [10,11,14]. Changes in ghrelin during IFN-α therapy have not been previously studied. Hence, in the present study, we measured changes in serum ghrelin levels and body weight during IFN-α2b therapy alone and in combination with ribavirin.

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doi:10.1016/j.hepres.2005.06.008

2. Patients and methods

2.1. Patients

Fourteen patients (nine males and five females) with chronic hepatitis C were enrolled in the study. Informed consent was obtained from each patient, and ethical committee approval was obtained before the study was undertaken. The patients were between 39 and 72 years of age (median age, 58) (Table 1). All patients were positive for anti-HCV antibody, as determined by a second-generation enzyme-linked immunosorbent assay (Ortho Diagnostic Systems, Raritan, NJ). HCV RNA in serum was detected by a quantitative polymerase chain reaction (PCR) assay (Amplicor HCV v2.0; Roche Diagnostics, Tokyo, Japan). The HCV genotype was 1b in 11 patients, 2a in 1, and 2b in 2. The median of the HCV RNA titer was 620 kIU/ml. Each patient received 6 million units (MU) of IFN-α2b every day for 2 weeks and then three times a week for a further 22 weeks. Seven patients of the 14 subjects consecutively received 800 mg of ribavirin per day orally for 24 weeks in combination with IFN-α2b (Rib+ group). The other seven patients consecutively received IFN-α2b alone (Rib− group).

On the day before initiation of IFN-α2b therapy, and on the 14th day and the last day of therapy, serum samples were obtained between 7:00 and 8:00 a.m. after an overnight fast. Body weight was determined at the same time.

2.2. Ghrelin assay

Serum immunoreactive ghrelin concentrations were measured in duplicate using a commercial radioimmunoassay kit (LINCO Research, St. Charles, MO). The range of detection level was 93–6000 pg/ml when using a 100 μl sample size.

2.3. Statistical analysis

Changes in serum ghrelin and body weight during IFN-α therapy were compared using a paired t-test. $P<0.05$ was chosen as the level of significance.

Table 1
Baseline characteristics in patients with chronic hepatitis C who underwent interferon-α2b therapy with or without ribavirin

Variable	With ribavirin	Without ribavirin	P
Patients no.	7	7	NS
Gender (male/female)	6/1	3/4	NS
Age (years)	54.7 ± 12.8	59.7 ± 10.1	NS
Body weight (kg)	66.7 ± 11.3	60.2 ± 15.7	NS
BMI	23.1 ± 4.8	22.9 ± 4.2	NS
HCV genotype			
1b/2a/2b	7/0/0	4/1/2	NS
HCV RNA (kIU/ml)	595 ± 282	294 ± 375	NS
Liver histology			
A0/A1/A2/A3	0/2/2/3	0/1/5/1	NS
F0/F1/F2/F3/F4	1/1/0/5/0	0/1/3/3/0	NS

Data were expressed as mean ± standard deviation. NS: not significant.

3. Results

3.1. Changes in serum ghrelin concentration and body weight

The serum ghrelin concentration significantly decreased 2 weeks after the beginning of IFN-α2b therapy ($P=0.0008$), and then returned to the level before therapy (Fig. 1A). Body weight also significantly decreased 2 weeks after the beginning of therapy ($P=0.0062$), and then returned to the level before therapy (Fig. 1B). The change in serum ghrelin concentration 2 weeks after the beginning of therapy (the serum concentration before therapy minus the concentration after 2 weeks; henceforth referred to as Δserum ghrelin) was significantly correlated with the change in body weight after 2 weeks (body weight before therapy minus body weight after 2 weeks; Δbody weight) ($r=0.726$, $P=0.023$) (Fig. 2). These results suggested a short-term reduction of the serum ghrelin concentration upon initiation of IFN-α2b therapy, and subsequent weight loss.

3.2. Comparison of ghrelin and body weight between the Rib+ and Rib− groups

The percentage reduction in serum ghrelin concentration 2 weeks after initiation of IFN-α2b therapy was significantly higher in the Rib+ group, compared to the Rib− group ($P=0.046$) (Fig. 3A). The percentage reduction of body weight showed a tendency to be greater in the Rib+ group, compared to the Rib− group ($P=0.057$). These results suggested that the short-term reduction of serum ghrelin and body weight was more severe in the Rib+ group than in the Rib− group.

4. Discussion

The efficacy of IFN-α therapy is largely dependent on compliance. Anorexia is one of important adverse effects of the IFN therapy in determining compliance. However, the mechanisms underlying anorexia remain to be determined. We have previously reported that upper abdominal side effects during IFN-α therapy may be attributable to disturbance of the motility of the upper gastrointestinal tract, accompanied by a delay in gastric emptying [15]. Ghrelin is a stomach-derived 28-amino acid peptide hormone that is structurally related to motilin [16,17]. Recently, several lines of evidence have shown that ghrelin can stimulate appetite by enhancing motility of the gastrointestinal tract and can control energy homeostasis [9–14,16,17]. Thus, it appears reasonable to examine whether ghrelin participates in appetite loss in chronic hepatitis C patients during IFN-α therapy.

In the present study, the 14 patients showed a significant decrease in serum concentrations of ghrelin 2 weeks after initiation of IFN-α2b therapy, whereas there was no difference between the concentrations before therapy and after 24

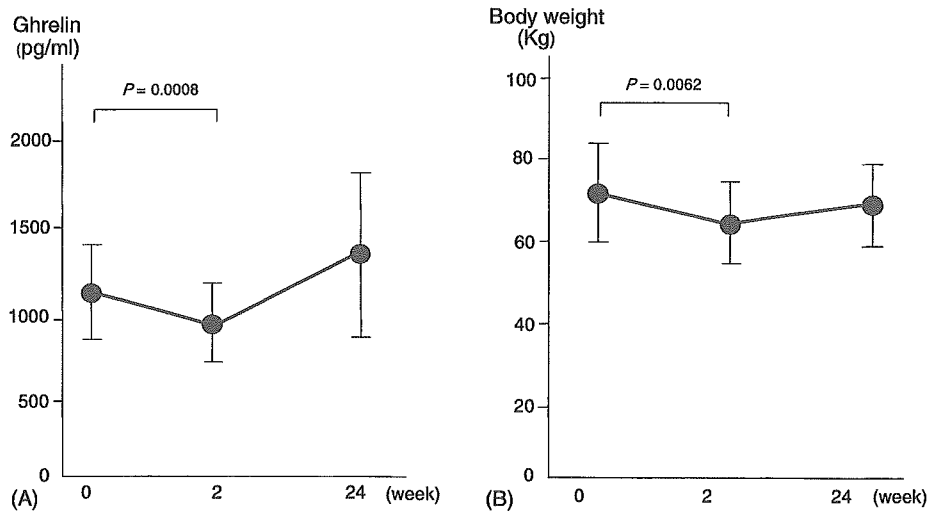

Fig. 1. Changes in serum ghrelin concentration (Panel A) and body weight (Panel B) during IFN-α2b therapy in patients with chronic hepatitis C.

weeks. This result suggests a short-term reduction in serum ghrelin caused by IFN-α2b therapy for chronic hepatitis C. In addition, the Δserum ghrelin concentration after 2 weeks was significantly correlated with Δbody weight, suggesting that reduction of serum ghrelin may lead to body weight loss.

The frequency of anorexia and weight loss has been reported to be higher with combination therapy using IFN-α and ribavirin than with IFN-α alone [18]. Hence, we examined the difference in the change in serum ghrelin concentrations in the Rib+ and Rib− groups. There were no statistically significant differences between the groups in basic characteristics such as age, gender, body weight, genotype distribution, viral load and histology, although viral loads tended to be higher in the Rib+ group. The percentage reduction of serum ghrelin concentrations and body weight 2 weeks after initiation of therapy was greater in the Rib+ group than in the Rib− group, although the difference in the percentage reduction of body weight was not statistically significant. This

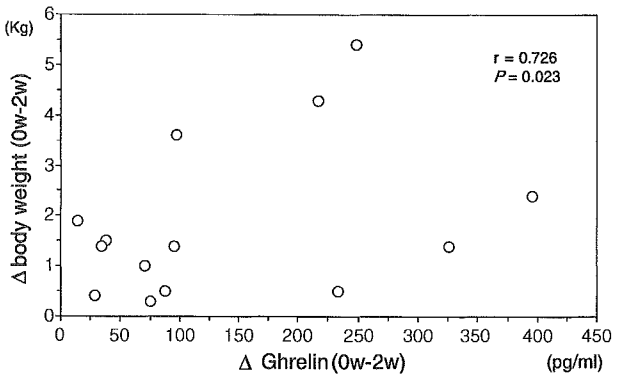

Fig. 2. Correlation between the Δserum ghrelin concentration and the Δbody weight 2 weeks after the start of IFN-α2b therapy in patients with chronic hepatitis C.

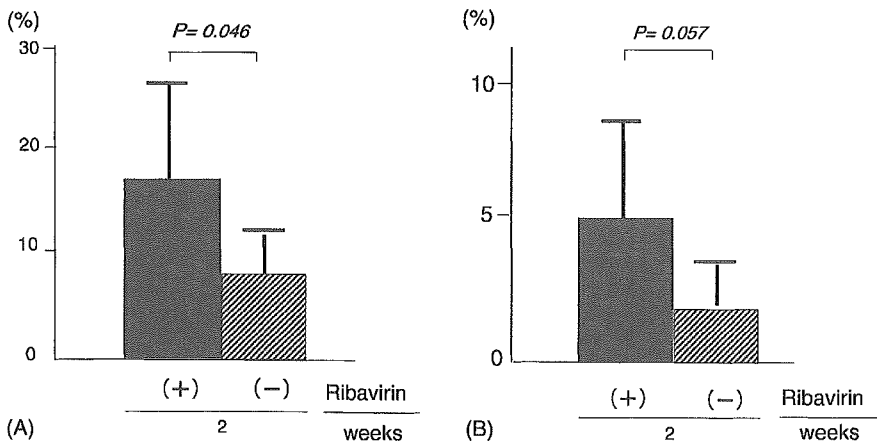

Fig. 3. Comparisons of percentage reductions in serum ghrelin concentration (Panel A) and body weight (Panel B) between patients receiving IFN-α2b alone and those receiving IFN-α2b and ribavirin, 2 weeks after initiation of IFN-α2b therapy.

suggests that a reduction in the serum ghrelin concentration profoundly contributes to appetite and body weight loss during IFN-α therapy.

Serum ghrelin concentrations are suppressed in obesity [19] and increased in emaciation [20,21]. Thus, reduction of serum ghrelin seems to cause weight loss, but not vice versa. Ghrelin also suppresses energy expenditure in addition to increasing food uptake [11], and it is possible that a reduction in serum ghrelin concentration may increase energy expenditure in patients receiving IFN-α therapy, which, together with a decrease in appetite, may lead to weight loss.

The regulation of gastric ghrelin secretion is poorly understood, and the mechanisms underlying the reduction of the serum ghrelin concentration during IFN-α therapy, with or without ribavirin, remain unknown. Ghrelin can negatively regulate expression of proinflammatory cytokines such as IL-1β, IL-6 and TNF-α [22], while IL-1β can suppress secretion of ghrelin from the gastric mucosa [17]. Hence, there appears to be crosstalk between ghrelin and several cytokines, and since expression of these proinflammatory cytokines is enhanced during IFN-α therapy, this may decrease gastric secretion of ghrelin, although such cytokines are also known to suppress appetite.

The concentrations of ghrelin restored around the baseline at 24 weeks after the beginning of therapy in this study. However, anorexia seems to usually continue during the IFN-α therapy. It remains unknown why the reduction of ghrelin concentration was short-term. It is possible that other hormones controlling appetite can explain the discrepancy between appetite and ghrelin concentration at a long-term period.

There are many hormones that can control appetite, in addition to ghrelin, and hormones such as leptin, glucagon-like peptide-1, oxyntomodulin, peptide YY and pancreatic polypeptide all inhibit appetite [23]. Thus, it is unlikely that only ghrelin participates in the development of anorexia during IFN therapy, and further studies on anorexogenic hormones participating in appetite regulation are needed to understand the mechanisms underlying anorexia and weight loss during IFN-α therapy.

The present study was open and longitudinal in nature, and limited in terms of patient number and in the time points at which the serum ghrelin was measured. Nonetheless, we were able to show that the serum ghrelin concentration substantially decreased 2 weeks after initiaton of IFN therapy, and that this reduction was correlated with a reduction in body weight. It will be important for our findings to be confirmed in a placebo-controlled study, and also for immunohistochemical data to be obtained from gastric biopsy specimens collected before and after IFN-α therapy alone and in combination with ribavirin.

In conclusion, IFN-α therapy caused short-term reduction of the serum ghrelin concentration and of body weight, and these changes were more severe in IFN-α combination therapy with ribavirin, compared to IFN-α therapy alone. Reduction of the serum ghrelin concentration may contribute partly to anorexia, leading to body weight loss, and the mechanisms underlying the reduction in serum ghrelin are under investigation in our laboratory.

Acknowledgements

This study was supported by a grant-in-aid (F-1) from the 21st Century Center of Excellence (COE) Program of the Japan Society for the Promotion of Science.

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J Gastroenterol 2005; 40:1075–1079

Journal of Gastroenterology

Letters to the editor

Successful percutaneous radiofrequency ablation of adrenal metastasis from hepatocellular carcinoma

To the Editor: Recently, radiofrequency ablation (RFA) has been widely used as therapy for hepatocellular carcinoma (HCC), because it is safe and only minimally invasive to the human body.[1,2] However, the therapeutic efficacy of RFA for extrahepatic metastasis of HCC is still unclear. The adrenal gland is one of the organs often affected by hematogenous metastasis of HCC. As a recent report has indicated that percutaneous RFA is a safe and well-tolerated procedure for the treatment of unresectable adrenocortical carcinoma,[3] it appears that RFA could also be applicable for the treatment of HCC metastasis to the adrenal gland. So far, surgical procedures have been employed for the removal of adrenal metastasis from HCC, but the optimal treatment regimen is still inconclusive. Here, we describe the use of ultrasonography (US)-guided percutaneous RFA for the treatment of a patient with adrenal metastasis of HCC. We found that this procedure allowed complete ablation of the metastasis without any severe adverse events.

The patient was a 69-year-old woman suffering from Child's class A, compensated cirrhosis due to hepatitis C. Computed tomography (CT) scan showed that an HCC, 20 mm in diameter, in the right lobe of the liver, had metastasized to the right adrenal gland and right portal vein, resulting in a 20-mm adrenal tumor mass and tumor emboli, respectively. The HCC was treated by arterial infusion of the chemotherapeutic agents, fluorouracil and cisplatin, according to the planned regimen, via a subcutaneously implanted injection port. Six months after the start of chemotherapy, CT imaging showed that both the primary HCC in the liver and the tumor emboli were reduced markedly, to an undetectable level, and the serum alpha-fetoprotein (AFP) level had a decreased from 29740 ng/ml before therapy to 6280 ng/ml after. However, the metastatic lesion in the adrenal gland was found to have increased in diameter, to 40 mm (Fig. 1a). Histological examination of a biopsy sample of the adrenal tumor, taken using a 21-gauge needle, showed the features of well-differentiated HCC. Immunohistochemical analysis of the tumor cells revealed positive staining for AFP. After local anesthesia was carried out with intradermal and subcutaneous lidocaine (1%), percutaneous transhepatic thermoablation of the metastatic tumor was performed twice, under US guidance, using a cool-tip needle radiofrequency system (Radionics, Burlington, MA, USA), comprising a 17-gauge, 3-cm active-tip RF electrode and radiofrequency generator, with an allowable output power of 120 W for 12 min. The first RFA was performed on the upper side of the tumor, and the second RFA was performed on the lower side of the tumor. There were no procedure-related complications. Both the plasma cortisol and catecholamine levels were measured 1 week before and after RFA. The plasma cortisol levels before and after RFA were 13.6 and 10.8 μg/dl, respectively. The plasma catecholamine levels before and after RFA were 16.0 and 10.0 pg/ml (adrenaline), 188.0 and 94.0 pg/ml (noradrenaline), and 8.0 and 5.0 pg/ml (dopamine), respectively. All levels of adrenal hormones were within the normal ranges. CT scan 6 months after the RFA showed that the adrenal metastatic HCC had been completely ablated and devascularized, with loss of contrast enhancement (Fig. 1b). The serum AFP level returned to the normal range, below 10 ng/ml, 4 months after the RFA.

To our knowledge, this is the first report of the successful treatment of adrenal metastasis of HCC using percutaneous RFA. This procedure was safe and well-tolerated and allowed complete ablation of the localized adrenal metastasis in this patient. Recently, it has been reported that unintended injury to normal adrenal tissue during RFA of adrenal tumors can lead to hypertensive crisis, a potentially catastrophic complication.[4] Therefore, we must pay sufficient attention to serious adverse

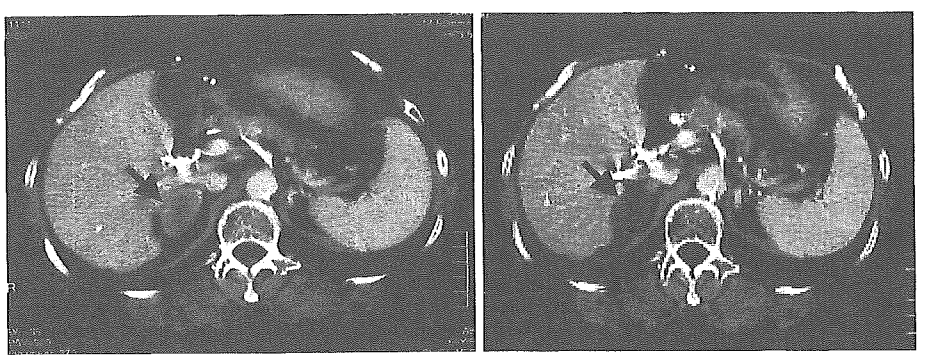

Fig. 1a,b. The early phase of dynamic computed tomography (CT), showing complete ablation of metastatic lesion of hepatocellular carcinoma in the right adrenal gland. **a** Before therapy, the metastatic tumor shows faint contrast enhancement with peripheral ring enhancement (*arrow*). Tumor biopsy showed the features of well-differentiated hepatocellular carcinoma. **b** Six months after radiofrequency ablation, the adrenal metastatic lesion has been completely ablated and devascularized, with loss of contrast enhancement (*arrow*)

events of the systemic circulation when we employ RFA for an adrenal tumor. Monitoring of the adrenal hormones should also be considered. Although considerable care is also required to avoid severe adverse events such as infarction[5] or tumor rupture[6] during the procedure, our experience suggests that percutaneous RFA is a useful approach for the control of metastatic HCC and prevention of localized tumor growth in the adrenal gland.

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Received: January 17, 2005 / Accepted: May 11, 2005
Reprint requests to: T. Saito
DOI 10.1007/s00535-005-1666-0

Partial splenic embolization facilitates completion of interferon therapy in patients with chronic HCV infection and hypersplenism

To the Editor: Hypersplenism with splenomegaly is occasionally observed in patients with chronic liver disease, including hepatitis C virus (HCV) infection.[1,2] Such HCV-infected patients suffering from cytopenia, especially leukocytopenia or thrombocytopenia, do not adequately tolerate interferon treatment, which itself can decrease peripheral leukocyte and platelet numbers,[3] even though it has been proven as a critical treatment to eliminate HCV, histologically reduce the stage of fibrosis, and decrease the risk of hepatocellular carcinoma (HCC).[4] Partial splenic embolization (PSE), an effective alternative to splenectomy for hypersplenism, has been widely used to reduce variceal bleeding episodes and correct cytopenia in patients with cirrhosis and hypersplenism due to portal hypertension.[5]

We performed PSE treatment to restore blood cell counts in five patients with chronic HCV infection associated with leukocytopenia and thrombocytopenia due to hypersplenism, so that 24-week interferon therapy could be better tolerated. All of the patients had splenomegaly. PSE was conducted using gelatin sponge as the embolization material; the average amount of devascularized parenchyma shown on computed tomography (CT) 1 week after PSE was 69.6%. Prophylactic antibiotics to prevent the development of splenic abscesses were used for 3 days following the procedure. Moderate-grade fever and left abdominal pain were seen in all patients for several days, but these symptoms decreased spontaneously without specific treatment. Liver biochemical functions did not worsen after PSE. Splenic abscesses and portal vein thrombosis, which have been reported to occur occasionally after PSE,[6] were not seen. PSE improved both the leukocyte number (before PSE, $3520 \pm 311/mm^3$ [mean ± SD]; after PSE, $4400 \pm 1111/mm^3$; $P < 0.05$; Wilcoxon signed-ranks test), and the platelet number (before PSE, $56400 \pm 15300/mm^3$; after PSE, $109600 \pm 27400/mm^3$; $P < 0.05$; Wilcoxon signed-ranks test). Interferon alfa-2b alone was used in two patients and consensus interferon was used in three patients. The type and amount of interferon, the differences in blood cell counts before and after PSE, and the viral response of each patient are shown in Table 1. Figure 1a,b shows the time course of the leukocyte and platelet counts during the treatment. In all patients, the treatment was well tolerated, and the 24-week interferon therapy was completed

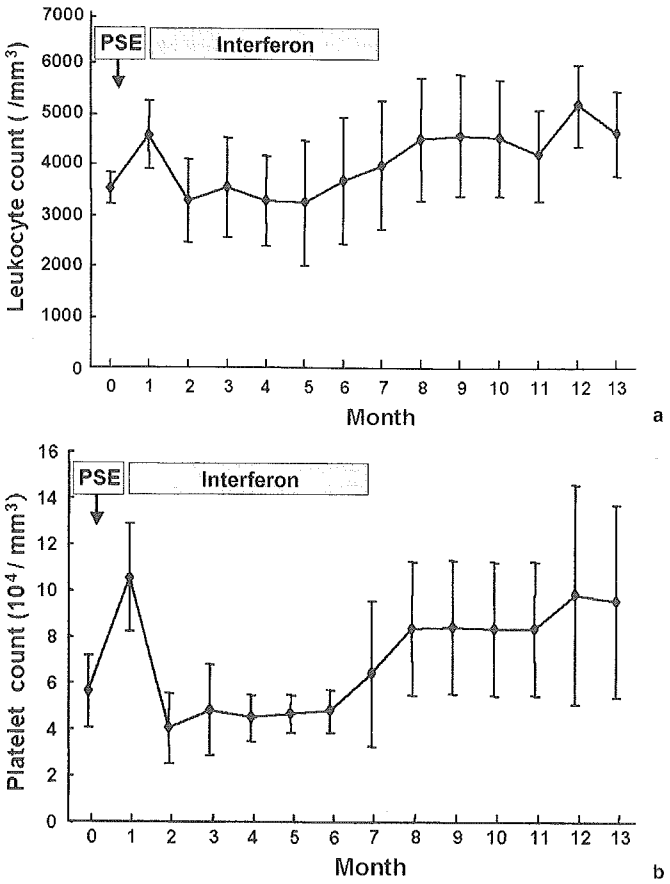

Fig. 1. **a** Leukocyte and **b** platelet counts were estimated during partial splenic embolization (*PSE*), during the 24-week interferon treatment period, and for 6 months after completion of the treatment. Data values are expressed as the mean and SD of five patients

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Hepatology Research 31 (2005) 211–216
HEPATOLOGY RESEARCH
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Efficacy of prolonged treatment following combination with ribavirin and interferon for chronic hepatitis type C: A pilot study

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Received 13 January 2004; received in revised form 27 May 2004; accepted 22 February 2005
Available online 29 April 2005

Abstract

The aim of the present study was to assess the efficacy of the prolonged interferon monotherapy following combination treatment. Seventy-six patients were enrolled. Of these, 7 were withdrawn while undergoing treatment with interferon combined with ribavirin, and 12 remained positive for HCV-RNA at the completion of the combination treatment. We studied 57 Japanese patients with chronic hepatitis C due to genotype 1b HCV of a high viral load. These patients tested negative for HCV-RNA at the completion of the combination treatment for 24 weeks. After the combination treatment, 29 patients of the prolonged treatment group successively received interferon-α monotherapy for 24 weeks, while 28 patients in the combination treatment alone group received no medication. The rate of a sustained virologic response (SVR) was higher in the prolonged treatment group (41%, 12/29) than in the combination treatment alone group (25%, 7/28), but not significantly. Patients who became HCV-RNA negative by 4 weeks after the start of the combination treatment showed an SVR rate of 86%. The prolonged treatment resulted in SVR in all five patients who newly became HCV-RNA negative at 12 weeks. In conclusion, the prolonged treatment was effective for patients who newly became HCV-RNA negative at 12 weeks.

Keywords: Ribavirin; Prolonged interferon monotherapy; Genotype; Chronic hepatitis C

1. Introduction

Interferon (IFN) is the only agent for treating chronic hepatitis C that eradicates the hepatitis C virus (HCV) [1,2]. However, IFN monotherapy is of limited effectiveness in patients who are infected with genotype 1b HCV of a high viral load

Abbreviations: IFN, interferon; HCV, hepatitis C virus; ALT, alanine aminotransferase; MU, million unit; HLBI, human lymphoblastoid interferon; SBR, sustained biochemical response; SVR, sustained virologic response

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doi:10.1016/j.hepres.2005.02.005

[3–5]. The combination treatment with ribavirin and IFN is currently used for patients with a high viral load, and has been found to be effective [6,7]. It has also been demonstrated that the combination treatment is more effective when the duration of the treatment is extended from 24 to 48 weeks [8]. The combination treatment with ribavirin and IFNα2b was started in Japan in December 2001 and was expected to be effective in patients with HCV of a high viral load. However, in Japan, where the IFN–ribavirin combination treatment is required by the Ministry of Health, Labour and Welfare to be completed within 6 months, the results are different from those of the 48-week treatment, which prevails in Western countries. Therefore, of patients who tested HCV-RNA negative after the 6-month combination treatment, 22% experienced relapse within several months, resulting in a final HCV-RNA negative rate of 31–35% [8,9]. At the completion of the 6-month combination treatment, 66% of patients with genotype 1b HCV with a high viral load became HCV-RNA negative. However, many of them experienced relapse afterwards, resulting in a sustained virologic response rate of 19% [10].

IFN monotherapy is reportedly effective when carried out long-term for 3 years [11]. It is believed that patients with genotype 1b of a high viral load need a prolonged treatment with IFN alone following the 24-week combination treatment with ribavirin. However, no studies have been reported for the efficacy of prolonged treatment with IFN alone after the combination treatment. The aim of this study was to assess the efficacy of the prolonged treatment with IFN alone for 24 weeks after the combination treatment with ribavirin and IFNα2b.

2. Patients and Methods

2.1. Patients

The combination treatment with ribavirin and IFNα2b was started between December 2001 and May 2002 in 76 patients with chronic hepatitis C with genotype 1b and had a viral load of 100 kIU/mL or higher determined by an Amplicor-HCV monitor assay (Roche Molecular Diagnostics, Tokyo, Japan) [12]. Seven of 76 patients dropped out during the combination treatment with ribavirin and IFNα2b. The reasons for dropout included a high-grade anemia, insomnia, and generalized malaise.

Of the 69 patients who completed the treatment, 57 patients who were HCV-RNA negative at the completion of the 6 months combination treatment with ribavirin and IFNα2b were participated in the study. Of these 57 patients, 22 patients had previously received IFN, and the remaining 35 patients were IFN-naive. These 57 patients were divided into two groups, comprising one group (the prolonged treatment group) of 29 patients who received the prolonged treatment with IFN alone for 24 weeks after the completion of the combination treatment, and another group (the combination treatment alone group) of 28 patients who received the 24-week combination treatment only. This study was not randomized. Six of the 12 patients who were HCV-RNA positive at the completion of the combination treatment also received the prolonged IFN treatment. Liver biopsy was performed within 12 weeks before starting the treatment. The severity of the findings was assessed according to a three-grade scheme, based on the extent of fibrosis (mild, periportal expansion; moderate, portal septa; severe, porto-central linkage or bridging fibrosis), using the classification system for chronic hepatitis proposed by Desmet et al. [13]. The baseline characteristics of the patients studied are shown in Table 1. There were no differences in age, sex, viral load before treatment, liver histology, or serum alanine aminotransferase (ALT) levels between the two groups.

Patients were excluded if they had cirrhosis, autoimmune hepatitis, or alcoholic liver damage, or if they tested positive for hepatitis B surface antigen by an enzyme-linked immunosorbent assay (Abbott Japan Co. Ltd., Tokyo, Japan).

2.2. Ribavirin–IFN combination treatment followed by prolonged IFN treatment

Ribavirin–IFN combination treatment consisted of intramuscular injection of 6 million units (MU) of IFN-α2b (Intron A, Schering-Plough K.K., Osaka, Japan) six times a week for the first 2 weeks and three times a week for a further 22 weeks, and ribavirin (Rebetol, Schering-Plough K.K., Osaka, Japan) taken orally at a dose of 600 mg/day if body

Table 1
Patient baseline characteristics

	Prolonged treatment group; $n=29$	Combination treatment alone group; $n=28$	p value
Mean age (years, mean ± S.D.)	58.1 ± 12.4	59.2 ± 13.4	0.748
Male/Female	17/12	17/11	0.543
HCV load (kIU/mL, mean ± S.D.)	696 ± 221	628 ± 188	0.216
100–300 (kIU/mL)	8	10	0.353
>300 (kIU/mL)	21	18	
Liver histology (fibrosis staging)			
Mild	19	16	0.353
Moderate/severe	10	12	
ALT (U/L, mean ± S.D.)	76 ± 29	67 ± 21	0.184

Prolonged treatment group: ribavirin–interferon combination treatment + prolonged interferon treatment; combination treatment alone group: ribavirin–interferon combination treatment only.

weight was less than 60 kg before treatment and at a dose of 800 mg/day if body weight was 60 kg or higher.

Prolonged treatment with IFN alone consisted of intramuscular or subcutaneous injection of one of three types of IFNα (HLBI, IFNα2b, and r-IFNαcon1), applied to 29 patients three times a week for 24 weeks, as follows: 16 patients received 6 MU of natural IFN-α (human lymphoblastoid interferon (HLBI), Sumiferon, Sumitomo Pharmaceuticals Co. Ltd., Osaka, Japan), eight patients received 6 MU of IFNα2b, and five patients received 18 MU of recombinant-IFN αcon-1 (r-IFN-αcon1, Advaferon, Yamanouchi Pharmaceutical Co. Ltd., Tokyo, Japan).

2.3. Blood testing

HCV-RNA was measured using an Amplicor-HCV assay version 2.0 (Roche Molecular Diagnostics Co. Ltd., Tokyo, Japan) [14]. Measurements were carried out eight times: before treatment and 4, 8, 12, 24, 36, 48, and 72 weeks after the start of the combination treatment. The white blood cell count, red blood cell count, hemoglobin concentration, platelet count, and serum ALT levels were measured before treatment and once every 4 weeks until 72 weeks after the start of the treatment. The upper limit of the normal range for ALT was 40 IU/L.

The efficacy of treatment was assessed based on HCV-RNA negativity and sustained normalization of hepatic function. Patients who tested HCV-RNA negative at 24 weeks after completion of the IFN treatment were considered to be in a sustained virologic response (SVR). Patients who tested HCV-RNA positive but showed sustained control of ALT below the upper limit of the normal range at 6 months after the completion of the IFN treatment were considered to have a sustained biochemical response (SBR). The remainder of the patients were considered to be non-responders.

2.4. Informed consent

The study protocol was approved by the institutional ethics committees and all patients gave informed consent to participate in this study. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and International Conference on Harmonization guidelines for good clinical practice.

2.5. Statistical analysis

Differences between the groups were analyzed using a Fisher's exact test. All tests were two-sided, and p values less than 0.05 were considered to be significant.

3. Results

3.1. Results of prolonged IFN treatment

The results of prolonged IFN treatment and combination treatment alone are shown in Table 2. At the completion of the prolonged treatment, the HCV-RNA negative rate was 25% in the combination treatment alone group. In the prolonged treatment group, the HCV-RNA negative rate was 76% (22/29); significantly higher than that of the combination treatment alone group ($p < 0.001$). During the prolonged IFN treatment, the relapse rate was significantly lower, demonstrating that the prolonged IFN treatment effectively prevented patients from testing HCV-RNA positive again.

The rate of SVR was higher in the prolonged treatment group (41%, 12/29) than in the combination treatment alone group (25%, 7/28), but not significantly. However, the SBR rate was 66% (19/29), significantly higher in the prolonged treatment group compared to 32% (9/28) in the combination treatment alone group ($p < 0.05$).

The IFN treatment was continued in 6 of the 12 patients who were HCV-RNA positive at completion of the combination treatment, but none of these six patients became HCV-RNA negative either during or after the prolonged treatment.

The percentage of HCV-RNA-negativity at the completion of the prolonged treatment did not vary among IFNs used in the study: 75% (12 out of 16 patients) for HLBI, 75% (6 out of 8 patients) for IFNα2b, and 80% (4 out of 5 patients) for r-IFN-αcon1. There were also no differences in the SVR rates: 44% (7 out of 16 patients) for HLBI, 38% (3 out of

Table 2
Results for prolonged interferon treatment vs. combination treatment alone

	Prolonged treatment group ($n=29$) n (%)	Combination treatment alone group ($n=28$) n (%)	p value
(1) HCV-RNA negativity			
At completion of combination treatment	29 (100)	28 (100)	
24 weeks after completion of combination treatment	22 (76)	7 (25)	0.0001
24 weeks after completion of prolonged treatment	12 (41)	–	–
(2) Sustained virologic response	12 (41)	7 (25)	0.151
(3) Sustained biochemical response	19 (66)	9 (32)	0.011

n: Number of HCV-RNA negative patients; (%): percentage of HCV-RNA negative patients; P value: prolonged treatment group vs. the combination treatment alone group; prolonged treatment group: ribavirin–interferon combination treatment + prolonged interferon treatment; combination treatment alone group: ribavirin–interferon combination treatment only.

Table 3
HCV-RNA negativity after start of the combination treatment and SVR rate

	Prolonged treatment group ($n=29$); SVR/HCV-RNA negativity (%)	Combination treatment alone group ($n=28$); SVR/HCV-RNA negativity (%)
4 weeks after start of combination treatment	7/8 (88)	6/7 (86)
8 weeks after start of combination treatment	0/1 (0)	0/1 (0)
12 weeks after start of combination treatment	5/5 (100)	1/5 (20)

SVR: sustained virologic response; prolonged treatment group: ribavirin–interferon combination treatment + prolonged interferon treatment; combination treatment alone group: ribavirin–interferon combination treatment only.

8 patients) for IFNα2b, and 40% (2 out of 5 patients) for r-IFN-αcon1.

3.2. HCV-RNA negativity after start of the combination treatment and SVR rate

Eight and seven patients who became HCV-RNA negative by 4 weeks received the prolonged treatment and combination treatment alone, respectively (Table 3). Of these, SVR was achieved in 7 of 8 (88%) patients receiving the prolonged treatment and in 6 of 7 (86%) patients receiving the combination treatment alone. One each patient who newly became HCV-RNA negative at 8 weeks received the respective treatments, although neither of them showed SVR. Five each patients who newly became HCV-RNA negative at 12 weeks received the respective treatments. Of these, SVR was achieved in all five patients receiving the prolonged treatment and in 1 of 5 patients receiving the combination treatment alone.

Seven patients who became HCV-RNA positive again during the prolonged treatment had tested HCV-RNA positive at 4, 8, and 12 weeks as well and had a baseline viral load of more than 850 kIU/mL. Of 10 patients experiencing relapse after the completion of the prolonged treatment, eight had remained HCV-RNA positive at 12 weeks, whereas two had become HCV-RNA negative at 12 weeks but had a baseline HCV load of more than 500 kIU/mL.

3.3. Sustained virologic response rate by each factor

SVR rates by each factor are shown in Table 4. The percentage of the HCV-RNA negative patients in week 12 of the combination treatment and the respective SVR rates were examined. The SVR rates in both the prolonged treatment group and the combination treatment alone group were significantly higher in the patients who were HCV-RNA negative in week 12 of the combination treatment than in the patients who were HCV-RNA positive ($p<0.01$ and $p<0.01$, respectively). In both groups, all of the patients who showed SVR were HCV-RNA negative by week 12 of the combination treatment. In the patients who were HCV-RNA negative in week 12 of the combination treatment, the SVR rate was higher in the prolonged treatment group than in the combination treatment alone group, but not significantly.

The patients with a moderate viral load (100–300 kIU/mL) before treatment showed a significantly higher SVR rate than the patients with a high viral load (>300 kIU/mL) in both groups ($p<0.05$). There were no differences in the SVR rate between the prolonged treatment and combination treatment alone groups as determined by the viral load. There was no

Table 4
Sustained virologic response rates

	Sustained virologic response rate after interferon treatment		p value
	Prolonged treatment group SVR/n (%)	Combination treatment alone group SVR/n (%)	
Week 12 of treatment			
HCV-RNA (−)	12/14 (86)[a]	7/13 (54)[b]	0.081
HCV-RNA (+)	0/15 (0)[a]	0/15 (0)[b]	
HCV load before treatment			
100–300 (kIU/mL)	6/8 (75)[c]	5/10 (50)[c]	0.278
>300 (kIU/mL)	6/21 (29)[c]	2/18 (11)[c]	0.172
Liver histology (fibrosis staging)			
Mild	9/19 (47)	4/16 (25)	0.155
Moderate/severe	3/10 (30)	3/12 (25)	0.582
Total	12/29 (41)	7/28 (25)	0.151

Prolonged treatment group: ribavirin–interferon combination treatment + prolonged interferon treatment; combination treatment alone group: ribavirin–interferon combination treatment only.

[a] $p<0.0001$ (HCV-RNA (−) vs. (+) in week 12: prolonged treatment group).

[b] $p<0.01$ (HCV-RNA (−) vs. (+) in week 12: combination treatment alone).

[c] $p<0.05$ (HCV load: 100–300 vs. >300 kIU/mL).

relationship between the fibrosis staging before treatment and the rate of SVR.

3.4. Univariate analysis of predictive factors of sustained virologic response

Univariate analysis showed that the predictive factors of SVR were: gender (male, $p=0.705$), age (56 years of age and older, $p=0.705$), liver histology (mild, $p=0.452$), and HCV load (100–300 kIU/mL, $p=0.003$), and the prolonged treatment (yes, $p=0.199$). HCV load was significant factor while gender, age, and liver histology were not significant.

3.5. Safety

No patients dropped out during the prolonged treatment.

4. Discussion

We assessed whether the 6-month IFN monotherapy following the 6-month combination treatment was effective in the treatment of patients with chronic hepatitis C due to genotype 1b HCV with a high viral load. In this study, the SVR rate was higher in the prolonged treatment group than in the combination treatment alone group, but not significantly. However, the HCV-RNA negative rate was higher during the IFN monotherapy following the combination treatment. This means that the successive IFN monotherapy effectively prevented relapse of HCV-RNA following the combination treatment.

In prolonged IFN monotherapy, the HCV-RNA negative rate is low even after 3-year treatment unless HCV-RNA negativity is achieved within 6 months after the start of treatment [11]. In this study, no patients who were HCV-RNA positive at the completion of the combination treatment became HCV-RNA negative after the successive treatment. Therefore, HCV-RNA negativity at the completion of the combination treatment was required to achieve SVR during the successive IFN monotherapy. In prolonged IFN monotherapy lasting 24 months or more, the SVR rate is high if HCV-RNA negativity is sustained for over 24 months [15]. In this study, because our patients received IFN monotherapy for only a short period of time (24 weeks), a high percentage of patients who remained HCV-RNA negative during the treatment tested positive again after the treatment. This result suggests that the prolonged treatment for 24 weeks is insufficient in patients who are genotype 1b with a high viral load. In addition, IFN monotherapy needs to be carried out over a longer period to maintain sustained HCV-RNA negativity.

Monitoring of HCV-RNA during the IFN treatment is of importance partly in predicting the therapeutic effect of the IFN treatment. The Peg-IFN–ribavirin combination treatment produced an SVR rate of 89% in patients who became HCV-RNA negative by 4 weeks and showed early virologic response, but no response in any patients who remained HCV-RNA positive at 12 weeks [16]. Our study revealed that patients who became HCV-RNA negative by 4 weeks had an SVR rate of 86% even with the 6-month IFN–ribavirin combination treatment alone. Consequently, HCV-RNA negativity achieved by 4 weeks of the combination treatment seems not to require the prolonged IFN treatment. Castro et al. state that testing negative for HCV-RNA by 12 weeks after the start of treatment is the most important factor in achieving SVR in the ribavirin–IFN combination treatment [17]. In our study also, the 6-month prolonged IFN treatment produced a high SVR rate in patients who newly became negative for HCV-RNA at 12 weeks but no SVR in patients who remained HCV-RNA positive at 12 weeks. Thus, the prolonged IFN treatment was effective for those who newly became HCV-RNA negative at 12 weeks, but was almost ineffective for those who remained HCV-RNA positive at that time point.

The 48-week Peg-IFN–ribavirin combination treatment is shown to produce high therapeutic effect [6,7]. The study of the combination of Peg-IFNα2a with ribavirin reported that 313 of 453 patients tested negative at the completion of treatment, and that 59 (19%) of them experienced relapse 6 months later [6]. The study of the combination of Peg-IFNα2b with ribavirin also showed a similar relapse rate of 18% [7]. Combined, these results show that 48-week Peg-IFN–ribavirin combination treatment results in relapse at a rate of not more than 20%. In our study, 59% of patients receiving the prolonged treatment experienced relapse. Patients who became HCV-RNA positive again during the prolonged treatment or those who experienced relapse after the completion of the prolonged treatment had either remained HCV-RNA positive at 12 weeks or had a baseline HCV load of more than 500 kIU/mL. In such cases, the prolonged IFN treatment seems not to exert efficacy, thus requiring the IFN–ribavirin combination treatment for 48 weeks or longer.

During the 24- and 48-week IFN–ribavirin combination treatments, 3.5 and 9.2% were discontinued, respectively; thus, the 48-week treatment caused more dropouts [9]. During the 48-week Peg-IFN–ribavirin combination treatment, the highest virological response (negative HCV-RNA) was achieved at 24 weeks, followed by a decline until 48 weeks. This decline in virological response was attributable to increased dropouts due to adverse drug reactions after 24 weeks [18]. Our study revealed no dropouts due to adverse drug reactions that newly emerged during the prolonged treatment. Ribavirin frequently causes anemia in patients aged 60 years or more and females, resulting in treatment discontinuation [20]. For patients with genotype 1 HCV of a high viral load, the 48-week IFN–ribavirin combination treatment is seen to be most effective. In such patients as the elderly or females who experienced adverse drug reactions due to ribavirin, however, the combination treatment may have to be replaced by a safer treatment involving the IFN monotherapy.

Three types of IFNα (HLBI, IFNα2b, and r-IFNαcon1) were used for prolonged treatment. R-IFNαcon1, which was used at a high dose (18 MU) and is reported to be effective in patients who are genotype 1b of a moderate

viral load (100–300 kIU/mL), [19] was expected to produce good results in the successive treatment in this study. However, the percentage of the HCV-RNA negative patients did not differ between r-IFNαcon1 and the other types of IFN (HLBI and IFNα2b) given at a dose of 6 MU. This result indicates that in the additional IFN monotherapy, IFN may effectively prevent relapse of HCV-RNA at a daily dose of 6 MU. The fact that there were no dropouts during the prolonged treatment indicates that the prolonged treatment is safe and could be carried out over a longer period.

After completion of prolonged treatment, some patients again tested positive for HCV-RNA and the SVR was not significantly higher than in the patients who received combination treatment alone. However, the SBR rate was significantly higher in the prolonged treatment group than in the combination treatment alone group. With prolonged IFN treatment, long-term sustained normalization of hepatic function is seen even after completion of treatment [11]. Many of our patients also showed sustained normalization of hepatic function as a result of additional IFN monotherapy. This indicates that prolonged IFN monotherapy not only prevents patients from testing HCV-RNA positive again during treatment but also useful for sustained improvement of hepatic function after treatment has stopped.

In conclusion, the prolonged treatment after the ribavirin–IFN combination treatment significantly reduced the percentage of patients who became HCV-RNA positive again during IFN monotherapy. A significantly greater percentage of patients showed sustained normalization of hepatic function as a result of prolonged IFN monotherapy, demonstrating the usefulness of this form of treatment. No patients dropped out of the prolonged treatment as a result of adverse reactions, indicating that the prolonged treatment was safe. HCV-RNA negativity achieved by 4 weeks of the combination treatment seems not to require much of the additional prolonged treatment. In contrast, the prolonged treatment was effective for patients who newly became HCV-RNA negative at 12 weeks, but was almost ineffective for those who remained HCV-RNA positive at that time point. In patients who experienced ribavirin-attributable adverse drug reactions during the combination treatment, it is necessary to consider a safer prolonged treatment involving the IFN monotherapy.

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