Efficacy and safety of 6-month iron reduction therapy in patients with hepatitis C virus-related cirrhosis: a pilot study

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Abstract

Background and Purpose

Iron reduction therapy (IRT) has been recognized as beneficial for chronic hepatitis C patients. However, its efficacy for hepatitis C virus-related liver cirrhosis (LC-C) has not been elucidated. We aimed to evaluate the efficacy and safety of IRT for LC-C patients.

Methods

Twenty-two LC-C patients were treated with biweekly phlebotomy and low iron diet for 6 months, in addition to regular hepatoprotective therapy. Nineteen sex- and age-matched patients who refused to receive IRT were used as controls. The efficacy of IRT was evaluated based on biochemical parameters.

Results

Of 22 patients receiving IRT, 19 completed the 6-month treatment. IRT significantly reduced serum levels of aspartate aminotransferase (from 89 to 57 U/L; $P = 0.003$), alanine aminotransferase (from 101 to 54 U/L; $P < 0.001$), and alpha-fetoprotein (from 28 to 12 ng/mL; $P = 0.003$). These changes were not observed in the controls. Two

patients whose serum albumin concentrations were less than 3.6 g/dL at the beginning of IRT withdrew from IRT because of a new appearance of ascites.

Conclusions

IRT improved the serum levels of aminotransferases and alpha-fetoprotein for LC-C patients, and was generally safe; however, IRT should be performed in patients who maintain serum albumin concentrations of more than 3.6 g/dL.

Introduction

Chronic hepatitis caused by persistent hepatitis C virus (HCV) infection may lead to the development of liver cirrhosis (LC) and eventually complication of hepatocellular carcinoma (HCC)[1]. LC has been recognized as one of the major risk factors of HCC and hepatic failure in patients with persistent HCV infection. To prevent the development of HCC and the progression to hepatic failure in HCV-related LC (LC-C), it is important to keep serum aminotransferase levels as low as possible[2].

Interferon therapy has diverse beneficial effects for HCV-related chronic liver diseases: promoting HCV elimination, suppressing HCV replication, ameliorating the activity of hepatitis, and eventually preventing the progression of hepatic fibrosis and the occurrence of HCC. However, its use is sometimes limited for elderly patients and for patients with marked thrombocytopenia, diabetes, severe systemic arteriosclerosis, or psychological disease, because of the increased risk of harmful or irreversible adverse effects (e.g., cerebral hemorrhage, depression). Therefore, the establishment of alternative strategies is required for such LC-C patients.

Iron reduction therapy (IRT) was first introduced by Hayashi et al.[3], based on the histochemical detection of iron deposits in the liver, and then on the detection of lysosomal iron stores in hepatocytes. Hepatic iron accumulation induces enhanced

generation of reactive oxygen species (ROS), thus damaging hepatocytes[4,5]. Recently, a randomized controlled study in Japanese chronic hepatitis C patients showed that biweekly phlebotomy for 3 months significantly reduced serum alanine aminotransferase (ALT) levels[6]. It has been reported that, in HCV-infected patients, intrahepatic iron accumulation and the resultant ROS production enhances as hepatic fibrosis progresses[7], suggesting that IRT might be useful for LC-C patients. However, for patients with compensated LC-C, the efficacy and safety of IRT, i.e. the combination of phlebotomy and a low iron diet, has not been previously elucidated. Therefore, we planned this pilot study to evaluate it.

Materials and Methods

Patients

From October 2003 to March 2005, 22 LC-C patients (10 men and 12 women, 66 $\pm$ 11 years old) entered this pilot study after informed consent was obtained. All patients were positive for serum HCV-RNA and had abnormal serum ALT levels. Serum aspartate aminotransferase (AST) and ALT levels of less than 35 U/L were defined as the normal ranges. Basically, the diagnosis of LC was made according to the histological findings of percutaneous liver biopsy, and was confirmed by imaging

findings (e.g., liver surface irregularity, swelling of the left lobe and caudal lobe, the presence of splenomegaly, and the development of esophageal and/or gastric varices) and by laboratory data such as platelet counts of less than 10.0 x $10^4/\mu L$[8] and serum hyaluronic acid levels of more than 237 ng/mL[9]. All 22 patients had splenomegaly, and 7 of these had esophageal varices. Twenty and 18 patients of these had platelet counts of less than 10.0 x $10^4/\mu L$, and serum hyaluronic acid levels of more than 237 ng/mL, respectively. Three of the 22 patients refused liver biopsy, so the diagnosis of LC was made using imaging findings and a formula for estimating liver cirrhosis proposed by Ikeda et al[7]. Its accuracy for diagnosing LC was as high as 91.2%[7]. The exclusion criteria at entry were: (1) previous interferon therapy within 6 months; (2) decompensated LC (Child-Pugh classification B and C); (3) hemoglobin values of less than 12 g/dL; (4) serum albumin concentrations of less than 3.3 g/dL; (5) severe complications such as cardiac, pulmonary, renal, or hematological disease, and (6) pregnancy. All patients underwent hepatoprotective therapy such as ursodeoxycholic acid and/or glycirrhycin injection for more than 6 months before entry. Of the 22 patients, 8 had been administered oral branched-chain amino acids for more than 6 months before entry. None had received regular administration of oral diuretics or albumin infusion. These therapies were not changed after this study was begun.

When informed consent was performed to LC-C patients, some of them refused the participation in this study. From them, 19 patients who matched the sex and age of the subjects were selected and were used as controls for comparison of biochemical markers. These patients underwent only hepatoprotective therapy for more than 6 months, and continued it.

When IRT was begun, body mass index (BMI) was calculated. Patients were considered to have hypertension if their systolic/diastolic pressure was greater than 140/90 mmHg, or if they were taking anti-hypertensive drugs. Patients were considered to have diabetes if they had a fasting glucose level equal to or higher than 126 mg/dL, or if they were taking insulin or oral hypoglycemic drugs.

Histological examination

Percutaneous ultrasonography-guided liver biopsy was performed before entry, and the liver specimens were fixed in 10% formalin and stained using hematoxylin-eosin and the Azan-Mallory methods. The presence of hepatic steatosis was defined as macrovesicular fat accumulation in more than 30% of the hepatocytes affected.

IRT

IRT consisted of periodical phlebotomy and a low iron diet. Phlebotomy of 200 mL in volume for female patients with body weight of less than 60 kg or of 400 mL for other patients was repeated biweekly. Phlebotomy was planned to be continued for 6 months, but was discontinued according to the following criteria: (1) hemoglobin values of less than 10 g/dL, (2) serum ferritin levels of less than 10 ng/mL, (3) the appearance of adverse effects, or (4) patient's refusal to continue phlebotomy. In addition, all patients in the IRT group were advised to reduce their iron-rich foods during the treatment and were instructed by a registered dietitian. To aid with compliance, each patient was given a comprehensive list of iron-rich foods that they were to avoid, as well as instructions on how to complete dietary records, which required a listing of all food and drink consumed over a 3-day period once every 3 months throughout the treatment. All patients in the IRT group were instructed to reduce their consumption of beans, shellfish, green vegetables, meat, and seaweed and replace them with refined carbohydrates. Dietary energy (30 kcal/kg of body weight/day), nutritional balance, and iron intake (6 mg/day) during the IRT were assessed based on the dietary records by using the nutrition-analyzed software BASIC-4 for Windows version 2.0 (Kagawa Nutrition University Publishing Division, Tokyo, Japan).

Laboratory tests

Complete blood counts, including hemoglobin values and platelet counts, and biochemical parameters such as serum AST, ALT, albumin, bilirubin, alpha-fetoprotein (AFP), hyaluronic acid, and ferritin levels were measured monthly using standard automated analyzers. The amounts of serum HCV-RNA were measured by the amplicore monitoring method (Roche Diagnostic System, Basel, Switzerland). In the IRT group, blood was drawn for tests just before phlebotomy was performed.

Ethics

This study was carried out in accordance with the World Medical Association Helsinki Declaration, and was approved by the ethics committee of Showa Inan General Hospital. All patients gave their informed consent to participate in the study.

Statistics

Statistical analyses were performed using SPSS software 11.0J for Windows (SPSS Inc., Chicago, Illinois, USA). Qualitative variables were expressed as percentages and were compared using the χ^2 test. Quantitative data were expressed as means and standard deviations and were compared using the unpaired or paired two-tailed t test. A

probability value of less than 0.05 was considered to be statistically significant.

Results

Effects of IRT on laboratory tests

Of the 22 patients receiving IRT, 19 patients completed the 6-month treatment. There were no differences in the patients' backgrounds between the IRT and the control groups (Table 1). Serum AST and ALT levels in the IRT group were significantly decreased after the treatment (from 89 $\pm$ 33 to 57 $\pm$ 19 U/L; $P = 0.003$, and from 101 $\pm$ 44 to 54 $\pm$ 24 U/L; $P < 0.001$, respectively) (Figures 1 and 2), but remained unchanged in the control group (from 84 $\pm$ 26 to 82 $\pm$ 23 U/L, and from 93 $\pm$ 26 to 91 $\pm$ 27 U/L, respectively) (Figure 2). Serum AFP levels in the IRT group were also significantly decreased after the treatment (from 28 $\pm$ 20 to 12 $\pm$ 6 ng/mL; $P = 0.003$), but were constant in the control group (from 32 $\pm$ 24 to 31 $\pm$ 24 ng/mL) (Figure 2). Due to IRT, the hemoglobin values and serum ferritin levels were decreased (from 14.5 $\pm$ 1.1 to 11.7 $\pm$ 1.9 g/dL; $P < 0.001$, and from 237 $\pm$ 145 to 26 $\pm$ 21 ng/mL; $P < 0.001$, respectively) (Figure 2). The platelet counts were significantly increased after the IRT (from 9.2 $\pm$ 2.9 to 11.7 $\pm$ 2.5 $x10^4/\mu L$; $P = 0.003$) (Figure 2). Serum albumin, bilirubin, and hyaluronic acid levels remained unchanged after the treatment (Figure 2).

Clinical parameters after 6 months of IRT

Several clinical parameters after 6 months of IRT were compared with those of the controls. Serum AST and ALT levels were significantly lower in the IRT group compared with the control group (57 ± 19 vs. 82 ± 23 U/L; $P = 0.001$, and 54 ± 24 vs. 91 ± 27 U/L; $P = 0.003$, respectively) (Figure 2). Serum AFP levels were also lower in the IRT group compared with the control group after 6 months (12 ± 6 vs. 31 ± 24 ng/mL; $P = 0.042$) (Figure 2). The hemoglobin values and serum ferritin levels significantly dropped in the IRT group compared with those in the control group (11.7 ± 1.9 vs. 13.8 ± 1.1 g/dL; $P < 0.001$, and 26 ± 21 vs. 223 ± 111 ng/mL; $P < 0.001$, respectively) (Figure 2). The platelet counts were higher in the IRT group than in the controls (11.7 ± 2.5 vs. 8.4 ± 3.7 $x10^4/\mu L$; $P = 0.004$) (Figure 2). On the other hand, serum albumin, bilirubin, and hyaluronic acid levels did not differ between the two groups after 6 months (Figure 2).

Association between improvement of clinical parameters and iron reduction

To examine whether iron reduction would contribute to the improvement of serum aminotransferase levels in this study, the patients receiving IRT were divided into two

groups according to their serum ferritin levels as measured after 6 months of treatment (more or less than 10 ng/mL), and the changes in their clinical parameters were compared. As shown in Table 2, 6 patients reached serum ferritin levels of less than 10 ng/mL after 6 months of IRT. Serum AST, ALT, and AFP levels after the treatment were similar in both groups, but the change in AST levels was significantly greater in patients with serum ferritin levels of less than 10 ng/mL than in patients with serum ferritin levels of more than 10 ng/mL (Table 2). The changes in ALT and AFP levels tended to be greater in patients with serum ferritin levels of less than 10 ng/mL (Table 2).

Adverse effects

IRT was generally tolerated in LC-C patients. However, 3 of 22 LC-C patients withdrew from IRT. One patient refused phlebotomy because of the pain experienced at the venous puncture. The remaining 2 patients showed a new appearance of ascites due to decreased serum albumin levels. One of these patients was a 41-year-old obese man suffering from diabetes, and the other was a 70-year-old woman with hypertension. The serum albumin levels of these patients at the beginning of IRT were 3.5 and 3.4 g/dL, respectively. Discontinuance of phlebotomy and additional oral administration of spironolactone resulted in the rapid disappearance of the ascites. Albumin infusion was

not performed. In the control group, a new appearance of ascites or HCC was not observed.

Discussion

This pilot study demonstrated that IRT significantly improved the serum levels of aminotransferases and AFP for patients with LC-C. Moreover, IRT can be performed safely in patients with compensated LC-C. As far as we know, this is the first study concerning the efficacy and safety of IRT for LC-C patients.

The remarkable characteristic of this study is that only compensated LC patients were allocated. Histological diagnosis of LC only by ultrasonography-guided liver biopsy sometimes leads to misinterpretation because of high sampling error rate[8,10]. In this study, the diagnosis of LC was made fundamentally according to the histological findings, and was confirmed by imaging and laboratory findings. Thus, the diagnosis of LC is considered to be valid in this study.

Six-month IRT significantly decreased serum aminotransferase levels, which may be caused by reduced iron-induced hepatotoxicity. As shown in Table 2, the efficacy of iron reduction is supported by the fact that the degree of improvement of serum AST levels was greater in patients who showed serum ferritin levels of less than 10 ng/mL

compared with in patients who showed those of more than 10 ng/mL after the treatment. The probability that the improvement of serum aminotransferase levels would result from other hepatoprotective therapies such as ursodeoxycholic acid and glycirrhycin injection is considered to be very low, because the dose of these hepatoprotective agents was unchanged during this study, and because these agents were also administered to the control group, in which the serum aminotransferase levels did not change.

Another notable finding in this study is that IRT significantly reduced serum AFP levels. Until now, it has been recognized that only low-dose long-term interferon injection can improve serum AFP levels[11]. Since a high level of AFP is suggested to be one of the major risk factors of the development of HCC[12,13], lowering serum AFP levels is important to prevent HCV-related hepatocarcinogenesis. Hepatic iron accumulation induces mitochondrial abnormalities and enhanced ROS generation[14], causing the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG)[14,15] and p53 mutation[16], which may eventually lead to the initial step of HCC. It has also been reported that hepatic iron accumulation disrupted balance between hepatocyte proliferation and apoptosis in transgenic mice expressing HCV polyprotein[14] or in patients with chronic hepatitis C[17]. Furthermore, in vitro study using mouse hepatocytes revealed that iron overload promoted *Cyclin D1* expression and accelerated hepatocyte

division[18]. In fact, it has been demonstrated that IRT significantly lowered hepatic 8-OHdG levels and reduced the incidence rate of HCC in patients with chronic hepatitis C[19]. Therefore, maintaining hepatic iron contents as low as possible by long-term IRT might be useful to prevent HCV-related hepatocarcinogenesis, especially for patients with LC-C. This requires a long-term follow-up observation.

There is a possibility that IRT, especially periodical phlebotomy, would decrease serum albumin concentrations. It has been unclear whether IRT is indicated or not for compensated cirrhotic patients with serum albumin levels of less than 3.7 g/dL. This study demonstrated for the first time that, even in compensated cirrhosis, regular repeated phlebotomy is risky in patients with serum albumin levels of less than 3.6 g/dL. Therefore, in such cirrhotic patients, adjustment of phlebotomy (e.g., reduction of removed blood volume, extension of interval of phlebotomy) is needed.

It is evident that a low iron diet plays an important role in ameliorating the effect of IRT[20]. Many compensated LC-C patients are in the condition of subclinical protein-energy malnutrition (PEM)[21,22]; excessive dietary iron restriction would worsen it. Careful nutritional evaluation and appropriate pharmacological interventions to improve PEM, such as regular branched-chain amino acid supplementation and a late-evening snack before starting IRT, and repeated education by dietitians are essential

if patients are to complete IRT safely and efficiently.

Long-term interferon therapy has been demonstrated to reduce development of HCC and improve survival of LC-C patients[23]. However, interferon therapy is not perfect with respect to its unpleasant adverse effects such as fever and appetite loss, and its expensiveness. In addition, interferon therapy is risky in elderly patients and in patients with severe thrombocytopenia or depression. On the other hand, IRT has few adverse effects and is not so expensive; IRT can perform safely in cirrhotic patients having thrombocytopenia or psychological diseases. Thus, we think that IRT might compensate imperfection of interferon therapy for LC-C patients.

Concerning IRT for LC-C patients, there are some points to be evaluated hereafter. Firstly, to confirm these beneficial effects of IRT, large-scale long-term prospective studies are needed for compensated LC-C patients. Secondly, since iron deposition enhances collagen synthesis in hepatic stellate cells and promotes the progression of hepatic fibrosis[24], it would be of great value to evaluate whether long-term IRT can regress hepatic fibrosis in patients with LC-C. Finally, the establishment of safe iron reduction strategies for LC-C patients having serum albumin levels of less than 3.6 mg/dL, e.g., the development of a new method of phlebotomy that removes only erythrocytes and transfuses the remainder into the vein, would be beneficial.

In conclusion, we demonstrated that IRT significantly reduced serum aminotransferases and alpha-fetoprotein levels for LC-C patients. IRT was generally safe; however, IRT should be performed in patients with serum albumin concentrations of more than 3.6 g/dL.

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Table 1. Baseline Characteristics of Study Groups

	IRT (n = 19)	Control (n = 19)	*P*
Age (years)	67 ± 9	65 ± 6	0.559
Gender (Male:Female)	8 : 11	8 : 11	1.000
BMI (kg/m^2)	23.3 ± 2.5	22.0 ± 2.5	0.343
Hypertension (n)	10	6	0.325
Diabetes (n)	4	2	0.660
Steatosis (n)	3	2	1.000
Hemoglobin (g/dL)	14.5 ± 1.1	14.0 ± 1.2	0.375
Platelet ($x10^4/\mu L$)	9.2 ± 2.9	8.5 ± 3.6	0.517
Albumin (g/dL)	4.0 ± 0.4	3.9 ± 0.3	0.378
Bilirubin (mg/dL)	0.8 ± 0.3	1.1 ± 0.4	0.519
AST (U/L)	89 ± 33	84 ± 26	0.609
ALT (U/L)	101 ± 44	93 ± 26	0.540
Iron (μg/dL)	161 ± 53	160 ± 45	0.935
Transferrin saturation (%)	45.0 ± 14.6	48.4 ± 15.6	0.583
Ferritin (ng/mL)	237 ± 145	222 ± 104	0.410
AFP (ng/mL)	28 ± 20	32 ± 24	0.642
Hyaluronic acid (ng/mL)	292 ± 203	286 ± 110	0.438
HCV-RNA (KIU/mL)	639 ± 416	672 ± 412	0.859

Quantitative data are expressed as means ± S.D. *P* values were calculated using the unpaired two-tailed t test and the χ^2 test, respectively. BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, alpha-fetoprotein, HCV-RNA; circulating hepatitis C virus RNA.

Table 2. Comparison of IRT Group Patients between Those with Serum Ferritin Concentrations More and Less Than 10 ng/mL after 6 Months of Treatment

	Ferritin <10 (n = 6)	Ferritin >10 (n = 13)	*P*
After 6 months of treatment			
Hemoglobin (g/dL)	10.2 ± 1.1	12.3 ± 1.9	**0.016**
Platelet ($x10^4/\mu L$)	12.7 ± 2.1	11.1 ± 2.7	0.244
Albumin (g/dL)	4.1 ± 0.8	3.9 ± 0.3	0.682
AST (U/L)	51 ± 13	59 ± 22	0.379
ALT (U/L)	55 ± 27	53 ± 24	0.882
Ferritin (ng/mL)	6 ± 2	35 ± 20	**<0.001**
AFP (ng/mL)	9 ± 6	13 ± 6	0.244
Changes (6 month/0 month)			
AST (%)	53 ± 15	78 ± 30	**0.045**
ALT (%)	43 ± 13	65 ± 28	0.056
AFP (%)	40 ± 14	58 ± 24	0.144

Quantitative data are expressed as means ± S.D. *P* values were calculated using the unpaired two-tailed t test. BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, alpha-fetoprotein.

Fig. 1. Changes in Serum Aminotransferase Levels during IRT.

Serum AST (A) and ALT levels (B) were significantly decreased by IRT. Data are expressed as means ± S.D.

Fig. 2. Comparison of Changes in the Representative Biochemical Markers.

Data are expressed as means ± S.D. #, $P < 0.05$ compared with the pretreatment (paired two-tailed t test).