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THE ADVANTAGE OF GASTRECTOMIZED PATIENTS IN MANAGEMENT OF THEIR CHRONIC HEPATITIS C

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ABSTRACT

Because the majority of patients with chronic hepatitis C do not respond to interferon, alternative treatments need to be established. Several lines of evidence suggest that iron depletion is beneficial for such patients. Thus, gastrectomized patients with a reduced capacity for iron absorption might have an advantage in treatment of their liver damage over patients with intact gastrointestinal tracts.

Four male gastrectomized patients had post-transfusion chronic hepatitis C. The iron load in three patients was adjusted below 10 ng/ml of serum ferritin level by phlebotomy. Subsequent interferon treatment for the four patients without iron load cleared circulating hepatitis C virus RNA in one patient only. However, serum ferritin concentrations were stabilized at low levels without maintenance phlebotomy, and sustained normalization of serum liver enzyme activities was obtained in all four patients. Similar treatments were done for 10 male patients with intact gastrointestinal tracts. The amount of removed iron from these patients was more than that from gastrectomized patients. Interferon also failed to clear circulating hepatitis C virus RNA except in one case. Low ferritin levels and sustained normalization of liver enzymes were seen in three patients. A transient elevation of ferritin levels with low enzyme activities was seen in two patients. Relapsing hepatitis was seen in five of the seven patients who needed maintenance phlebotomy due to a rebound in serum ferritin levels, probably because of active iron absorption from the intestine.

Our data suggest that depletion of cytotoxic iron is a key to managing patients with chronic hepatitis C.

Key Words: Interferon, Iron, Phlebotomy

INTRODUCTION

A high prevalence of hepatitis C virus (HCV) has been reported in hemosiderosis due to thalassemia major and porphyria cutanea tarda, and idiopathic hemochromatosis.[1-3] A good correlation between serum levels of ferritin and aminotransferases in male blood donors infected by HCV suggests participation of iron hepatotoxicity in the pathogenesis of their liver injury.[4] Effective iron removal supports iron cytotoxicity in patients with chronic hepatitis C (CHC).[5,6] Because gastrectomized patients have reduced iron absorption capability from the intestine due to insufficient gastric acid secretion, their CHC should be modified to some extent, and might respond differently to treatment. Thus, we tested whether gastrectomized patients have any advantage in treatment for CHC.

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MATERIALS AND METHODS

Four male patients with gastrectomy and 10 male patients with intact gastrointestinal (GI) tracts as controls were included in the study. All patients, positive for anti-HCV antibody by the 2nd generation test, had biopsy-proven chronic hepatitis. Pre-treatment liver specimens were processed for routine histology and histochemistry for iron. When histochemical iron was negative, a sensitive method, X-ray microanalysis,[7] was done to determine subcellular iron localization in hepatocytes. Iron, when concentrated in the lysosomal matrix, was removed by phlebotomy prior to interferon (IFN) treatment. The hemoglobin concentration and serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined monthly using standard laboratory methods. Serum ferritin concentrations were determined by radioimmunoassay. The hemoglobin concentration and serum levels of ferritin and aminotransferase activities at the pre-treatment, post-phlebotomy and post-IFN stages were expressed as mean values of data obtained over three months, and the values associated with the maintenance stage were mean values of data collected over the last 6 months of the one-year maintenance phlebotomy treatment period after IFN therapy. The biochemical parameters at each stage were compared by Student's t test. These parameters were also compared between gastrectomized patients and patients with intact GI tracts. The rebound of serum ferritin levels during the maintenance stage was tested in the patient groups using X^2 with continuity correlation.

Circulating HCV-RNA was measured using reverse transcription and nested polymerase chain reaction with primers of the 5′-noncoding region of the HCV[8] in sera obtained before phlebotomy and 6 months after IFN treatment. When it was negative after 6 months of observation, the test was repeated in sera at the end of the one year maintenance phlebotomy period. The HCV genotype was determined by two-stage polymerase chain reaction using type-specific primer[9] and the nomenclature was based on the system proposed by Simmonds et al.[10] Levels of circulating HCV RNA were measured with branched-DNA amplification assay in sera obtained at pre-treatment, post-phlebotomy and the end of the observation period.[11] These samples were stored at −70°C without thawing until testing.

The clinical and laboratory data of the patients are summarized in Table 1. Four patients had blood transfusions at gastrectomy and late complication with CHC. Three patients were infected with the HCV genotype 1b, and one patient with the HCV genotype 2a. Their circulating HCV-RNA titers were $1.8 \pm 1.6 \times 10^6$ eq/ml. The serum ferritin concentrations were less than 50 ng/ml. One patient was considered free from iron overload. His serum ferritin concentration was 4 ng/ml with minimal elevations of liver enzyme activities. Liver histology showed chronic persistent hepatitis without histochemical iron deposits. X-ray microanalysis confirmed the absence of iron concentrated in hepatocellular lysosomes. Another patient had chronic active hepatitis with cirrhosis and a negative test result for histochemical iron. X-ray microanalysis, however, showed iron deposits in hepatocellular lysosomes. His serum ferritin concentration was 11 ng/ml. Two other patients had chronic active hepatitis with histochemical iron deposits.

The patients with intact GI tracts differed in iron indices from gastrectomized patients. The serum ferritin levels of patients with intact GI tracts ranged between 48 and 777 ng/ml. Histochemical iron analysis of the liver was positive in eight of the 10 patients. The remaining two patients showed negative test results for histochemical iron but their hepatic iron overload was confirmed by X-ray microanalysis. Nine patients were infected with the HCV genotype 1b, and one patient with the HCV genotype 2a. Circulating HCV-RNA titers were $4.8 \pm 4.3 \times 10^6$ eq/ml. There was no difference in HCV RNA levels between the patient groups.

The protocol for iron removal from patients with CHC was approved by the ethics committee

Table 1. Clinical and laboratory data for gastrectomized patients and patients with intact GI tracts

Gastrectomized patients	Age	Alcoholic	Blood transfusion	HCV RNA (10^6 eq/ml)	Serum ferritin (μg/ml)	Liver*1 hisology	Histological iron
1	35	−	+	1.6	4	1	−*2
2	58	−	+	<0.5*3	11	3	−
3*4	46	+	+	4.1	34	2	+
4	60	+	+	0.9	45	2	+
Patients with intact GI tracts	**Age**	**Alcoholic**	**Blood transfusion**	**HCV RNA (10^6 eq/ml)**	**Serum ferritin (μg/ml)**	**Liver histology**	**Histological iron**
1	54	−	−	2.3	48	2	−
2	35	−	+	2.1	96	2	+
3	29	−	−	11	97	2	−
4	41	−	+	3.3	158	1	+
5	53	−	−	<0.5	158	2	+
6	43	−	−	6.0	218	3	+
7	45	−	−	8.0	226	2	+
8	42	+	−	<0.5*3	295	3	+
9	53	+	−	1.8	305	2	+
10	56	+	+	12.0	777	3	+

*1; 1; chronic persistent hepatitis, 2; chronic active hepatitis, 3; chronic active hepatitis with cirrhosis.
*2; X-ray microanalysis showed no iron concentration in hepatocellular lysosomes. Other three cases with negative test results (gastrectomized patient No. 2, patients with intact GI tracts No. 1 and 2) had iron concentrated in the lysosomes.
*3; Two patients were infected with genotype 2a, all other patients with genotype 1b.
*4; Gastrojejunostomy was done for this patient. The other three patients had gastroduodenostomy.

Table 2. Treatments of gastrectomized patients and patients with intact GI tracts

Gastrectomized patients	Phlebotomy (Amount removed; ml)	Interferon*1	Maintenance*3
1	0	α	UDCA
2	400	α*2	no (responder)
3	2400	β	UDCA
4	2400	α	UDCA
Patients with intact GI tracts	**Phlebotomy (Amount removed; ml)**	**Interferon*1**	**Maintenance*3**
1	2800	α	UDCA
2	3400	β	no (responder)
3	2800	α	UDCA/Phlebotomy
4	4000	α	UDCA
5	4000	α	UDCA/Phlebotomy
6	3100	β	UDCA/Phlebotomy
7	3200	β	UDCA/Phlebotomy
8	5200	α	UDCA/Phlebotomy
9	3200	α	UDCA/Phlebotomy
10	6600	β	UDCA/Phlebotomy

*1; A total dose of 288 × 10^6 U during 8 weeks for IFN-β, 500 × 10^6 U during 6 months for IFN α except for one patient.
*2; A total dose of 250 × 10^6 U was administered because of malaise.
*3; Two complete responders did not need any treatment. Viremic patients received a regimen of UDCA 600 mg per day. The drug treatment was combined with a maintenance phlebotomy when ferritin levels rebounded.

of Nagoya University Hospital. Informed consent was obtained from each patient who understood that IFN treatment would be delayed a few months because of iron removal by phlebotomy. Treatments are summarized in Table 2. Iron load in each of the 13 patients was adjusted below 10 ng/ml of serum ferritin concentration by repeated phlebotomies as reported previously.[5] Phlebotomy of 200 ml was done twice for a histochemical iron-negative patient with gastrectomy. Twenty-four phlebotomies during 12 months were needed to remove the maximal volume of 6600 ml from a non-gastrectomized patient with a serum ferritin level of 777 ng/ml. Mean blood volume removed was 1300 ml for gastrectomized patients and 3800 ml for patients with intact GI tracts. To clear circulating HCV, either a total dose of 500×10^6 U of IFN-α (Sumiferon, Sumitomo Pharmaceuticals, Tokyo) over 6 months, or 288×10^6 U of natural IFN-β (Feron, Toray Medical, Tokyo) for 8 weeks was administered. A total dose of 250×10^6 U of IFN-α was administrated to a gastrectomized patient who complained of malaise with the standard dose. A hepatoprotective drug, ursodeoxycholic acid (UDCA) 600 mg/day,[12] was allowed when HCV RNA reappeared. When serum levels of ferritin rebounded over 10 ng/ml with or without exacerbation (ALT above 50 U/L and reappearance of HCV RNA), iron removal by maintenance phlebotomy was combined with the medical treatment.

RESULTS

Changes in hemoglobin concentrations and serum ferritin levels are summarized in Table 3, and those of serum aminotransferase activities are in Table 4. Compared to the pre-treatment levels, both hemoglobin concentrations and serum ferritin levels at the three stages, post-phlebotomy, post-IFN and maintenance phlebotomy, remained low in gastrectomized patients (Fig. 1). In contrast, 8 of the 10 patients with intact GI tracts showed a rebound of serum

Table 3. Effect of treatments on the hemoglobin concentration and serum ferritin level

	Patient group	Pre-treatment	Post-phlebotomy	Post-interferon	Maintenance treatment
Hemoglobin	gastrectomized patients (n=4)	13.7 ± 1.7	8.9 ± 0.8	10.0 ± 1.5	10.1 ± 1.5
	patients with intact GI tracts (n=10)	14.9 ± 1.1	10.2 ± 1.6*1	11.7 ± 0.9*2	12.5 ± 0.8*3
	p value*4	0.03	nd	nd	0.01
Ferritin	gastrectomized patients (n=4)	24 ± 19	7 ± 2	7 ± 4	7 ± 2
	patients with intact GI tracts (n=10)	238 ± 208	7 ± 3*1	8 ± 3*1	13 ± 5*3
	p value*4	0.01	nd	nd	0.02

*1; different compared with pretreatment, *2; different compared with pretreatment and post-phlebotomy,
*3; different compared with pretreatment, post-phlebotomy and post-interferon,
*4; gastrectomized patients vs. patients with intact GI tracts, nd; no difference.

Table 4. Effect of treatments on serum aminotransferase activities

	Patient group	Pre-treatment	Post-phlebotomy	Post-interferon	Maintenance treatment
AST (U/L)	gastrectomized patients (n=4)	94 ± 46	75 ± 31	56 ± 22	34 ± 5
	patients with intact GI tracts (n=10)	74 ± 40	51 ± 22*	55 ± 20*	47 ± 25*
ALT (U/L)	gastrectomized patients (n=4)	126 ± 66	93 ± 56	63 ± 31	35 ± 8
	patients with intact GI tracts (n=10)	107 ± 54	59 ± 24*	68 ± 39*	53 ± 32*

*; different compared with pretreatment.

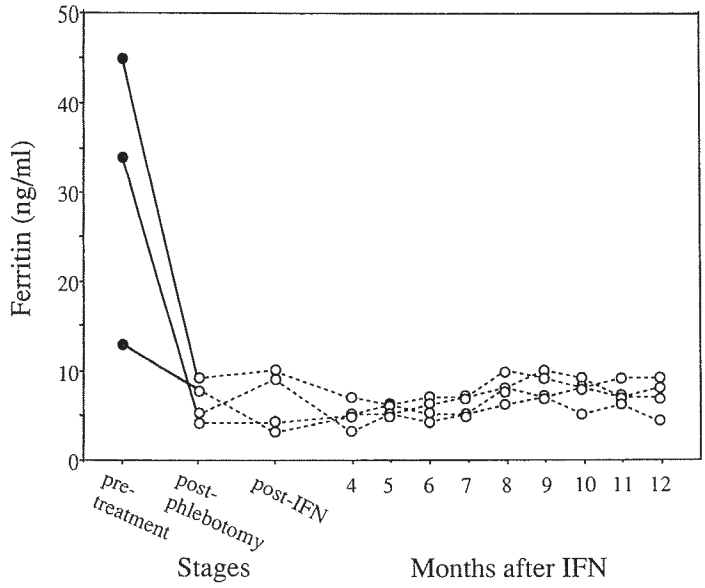

Fig. 1. Changes in serum ferritin levels in gastrectomized patients
Serum ferritin was stabilized at a low level during the one-year observation period so that no maintenance phlebotomy was required.

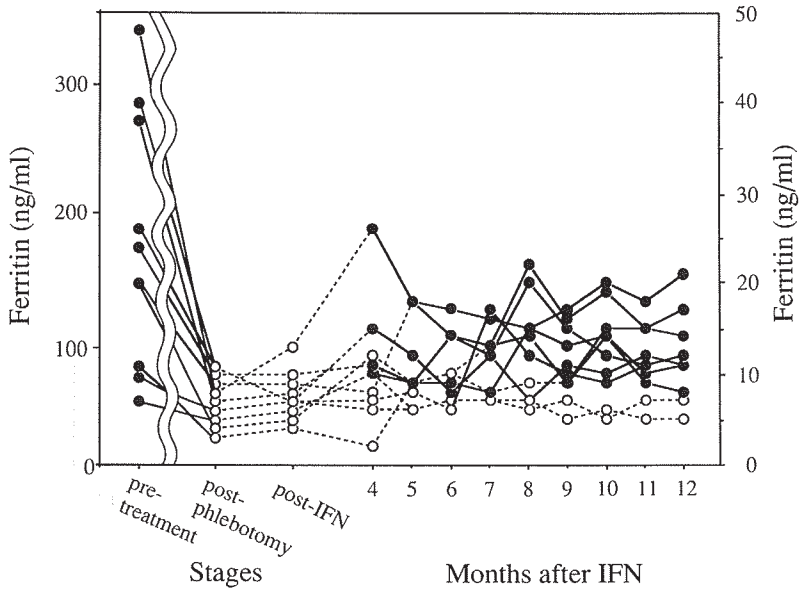

Fig. 2. Changes in serum ferritin levels in patients with intact GI tracts
Serum ferritin levels rebounded in 7 of the 10 patients and remained high in all but one patient even after maintenance phlebotomy. The solid line during the observation period indicates the level after maintenance phlebotomy.

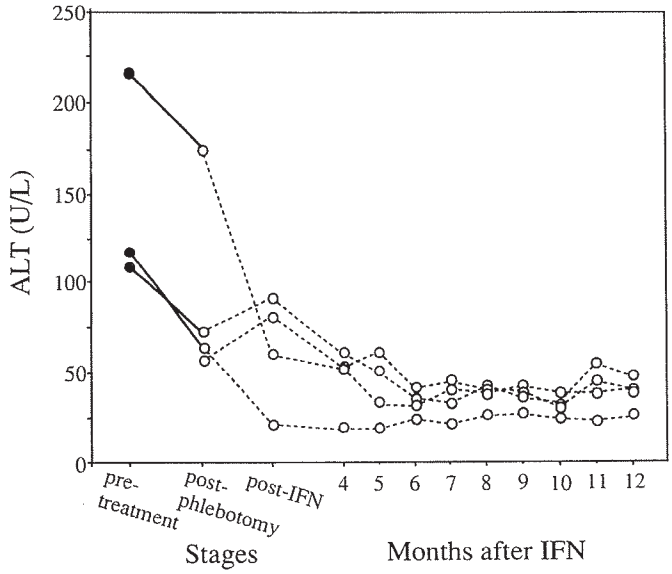

Fig. 3. Changes in serum ALT activities in gastrectomized patients
UDCA 600 mg/day alone almost normalized serum levels of ALT activities during the observation period.

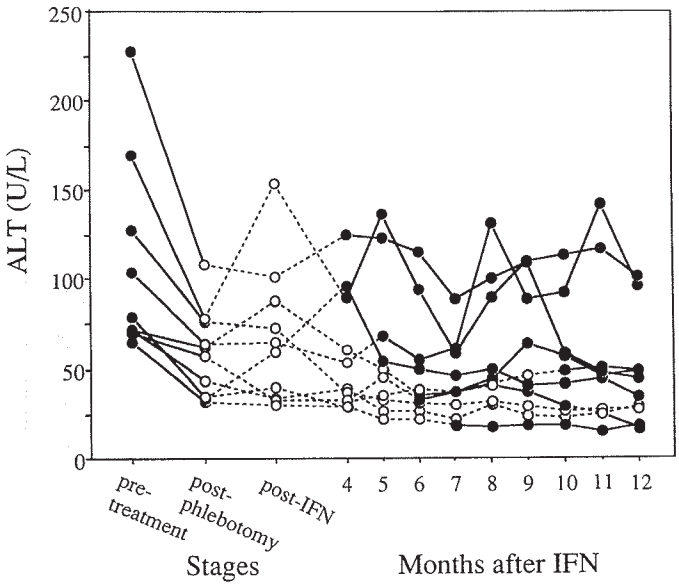

Fig. 4. Changes in serum ALT activities in patients with intact GI tracts
All the patients responded to the initial phlebotomy. Their enzyme levels fluctuated during the maintenance phlebotomy period. The solid line during the observation period indicates the level after maintenance phlebotomy.

ferritin levels over 10 ng/ml which required maintenance phlebotomy during the late observation period (Fig. 2). The rebound of serum ferritin levels in patients with intact GI tracts was significant compared to that in gastrectomized patients ($p < 0.04$ by X^2 with continuity correlation). Circulating HCV-RNA remained positive without a change in serum levels after iron removal ($1.4 \pm 1.1 \times 10^6$ eq/ml in gastrectomized patients and $3.9 \pm 6.1 \times 10^6$ eq/ml in patients with intact GI tracts). IFN cleared HCV-RNA from each one of the gastrectomized patients and patients with intact GI tracts. Responders from the different patient groups showed stabilization of the serum ferritin level with sustained normalization of liver enzyme activities. Iron removal by the initial phlebotomy significantly reduced serum AST and ALT activities in patients with intact GI tracts, but was not statistically significant in the gastrectomized patients, possibly because the number of gastrectomized patients studied was small. During the maintenance phlebotomy period, serum enzyme levels in patients with intact GI tracts remained low compared to pretreatment levels. Again there was no difference in the enzyme levels between the pretreatment stage and the maintenance phlebotomy period of gastrectomized patients. UDCA 600 mg/day stabilized the serum enzyme levels within a normal range in all three gastrectomized patients in spite of persistent viremia (Fig. 3). In contrast, stabilization of AST and ALT activities below 50 U/L was seen in only two of seven patients with intact GI tracts when the medication was combined with maintenance phlebotomy. The remaining five patients had an active form of CHC despite combined management. Over all, complete response was seen in one, remission in four and relapsing hepatitis in five patients with intact GI tracts at the end of the maintenance phlebotomy stage (Fig. 4).

Circulating HCV RNA levels during the late observation period were $2.2 \pm 1.4 \times 10^6$ eq/ml in RNA-positive gastrectomized patients and $4.5 \pm 4.5 \times 10^6$ eq/ml in HCV RNA-positive patients with intact GI tracts. At the end of the one-year maintenance phlebotomy period, mild anemia remained in two gastrectomized patients but their daily activities were fairly well preserved. Otherwise, untoward side effects were not reported.

DISCUSSION

This study clarifies the clinical significance of iron depletion in CHC. Based on the finding that iron was concentrated in hepatocellular lysosomes, most of our patients were loaded with iron. Only one exceptional case involved chronic persistent hepatitis with a serum ferritin level of 4 ng/ml. The iron load of gastrectomized patients was less, and its removal by phlebotomy was easier to complete than that of patients with intact GI tracts. But, it is unlikely that gastrectomized patients were not affected by iron hepatotoxicity. Ferrous ions can mediate free radical generation, which causes lipid peroxidation.[13] Therefore, iron load, even though it is slight, could be hepatotoxic in the subjects infected with HCV. In fact, serum ALT activities were a more reliable index of predicting iron removal treatment in CHC than iron index such as serum ferritin level.[14] Cytotoxic iron may be in the form of reactive metabolites rather than stored as iron proteins, but iron storage, since it is a source of such reactive iron species, should be as small as possible in management of CHC.

To determine whether iron removal prior to IFN is beneficial or not, we need further study of a large group of patients. Low hepatic iron content was found to be an index for IFN effect in CHC.[15,16] But, it seems likely that iron removal does not affect viremia nor enhance the antiviral effect of IFN to clear circulating HCV RNA. Observations in the USA indicated that iron removal improved serum levels of aminotransferases but did not affect circulating HCV RNA.[17] In Italy, there was no enhancement of the response to the second IFN treatment after iron

removal in patients who had responded poorly to the first trial.[18] Hepatic iron may predict the response of CHC infection to IFN therapy, but it does not determine the anti-viral effect of IFN.

Interferon therapy induces a long-term carrier state of HCV infection with normal ALT levels in some patients.[19] The underlying mechanisms involved here are not clear as yet. In our patients, reduced iron absorption may be a possile explanation for normalization of liver enzyme activities. Neither relapsing hepatitis nor hyperferritinemia was seen in all three gastrectomized patients in spite of persistent viremia during the post-IFN observation period. In contrast, rebound of serum ferritin levels was associated with relapsing hepatitis in patients with intact GI tracts. Hyperferritinemia may not only be the result of relapsing hepatitis, but may also be closely related to restored iron which could be a trigger for liver damage during the post-IFN observation period. One of the characteristics of gastrectomized patients is poor iron absorption from GI tracts because of insufficient acidification of ingested nutrients. Thus, their pretreatment iron storage was small, and iron absorption was limited even in an iron-depleted state. In patients with an intact GI tract, gastric acid is secreted normally, resulting in large iron absorption and storage. After iron depletion, much iron may be absorbed effectively and reach the liver. Reactive iron species, either derived from iron stores or absorbed from dietary nutrients, can mediate free radical generation, which causes lipid peroxidation.[13] Thus, active absorption from the GI tracts may be a route for supplying cytotoxic iron in iron-depleted CHC patients with intact GI tracts. For better treatment of such patients, medication and maintenance phlebotomy should be combined with some efforts to limit iron absorption in the intestine.

Our data suggest that gastrectomized patients have an advantage in management of iron-induced hepatotoxicity complicated by CHC over patients with intact GI tracts because of their relatively easy transition to an iron depletion state.

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