J Gastroenterol 2004; 39:601–603
DOI 10.1007/s00535-004-1372-3

Journal of Gastroenterology



*Editorial*

# Phlebotomy: a promising treatment for chronic hepatitis C

Article on page 570
**A significant reduction in serum alanine aminotransferase levels after 3-month iron reduction therapy for chronic hepatitis C: a multicenter, prospective, randomized, controlled trial in Japan**
YANO M, HAYASHI H, YOSHIOKA K, et al.

## Iron overload and chronic liver disease

The liver is an iron-rich organ, which contains approximately 30% of the total iron storage for the body.[1] Hereditary hemochromatosis, which is a common genetic disorder of iron metabolism, leads to liver injury and fibrosis, and eventually to hepatic failure and hepatocellular carcinoma.[2] Acquired excessive iron storage in the liver is known to be associated with several types of chronic liver disease such as chronic viral hepatitis, porphyria cutanea tarda, postportocaval shunting, alcoholic liver disease, and nonalcoholic steatohepatitis. These diseases are classified as iron overload disorders/syndromes.[2]

Iron overload results in hepatocyte injury. For example, in mice that are chronically fed an iron-rich diet, an elevation in serum alanine aminotransferase (ALT) levels was observed.[3] In patients receiving frequent blood transfusions because of acquired anemia, elevated ALT levels were seen only at hepatic iron concentrations of more than 300 μM/g.[4] These findings demonstrate that iron itself possesses hepatotoxicity, and iron overload may aggravate several chronic liver diseases.

## How does iron overload induce hepatotoxicity?

In hepatocytes, the most toxic type of reactive oxygen species (ROS), the hydroxy radical (·OH), appears in the presence of ferrous iron (Fenton reaction). Once this ROS is generated in hepatocytes, the levels of a number of antioxidants (catalase, glutathione peroxidase, superoxide dismutase, etc.) increase, which leads to a decrease in ROS.[5,6] However, when the formation of ROS exceeds the capacity of the antioxidant system, the lipid membranes of organelles are oxidized by the ROS, cell function is impaired, and subsequent apoptosis/necrosis takes place.[5,6] Forced iron overload results in increased hepatic hydroperoxides, malondialdehyde and hydroxynonenal, which are markers of lipid peroxidation.[7] The presence of ROS also modulates inflammatory responses through the activation of nuclear factor kappa B.[5] Moreover, increases in lipid peroxidation due to chronic iron overload lead to the formation of 8-hydroxy-2′-deoxyguanosine,[8,9] mitochondrial DNA aberration,[10] and p53 or c-*myc* mutation,[11] which may eventually lead to hepatocarcinogenesis. Thus, hepatocyte toxicity caused by iron overload is primarily attributed to the enhancement of ROS formation and resultant lipid peroxidation.

Iron also activates Kupffer cells, promotes the release of proinflammatory cytokines and ROS generation, and damages hepatocytes in a paracrine manner.[6,12] In hepatic stellate cells, iron enhances collagen synthesis and promotes the progression of hepatic fibrosis.[6,12] It is thought that these iron-induced pathological changes are deeply related to an increased formation of ROS. In animal models employing the chronic administration of an iron-containing diet, antioxidant supplementation significantly prevents the progression of hepatic fibrosis.[13,14]

## The relationship between iron storage and HCV

It has already been demonstrated that hepatic iron accumulation is strongly associated with the pathogenesis of chronic hepatitis C. Serum ferritin levels and hepatic iron concentrations were significantly higher in hepatitis C virus (HCV)-positive patients than in hepatitis B virus (HBV)-positive patients.[15] Successful interferon therapy reversed enhanced hepatic iron accumulation and lipid peroxidation.[16] These findings support the

*Reprint requests to:* N. Tanaka

hypothesis that HCV itself might contribute to hepatic iron accumulation. Although the precise mechanism remains unclear, HCV may directly or indirectly influence the expression of iron regulatory proteins, which in turn play a central role in the maintenance of iron intracellular homeostasis.[17]

Because the HCV core protein enhances the formation of ROS,[10,18] hepatic iron overload may accelerate ROS-induced hepatocyte injury caused by persistent HCV infection. In fact, in chronic hepatitis C patients carrying the hereditary hemochromatosis gene (*HFE*) mutation referred to as C282Y, serum ferritin levels were found to be higher, hepatocyte iron staining was more commonly observed, and hepatic fibrosis was more advanced than in homozygous normal patients with chronic hepatitis C.[19–22] Therefore, it is quite reasonable to hypothesize that iron reduction therapy, including phlebotomy and dietary iron restriction, may ameliorate the activity of chronic hepatitis C and prevent its progression to cirrhosis.

## Phlebotomy for chronic hepatitis C

The beneficial effects of phlebotomy for patients with chronic hepatitis C have been previously reported. Hayashi et al.[23] reported that in 10 patients who underwent phlebotomy, serum ALT levels decreased in all patients (from 152 ± 49 to 55 ± 32 U/l). According to a report by Kato et al.,[24] serum ALT levels significantly improved in 34 patients after 6-year iron reduction therapy (from 150 ± 73 to 35 ± 11 U/l). A randomized controlled study reported by Yano et al.[25] (in this issue of the *Journal of Gastroenterology*) is noteworthy due to its clarification of the efficacy of phlebotomy for the improvement of serum ALT levels in Japanese patients with chronic hepatitis C. Although long-term histological changes were not investigated in the present study, it is expected that sustained improvement in ALT levels would reverse the progression of fibrosis.

This study[25] also demonstrated the high level of safety of phlebotomy for chronic hepatitis C patients. Clinicians occasionally hesitate to introduce interferon therapy, especially in elderly chronic hepatitis C patients and patients with disease complicated by hematological abnormalities, diabetes mellitus, severe systemic arteriosclerosis, and other disorders. The results of 6-month interferon/ribavirin combination therapy remain unsatisfactory for Japanese patients suffering from chronic HCV infection. Therefore, we expect that phlebotomy would be a useful and safe therapy to employ as a substitute for long-term interferon administration.

## Further perspectives

It will be important to investigate whether long-term phlebotomy might prevent the progression of hepatic fibrosis and the emergence of hepatocellular carcinoma. A related issue would be the potential of phlebotomy therapy to induce the regression of hepatic fibrosis and to prevent progression to hepatocellular carcinoma in patients with HCV-related cirrhosis. To solve these questions, further long-term randomized controlled studies are needed. Moreover, in Japanese patients with chronic hepatitis C, the association between *HFE* gene mutation and the development of hepatic fibrosis and hepatocellular carcinoma should be investigated.

Naoki Tanaka, M.D., Ph.D.
Department of Internal Medicine, Showa Inan General Hospital, 3230 Akaho, Komagane 399-4191, Japan

Kendo Kiyosawa, M.D., Ph.D.
Division of Gastroenterology, Department of Internal Medicine, Shinshu University School of Medicine, Matsumoto, Japan

## References

1. Bonkovsky HL. Iron and the liver. Am J Med Sci 1991;301:32–43.
2. Bacon BR. Hemochromatosis: diagnosis and management. Gastroenterology 2001;120:718–25.
3. Tjalkens RB, Valerio LG Jr, Awasthi YC, Petersen DR. Association of glutathione *S*-transferase isozyme-specific induction and lipid peroxidation in two inbred strains of mice subjected to chronic dietary iron overload. Toxicol Appl Pharmacol 1998; 151:174–81.
4. Jensen PD, Jensen FT, Christensen T, Nielsen JL, Ellegaard J. Relationship between hepatocellular injury and transfusional iron overload prior to and during iron chelation with desferrioxamine study in adult patients with acquired anemias. Blood 2003;101:91–6.
5. Loguercio C, Federico A. Oxidative stress in viral and alcoholic hepatitis. Free Radic Biol Med 2003;34:1–10.
6. Parola M, Robino G. Oxidative stress-related molecules and liver fibrosis. J Hepatol 2001;35:297–306.
7. Sochaski MA, Bartfay WJ, Thorpe SR, Baynes JW, Bartfay E, Lehotay DC, et al. Lipid peroxidation and protein modification in a mouse model of chronic iron overload. Metabolism 2002; 51:645–51.
8. Kappus H. Oxidative stress in chemical toxicity. Arch Toxicol 1987;60:144–9.
9. Farber JL, Kyle ME, Coleman JB. Mechanisms of cell injury by activated oxygen species. Lab Invest 1990;62:670–9.
10. Moriya K, Nakagawa K, Santa T, Shintani Y, Fujie H, Miyoshi H, et al. Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. Cancer Res 2001;61:4365–70.
11. Hussain SP, Raja K, Amstad PA, Sawyer M, Trudel LJ, Wogan GN, et al. Increased p53 mutation load in nontumorous human liver of Wilson disease and hemochromatosis: oxyradical overload diseases. Proc Natl Acad Sci U S A 2000;97:12770–5.
12. Pietrangelo A. Iron-induced oxidant stress in alcoholic liver fibrogenesis. Alcohol 2003;30:121–9.

13. Pietrangelo A, Borella F, Casalgrandi G, Montosi G, Ceccarelli D, Gallesi D, et al. Antioxidant activity of sylibin in vivo during chronic iron overload in rats. Gastroenterology 1995;109:1941–9.
14. Pietrangelo A, Gualdi R, Casalgrandi G, Montosi G, Ventura E. Molecular and cellular aspects of iron-induced hepatic cirrhosis in rodents. J Clin Invest 1995;95:1824–31.
15. Farinati F, Cardin R, De Maria N, Della Libera G, Marafin C, Lecis E, et al. Iron storage, lipid peroxidation and glutathione turnover in chronic anti-HCV positive hepatitis. J Hepatol 1995; 22:449–56.
16. Kageyama F, Kobayashi Y, Kawasaki T, Toyokuni S, Uchida K, Nakamura H. Successful interferon therapy reverses enhanced hepatic iron accumulation and lipid peroxidation in chronic hepatitis C. Am J Gastroenterol 2000;95:1041–50.
17. Cairo G, Recalcati S, Pietrangelo A, Minotti G. The iron regulatory proteins: targets and modulators of free radical reactions and oxidative damage. Free Radic Biol Med 2002;32:1237–43.
18. Okuda M, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, et al. Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. Gastroenterology 2002;122:366–75.
19. Smith BC, Gorve J, Guzail MA, Day CP, Daly AK, Burt AD, et al. Heterozygosity for hereditary hemochromatosis is associated with more fibrosis in chronic hepatitis C. Hepatology 1998;27: 1695–9.
20. Diwakaran HH, Befeler AS, Britton RS, Brunt EM, Bacon BR. Accelerated hepatic fibrosis in patients with combined hereditary hemochromatosis and chronic hepatitis C infection. J Hepatol 2002;36:687–91.
21. Tung BY, Emond MJ, Bronner MP, Raaka SD, Cotler SJ, Kowdley KV. Hepatitis C, iron status, and disease severity: relationship with HFE mutations. Gastroenterology 2003;124:318–26.
22. Pietrangelo A. Hemochromatosis gene modifies course of hepatitis C viral infection. Gastroenterology 2003;124:1509–23.
23. Hayashi H, Takikawa T, Nishimura N, Yano M, Isomura T, Sakamoto N. Improvement of serum aminotransferase levels after phlebotomy in patients with chronic active hepatitis C and excess hepatic iron. Am J Gastroenterol 1994;89:986–8.
24. Kato J, Kobune M, Nakamura T, Kuroiwa G, Takada K, Takimoto R, et al. Normalization of elevated hepatic 8-hydroxy-2′-deoxyguanosine levels in chronic hepatitis C patients by phlebotomy and low iron diet. Cancer Res 2001;61:8697–702.
25. Yano M, Hayashi H, Yoshioka K, Kohgo Y, Saito H, Niitsu Y, et al. A significant reduction in serum alanine aminotransferase levels after 3-month iron reduction therapy for chronic hepatitis C: a multicenter, prospective, randomized, controlled trial in Japan. J Gastroenterol 2004;39:570–4.