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Editorial

Molecular mechanism of iron metabolism and overload in chronic hepatitis C

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Efficacy and safety of 6-month iron reduction therapy in patients with hepatitis C virus-related cirrhosis: a pilot study
Tanaka N, Horiuchi A, Yamaura T, et al.

Iron is a ubiquitous element in the environment and plays an important role in several biological conditions. It is an essential element for all living organisms, because it is required in several metabolic processes, including DNA synthesis, oxygen transport, and energy production.[1] However, excess iron can be harmful to the organism, in part through the generation of free radicals. Excess divalent iron generates free radicals, mainly via Fenton chemistry,[2] that is, the formation of highly reactive hydroxyl radicals through its reaction with H_2O_2. Recent works have demonstrated the molecular mechanisms of iron metabolism (see Fig. 1 for a map of iron metabolism). Dietary trivalent iron is reduced to divalent iron by the duodenal cytochrome b.[3] Divalent iron is absorbed by intestinal cells via the divalent metal transporter (DMT1)[4] in the brush-border membrane, and then transferred to the portal blood flow via ferroportin 1 (FP1)[5] in the basolateral surface. It is oxidized to trivalent iron by hephaestin[6] on the basolateral surface. Trivalent iron binds to transferrin and then is transported to the liver and bone marrow. On the hepatocyte membrane, transferrin receptor (TfR) 1[7,8] and TfR2[9] bind to two diferric transferrin molecules, leading to internalization of the complex into endosomes. Trivalent iron is released from the complex in the endosome at low pH. It is reduced to divalent iron in the endosome, and then this divalent iron is absorbed by hepatocytes via DMT1. Hydroxyl radicals are formed via Fenton chemistry. Excess trivalent iron is stored as ferritin in the cytoplasm or as hemosiderin in lysosomes. Iron homeostasis is tightly regulated in all organisms. The peptide hormone hepcidin, which plays a central role in the regulation of iron homeostasis, derives from hepatocytes; hepcidin is believed to be the central iron sensor in mammals.[10–12] Hepcidin binds to FP1 and inhibits cellular iron efflux by inducing internalization of FP1.[13] Iron-induced oxidative stress may be negligible in healthy persons, but it can induce organ damage in some sensitive hosts.

Some patients with chronic hepatitis (CH), regardless of etiology,[14] have iron overload, particularly those with chronic hepatitis C (CHC).[15] Electron microscopic studies have shown that most patients with CHC have iron-induced oxidative stress, as demonstrated by the presence of lysosomal iron stores detected by X-ray microanalyzer.[16] An iron-rich diet provokes biochemical and pathological exacerbation of liver injury in chronic hepatitis C virus (HCV)-infected chimpanzees.[17] In line with this finding, iron reduction therapy by phlebotomy[18,19] or dietary iron restriction[20,21] has been found to reduce hepatic inflammation and to lower aminotransferase levels in CHC patients.

What are the molecular mechanisms underlying hepatic iron overload in CHC patients? We investigated the expression of TfR1, TfR2, FP1, and hepcidin messenger RNA in the livers of patients with chronic hepatitis B (CHB), CHC, and controls. TfR2 was higher in the livers of CHC patients that in those of CHB patients.[22] Hepcidin was lower in CHC patients than in CHB patients.[23] Hepcidin and TfR2 may play a role in the pathogenesis of iron overload in CHC patients. Decreased hepcidin levels and increased TfR2 levels contribute to delivery of iron to hepatocytes from macrophage iron stores and intestinal mucosa. Iron homeostasis is disturbed in CHC patients

Hydroxyl radicals cause accumulation of 8-hydroxy-2′-deoxyguanosine (8-OHdG)[24] during DNA and lipid peroxidation[15,25] in the cytoplasm of hepatocytes. This is believed to contribute to the pathogenesis of carcinogenesis and liver injury progression in CHC patients. Nitric oxide is also involved in the process of chronic inflammation-mediated carcinogenesis.[26] Nucleic acid damage by reactive nitrogen and oxygen species may also constitute important causative factor of HCV-related hepatocarcinogenesis. We measured 8-

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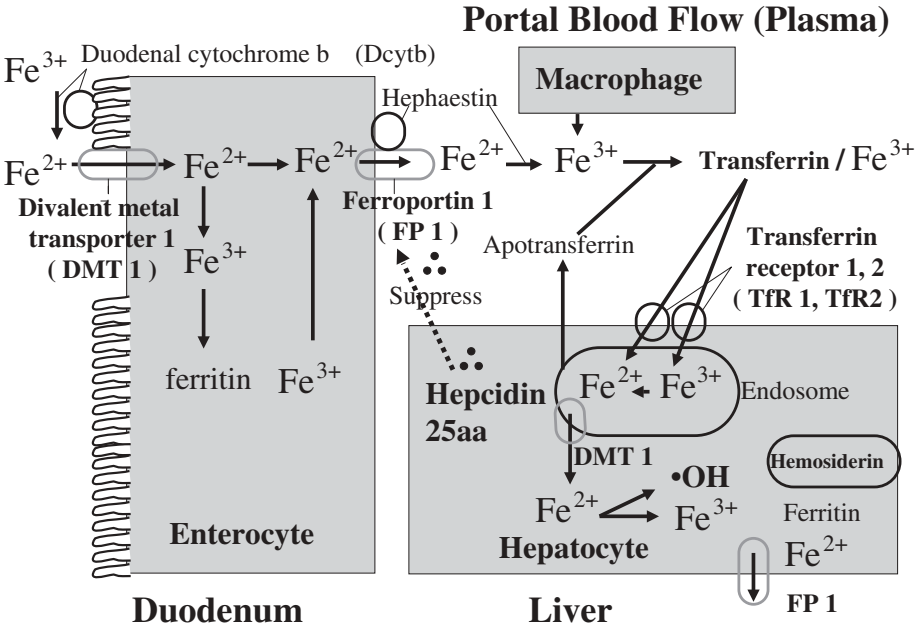

Fig. 1. Metabolic map of iron

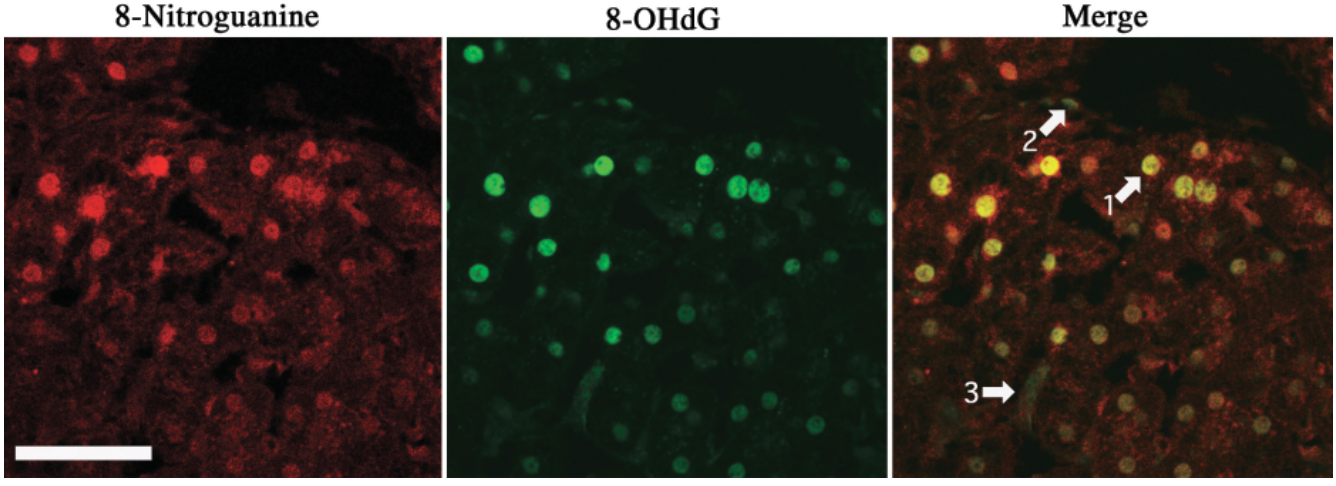

Fig. 2. 8-Nitroguanine and 8-hydroxy-2′-deoxyguanosine (*8-OHdG*) accumulation in the liver tissue from a chronic hepatitis C patient. 8-Nitroguanine immunoreactivity is strongly observed in the nuclei and weakly in the cytoplasm of hepatocytes, whereas 8-OHdG immunoreactivity is observed mainly in the nuclei (*arrow 1*, hepatocyte; *arrow 2*, lymphocyte; and *arrow 3*, Kupffer cell). Scale bar = 100 μm

nitroguanine and 8-OHdG in the livers of CHC patients before and after interferon therapy.[27] We found strong immunoreactivity of 8-nitroguanine and 8-OHdG in the livers from CHC patients (Fig. 2). 8-Nitroguanine is extremely unstable on DNA, and depurination easily occurs, leading to formation of apurinic sites.[28] Apurinic sites and 8-OHdG on DNA can cause G to T transversions.[29] Indeed, mutations of p53, β catenin, and other proto-oncogenes and tumor suppressor genes are frequently observed in HCV-associated hepatocellular carcinoma (HCC).[30] Recent studies have also demonstrated a causative link between moderate iron overload by iron supplementation and HCC in mice transgenically expressing the full HCV polyprotein.[31] Hepatic iron accumulation, by feeding a diet containing carbonyl iron, induces mitochondrial alterations, hepatic steatosis, fatty acid oxidation, formation of 8-OHdG, and tumor development in mice. This study demonstrated the existence of a critical interaction between HCV proteins and iron in the development of HCV-related HCC.[31]

As a result of the publication of a multicenter, prospective, randomized, controlled clinical trial showing

the usefulness of phlebotomy for the treatment of CHC patients,[32] Japanese national health insurance started covering phlebotomy for CHC patients in April 2006. Iron reduction therapy by phlebotomy has become the standard treatment for CHC patients in Japan, and it has been reported to definitely reduce lipid peroxidation,[33] hepatic content of 8-OHdG,[24] and oxidative stress in CHC patients. The study by Tanaka et al.[34] in this issue of the *Journal of Gastroenterology* shows the efficacy of this therapy for compensated HCV-related liver cirrhosis (LC-C) patients. The authors demonstrate that iron reduction therapy by phlebotomy and dietary iron restriction significantly reduces serum aminotransferase and α-fetoprotein (AFP) levels in LC-C patients. High AFP is a major risk factor for the development of HCC.[35] Therefore, decreasing serum AFP levels by iron reduction therapy may be an important means of preventing the development of HCV-related HCC. The authors also show adverse effects of iron reduction therapy. Two of 22 LC-C patients developed ascites, probably owing to decreased serum albumin levels. Tanaka et al.[34] believe that iron reduction therapy should be performed only in patients with a serum albumin concentration of more than 3.6 g/dl. To avoid adverse effects in LC-C patients treated with phlebotomy, the reduction of blood volume and interval between phlebotomies should be taken into account in patients with hypoalbuminemia. Small-volume (100-ml) phlebotomies should be repeated monthly. Clinical application of erythrocytapheresis to decompensated LC-C patients should also be considered as an alternative therapy to deplete excessive iron accumulation.

Iron homeostasis in patients with HCV-related chronic liver diseases is unbalanced. Therefore, patients with this disease should avoid ingestion of foods with excessive iron content[21] or commercially available high-energy "healthy foods"[36] rich in protein and vitamins, as reported by Patek et al.[37] Dietary iron restriction[20,21] (an iron intake of 6 mg/day or less), an energy intake of 30 kcal/kg per day, a protein intake of 1.1–1.2 g/kg per day, and 20% of energy derived from fat are recommended for patients with HCV.

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