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Case report

Activated hepatic stellate cells participate in liver fibrosis in a patient with transfusional iron overload

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Abstract: We describe liver fibrosis caused by iron overload after a long history of blood transfusion in a patient with chronic renal failure. Pertinent laboratory data were: serum (s)-Fe 148 μg/dl; unsaturated iron binding capacity (UIBC) 14 μg/dl; s-ferritin 9350 ng/ml; human leukocyte antigen (HLA) A2, A24, B39, B55, Cw1, Cw7. Computed tomography revealed a high density in the liver, and laparoscopy revealed a brown liver. Liver histology showed bridging fibrosis from portal tracts. A heavy iron deposit was seen in Kupffer cells as well as in hepatocytes surrounded by fibrosis around the portal tracts. Immunocytochemistry revealed α-smooth muscle actin in many stellate cells distributed along the fibrotic area, and electron microscopy revealed infiltrating myofibroblastic stellate cells coexisting with collagen fibers around degenerated hepatocytes containing iron deposits. The findings are consistent with the notion that stellate cells play an important role in liver fibrogenesis in both genetic and transfusional iron overload hemochromatosis.

Key words: liver fibrosis, iron, transfusion, hepatic stellate cell

Introduction

In patients with hereditary hemochromatosis the clinical and pathological features of iron overload are well known.[1,2] In patients with chronic renal failure, iron overload is often caused by intravenous blood transfusion or iron dextran,[3] and there are common liver pathology findings in hereditary hemochromatosis and heavy parenteral iron overload.[4,5] After a long history of blood transfusion, iron accumulates not only in the reticuloendothelial system but also in the parenchymal cells.[4] In hereditary hemochromatosis, liver fibrosis occurs with increasing hepatic iron concentration, and hepatic stellate cells are recognized to play a central role in this liver fibrosis.[6,7] We demonstrated liver fibrosis in a renal transplant recipient with a long history of blood transfusion. We investigated the ultrastructural features of stellate cells and the presence of α-smooth muscle actin (α-SMA) in these cells, to elucidate the role of these cells in liver fibrosis caused by transfusional iron overload. We also present a review of the literature.[8–10]

Case report

A 39-year-old man with chronic renal failure was referred to our department on December 4, 1994 for evaluation of abnormal liver function test results. Proteinuria had been shown in 1965. He had been receiving long-term hemodialysis since 1972 and received the first renal allograft in our hospital on December 12, 1973. However, the cadaveric transplant failed because of immunological rejection, and he had a retransplant in 1984. He had received 300 U of packed red blood cells during 22 years, but no iron dextran had been administered. He had been taking prednisone and azathioprine since 1973, but he did not drink alcohol. On admission, his skin was pigmented, and elastic liver was palpable 5 cm beneath the right costal margin. Laboratory findings on admission are shown in Table 1. Computed tomography (CT) scan revealed high density of the liver, spleen, and pancreas. The CT density of the liver was 106 Hounsfield units (HU) (Fig. 1). Magnetic resonance (MR) revealed low intensity in the liver on T1- and T2-weighted images. Laparoscopy revealed an enlarged brown liver, and, histologically, bridging

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Table 1. Laboratory findings on admission

RBC	3.28×10^6/mm^3
Hb	9.7 g/dl
Ht	29.6%
PLT	12×10^4/mm^3
TP	5.7 g/dl
T-Bil	0.89 mg/dl
AST	41 IU/l
ALT	52 IU/l
ALP	357 IU/l
γ-GTP	156 IU/l
ChE	3.62 IU/ml
T-Cho	132 mg/dl
hyaluronate	424 ng/ml
desferal test	5.7 mg/dl
BUN	48 mg/dl
Cr	2.9 mg/dl
s-Fe	148 μg/dl
UIBC	14 μg/dl
s-ferritin	9350 ng/ml
HLA A2, A24, B39, B55, Cw1, Cw7	
HbsAg, HCV-RNA	—

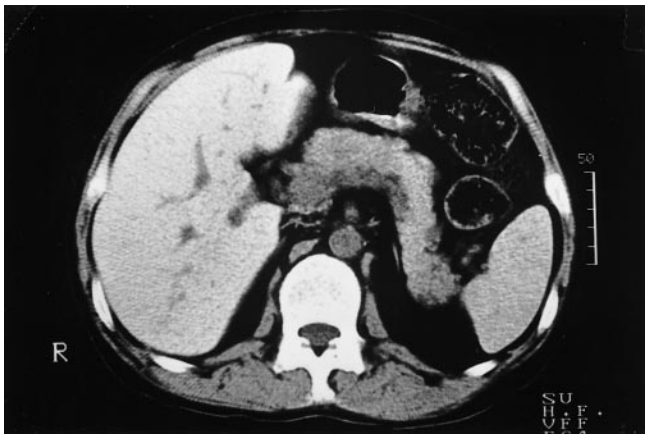

Fig. 1. High density of the liver, spleen, and pancreas was revealed on computed tomography (CT). The CT density of the liver was 106 Hounsfield units

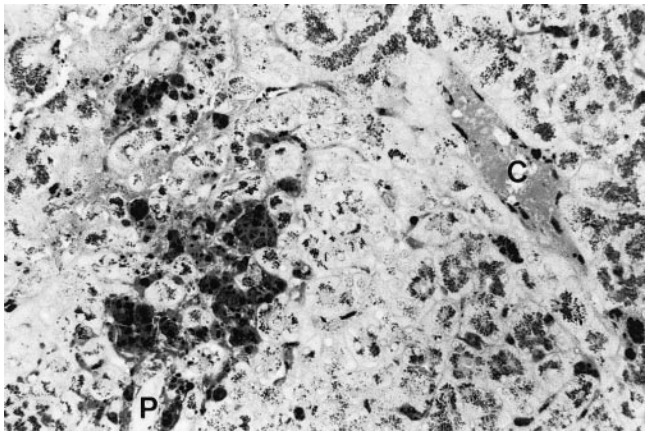

Fig. 2. Iron was heavily deposited in Kupffer cells and hepatocytes around the portal tracts (*P*) and along the fibrotic area. Kupffer cells and hepatocytes around the central vein (*C*) and in the middle zone contained fine granules of iron. Prussian blue, ×130

fibrosis was seen in the liver. Iron deposits were found in Kupffer cells and parenchymal cells, and hepatocytes with heavy iron loading were distributed mainly in the periportal area (Fig. 2). Masson's Trichrome staining showed fibrotic changes co-localized with heavy iron-laden hepatocytes (Fig. 3A). Immunocytochemical study of a serial section revealed α-SMA in proliferated stellate cells near collagen fibers surrounding the heavy iron-laden hepatocytes, the stellate cells formed a continuous network with the collagen fibers (Fig. 3B). Electron microscopy revealed degenerated hepatocytes laden with iron deposits surrounded by collagen fibers, Kupffer cells, and myofibroblastic stellate cells (Fig. 4).

Discussion

Iron is essential for life, but excessive intake is toxic to many organs. The liver is the major organ for iron storage and is susceptible to iron's toxic effect. Our patient had received 300 U of packed red blood cells over a period of 22 years. His serum ferritin level was very high. The CT density of the liver was 106 HU; an upper limit of 70 HU for coefficient attenuation provided 64% sensitivity and 87% specificity for hepatic iron overload.[11] Hepatic MR revealed a low intensity. These image analyses, together with the elevated serum ferritin, indicated heavy iron deposition in the liver.[12] Laparoscopy revealed a brown liver, and biopsy showed bridging fibrosis and heavy iron deposition. These findings were consistent with the diagnosis of liver fibrosis due to heavy parenteral iron overload.

Iron deposition is often seen in both inherited and acquired disorders,[13] and iron deposition caused by transfusion of large amounts of blood saturates Kupffer cells and parenchymal cells.[4] The precise mechanism of hepatocellular injury in iron overload is still unknown, but iron has a catalytic action in the generation of free radicals, which promotes lipid peroxidation of hepatocytes. This is considered to be the main mechanism of cell injury in iron overload,[14,15] eventually leading to liver fibrosis.

Recent advances in studies of liver fibrosis have determined that hepatic stellate cells play a central role in the fibrorgenesis,[8,16] and activated stellate cells

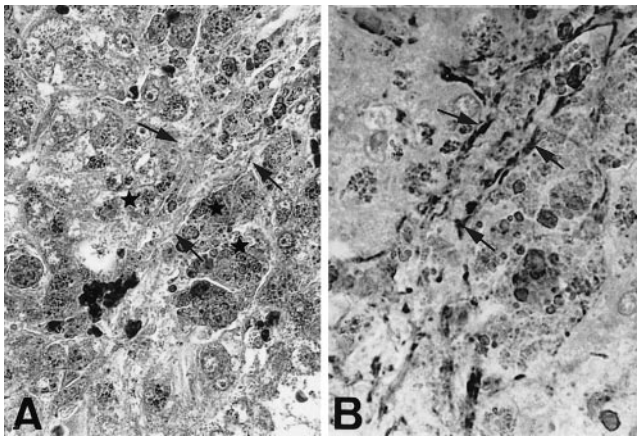

Fig. 3. A Masson's Trichrome staining. Fibrotic areas (*arrows*) were co-localized with heavy iron-laden hepatocytes (*asterisks*) around the portal tracts. **B** Immunocytochemical study of serial section stained for α-smooth muscle actin (SMA). α-SMA Immunoreactivity (*arrows*) was seen in stellate cells along the fibrotic area. **A,B,** ×530

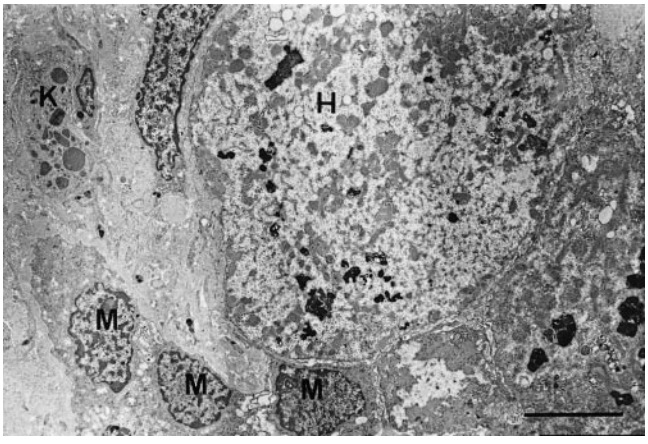

Fig. 4. Electron microscopic findings. Degenerated hepatocytes (*H*) around the portal tract contained iron granules, and were surrounded by collagen, fibers Kupffer cells (*K*), and myofibroblastic stellate cells (*M*). *Bar*, 5 μm

have been reported to participate in liver fibrosis in necro-inflammatory injury,[17,18] as well as hereditary hemochromatosis,[9,19] expressing collagens and other extracellular matrix constituents. Current studies also indicate that the proliferation of hepatic stellate cells is associated with a change in cellular phenotype to that of activated myofibroblasts, characterized by loss of retinoid droplets, increase in rough endoplasmic reticulum, and the expression of α-SMA.[10] In our patient, stellate cells with α-SMA proliferated in the portal area with sideronecrotic injury, coexisting with fibrosis. Ultrastructurally, we observed myofibroblastic stellate cells, Kupffer cells, and increased collagen fibers around the iron-laden degenerated hepatocytes.

Although the precise mechanism by which stellate cells are activated in transfusional iron overload is not known, stellate cells may be activated by degenerated hepatocytes and Kupffer cells through soluble factors, such as insulin-like growth factor-1, fibroblast growth factor, transforming growth factor (TGF)-α, and TGF-β.[6,20,21] In our patient, there was a common association between activated stellate cells and fibrosis. Activated stellate cells are thought to play an important role in liver fibrosis in both genetic and transfusional iron overload hemochromatosis.

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