Ultrastructural localization of Cu, Zn-SOD in hepatocytes of patients with various liver diseases

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Summary. The ultrastructural localization of copper, zinc-superoxide dismutase (Cu, Zn-SOD) in the liver of patients with acute hepatitis, chronic hepatitis, liver cirrhosis and alcoholic fatty liver was studied by means of the indirect immunoperoxidase technique. In hepatocytes Cu, Zn-SOD was found to be localized in perinuclear cisternae, rough endoplasmic reticulum (rER), vesicles and Golgi apparatus. The Cu, Zn-SOD was also detected around the lipid droplets in hepatocytes as well as on the cytoplasmic membrane in cases of liver cirrhosis.

These findings suggest that Cu, Zn-SOD is produced in the rER in hepatocytes and protects the cells from cellular injury caused by superoxide anion radical in various disorders of the liver.

Key words: Superoxide, SOD, Human, Liver, Ultrastructure

Introduction

Superoxide dismutase (SOD) is an enzyme which catalyzes the dismutation of superoxide anion radical (O_2^-) leading to the formation of molecular O_2 and H_2O_2 (Klung et al., 1972; Forman and Fridovich 1973). O_2^- is one of the reactive oxygen species which is formed through the addition of one electron to an oxygen molecule (Niki, 1987). O_2^- is believed to be produced in a number of normal endogenous processes, including autoxidation of hemoglobin, microsomal mixed-function oxidase hydroxylations, and in association with granulocytic phagocytosis (Babior et al., 1973; Fridovich, 1975; Lynch et al., 1976). This highly reactive radical has been suggested to be responsible for some kind of arthritis (Munth et al., 1982) and gastric ulcer (Ogino et al., 1987).

SOD appears to play a primary biological role as a defense mechanism against O_2^- through this dismutation (Fridovich, 1975). SOD is contained richly in the liver of humans (Peeters et al., 1975) and biochemically two forms have been clarified in mammalian tissues. The first form containing copper and zinc (Cu, Zn-SOD) is localized in the cytosolic compartment and the second form containing manganese (Mn-SOD) is localized in the mitochondrial matrix (Fridovich, 1974).

The amount of lipidperoxide, an endproduct of O_2^-, is increased in the serum and liver tissues of patients with viral hepatitis and alcoholic liver disease (Matsumura et al., 1981). On the other hand, the activity of SOD is also increased in the liver tissues of patients with viral hepatitis and alcoholic liver disease (Togashi et al., 1987 a,b).

The histopathological study with regard to the localization of SOD in liver tissue is very useful to clarify the problems of liver damage. However, no reports about the electron microscopical localization of SOD in human liver have been published. In the present paper, we clarify the electron microscopic localization of Cu, Zn-SOD in hepatocytes of the patients with various liver diseases.

Materials and methods

1. Subjects

The subjects were obtained from 7 patients: two with acute hepatitis (AH), one with chronic persistent hepatitis (CPH), one with chronic active hepatitis (CAH), two with liver cirrhosis (LC) and one with alcoholic fatty liver. The diagnosis of CPH and CAH was made according to the diagnostic criteria of the International group (Bianchi et al., 1977). Blood HBsAg was negative in all cases. Hepatitis A virus, Epstein-Bar virus, Cytomegalo virus and drugs were not found as the cause of hepatitis in two patients with AH. All cases of CPH, CAH and LC had neither a drug history nor drinking habit. Thus the causes of AH, CPH, CAH and LC were thought to be the non-A, non-B hepatitis virus.

2. Preparation of liver tissues and immunoelectron procedures

The liver tissues were removed under laparoscopy. Two cases with AH were biopsied 4 weeks after the GOT peak. Immediately following the extirpation, liver tissues were fixed in a mixture of 0.2% glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) at 4°C for 2 hours. The fixed materials were sliced into approximately 25 microns with a Microslicer (DOSAKA EM Co., LTD.). The floating sections were treated with 5 mM periodic acid for 10 minutes, according to a modified Isobe method (Isobe et al., 1977) to inhibit endogenous peroxidase activity. Then the sections were incubated with 3% bovine serum albumin for 10 minutes at room temperature. After washing, the sections were incubated with anti-human Cu, Zn-SOD rabbit IgG antibody as primary antibody at 4°C overnight and subsequently with peroxidase-labelled anti-rabbit swine IgG (DAKO) as the secondary antibody at room temperature for one hour. Finally the sections were immersed in a solution consisting of 0.03% DAB, 0.05M Tris-HCl buffer (pH 7.4), and 0.01% H_2O_2 for 10 minutes. Immediately following color development, the sections were postfixed with 1% osmium tetraoxide, dehydrated with gradient ethanol and embedded in Epon 812 according to the routine methods. Ultrathinsections were stained with uranyl acetate and lead citrate and examined by a Hitachi HS-9 electron microscope.

3. Production and purification of anti-human Cu, Zn-SOD antibody

Anti-human Cu, Zn-SOD rabbit IgG antibody, used as the primary antibody, was raised by immunizing rabbit with Cu, Zn-SOD derived from human erythrocytes (Wako Pure Chemicals). Rabbit serum IgG fractions were precipitated with ammonium sulphate and purified with affinity chromatography. The specificity was confirmed by immunodiffusion and by an absorption test with sufficient controls.

Results

In the semiultrathin sections with toluidine blue staining, the reaction products against Cu, Zn-SOD appeared to be dark brown color and localized in the cytoplasm of hepatocytes. This intra-hepatocytic reactivity was varied in all 7 patients. Furthermore the degree of reactivity was different from hepatocyte to hepatocyte in each section. Even in one hepatic lobule some cells displayed a strong diffuse reactivity and the others showed slight or no reactivity.

Immunoelectron microscopically, the reaction products were detected to be fine granular black particles which meant the localization of Cu, Zn-SOD. In many cases showing moderate degree of reaction products, the SOD was found on the rough endoplasmic reticulum (rER) and on the membrane of various sized vesicles

(Fig. 1). Generally the amount of the reaction products seemed to depend on the reactivity of vesicles. Intra-hepatocytic localization of SOD showed an uneven distribution and often clustered on one side of the cytoplasm. In each hepatocyte, the amount of reaction products were limited and their reactivity was poor.

In a case of a hepatocyte which showed diffuse reactivity, it was rather difficult to clarify the definite localization of SOD because of the covering up to the cytoplasm by excessive reaction products. Sometimes the SOD was observed in the perinuclear cisternae which continued to rER (Fig. 2). The deposits of reaction products were recognized on the circumference of perinuclear cisternae in the form of electron high density line of continuity or discontinuous partial density.

The reaction products were also found in a part of the lamellae of Golgi apparatus (Fig. 3). These forms of ultrastructural localization of Cu, Zn-SOD were observed in the hepatocytes of all patients with AH, CPH, CAH and LC.

The following two findings were also cited as interesting examples which attracted our attention. One was the massive concentration of Cu, Zn SOD around the fatty droplets (Fig. 4) and another was continuous deposition of the SOD along the cell membrane of hepatocytes in LC patients (Fig. 5).

Discussion

In the present study, it was clarified that Cu, Zn-SOD localized on rER, perinuclear cisternae continuous with rER, membrane of vesicles and lamellae of the Golgi apparatus. These findings are in accordance with the fact that a large quantity of Cu, Zn-SOD is biochemically detected in the microsomal fraction of the cytosol (Peeters et al., 1975). Therefore it is suggested that Cu, Zn-SOD is assumed to be generated in rER of hepatocytes and to be released through tubular endoplasmic reticulum, vesicles and Golgi apparatus outside the cells. Matsumura et al. (1981) pointed out that lipid peroxide increased in hepatic tissue of the patients with fatty liver. Therefore it is supported that lipid peroxidation is accelerated in the cells with fatty degeneration. Togashi et al. (1987a) reported that Cu, Zn-SOD protected the hepatocytes against lipidperoxide, which increased in

Fig. 1. Cu,Zn-SOD is observed on the tubular endoplasmic reticulums and vesicles of the hepatocyte (H) as electron dense materials. Immunoelectron microscopy of the liver in chronic persistent hepatitis (CPH). × 2,000

Fig. 2. Cu,Zn-SOD is also observed on the rER (arrowheads) and perinuclear cisternae (arrows) of hepatocyte in CPH. × 7,000

Fig. 3. The photograph shows the immunoelectron microscopy of CPH liver. Cu,Zn-SOD is localized on the lamellae of Golgi apparatus. × 10,000
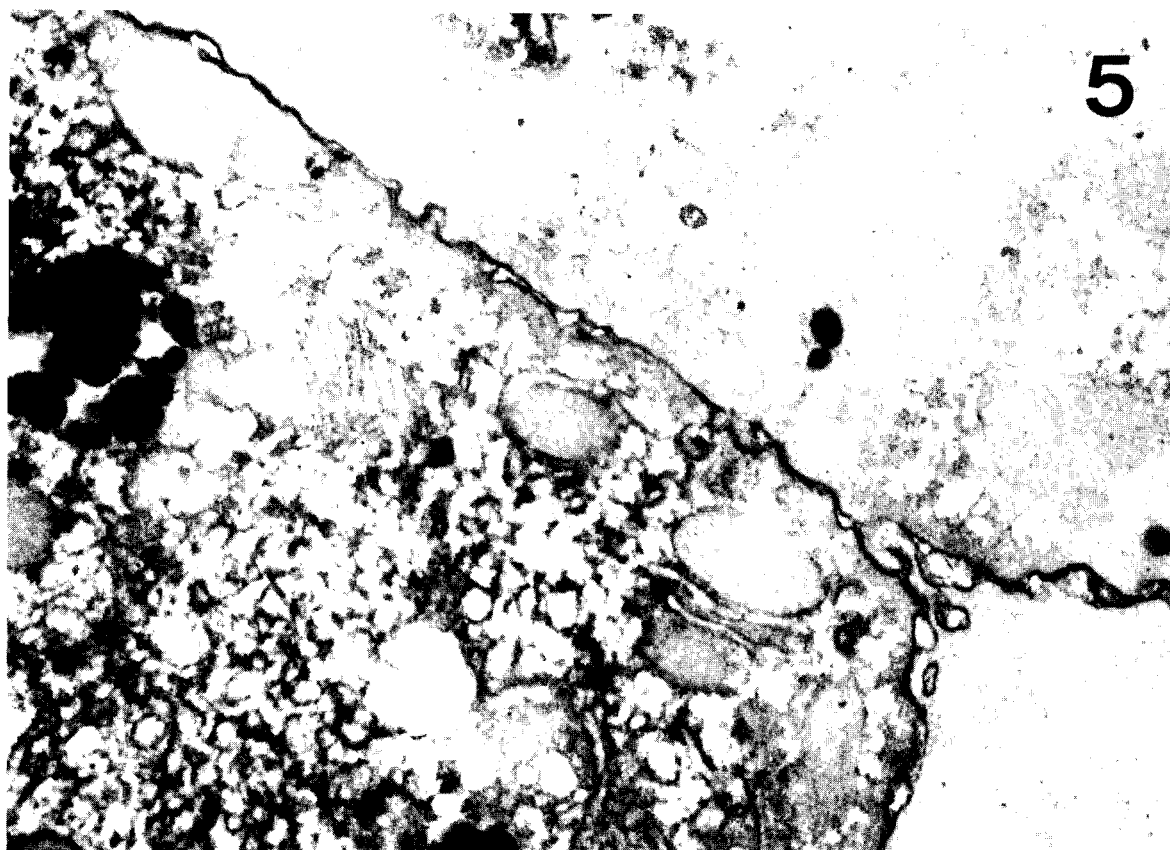
Fig. 4. This photograph shows immunoelectron microscopy of alcoholic fatty liver. Cu,Zn-SOD is accumulated around the lipid droplet (L) in the hepatocyte. × 3,000

Fig. 5. In the case of liver cirrhosis. Cu,Zn-SOD is continuously observed along the cell membrane of the hepatocytes. × 10,000
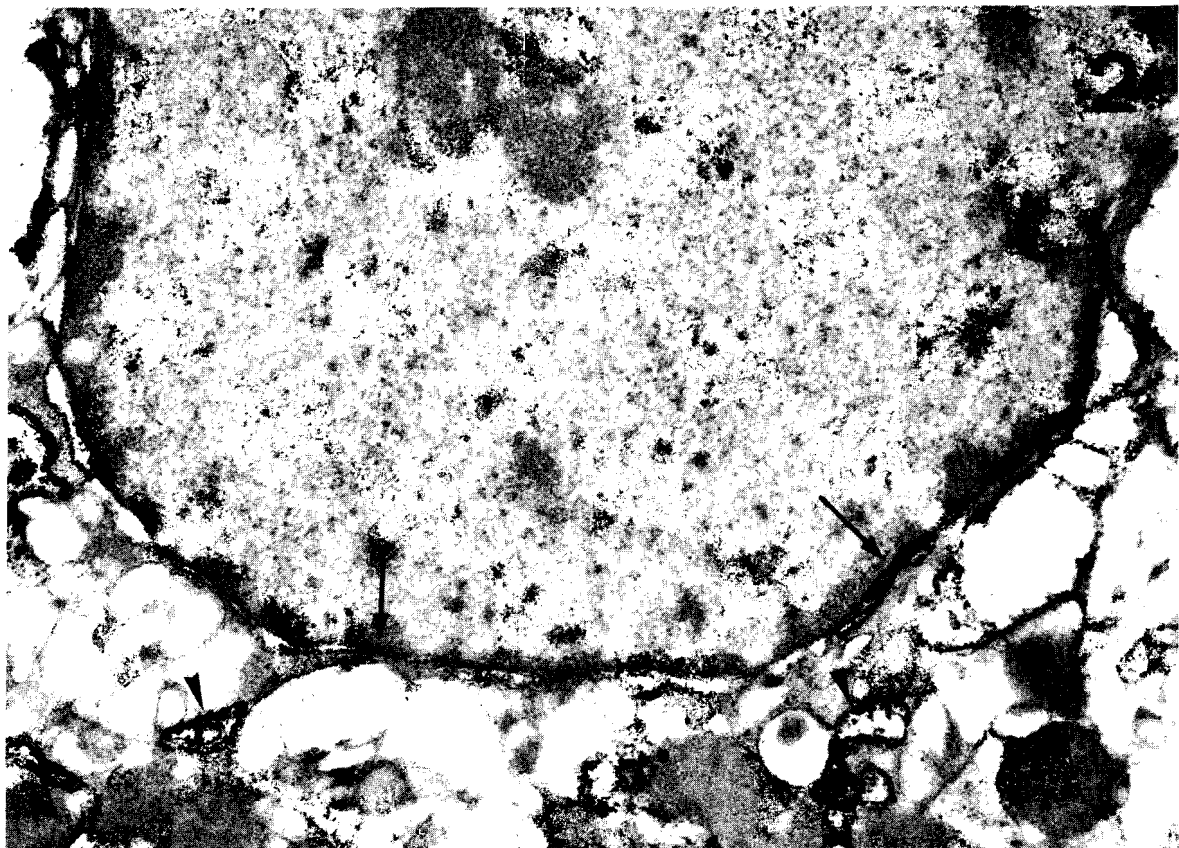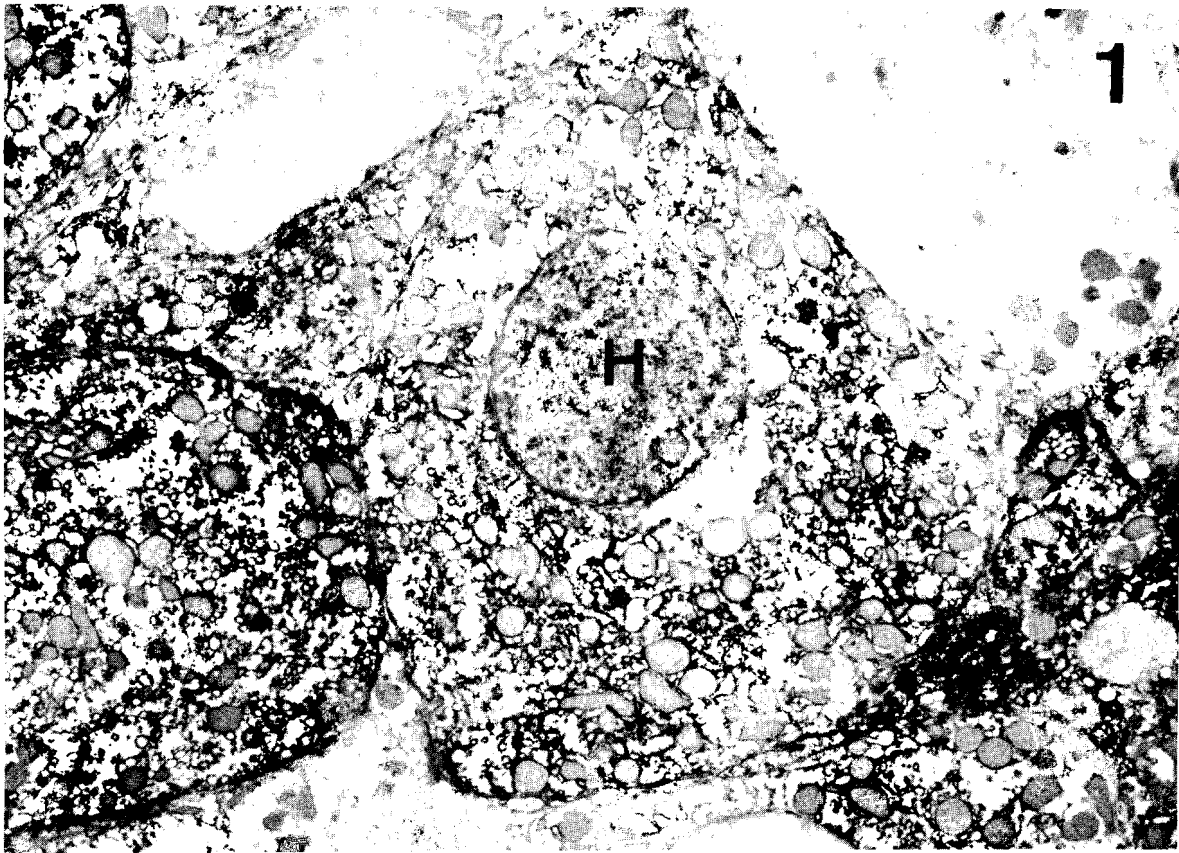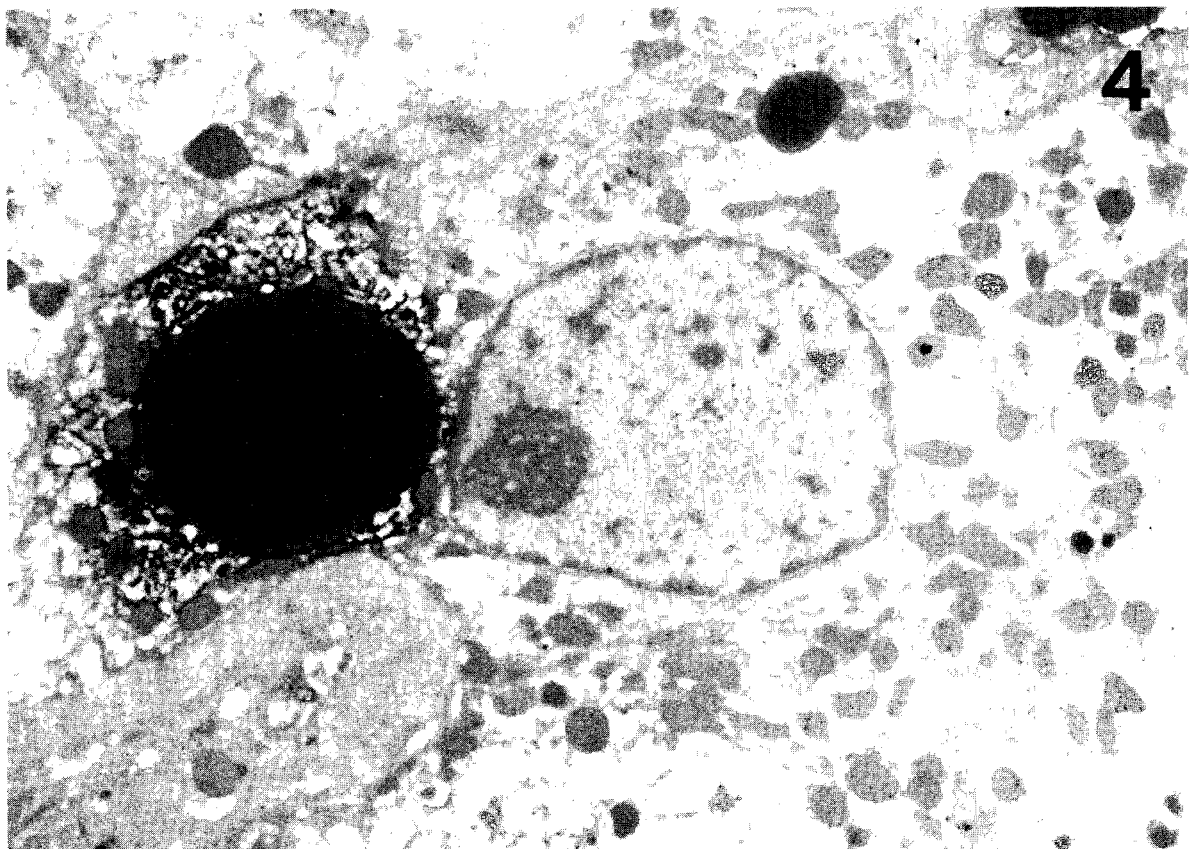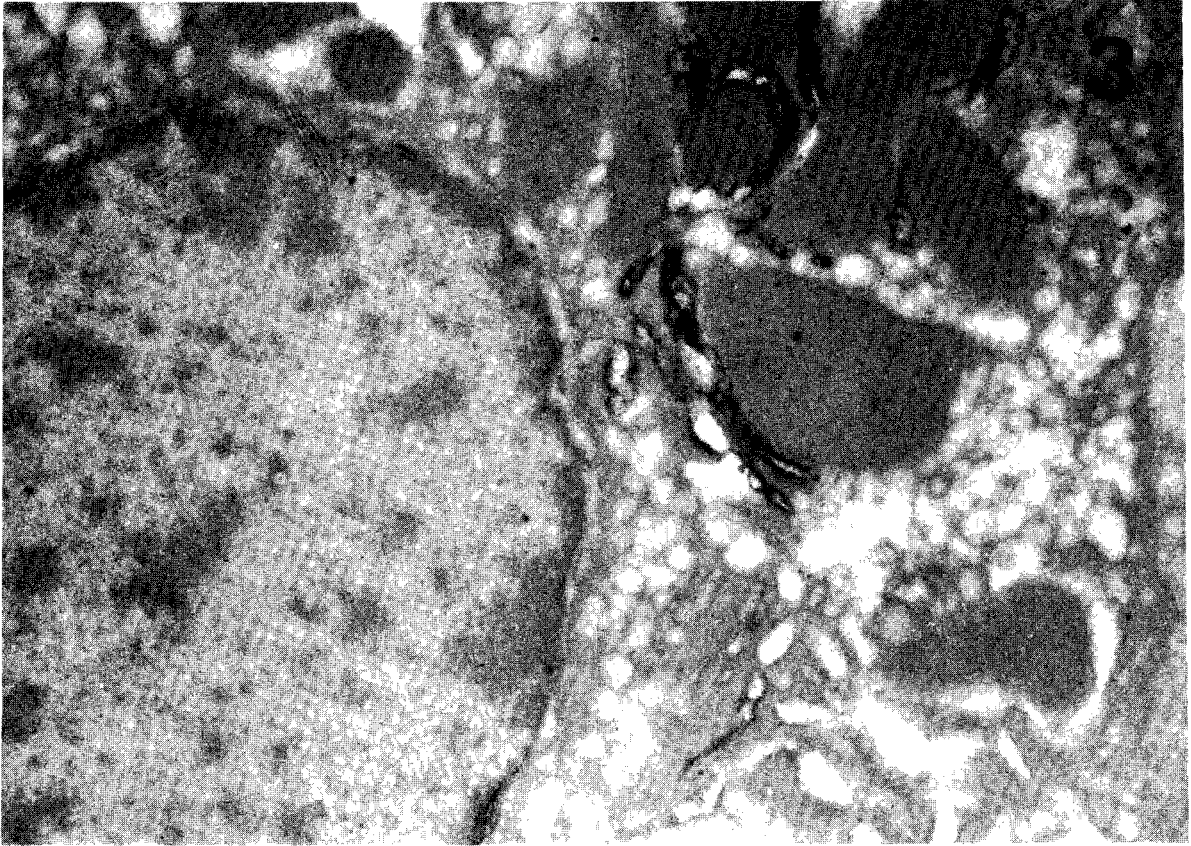
the hepatic tissues of a patient with fatty liver. In our present study Cu, Zn-SOD was observed around the lipid droplets in the hepatocytes. The abundant presence of Cu, Zn-SOD around lipid droplets suggests an accelerating production of the SOD which plays the role of inhibiting the formation of lipidperoxide, induced by O_2^-.

Esterbauer (1985) reported that the cell membrane became fragile with the progression of liver disorders. These phenomena are able to explain the accumulation of lipid peroxide on hepatocyte membrane caused by superoxide radicals (Fridovich, 1978). Immunoelectron microscopically, Cu,Zn-SOD was continuously observed on the cell membrane of hepatocytes of patients with LC. The abundant Cu, Zn-SOD observed on the hepatocyte membrane suggested that the SOD worked protectively for the injury of the hepatocyte membrane.

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