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Copper/zinc and manganese superoxide dismutases in alcoholic liver disease: immunohistochemical quantitation

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Summary. Alcohol damage to the liver can, among other factors, be mediated through the action of toxic oxygen radicals generated by ethanol. Major antioxidants in the liver are copper/zinc and manganese superoxide dismutases (Cu/Zn- and Mn-SODs). In order to test whether SODs may be differentially expressed in alcoholic liver disease (ALD), biopsies from 45 patients with ALD were analyzed for qualitative and quantitative immunoreactivity of Cu/Zn- and Mn-SOD in hepatocytes. The overall amount of Cu/Zn-SOD reactivity was significantly lower in ALD than in control biopsies, whereas no difference was found for Mn-SOD. Staining for both enzymes was decreased in ballooned hepatocytes. Low Cu/Zn-SOD was correlated with advanced lattice-like perisinusoidal fibrosis. In hepatocytes forming cirrhotic nodules, SOD reactivity was similar to that of control cells. The results suggest that SODs may be differentially regulated in ALD, and that Mn-SOD, an inducible enzyme, may be involved in recovery and cell protection in ALD.

Key words: Superoxide dismutases, Alcoholic liver disease, Alcohol toxicity, Liver

Introduction

Alcohol toxicity to the liver is mediated by several pathogenic pathways. One of them appears to act via the production of cytotoxic reactive oxygen species (Suematsu et al., 1981; Dianzani, 1985; Kawase et al., 1989; Nordmann, 1994; Lieber and Decarli, 1994; Reinke et al., 1994). During the metabolism of alcohol to acetaldehyde and the further metabolism of the latter by cytochrome P450IIE1, toxic-free radicals are formed (Ingelman-Sundberg and Johansson, 1984; Lieber, 1988). These highly reactive agents can cause lipid peroxidation in several cellular membrane systems, demonstrable by an increased production of hepatic malonaldehyde (Teare et al., 1994), thiobarbituric acid reactive substances (Ekstrom and Ingelman-Sundberg, 1989), hydroxyl radicals (Cederbaum, 1989), diene conjugates (Situnayake et al., 1990), and superoxide anion (Williams and Barry, 1987). Defense against such damaging oxidants in the liver is provided by non-enzymatic and enzymatic systems, the former mainly comprising the endogenous tripeptide, glutathione, and the latter superoxide dismutase (SOD; E.C. 1.15.1.1). SOD catalyzes dismutation or destruction for most superoxide anions produced under physiological and pathological conditions, and is considered a key enzyme in protecting cells against oxidative injury (Gregory and Fridovich, 1973; Fridovich, 1975; Bannister et al., 1987). SOD is widely distributed in eukaryotic cells and occurs in two major species, i.e. copper/zinc SOD (Cu/Zn-SOD), expressed throughout the cell, and manganese SOD (Mn-SOD), located mainly in the mitochondrial matrix (Bannister et al., 1987; Dobashi et al., 1989; Crapo et al., 1992).

Glutathione concentrations are normally lowest in centrilobular hepatocytes where part of the early lesions in alcoholic liver disease (ALD) ensue, and it has been demonstrated that liver tissue in ALD contains lower levels of glutathione (Shaw et al., 1983) and that experimental reduction of hepatic glutathione increases hepatic damage by ethanol (Strubelt et al., 1987). Conversely, the role of SOD in ALD has not yet been clarified. The aim of the present study was, therefore, to systematically analyze the patterns of Cu/Zn-SOD and Mn-SOD immunoreactivity in ALD by qualitative and quantitative methods, and to correlate SOD reactivity patterns with type and grades of ALD-associated liver lesions.

Materials and methods

Biopsies

Liver biopsies were obtained from 45 patients with alcoholic liver disease (ALD; 39 males, 6 females; age

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range: 21 to 79 years). Diagnosis of ALD was based on the patient's history, on clinical and biochemical findings, and on histological patterns, in accordance with published criteria (Christoffersen and Nielsen, 1972; Edmondson, 1980; Baptista et al., 1981; French et al., 1993). Biopsies of livers showing no relevant histological change (staging biopsies without evidence of malignant disease), obtained from 13 patients covering a similar age range, served as normal controls.

For light microscopy including immunohistochemistry, biopsies were fixed in 4% neutral-buffered formalin for 2 to 4 hours and embedded in paraffin. 4 μm-thick sections were processed for routine staining (hematoxylin-eosin, PAS, iron, van Gieson's, reticulin and chromotrope aniline stains) and for immunohistochemistry (see below).

Immunohistochemistry of SOD

Immunoreactive SODs were detected by use of a modified alkaline phosphatase-anti-alkaline phosphatase (APAAP) procedure. Sections were deparaffinized and rehydrated in Tris-NaCl buffer (0.1% Tris and 1% NaCl, pH 7.4). and preincubated with 3% bovine serum albumin (BSA; Serva) in Tris buffer with 2% normal rabbit serum (Dako) for 30 min. Primary antibodies, mouse monoclonal antibody directed against human Cu/Zn-SOD (Sigma Immunochemicals) at a dilution of 1:200, and sheep polyclonal antibody directed against human Mn-SOD (The Binding Site, Birmingham, UK) at a dilution of 1:20, were applied on the sections and incubated for 1 hour. For Cu/Zn-SOD, monoclonal rabbit anti-mouse antibody at a dilution of 1:30 and monoclonal antibody APAAP complex at a dilution of 1:50 were used for incubating the sections for 45 min. For Mn-SOD, incubation with an alkaline phosphatase-conjugated donkey anti-sheep antibody (The Binding Site, Birmingham, UK) at a dilution of 1:50 and for 30 min was employed.

Alkaline phosphatase (New fuchsin substrate solution, Dako) was developed for 20 min, and sections were counterstained with hematoxylin (Merck) and mounted with aquadex. For immunohistochemical quantitation, all procedures were performed under strictly controlled conditions, and both ALD biopsies and control biopsies were processed in parallel using the same batches of antisera, substrate buffers, and washing solutions. Stained sections were kept in the dark until use.

Histopathological assessment and grading of lesions

The amount of macrovesicular fatty change was estimated as percentage of parenchymal surface involved (classes: 0; <10%; ~10-~25%; ~25%-~50%; >50%). Other parenchymal changes associated with ALD (microvesicular fatty change, Mallory bodies, ballooning degeneration of hepatocytes, cholestasis, and cellular infiltrates) were registered as either present or absent (Table 1). Ductular proliferations (DP) were graded by use of a system previously reported (Zhao et al., 1993; grade 0, normal amount of ductules; grade 1, minor increase of ductules in few portal tracts; grade 2, DP easily detectable in most portal tracts, but not involving the parenchyma; grade 3, same as 2, but clearly involving the parenchyma; grade 4, numerous DP in all portal tracts, involving the parenchyma and extending along fibrous septa). Hepatic fibrosis was evaluated as previously described (Knodell et al., 1981), but with some modifications. Perisinusoidal/pericellular fibrosis (PSF) was graded based on Van Gieson-stained sections (grade 0, none; grade 1, slight perisinusoidal fibrosis in the pericentral area, but without lattice formation; grade 2, lattice-like perisinusoidal fibrosis, but still restricted to the pericentral area; grade 3, lattice-like fibrosis extending into midzonal and/or periportal areas). For grading of portal tract and septal fibrosis (SF), the system recently proposed by Schmid and coworkers (Schmid et al., 1994) was employed (grade 0, none; grade 1, mild only portal tract fibrosis; grade 2, portal tract fibrosis plus incomplete septa; grade 3, fibrous septa bridging portal-portal; grade 4, fibrous septa bridging portal-central, and/or focal incomplete cirrhosis; grade 5, diffuse incomplete and/or focal complete cirrhosis; grade 6, diffuse complete cirrhosis). Fibrosis grading according to these two systems (Knodell et al., 1981; Schmid et al., 1994) refer to general fibrosis patterns and can, therefore, be applied for ALD, even though they had originally been designed for fibrosis in viral hepatitis.

Microphotometry of Cu/Zn-SOD and Mn-SOD immunoreactivity

Reaction products were measured by use of the two solid-state image-sensing channels of the CAS 200 image analysis system (CAS Cell Analysis System, Inc., Becton Dickinson, Elmhurst, Illinois). The CAS 200 APAAP immunostain software program (Bacus and Grace, 1987; Bacus et al., 1988, 1990) employs two video cameras which are matched to the two components of the stains used (first camera: at 260 nm; second camera: at 500 nm). The program, originally set up to measure the 2/Neu oncogene product, and requiring cells with known amounts of DNA and oncogene product to calibrate the system, can also be used for conventional APAAP immunostained preparations by setting the calibration values manually. Thus, all measurements are done under the same modified conditions. The reaction product/DNA ratio (calculated as «picogram (pg) immunoreactive protein/pg DNA») provides appropriate values for the amount of reaction product.

Video images, seen via a x40 objective, resulted in an area of 0.01 mm^2 per field. Of each field measured, the total optical density of epithelial cell nuclear components was obtained from the first sensor, whereas the total optical density of reaction product was obtained from the second. At least 20 fields per sample covering

hepatocyte parenchyma were randomly chosen and measured. Fat-containing hepatocytes were included in the analysis, whereas hepatocytes containing Mn-SOD-immunoreactive nuclei were excluded. Care was taken to analyze cells whose nucleus was in the plane of section (also for ballooned hepatocytes, which may have contained nuclei of decreased size). It was assumed that the nuclear DNA distribution of all specimens tested was constant.

Table 1. Alcoholic liver disease: histological changes,

CASE	AGE/SEX	FC(%)	MB	BD	CH	PTI	DP	PSF	SF
1	52/M	Ma/>50	+			+		2	2
2	61/M	Ma/>50	+*	+	+		1	3	4
3	51/M	Ma/>50	+		+	+	1	2	5
4	63/M	Ma/10-25	+	+		+		1	3
5	60/M	Ma/>50	+	+	+	+	1	2	4
6	56/F	Ma/<10	+				1	2	5
7	62/M	Ma>50	+*	+		+	1	3	5
8	50/M	Ma/>50	+	+	+	+		3	3
9	46/M	Ma/>50	+					1	0
10	48/M	Ma/10-25	+*	+		+		3	3
11	31/M	Ma/>50	+*	+	+	+		3	4
12	44/M	Ma/>50	+	+	+			3	5
13	68/F	Ma/10-25	+	+	+	+	1	3	5
14	58/M	Ma/>25-50	+*	+		+	1	3	3
15	64/M	Ma/<10	+*	+			1	3	5
16	59/M	Ma/<10	+	+		+	1	3	5
17	64/M	Ma/<10	+			+	1	2	5
18	56/F	Ma/>50	+			+	1	3	5
19	45/M	Ma/<10	+			+	1	3	5
20	55/M	Ma/<10	+*	+		+	1	2	3
21	56/M	Ma/<10	+*	-				1	0
22	47/M	Ma/>25-50	+*	+				1	0
23	56/M	Ma/>25-50	+	+	+		1	2	5
24	49/M	Ma/>50	+	+				2	4
25	47/F	Ma/>25-50	+*			+		2	3
26	33/M	Ma/>50				+	1	1	3
27	46/M	Mi+Ma/>50		+	+		1	2	5
28	63/M	Ma/>50		+		+	1	3	5
29	43/M	Mi+Ma/>25-50						2	3
30	31/M	Ma/>25-50			+		1	3	4
31	44/M	Ma/25-50		+		+	1	3	5
32	43/F	Ma/>50						0	0
33	59/M	Ma/>50				+		1	3
34	70/M	Ma/>50				+		2	2
35	40/M	Ma/10-25				+		2	2
36	52/M	Ma/>25-50				+		2	2
37	60/M	Ma/10-25			+	+		3	2
38	38/M	Mi+Ma/>10-25				+		1	0
39	49/F	(-)						0	1
40	58/M	Ma/25-50				+	1	1	0
41	45/M	Ma<10			+	+	1	2	5
42	79/M	Mi/<10				+		2	0
43	21/M	Mi/<10						2	0
44	72/M	Mi/<10						2	0
45	70/M	(-)						1	0

FC%: fatty change (Ma, macrovesicular; Mi, microvesicular: %, surface involved); MB: Mallory body; BD: ballooning degeneration; CH: cholestasis; PTI: portal tract infiltrates; DP: ductular proliferations; PSF: perisinusoidal (pericellular) fibrosis; SF: portal tract and septal fibrosis; MB*: MB-containing hepatocytes with neutrophil satellitosis; +: histological change present. For grading of PSF and SF, see materials and methods.

Statistical analysis

All results are expressed as mean ± SD. T test, Pearson's correlation, and Chi-square analyses were employed. $p<0.05$ was considered to be statistically significant.

Results

Histopathological features of ALD biopsies

Histopathological changes and grading are compiled in Table 1. It is seen that the test group of ALD is histologically heterogeneous. The majority of biopsies disclosed macrovesicular fatty change (84.4%), involving fifty percent or more of the parenchymal surface in about a third of cases. Based on H-E stained sections, we noted a microvesicular component of fatty change in 6/45 biopsies only; no foamy degeneration of hepatocytes (Uchida et al., 1983) was observed. Mallory bodies (MBs), as visualized on H-E sections, were detected in 55.5% of preparations, frequently associated with neutrophil granulocytes in the vicinity, but with neutrophil satellitosis in only 10/25 cases showing MBs. Most biopsies showing MBs also disclosed ballooning degeneration of hepatocytes. A slight cholestasis was found in almost a fourth of the biopsies. A ductular reaction (ductular proliferations) occurred in 20/45 biopsies (44.4%), but was of grade 1 only throughout.

With two exceptions, PFS was present in all biopsies. It presented the lattice-like pattern typical for ALD in 71.1% involving more than the pericentral area in 35.5% of cases. Incomplete and complete fibrous septa not associated with nodular change occurred in 26.6%, whereas liver cirrhosis (either incomplete or focal complete) was noted in 44.4% of biopsies. There was no case of diffuse complete cirrhosis.

No relevant histological changes were detected in control biopsies.

Qualitative distribution of Cu/Zn- and Mn-SOD immunoreactivity

In the cytoplasm of hepatocytes, granular immunoreactivity for both Cu/Zn-SOD and Mn-SOD was observed (Fig. 1). The overall intensity of staining for both enzymes varied considerably, but immunoreactivity was found in all biopsies with ALD and in all normal control biopsies. Mn-SOD staining was more frequently encountered and seemed to be focally more intense than that of Cu/Zn-SOD. Reactivity for both enzymes exhibited a zonal distribution in 55.5% of ALD biopsies not showing nodular change. Mostly, periportal and pericentral areas were more strongly stained than the midzonal area. Staining also occurred in ballooned cells with or without MBs; however, the pattern, frequency and intensity of staining differed (Fig. 2): whereas estimated intensity of Cu/Zn- and Mn-SOD staining in normal-looking hepatocytes was 1+ or more in 44.4%

and 66.6% of ALD biopsies, respectively, staining of this degree for the two enzymes in MB-positive cells was found in 28% and 64% of cases, i.e. almost the same for Mn-SOD, but considerably less for Cu/Zn-SOD. In ballooned hepatocytes without MBs, staining for Cu/Zn-SOD and Mn-SOD appeared to be weaker, i.e. either slight or frankly negative (Fig. 3). In contrast to damaged hepatocytes with or without MBs, hepatocytes in part of the cirrhotic nodules were SOD immunoreactive similar to normal hepatocytes (Fig. 4).

Microphotometry of Cu/Zn- and Mn-SOD immunoreactivity in ALD and control biopsies

Data for the measurement of overall enzyme immunoreactivity in ALD biopsies are compiled in Table 2 (calculated as «pg immunoreactive protein/pg DNA»). For Cu/Zn-SOD, the mean value was 40.3, which is significantly lower than in normal control biopsies (94.0). In contrast, no significant difference between ALD and control biopsies was found for Mn-

Table 2. Quantitative immunohistochemistry of Cu/Zn-SOD and Mn-SOD ina ALD and normal control biopsies (mean values ± SD).

	Cu/Zn-SOD	Mn-SOD
Controls (n=13)	94.0±55.6	59.9±23.3
ALD (n=43)	40.3±55.4*	50.1±33.1**

Values are expressed as the reaction product/DNA ratio (calculated as pg immunoreactive protein/pg DNA). *: $p<0.01$ vs controls; **: $p>0.05$ vs controls.

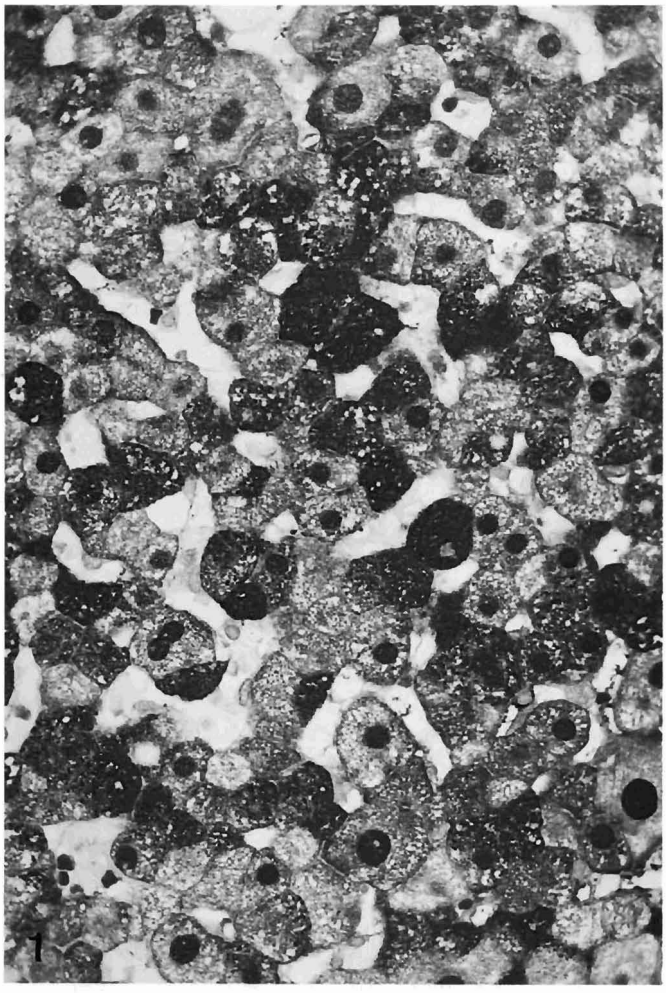

Fig. 1. Hepatocyte parenchyma of control liver, immunostained for Mn-SOD. Hepatocytes exhibit a granular reaction product, which is also visualized in nuclei of these cells, but not in nuclei of sinusoidal cells. Note that immunoreactivity in cytoplasm is heterogeneous, some hepatocytes showing a much more intense reaction than others. APAAP immunostain. x 300

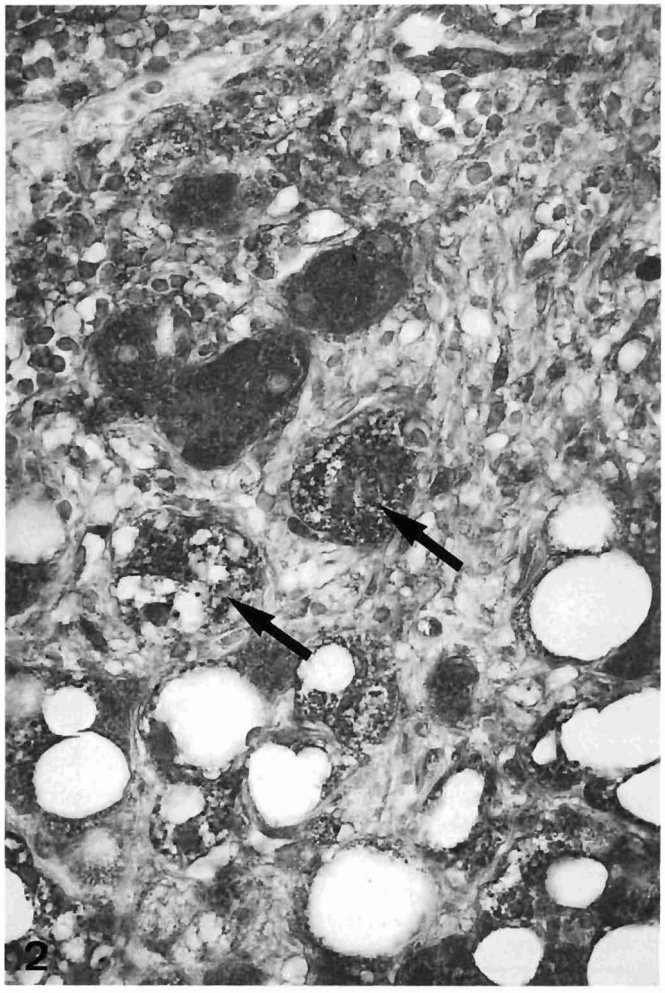

Fig. 2. Liver tissue in ALD, with macrovesicular fatty change and pericellular fibrosis, immunostained for Cu/Zn-SOD. In contrast to a small group of morphologically intact hepatocytes (top of figure), showing a diffuse and strong reaction product in cytoplasm, cells containing Mallory bodies (arrows) exhibit an irregular and loose immunostaining for the enzyme. APAAP immunostain. x 400

SOD, even though qualitative assessment of immunostained sections had led to an opposite impression (see above). In contrast to qualitative analysis, reaction products were measured on section areas that had been randomly chosen.

Comparative analysis of quantitative SOD immunohistochemistry with respect to histopathological parameters showed that, for hepatocytes containing MBs, measured values for Cu/Zn-SOD were significantly lower than for cells without MBs ($p<0.05$; non-random measurement), whereas no difference was found for Mn-SOD when comparing these two cell populations. Cu/Zn-SOD, but not Mn-SOD, levels were also significantly lower in cells showing ballooning degeneration ($p<0.01$).

Cu/Zn-SOD staining tended to be higher in grade 1 perisinusoidal fibrosis (slight pericellular fibrosis in the pericentral area, without lattice formation), and was significantly higher in grade 2 perisinusoidal fibrosis (i.e., with pericentral lattice formation; $p<0.05$). Conversely, it was lower in advanced lattice-like fibrosis (grade 3) involving midzonal and/or periportal areas. No significant correlations were found between Cu/Zn-SOD levels and the extent of fatty change, cholestasis, the presence of DP, portal tract inflammation, portal tract fibrosis, and all grades of SF with or without nodular change. No relationship was found between the amount of Mn-SOD reactivity and any histopathological parameter.

Discussion

There is evidence to suggest that one of the mechanisms of ethanol toxicity to the liver is related to the production of excess reactive oxygen species (ROS)

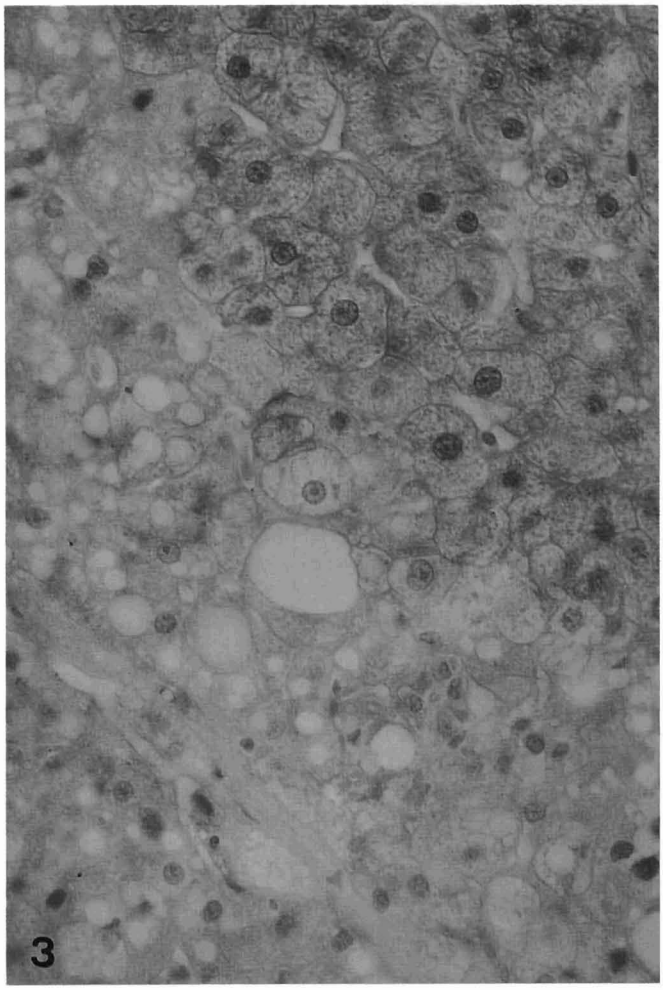

Fig. 3. Liver tissue in ALD, immunostained for Cu/Zn-SOD. Part of the hepatocyte population shows macro- or microvesicular fatty change. Hepatocytes appear enlarged, and mostly exhibit a weak cytoplasmic enzyme reaction only. In the center, a ballooned hepatocyte fails to stain for Cu/Zn-SOD. APAAP immunostain. x 400

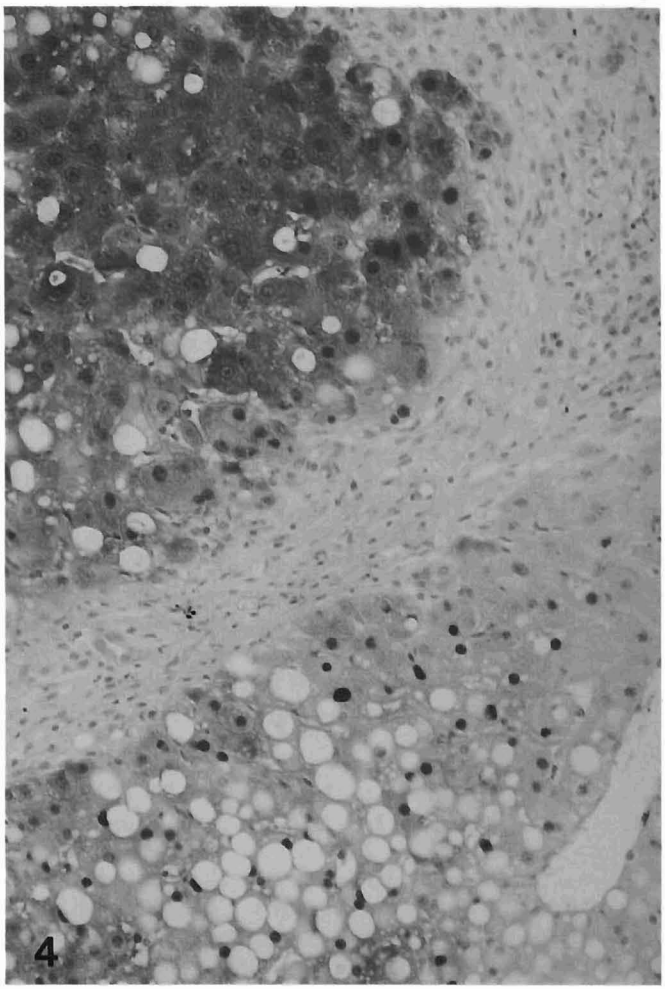

Fig. 4. Liver cirrhosis in ALD. Parts of two hepatocyte nodules are represented, one of them exhibiting advanced macrovesicular fatty change. Hepatocytes forming cirrhotic nodules reveal strong and homogeneous immunoreactivity for Mn-SOD. APAAP immunostain. x 200

including cellular damage via membrane lipid peroxidation (Suematsu et al., 1981; Videla and Valenzuela, 1982; Ingelman-Sundberg and Johansson, 1984; Dianzani, 1985; Dicker and Cederbaum, 1987, 1988; Williams and Barry, 1987; Lieber, 1988; Cederbaum, 1989; Ekstrom and Ingelman-Sundberg, 1989; Kawase et al., 1989; Situnayake et al., 1990; Koch et al., 1991; Bondy, 1992; Bondy and Pearson, 1992; Nordmann et al., 1992; Devi et al., 1993; Lieber and Decarli, 1994; Nordmann, 1994; Reinke et al., 1994; Teare et al., 1994). The cytochrome P450-dependent microsomal monoxygenase system is involved in these pathways (Ingelman-Sundberg and Johansson, 1984; Lieber, 1988). In the liver, effects of ROS can be counteracted by antioxidant protective mechanisms, of which glutathione and SODs are key factors (Gregory and Fridovich, 1973; Fridovich, 1975; Shaw et al., 1983; Bannister et al., 1987; Strubelt et al., 1987; Dobashi et al., 1989; Crapo et al., 1992). In the present study, immunoreactivity for both Cu/Zn-SOD and Mn-SOD was observed in hepatocytes of all biopsies with histologically verified ALD, and of all normal control biopsies, even though reactivity for Mn-SOD was less intense. Presence of both species of SOD in liver cells has previously been demonstrated and has been shown to be developmentally regulated (Slot et al., 1986; Munin et al., 1992). Mn-SOD is inducible by several factors, such as hyperoxia, tumor necrosis factor, interleukin-1, lipopolysaccharides and active phorbol esters (Houssett and Junod, 1981; Wong and Goeddel, 1988; Visner et al., 1990; Fujii and Taniguchi, 1991; Mokuno et al., 1994), and Cu/Zn-SOD expression has been shown to be influenced by epidermal growth factor in rat fibroblasts (Nishiguchi et al., 1994). As inducible Mn-SOD is considered to be the key SOD species in protective defense pathways (Yoneda et al., 1992), and as there is evidence suggesting that both SODs are distributed preferentially at sites that are vulnerable to attack by ROS (Dobashi et al., 1991), we qualitatively assed SOD immunoreactivity in ALD with respect to visibly damaged hepatocytes and to regenerating hepatocytes occurring in nodules. The interpretation of the results of such a study is based on the assumption that immunohistochemistry is a useful means for detecting SOD patterns in the liver. That SODs can reproducibly be detected by this method has, in fact, previously been documented (Fridovich, 1975; Slot et al., 1986; Chan et al., 1988; Dobashi et al., 1989; Wakai et al., 1994; Zhao et al., 1995). The amount of immunoreactive SOD in a biopsy at a given time point, however, may not closely mirror the bioactivity of these defense systems, and enzymes are known to have a rapid cellular turnover. But we are not aware of any study that has provided conclusive evidence against a possible relationship between immunoreactive SOD and its function in vivo.

In hepatocytes which had undergone ballooning degeneration, but did not contain MBs, staining for Cu/Zn-SOD species was either weak or negative. Ballooning degeneration is usually present in liver biopsies at all stages of ALD (French et al., 1993). The pathogenesis of this mostly pericentral change is not clear. Morphometric quantitation of cytoplasmic structures has revealed a large increase in nonorganelle cytoplasmic volume in all degrees of severity of ALD, associated with an increase in mitochondrial volume and in smooth endoplasmic reticulum (Chen et al., 1987). The increase in volume may be due to reversible cell swelling (hydropic change) because of failure of the sodium potassium pump (review: French et al., 1993). Quantitative assessment confirmed that Cu/Zn-SOD levels were significantly lower in ballooned cells in comparison with normal-looking hepatocytes, whereas Mn-SOD levels were in the same range as controls. This finding suggests that cell swelling alone and, therefore, dilution of enzyme is not the major pathogenetic factor, and is of interest in the light of observations from other cell systems. Thus, results from a rat model of nerve damage have suggested that the induction of Mn-SOD, but not of Cu/Zn-SOD, may be involved in recovery and cell protection against toxicity of superoxide radicals (Yoneda et al., 1992). It is, therefore, tempting to assume that measurable expression of Mn-SOD in damaged, ballooned hepatocytes indicates preservation or even induction of this enzyme as a defense mechanism against further ROS-mediated cell injury. However, several ALD biopsies showed no SOD at all in ballooned cells. In analogy, SOD was significantly decreased in human eye lenses with cataracts induced by the action of free oxygen radicals (Fujiwara et al., 1992). Cu/Zn-SOD expression in the human central nervous system has recently been shown to be correlated with selective neuronal vulnerability (Bergeron et al., 1996). Therefore, the absence of SOD immunoreactivity in some of the ballooned hepatocytes in ALD may be related to a greater vulnerability of these cells to the action of ROS.

A similar situation was encountered when analyzing cells harboring MBs. The amount of Cu/Zn-SOD was significantly lower than for cells without MBs, whereas no difference was found for Mn-SOD between MB-containing and normal-looking hepatocytes. These results are not surprising, because there is a clear relationship between ballooned cells and hepatocytes containing MBs. Ballooned hepatocytes which appear «empty» and may remain unstained for cytokeratins, in fact exhibit a conformational change in the cytokeratin proteins which is followed, in a subset of cells, by the formation of MBs (French et al., 1993). The SOD immunoreactivity found in the present study further supports that ballooned cells and cells with MBs reflect a similar pattern of injury. However, we do not know whether a decreased Cu/Zn-SOD immunoreactivity in these cells represents enzyme downregulation or non-specific loss of enzyme protein due to cell damage, or loss of enzyme through a radical-induced mechanism. It has recently been shown that Cu/Zn-SOD itself can be inactivated by free radicals derived from ethanol metabolism (Santiard et al., 1995). But, even though

ballooned cells appear to undergo regressive change, they express Mn-SOD as normal cells do. Our finding of preserved levels of Mn-SOD in damaged hepatocytes in ALD is in line with observations in primary biliary cirrhosis, where immunostaining revealed increased expression of Mn-SOD in damaged epithelial cells of interlobular bile ducts, ductules and damaged hepatocytes (Ono et al., 1991). Overall hepatic levels of Mn-SOD in ALD are different from those of Cu/Zn-SOD: this observation suggests that differential regulation of SODs may occur, possibly in relation to the inducibility of Mn-SOD (Houssett and Junod, 1981; Wong and Goeddel, 1988; Visner et al., 1990; Fujii and Taniguchi, 1991; Mokuno et al., 1994). In contrast to ballooned hepatocytes with or without MBs, regenerating hepatocytes forming cirrhotic nodules exhibited levels for both enzymes similar to those of morphologically normal hepatocytes. The reason for this phenomenon is not clear. Theoretically, Cu/Zn-SOD, and to a lesser extent, Mn-SOD, may have been altered in ballooned cells through the action of toxic metabolites, in particular acetaldehyde, whereas such an effect may no longer occur in regenerating cells. It has been shown that long-term acetaldehyde poisoning changes the activities of antioxidant enzymes (Holownia et al., 1992). Acetaldehyde has been shown to form protein adducts exhibiting a zonal distribution in alcohol-fed rats and micropigs, the centrilobular zone being preferentially involved (Halsted et al., 1993; Lin et al., 1993). In our material, SOD immunoreactivity showed a zonal pattern in those biopsies where nodular change was not present: it was more pronounced in periportal and pericentral areas, and less in the midzonal area. However, Cu/Zn-SOD-deficient ballooned cells were localized in the centrilobular zone in active alcoholic hepatitis. This finding suggests that a subset of hepatocytes in the centrilobular zone may undergo acetaldehyde-induced depletion of some of the SOD proteins, rendering the cells more vulnerable to the action of ROS, whereas the regenerative offspring of cells located in more peripheral parts of the lobule may not, or to a lesser degree, be damaged by ROS. This view is in line with the observation that low Cu/Zn-SOD was correlated with lattice-like perisinusoidal fibrosis, typically starting in the centrilobular area, whereas fibrosis associated with formation of cirrhotic nodules did not show such a correlation.

In conclusion, liver tissue in ALD appears to show a differential expression of the two SOD species. Immunohistochemical quantitation reveals an overall decrease of Cu/Zn-SOD, but not of the inducible Mn-SOD. Depletion of Cu/Zn-SOD is particularly prominent in the damaged pericentral population of ballooned cells with or without MBs, and this phenomenon is correlated with extensive pericellular fibrosis as an indicator of active fibrosing ALD. The findings suggest that Mn-SOD, but not Cu/Zn-SOD, is involved in recovery and cell protection in ALD.

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