HEPATOCYTE PROLIFERATION AND APOPTOSIS IN RELATION TO OXIDATIVE DAMAGE IN ALCOHOL-RELATED LIVER DISEASE

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Abstract — In alcohol-related liver disease, free radicals play a part in the pathogenesis of liver damage and may influence cell turnover. The aims of this study were to correlate lipid peroxidation, antioxidant defence and iron metabolism with cell proliferation and apoptosis in alcoholic liver injury, and also in comparison with virus-related liver disease. In 45 patients [10 with chronic alcoholic liver damage (CALD), 24 with HCV-related (HCV) and 11 with HBV-related chronic hepatitis (HBV)], and 10 control subjects, we investigated serum ferritin, liver tissue iron, cysteine, reduced/oxidized glutathione, malondialdehyde, histology with hepatocyte proliferation and the apoptotic index. Ferritin, iron levels and malondialdehyde were significantly higher in HCV and CALD than in HBV, and malondialdehyde correlated with both iron and ferritin. Glutathione levels were significantly lower in CALD than in HCV, HBV and control subjects, whereas cysteine levels were significantly higher. The apoptotic index was slightly lower in CALD, with apoptosis occurring more frequently in the centrifibular area, while CALD had fewer proliferating hepatocytes, both overall and in the periportal and centrilobular areas. This study confirms that chronic alcohol intake: (1) induces more peroxidative damage, which correlates with iron loading; (2) reduces antioxidant defence, lowering reduced glutathione liver availability; (3) induces an accumulation of cysteine, a glutathione precursor/metabolite in the liver, probably due to gamma-glutamyltransferase induction; (4) correlates with a lesser extent and different distribution of hepatocyte proliferation and apoptosis than in viral liver damage. This last finding may explain the different types of liver cirrhosis deriving from alcoholic liver damage and the lower cancer risk.

INTRODUCTION

Alcohol ingestion is known to produce a variety of metabolic and pathological alterations in the liver, both in alcoholic patients and in experimental animals exposed to alcohol (Lieber, 1994; Seitz et al., 1998), which are especially due to cell metabolic disturbances associated with ethanol oxidation and oxidative stress (Lieber, 1993). Some of these alterations indeed depend on changes in the redox state due to NADH generation via the liver’s alcohol dehydrogenase (ADH) pathway, which in turn affects the metabolism of lipids, carbohydrates, proteins and purines (Lieber, 1992). Furthermore, induction of the microsomal pathway involving cytochrome P450 2E1 contributes to increasing acetaldehyde generation, with protein adduct formation, enzyme inactivation, decreased DNA repair, reduced liver glutathione (GSH) depletion, free radical-mediated toxicity and lipid peroxidation.

Alcohol intake is also described as being one of the dietary factors epidemiologically linked to a greater risk of cancer, and numerous studies investigating the correlation between ethanol consumption and the risk of hepatocellular carcinoma (HCC) have been published (Farinati et al., 1992; French, 1996; Seitz et al., 1998). Several plausible mechanisms have been suggested by various investigators for the link between excessive consumption of alcoholic beverages and the increased risk of liver cancer, including: (1) the induction of cirrhosis; (2) ethanol’s solvent effect; (3) exposure to carcinogens in alcoholic beverages; (4) dietary deficiencies and decreased immunological responsiveness, commonly associated with heavy drinking; (5) ethanol itself acting as a co-carcinogen at one or more stages in the multiphase process of carcinogenesis (Horie et al., 1965; Walker et al., 1979; Lieber et al., 1986; Farinati et al., 1991; Seitz et al., 1998). We have also focused our attention in the past on this latter aspect of the problem, reporting that a co-carcinogenic activity of ethanol is also supported by ethanol’s ability to increase the organism’s capacity to activate environmental carcinogens, by inducing the cytochrome P450-dependent mixed function oxidase systems, and to interfere with DNA repair mechanisms (Farinati et al., 1989).

Alterations in cell turnover, as expressed by changes in the cytoproliferation rate and/or in the process of apoptosis (programmed cell death) are also highly relevant in the process of carcinogenesis (Svegliati Baroni et al., 1994). Several papers suggest an increase in apoptosis (Higuchi et al., 1996; Kurose et al., 1997; Nanji, 1998; Pianko et al., 2000) or changes in cell growth rate in the liver of patients exposed to alcohol abuse (Hillan et al., 1996; Zhang and Farrell, 1999), but none has compared what happens in chronic alcohol-related liver disease with findings in patients with other types of liver disease, nor have they fully explored the link between free radical-mediated damage, iron metabolism and changes in cell growth rate. This paper reports the data we obtained from studying patients with alcohol-related liver damage in terms of the lipid peroxidation process, the levels of antioxidant defence and iron metabolism in relation to cytoproliferation and apoptosis, comparing them with the situation observed in chronic hepatitis C or hepatitis B virus-mediated damage.

MATERIALS AND METHODS

Patients

The study involved 45 consecutive patients (29 males/16 females, mean age of 47 years, range 20–67) with liver disease,
characterized by higher than normal serum alanine aminotransferase levels for more than 6 months and 10 control subjects (Table 1). This group included patients undergoing laparotomy for appendectomy, cholecystectomy, or other minor surgery, from whom a liver biopsy was obtained prior to surgery (Table 1). Informed consent was obtained from all patients and none experienced any complications after the procedure. None of the patients had received blood transfusions prior to the study. Any patient presenting overt acute or chronic causes of haemolysis was excluded from the study.

Before biopsy, each patient was tested to measure the panel of markers for HBV infection and HCV antibodies. HBV serum markers and anti-HDV were tested by radioimmunoassay and enzyme-linked immunosorbent assay (ELISA; Abbott commercial kits, Chicago, IL, USA). HBV-DNA was tested by a commercially available fluid-phase hybridization assay (Hepatitis B Viral DNA; Abbott). All HBsAg-positive patients were anti-HBe/HBV-DNA positive and none had anti-HDV positivity in the serum. Anti-HCV was tested by a second-generation ELISA, all positive sera being confirmed by a RIBA II assay. Serum transaminases were determined by routine clinical laboratory methods.

On the basis of their clinical history, biochemical and virological data, and histological examination, the patients were grouped as follows: (1) 10 patients (seven males and three females) with chronic alcoholic liver disease (CALD) (defined as chronic liver damage in patients who were both HBsAg- and HCV-negative, with a history of ethanol intake in excess of 80 g/day for males or 40 g/day for females for more than 10 years and lasting up until hospital admission, with no signs of autoimmunity (absence of any organ- and non-organ-specific autoantibody); (2) 24 patients (14 males and 10 females) were HCV-positive [HCV-related chronic hepatitis (HCV)]; (3) 11 patients (eight males and three females) were HBsAg-positive and anti-HCV-negative [HBV-related chronic hepatitis (HBV)]; (4) 10 control subjects (six males and four females).

The mean ethanol intake over the previous 10 years was 8 g/day in HCV-positive patients, 7 g/day in HBV-positive patients, 88 in CALD patients and 5 g/day in control subjects. All the following studies were performed prior to any treatment.

**Morphological evaluation**

Biopsies (one per patient) were taken using a 16–17 gauge modified Menghini needle under ultrasound guidance and local anaesthesia. Only patients whose biopsy material was adequate (i.e. ~4 cm in length) were included in the study, to avoid taking a second biopsy. At least 2 cm of biopsy material were cut, fixed in 10% buffered formaldehyde and handed over to the pathologists. The tissue was embedded in paraffin, cut and routinely stained with haematoxylin and eosin (Fig. 1) and periodic acid–Schiff for routine evaluation, and Perl’s stain for siderosis. Histological findings were classified as mild or moderate–severe chronic active hepatitis, with or without cirrhosis.

Together with the overall diagnosis, the pathologist (who was unaware of the clinical diagnosis) also indicated a semi-quantitative (0–3) score for the presence and extent of steatosis, hepatocyte and Kupffer cell siderosis, total, portal and lobular inflammation, and the Knodell index (Knodell et al., 1981) as modified by Ishak et al. (1995), which includes both a grading and a staging classification (Table 1). In summary, the grading was not significantly different in the three study groups, and so was the staging, even though HBV-positive patients tended to be more frequently in a cirrhotic or pre-cirrhotic stage.

**Biochemical tests**

The tissue for biochemical determination was ~15 mg in wet weight. Samples were either processed immediately or stored at ~80°C for up to 2 weeks.

**Serum ferritin.** Serum ferritin (µg/l) levels were determined by the standard technique used at our clinical chemistry laboratory, the normal range of which is 31–290 (for males) or 4–233 (for females).

Table 1. Characteristics of the study groups

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>Male/female</th>
<th>Age (years)</th>
<th>ALT (SI)</th>
<th>Staging (median score)</th>
<th>No. of patients</th>
<th>Grading (median score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALD (<em>n</em> = 10)</td>
<td>7/3</td>
<td>51</td>
<td>33–68</td>
<td>120</td>
<td>2</td>
<td>0/10</td>
</tr>
<tr>
<td>HCV (<em>n</em> = 24)</td>
<td>14/10</td>
<td>48</td>
<td>19–57</td>
<td>128</td>
<td>3</td>
<td>8/34</td>
</tr>
<tr>
<td>HBV (<em>n</em> = 11)</td>
<td>8/3</td>
<td>48</td>
<td>20/67</td>
<td>98</td>
<td>5</td>
<td>4/11</td>
</tr>
<tr>
<td>C (<em>n</em> = 10)</td>
<td>6/4</td>
<td>53</td>
<td>22/64</td>
<td>33</td>
<td>0</td>
<td>0/10</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; CALD = chronic alcoholic liver disease; HCV/HBV = HCV and HBV chronic hepatitis; C = control subjects. M/F, male/female; ALT, alanine aminotransferase.

Fig. 1. Haematoxylin and eosin staining (×40 magnification) in a patient with mild chronic active hepatitis. Portal inflammation can be observed together with interface hepatitis and several Councilman bodies indicating an apoptotic process.
**Tissue iron determination.** Liver iron concentrations were measured by atomic absorption spectrophotometry (Bassett et al., 1986), in a 4 mg wet weight piece of a needle biopsy specimen. The liver tissue was placed in a clean tube and dried at 37°C for 24 h. After cooling, the sample was transferred to volumetric flasks, adding 3 ml of concentrated nitric acid and incubating at 37°C for 24 h. The samples were then made up with de-ionized water. Iron standard solutions were prepared by dilution of a concentrated iron stock solution (Titrisol; Merck, Darmstadt, Germany) with de-ionized water. A Perkin Elmer atomic absorption spectrophotometer with a simultaneous background corrector was used with an acetylene/air mixture. A lean blue (oxidizing) flame was used with a cathode lamp current of 5 mA, a monochromator wavelength of 248.3 nm and a slit width of 0.2 mm. Under these conditions, the detection limit for iron was 0.005 μg/ml. The results were expressed as μmol/g tissue (dry weight), the normal range being <30 μmol/g.

**Malondialdehyde (MDA) determination.** Liver lipoperoxide determination was performed by the thiobarbituric acid reaction using a modified version of Masugi’s method (Masugi and Nakamura, 1977). The method identifies TBARS (thiobarbituric acid-reactive substances), i.e. aldehydes, in particular MDA. Liver biopsy samples weighing ~5 mg were homogenized with a Teflon pestle in 0.5 ml of cold 50 mM phosphate buffer (pH 7.4). This homogenate was made up with 7% sodium dodecyl sulphate (SDS), 0.1 M HCl, phosphotungstic acid and 0.67% thiobarbituric acid aqueous solutions. The mixture was heated for 60 min in a boiling water bath. After cooling, 5 ml of n-butanol were added and the mixture was stirred vigorously, then spun at 3000 r.p.m. for 10 min. The separated n-butanol layer was assayed fluorometrically with excitation at 515 nm and emission at 553 nm. MDA tissue levels were expressed in nmol/g of wet tissue.

**Liver glutathione and cysteine determination.** The liver tissue was homogenized in ice-cold 0.15 M KCl and the homogenate was treated with 5% (v/v) perchloric acid for protein precipitation. The samples were spun at 3000 r.p.m. for 10 min and the supernatant was retained. Liver glutathione in its reduced (GSH) and oxidized (GSSG) forms and cysteine (CYSH) were determined according to Reed’s method (Reed et al., 1980). This method of analysis is based on the reaction of iodoacetic acid (80 mM) with thiols, in a solution containing excess sodium bicarbonate, to form S-carboxymethyl derivatives, followed by chromophore derivation of the amino groups with Sanger’s reagent (1-fluoro-2,4-dinitrobenzene) 1.5%. These derivatives were rapidly separated by high-performance liquid chromatography, using a Shimadzu gradient liquid chromatograph equipped with an aminopropyl-NH2 column, to permit nanomol level analysis of GSH, GSSG and CYSH. Results were calculated in relation to peak areas of freshly prepared standards. GSH, GSSG and CYSH levels were expressed as nmol/mg of wet tissue.

**In situ end-labelling (ISEL)**

The In Situ Cell Death Detection Kit (Boehringer, Indianapolis, IN, USA) was used to recognize DNA strand breaks (Gavrieli et al., 1992). Briefly, residues of digoxigenin-dUTP were catalytically added to 3'-OH ends of double- or single-stranded DNA by terminal deoxynucleotidyl transferase (TdT). The incorporated nucleotides were detected by a peroxidase-conjugated anti-digoxigenin antibody and visualized with 3,3'-diaminobenzidine (DAB) (Sigma Chemicals, St Louis, MO, USA). For negative control purposes, TdT was excluded from the reaction buffer. The apoptosis (APO) index (number of ISEL-positive cells/1000) was determined by counting 2000 cells per section (Fig. 2).

**Liver cytoproliferation determination**

The proliferative index was obtained using the Ki67 monoclonal antibody, clone MIB-1 (Biogenex, San Ramon, CA, USA) (MIB-1) (Sternberger, 1979). Briefly, 5 μm paraffin-embedded sections were deparaffinized and treated with four microwave cycles (750 W) for 5 min each in citrate buffer (pH 6). Sections were then incubated overnight with Ki67 MoAb diluted 1:80. The immunoreaction was revealed using the labelled streptavidin–biotin (LSAB) kit (Dako, Carpinteria, CA, USA). The proliferation rate was assessed in zone 1 (the perportal area) and in zones 2 and 3 together (intermediate and perivenular). Only clearly labelled nuclei were considered. The MIB-1 labelling index was calculated as the number of labelled cells divided by the total number of hepatocytes counted by two observers, who counted at least 1000 cells.

**Statistics**

The results obtained were analysed statistically using Student’s t-test, one-way analysis of variance (ANOVA), the Mann–Whitney U-test and the Kruskal–Wallis test; linear regression analysis was also used, together with the χ² and Fisher’s exact tests.

**RESULTS**

**Serum ferritin, tissue iron, degree of steatosis and siderosis, and malondialdehyde tissue levels**

Serum ferritin and liver iron levels did not differ significantly between CALD and HCV, but both were significantly higher in these two groups than in HBV and in control subjects (P < 0.05) (Table 2). The degree of steatosis did not differ significantly in the three patient groups, though there was a trend towards higher levels in CALD and HCV patients.

![Fig. 2. In situ end-labelling technique (×60 magnification) in the same patients, selectively indicating some apoptotic bodies and several hepatocytes undergoing the initial phase of apoptosis.](image-url)
Liver MDA was significantly higher in all patient subgroups, than in control subjects ($P < 0.05$). Both CALD and HCV patients had significantly higher MDA levels than HBV patients ($P < 0.0001$ and $P < 0.05$ respectively, Mann–Whitney $U$-test). MDA tissue levels were slightly, but not significantly, higher in CALD than in HCV. MDA tissue levels correlated with both tissue iron ($r = 0.597$, $P < 0.0001$) and serum ferritin ($r = 0.437$, $P < 0.0025$).

Hepatocellular siderosis was detected in none of the HCV-positive patients, in one of 11 HBV-positive, in three of 10 CALD and in none of the control subjects (not significant). Kupffer cell siderosis on the other hand was documented in two of 24 HCV-positive, none of 11 HBV-positive and five of 10 CALD patients, but in none of the control subjects ($P < 0.0005$, Fisher’s exact test).

Reduced/oxidized glutathione and cysteine

Liver GSH levels were significantly lower in CALD and HBV patients and higher in HCV and control subjects by one-way ANOVA ($P < 0.025$), with a significant difference between HCV and CALD ($P < 0.01$ by Student’s $t$-test) and between HCV and HBV ($P < 0.05$ by Student’s $t$-test), when these subgroups were analysed separately (Table 3). No significant differences were detected in tissue GSSG levels (Table 3). The results in Table 3 also show that CYSH levels were significantly higher in CALD than in the other three groups ($P < 0.05$ by one-way ANOVA). In summary, all of the above parameters of iron overloading and oxidative damage were higher in CALD and HCV, while only in CALD were GSH levels significantly lower than in the other patient groups.

Liver cell apoptosis and cytoproliferation

The data on the APO (apoptotic) index in CALD, HCV, HBV and control subjects are given in Table 4, whereas the results of haematoxylin and eosin staining and of the ISEL method are shown in Figs 1 and 2. All patient groups had a higher APO index, compared to the control group. However, although the APO index was lower in CALD patients than in the other groups, the difference failed to reach statistical significance ($P = 0.07$). When patients with virus-related liver disease were considered as a whole, HCV and HBV-related hepatitis patients had higher APO indices than CALD, but here again, the difference did not reach statistical significance ($P = 0.058$).

The data on the number of proliferating hepatocytes (MIB-1) in the three patient groups are also shown in Table 4. All patient groups had higher MIB-1 cell numbers than controls. CALD patients, however, had fewer MIB-1-labelled hepatocytes ($P < 0.02$ one-way ANOVA) than HCV or HBV patients.

When the data on apoptosis and cytoproliferation were considered separately for the perportal (zone 1) and centrolobular areas (zones 2 and 3), we obtained the following results:

1. patients with CALD had apoptotic cells more frequently in the centrolobular area (60%). The opposite was true for patients with HCV- or HBV-related hepatitis in whom the percentage of apoptotic cells in this area was only of 32 and 36%, respectively; (2) patients with CALD had cytoproliferation less frequently in zones 2 and 3 (15% of cases) than HCV (50%), but as frequently as HBV (18%) patients.

### Table 2. Serum ferritin, tissue iron, degree of steatosis and malondialdehyde (MDA) tissue levels

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>Serum ferritin (μg/l)</th>
<th>Tissue iron (μmol/g tissue)</th>
<th>Steatosis score</th>
<th>MDA (nmol/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALD</td>
<td>270 ± 116ab</td>
<td>18.9 ± 10.0ac</td>
<td>1.70 ± 0.20</td>
<td>101 ± 53ac</td>
</tr>
<tr>
<td>HCV</td>
<td>184 ± 113ab</td>
<td>14.7 ± 13.0c</td>
<td>1.68 ± 1.40</td>
<td>92 ± 51ac</td>
</tr>
<tr>
<td>HBV</td>
<td>140 ± 86ab</td>
<td>5.6 ± 2.5ac</td>
<td>1.10 ± 2.10</td>
<td>57 ± 11ac</td>
</tr>
<tr>
<td>C</td>
<td>153 ± 77c</td>
<td>6.8 ± 3.8c</td>
<td>0.60 ± 0.30</td>
<td>38 ± 9c</td>
</tr>
</tbody>
</table>

Values are means ± SD.

Ferritin: *$P < 0.05$ by one-way ANOVA, *$P < 0.02$ versus HCV, *$P < 0.005$ vs HBV.

Iron: *$P < 0.05$ by one-way ANOVA, *$P < 0.025$ CALD vs HBV.

MDA: *$P < 0.05$ by one-way ANOVA, *$P < 0.02$ CALD vs HBV.

For abbreviations, see Table 1.

### Table 3. Liver reduced (GSH) and oxidized (GSSG) glutathione and cysteine (CYSH) in the three groups of patients

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>GSH (nmol/mg tissue)</th>
<th>GSSG (nmol/mg tissue)</th>
<th>CYSH (nmol/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALD</td>
<td>0.8 ± 0.5ab</td>
<td>0.13 ± 0.01</td>
<td>1.2 ± 0.3ab</td>
</tr>
<tr>
<td>HCV</td>
<td>2.2 ± 0.8ab</td>
<td>0.2 ± 0.1</td>
<td>0.7 ± 0.6ab</td>
</tr>
<tr>
<td>HBV</td>
<td>1.4 ± 0.7ab</td>
<td>0.1 ± 0.02</td>
<td>0.5 ± 0.2ac</td>
</tr>
<tr>
<td>C</td>
<td>2.8 ± 1.2a</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.2a</td>
</tr>
</tbody>
</table>

Values are means ± SD.

GSH: *$P < 0.025$ by one-way ANOVA, *$P < 0.05$ CALD vs HCV and HBV.

CYSH: *$P < 0.05$ by one-way ANOVA, *$P < 0.05$ CALD vs HCV, *$P < 0.02$ CALD vs HBV.

For abbreviations, see Table 1.

### Table 4. Liver cell apoptosis and cytoproliferation in the three groups of patients

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>Apoptotic index (ISEL-positive cells/1000)</th>
<th>Proliferating hepatocytes (MIB-1 cells/1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALD</td>
<td>7.14 ± 6.7a</td>
<td>1.4 ± 0.5ab</td>
</tr>
<tr>
<td>HCV</td>
<td>10.6 ± 6.7a</td>
<td>3.2 ± 2ab</td>
</tr>
<tr>
<td>HBV</td>
<td>13.8 ± 6.9a</td>
<td>2.3 ± 1.5a</td>
</tr>
<tr>
<td>C</td>
<td>2 ± 1.3a</td>
<td>0.4 ± 0.02a</td>
</tr>
</tbody>
</table>

Values are means ± SD.

Apoptosis index: *$P < 0.02$ by one-way ANOVA, $P = 0.2$ not significant between groups.

Proliferating hepatocytes: $P < 0.04$ by one-way ANOVA, $P < 0.05$ CALD vs HCV.

ISEL, in situ end-labelling; for other abbreviations, see Table 1.
Correlation studies

APO index and cytoproliferation correlated one with the other (r = 0.51, P < 0.001) and both with ALT levels (r = 0.47, P < 0.005 and r = 0.84, P < 0.001 respectively). Similarly, both APO and MIB1 correlated with grading (r = 0.495, P < 0.025 and r = 0.4, P < 0.02). Serum ferritin and iron were highly correlated (r = 0.848, P < 0.00001) and MIB1 correlated with tissue iron (r = -0.300, P < 0.05). MDA correlated with tissue iron and with ferritin (r = 0.597, P < 0.001 and r = 0.437, P < 0.004 respectively). GSH, GSSG and CYSH were not significantly correlated with other parameters.

DISCUSSION

It is now clear that there are several ways in which ethanol and its metabolic products can damage cells, and an aspect of alcohol toxicity that has received increasing attention in recent years concerns the contribution of free radical intermediates in the pathogenesis of liver injury (Nordmann et al., 1992; Lieber, 1997). In fact, sophisticated techniques — such as electron spin resonance — have recently confirmed the formation of free radicals during alcohol metabolism (Albano et al., 1999). In addition, a number of experimental and clinical studies have demonstrated indirectly that both acute and chronic alcohol intake increase the formation of lipid peroxidation products, such as liperoxides, conjugated dienes and MDA, and reduce tissue levels of antioxidants, such as glutathione and vitamin E (Altomare et al., 1988; Nordmann, 1994; Grattagliano et al., 1996; Niemela et al., 1999).

The causal relationship between oxidative events and the onset of alcoholic liver damage is supported by immunohistochemical analysis showing the presence of aldehydes derived from lipid peroxidation in the areas of fatty infiltration, focal necrosis and fibrosis (Tsukamoto et al., 1995).

While several mechanisms involved in the pathogenesis of alcohol-mediated liver damage, such as oxidative stress, have been studied in detail, little is known about some other potential mechanisms, e.g. apoptosis and cytoproliferation in alcoholic liver disease. Cytoproliferation has been evaluated only rarely in humans chronically exposed to ethanol (Zhang and Farrell, 1999), while several studies have demonstrated that apoptosis occurs in both clinical and experimental alcoholic liver disease, though the mechanisms involved are not entirely clear (Amin, 1998). For instance, it has been suggested that oxidative stress may be one of the mechanisms underlying ethanol-induced apoptosis (Kurose et al., 1997).

Our aim was to evaluate all the above parameters in the same group of CALD patients and to compare this ‘scenario’ with findings obtained in other chronic liver diseases, such as HBV- or HCV-mediated liver damage and with data derived from control subjects. However, we will discuss our results focusing on the comparison with patients affected by other types of chronic liver damage, since we consider this as the more relevant point of our study. Our data confirm that, in comparison with HCV- and HBV-related hepatitis, chronic alcohol intake increases tissue MDA levels, a product of lipid peroxidation, and reduces GSH availability in the liver. This latter alteration indicates a progressive decline in the liver’s capacity to provide an adequate scavenging response and is associated with a build-up of hepatic cysteine — a finding that is more difficult to explain. The accumulation of this glutathione precursor/metabolite in the liver might stem from an excess GSH catabolism, also due to the induction of gamma-glutamyltransferase (an enzyme involved in GSH catabolism), as demonstrated by studying cysteine erythrocyte levels (Loguercio et al., 1999).

Alcohol intake therefore correlates inversely with GSH levels and directly with lipid peroxidation products. In addition, liver iron levels were found to be higher in patients with alcohol misuse, due to iron accumulation in Kupffer cells, as reported elsewhere (Fletcher et al., 1999), and were found to correlate with lipid peroxidation. We have already reported that the increased hepatic oxidative disease accompanied by iron overloading in HCV-related liver damage is accompanied by a build-up of DNA oxidative damage, a fact that is also thought to be relevant to liver carcinogenesis and which may well be important in alcohol-related liver carcinogenesis (Farinati et al., 1995). On the whole, the type of damage found in HCV-related liver disease in many ways resembles the situation in CALD, with the exception of liver GSH and CYSH levels, thus suggesting that oxidative damage is important in both, and that HBV-related liver damage definitely follows different pathways.

As for hepatocellular proliferation and the apoptosis rate (factors that may be quite important in carcinogenesis), we found that, in chronic alcohol-mediated liver damage, both MIB-1-positive, proliferating hepatocytes and apoptotic, in situ end-labeling-positive cells are less frequently detectable than in viral liver damage. While inducing cell proliferation in the digestive tract (Seitz et al., 1998), ethanol exposure is generally linked to an impaired cell proliferation in the liver (Zhang and Farrell, 1999), though data on chronic exposure in humans are apparently very scarce (Lieber and Leo, 1992). With respect to cell proliferation, therefore, our data are consistent with other reports. On the other hand, our data do not confirm previous reports with respect to apoptosis, or at least they show that any increase in apoptosis in CALD is definitely of a lower extent than in viral hepatitis. Finally, the distribution of proliferating and apoptotic cells in the lobule is very different in CALD from that observed in chronic viral liver damage. In particular, apoptotic cells are found more frequently in the perivenular area in CALD, where alcohol exerts its damaging effect (Lieber, 1994), whereas proliferating hepatocytes are observed in the perportal tract, i.e. at a more physiological site, which is not the case in HCV-related damage. The two observations are not significantly different due to the relatively small sample size.

Since oxidative damage is more relevant in patients with CALD and changes in cell turnover are less distinct than in patients with HBV- or HCV-mediated damage, the above findings suggest that the role of oxidative damage in modulating cell turnover in alcoholic liver disease is more limited than was previously reported (Higuchi et al., 1996), and that the turnover is lower and involves different functional areas of the liver. Whether the above aspects are also relevant in relation to the different prognosis and lower neoplastic risk for patients with alcoholic liver damage is open to debate.

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REFERENCES


