Intense of inflammatory damage and serum lipid peroxide concentrations in liver disease

C Fabris, M Pirisi, M P Panozzo, G Soardo, P Toniutto, V Hozca, E Bartoli

Abstract

Aims—To investigate variations in serum lipid peroxide activities in relation to various clinical entities of liver disease.

Methods—Serum lipid peroxides were measured fluorometrically in eight patients with acute hepatitis, six with liver steatofibrosis, five with chronic persistent hepatitis, 15 with chronic active hepatitis, 28 with liver cirrhosis, 22 with hepatocellular carcinoma; 19 patients with extrahepatic disease (six malignant, 13 benign) were used as controls.

Results—Higher serum lipid peroxide concentrations were found in patients with acute hepatitis (4.52 (SEM 0.56) nmol/ml than in all other groups of patients (p < 0.01). No significant difference was found among the mean values detected in the groups of patients affected by chronic liver disease and extrahepatic diseases. A history of chronic alcohol consumption was not associated with higher lipid peroxide concentrations. A significant correlation (R² = 0.4538, R = 0.6737, F = 7.617, p = 0.0000) was found between serum lipid peroxides and a set of indices of inflammation (ESR, total leucocyte count, C-reactive protein) and of hepatic function (aspartate aminotransferase (AST) or alkaline phosphatase (ALP) or bilirubin). Of these, bilirubin was the most significant indicator of inflammation. Analysis of covariance showed a significant difference in lipid peroxide values among groups, even when bilirubin was chosen as an independent variable.

Conclusions—Raised serum lipid peroxide concentrations can be found during acute inflammatory liver disease. Acute change in liver function, reflected by high bilirubin concentrations, seems to be more important for intravascular liberation of lipid peroxides than existence of specific aetiological factors or of severe longstanding global liver damage.

Oxygen radicals are important mediators of host defense and tissue damage. They are produced in conditions characterised by oxidative stress and determine peroxidation of polyunsaturated fatty acids. Determination of lipid peroxide products has therefore frequently been used as a measure of oxygen radical activation both in animal studies and in patients with a variety of clinical disorders. Overall, a high lipid peroxide concentration can be measured whenever intense inflammation occurs, often heralding unfavourable outcomes. Oxygen radical activation has been suggested to be involved in the pathogenesis of hepatocellular damage, especially of toxic origin; alcohol and xenobiotics might produce oxygen radicals via their metabolic pathways involving the microsomal oxidising system (MEOS). In particular induction of cytochrome P450 II E1 occurs during chronic alcohol consumption, determining a shift towards this metabolic pathway in chronic alcoholics.

No exhaustive information is available on variations in serum lipid peroxide concentrations in various clinical entities of liver disease of different aetiology and severity, nor of the possible mechanisms involved. To address these issues, we measured serum lipid peroxides in patients affected by a wide spectrum of liver diseases and analysed them in respect to commonly used indices of inflammation and liver function.

Methods

A total of 105 patients referred electively to our institution for complete diagnostic work ups were studied. Their demographic details are presented in table 1. The aetiology of acute hepatitis was viral (hepatitis B and hepatitis D virus co-infection) in one case, alcohol induced in five, and drug induced in two. Chronic persistent hepatitis was associated to hepatitis B virus (HBV) infection in one patient and to hepatitis C virus (HCV) infection in the other four. Chronic active hepatitis was related to HBV infection in six cases, to HCV infection in eight while it was cryptogenic in one. Liver cirrhosis was alcoholic in origin in 18 patients, and due to hepatitis virus infection in 10 (one HBV, nine HCV). In all patients with hepatocellular carcinoma neoplasia developed in a cirrhotic liver: three patients were HBsAg positive, nine were anti-HCV positive, and 10 had a history of chronic alcohol consumption. Of the six patients with steatofibrosis, the four men had a history of higher than average ethanol consumption; the two women were obese. The specific diagnoses of the six patients with malignant extrahepatic disease were as follows: adenocarcinoma of the pancreas in four; carcinoma of the oesophagus in one; mesenchymal hepatic tumour in one; angiosarcoma of the liver in one; and adenocarcinoma of the colon in one.


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Cattedra di Medicina Interna, Università degli Studi, Udine C Fabris M Pirisi G Soardo P Toniutto V Hozca E Bartoli

Cattedra di Malattie dell’Apparato Digerente, Università degli Studi, Padua, Italy M P Panozzo

Correspondence to: Dr Mario Pirisi, Cattedra di Medicina Interna, Università degli Studi, P.le SM della Maestria, 33100 Udine, Italy
one; and adenocarcinoma of the colon in one; three of these patients had secondaries in the liver. Detailed diagnoses of the 13 patients with benign extrahepatic disease were: mesenteric vein thrombosis, benign stenosis of the main bile duct, sideroblastic anaemia, aortic valve insufficiency, cystinuria, extra-capillary glomerulonephritis, type I diabetes mellitus, coeliac disease, coelitithiasis, epilepsy, hyperlipidaemia, varicocele, and hypertension. Diagnoses of chronic persistent and chronic active hepatitis, liver steatofibrosis, primary biliary cirrhosis, liver adenoma and extrahepatic malignancy were all confirmed histologically or at necropsy. Cirrhosis was diagnosed clinically on the basis of evidence of portal hypertension, ascites, hypoalbuminaemia, hypergamma globulinaemia and confirmed histologically in most cases. Hepatocellular carcinoma was diagnosed in the presence of raised (> 400 ng/ml) serum concentrations of α-1-fetoprotein or suggestive radiological imaging; it was always confirmed histologically or at necropsy. Acute hepatitis was diagnosed clinically, and confirmed histologically in seven patients out of eight; extrahepatic diseases were each diagnosed on the basis of accepted diagnostic standards.

Lipid peroxides were measured in fasting serum samples according to Yagi’s method, which fluorimetrically determines the reaction occurring between lipid peroxidation products and thiobarbituric acid (TBA). Biohumoral variables were measured using commercial kits. Statistical analysis of data was performed using Student’s t-test, analysis of variance (one-way ANOVA), Bonferroni’s test for pairwise comparisons, analysis of covariance (one-way ANCOVA), multiple regression analysis and canonical correlation, using the BMDP statistical software package.

Results

The figure illustrates the individual values of serum lipid peroxides observed in the various groups of patients. Patients with acute hepatitis had higher mean serum concentrations of lipid peroxides compared with those with chronic active hepatitis, cirrhosis, hepatocellular carcinoma, steatofibrosis, extrahepatic malignant diseases, and extrahepatic benign diseases. Two individual patients (not shown in the figure), affected by primary biliary cirrhosis and liver adenoma, presented with a high lipid peroxide value (4.61 and 5.39 mmol/ml, respectively). Table 2 shows the results of the multiple regression analysis. Lipid peroxide values were chosen as the dependent variable and common indices of inflammation (ESR, total leucocyte count, C-reactive protein) and of liver function (total bilirubin, aspartate aminotransferase (AST), alkaline phosphatase (ALP)) as predictor variables. The analysis was significant; only total bilirubin showed a significant correlation with lipid peroxides. Analysis of covariance (one-way ANCOVA) taking serum lipid peroxides as the dependent variable and total bilirubin as the independent variable, confirmed the significance obtained by ANOVA (equality of adjusted means F = 2.987, p < 0.01; zero slopes F = 2.749, p < 0.001; equality of slopes 1.391, p = NS).

Table 3 shows the results of canonical correlation analysis. Once again, the six parameters cited above (ESR, C-reactive protein, total leucocyte count, ALP, AST, total bilirubin) were analysed, identifying in this case two sets of three variables each: the first set comprised the inflammation indices and the second the selection of liver function tests. Similar results were obtained when lipid peroxides were allocated alternatively to the first set.
Table 3 Canonical correlations performed allocating lipid peroxides alternatively to a set of variables comprising inflammation indices and a set of variables including liver function test

<table>
<thead>
<tr>
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<th>Squared multiple correlations of each variable in first set with all variables:</th>
<th>Squared multiple correlations of each variable in second set with all variables:</th>
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<tr>
<td></td>
<td>In first set</td>
<td>In second set</td>
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<tr>
<td>Bilirubin</td>
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<td>C-reactive protein</td>
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</table>

or to the second set of variables. Bilirubin concentration and total leucocyte count emerged as the most significant parameters.

Finally, when all 105 patients were divided into two groups, according to a history of ethanol consumption of more or less than 40 g per day, no significant difference in mean lipid peroxide concentration was found between these groups (2.53 ± 2.24 nmol/ml, respectively; p = 0.47).

**Discussion**

Lipid peroxide mean values differed among the groups studied, being significantly higher in patients with acute hepatitis; no significant difference was found among the groups of patients with chronic hepatic and extrahepatic disease of any kind and aetiology. Lipid peroxide values correlated with a set of variables including tests of liver function and inflammation indices; serum total bilirubin showed the closest correlation with lipid peroxides.

In previous studies, high lipid peroxide concentrations have been suggested to be indicative of alcohol related liver disease, emphasising the part played by ethanol in the generation of oxygen radicals. Others have found that high lipid peroxide concentrations can be measured when liver failure is most severe, independently of the aetiology. In our study, patients with acute hepatitis had significantly higher concentrations of lipid peroxide compared with patients with chronic liver disease of whatever aetiology. This suggests that the intensity of the inflammatory response is strictly related to the oxygen radical production. In hepatocellular carcinoma, certainly the most advanced chronic liver disease, we could find no evidence of lipid peroxide liberation greater than that found in mild forms of liver disease. We confirm that aetiology seems to be important in this setting: we found the highest lipid peroxide concentrations in patients with severe acute hepatitis due to drugs or alcohol. However, in our hands the rapid occurrence of liver disease, more than the aetiological factor or the severity of liver damage, seems to influence lipid peroxide values. Chronic alcohol consumption per se did not explain differences in lipid peroxide concentrations. Lipid peroxides are by no means specific to any condition and high concentrations can be found in a variety of unrelated clinical states. Because lipid peroxide values were high in acutely ill patients, we found it reasonable to assume they could correlate both to inflammation indices and liver function indicators. This hypothesis was only partially confirmed by multiple regression analysis, which documented a strong correlation between lipid peroxides and the variables of inflammation and liver function we chose. However, only the standardised regression coefficient of total bilirubin was significant. Therefore, acute inflammation leading to a change in liver function and jaundice seems to be highly predictive of lipid peroxide release in our patients. Canonical regression analysis was almost equally significant in allocating lipid peroxides to the set of inflammation indices or to one of the liver function variables. Once again, one of the two most relevant variables able to predict lipid peroxide liberation was bilirubin, the second being total leucocyte count. As raised bilirubin concentrations have traditionally indicated a poor outlook in liver disease, their correlation with serum lipid peroxides would confirm this suggestion. Among the inflammation indices, total leucocyte count correlated with lipid peroxide, ESR, and C-reactive protein did not. These two parameters were increased in patients with advanced chronic rather than acute liver disease (data not shown), demonstrating that the mechanisms governing their increase might be different compared with lipid peroxides, at least in liver disease. Nevertheless, lipid peroxide liberation is clearly governed by multiple factors, as indicated by the fact that ANCOVA was still significant when bilirubin was chosen as an independent variable. Therefore, this does not explain the wholesale variability observed in lipid peroxide values. Another note of caution is warranted in interpreting the present results because of the relatively small size of some of the groups studied, particularly the one comprising patients with acute hepatitis, which was predominantly of toxic type.

In conclusion, our study shows that raised lipid peroxide concentrations can be found in...
acute inflammatory liver disease; acute changes in liver function, reflected by high bilirubin concentrations, seem to be more important for intravascular liberation of lipid peroxides than the existence of specific aetiological factors, or of severe, longstanding global liver damage.
