Oxidant stress in type I autoimmune hepatitis: the link between necroinflammation and fibrogenesis?

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Abstract

Autoimmune hepatitis (AIH) is a chronic liver disease of unknown aetiology characterized by circulating autoantibodies, hyperglobulinaemia and interface hepatitis. The mechanisms of progression from initial autoimmune attack to fibrosis and cirrhosis are unclear but oxidant stress may be involved. Markers of lipid peroxidation, antioxidant status, hepatic fibrogenesis and liver function were measured in blood and urine in 35 controls and in 33 patients with type-1 AIH; histology was assessed in 18 patients. In AIH, markers of lipid peroxidation were significantly elevated (8-isoprostane in both plasma and urine \( P < 0.001 \); plasma malondialdehyde \( P = 0.017 \)). Total antioxidant capacity in protein-free serum and total glutathione in both whole blood and plasma were significantly reduced (\( P = 0.007 \), \( P = 0.037 \), \( P < 0.001 \), respectively). The antioxidants selenium, vitamin A and vitamin E were significantly decreased (\( P = 0.007 \), \( P < 0.001 \), \( P = 0.025 \), respectively); vitamin C was unchanged. Urinary 8-isoprostane correlated positively with interface hepatitis and necroinflammatory score and with hepatic fibrogenesis (type III procollagen peptide). Interface hepatitis correlated negatively with vitamin A and whole blood total glutathione. Oxidant stress, as reflected in blood and urine by a wide range of pro- and antioxidant markers, is a significant feature of AIH and provides a probable mechanism linking hepatic necroinflammation to fibrogenesis and disease progression.

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1. Introduction

Autoimmune hepatitis (AIH) can be defined as “an unresolving, predominantly periportal hepatitis, usually with hypergammaglobulinaemia and tissue autoantibodies, which is responsive to immunosuppressive therapy in most cases” [1]. It typically fluctuates clinically and biochemically, and can apparently resolve temporarily despite continuing histological activity [2]. AIH is diagnosed after exclusion of possible viral, toxic, genetic and metabolic aetiologies.

Histology characteristically reveals portal and periportal lympho-plasmacytic infiltration with disruption of the limiting plate. “Piecemeal necrosis” of periportal hepatocytes is the hallmark of the moderate and severe forms of AIH [3] and, recently, a histological grading system (degree of inflammatory activity) and staging (extent of fibrosis) has been introduced [4]. Regardless of aetiological agent, the most important factor determining disease activity is the extent of inflammatory activity at the portal tract/hepatic parenchyma interface; this “piecemeal necrosis” is now more accurately described as interface hepatitis, being an example of apoptosis rather than necrosis [5]. Interface hepatitis is a major component of the diagnostic scoring system introduced by the International Autoimmune Hepatitis Group [6,7].

Subclassifications of AIH are made on the basis of the autoantibody profile present [2,6,8]. Type 1, 70–80% of
AIH, is positive for antinuclear (ANA) or anti smooth muscle antibodies (SMA) or both; type 2, 3–4% of AIH, is positive for liver-kidney-microsomal antibodies (anti-LKM1) reacting with a cytochrome isoenzyme P450 2D6; most of the remaining 10–20% of AIH patients are positive for soluble liver antigen (SLA) or a liver-pancreas (LP) antigen and are classified as type 3. SLA and LP have recently been shown to be identical (anti-SLA/LP [9]). None of the major autoantigens detected in AIH is liver-specific, and only very few are disease specific [8] and therefore cannot be used diagnostically.

The autoimmune response may arise in genetically predisposed individuals subsequent to a “triggering” environmental agent, such as the drug monocycline [10] or the HCV virus [11]. Although most AIH (87%) responds to immunomodulatory therapy, only 15% of individuals have complete resolution for at least 5 years; 40% progress to cirrhosis after 10 years [12]. Since the pathophysiological basis underlying progression from inflammation to fibrosis is incompletely understood, we have investigated the possible role of oxidative stress in progression of AIH.

Oxidant stress has been observed in chronic viral hepatitis [13,14], primary biliary cirrhosis [15,16] and alcoholic liver disease [17]. Reactive oxygen species generated during inflammation and lipid peroxidation products, such as malondialdehyde (MDA) or 4-hydroxynonenal (HNE) (or their adducts), can directly activate hepatic stellate cells to their adducts, leading to fibrosis and cirrhosis. The inflammatory and pro-fibrogenic cytokine TGFβ, released by Kupffer cells and other liver macrophages as a result of redox-sensitive reactions, may also play a key role [20].

This study examines oxidant stress in type I AIH by measuring, in blood and urine, a wide range of markers reflecting both pro- and antioxidant facets of the process. Although oxidant stress is now a recognized feature of decompensated cirrhosis, we wished to determine whether oxidant stress is also a feature of early disease and may therefore be implicated in the progression to fibrosis and cirrhosis, suggesting a potential therapeutic role for antioxidants.

2. Materials and methods

2.1. Study groups

The study was approved by the Central Manchester Health Authority Research Ethics Committee and all patients gave written informed consent. All subjects were nonsmokers, took no vitamin supplements and had a normal nutritional status. All AIH patients fulfilled conventional diagnostic criteria (raised serum IgG levels; presence of circulating antibodies to nuclei and/or smooth muscle) and a liver biopsy compatible with this diagnosis. Patients were also classified using the International AIH Group scoring system [7] for the diagnosis of AIH; “definite” score: pretreatment >15, posttreatment >17. “Probable” score: pretreatment 10–15, posttreatment 12–17. Of the 33 patients, 20 had “definite” AIH and 13 had “probable” AIH. Thirteen patients were untreated (11 naïve, 2 off treatment for at least 3 years); the remainder were currently receiving prednisolone (2–40 mg daily) and/or azathioprine (50–100 mg daily). As the AIH patients were predominantly older females, care was taken to ensure that patient and control groups were age- and sex-matched (control group n = 35, 7♂ 28♀, age = 52.2 ± 12.9 years, range 21–79 years; AIH group n = 33, 5♂ 28♀, age = 46.5 ± 17.2 years, range 15–79 years).

2.2. Liver biopsy

A subset of AIH patients (n=18) had undergone liver biopsy within 10 months of entry to this study (mean time = 70 days). Needle biopsies were assessed by the standard Ishak scoring method [4] for necroinflammation and fibrosis by an experienced hepatopathologist. The total necroinflammatory score is the sum of the scores for A—interface hepatitis; B—bridging necrosis; C—lobular inflammation; and D—portal inflammation.

2.3. Specimen processing

Blood and urine samples were collected after overnight fasting. For measurement of MDA and 8-isoprostanate, blood was collected into ice-cold tubes containing EDTA (1 mg/ml) and GSH (1 mg/ml) and, after centrifugation (800 × g, 10 min), the plasma was protected from oxidation during storage by addition of the chain-breaking antioxidant butylated hydroxytoluene (200 μg/ml). Urine was protected by addition of indomethacin (0.001% w/v) to prevent in vitro formation of prostanoids due to any leucocyte contamination [21]. Whole blood for glutathione assay was kept on ice and analysed on the morning of collection. All other samples were stored at −70 °C prior to assay.

2.4. Analytical procedures

A more detailed description of the assays employed can be found in a previous publication [16].

Lipid peroxidation markers: Plasma and urine samples were assayed [21] for 8-iso-PGF2α using an enzyme-linked immunoassay kit (Cayman Chemicals, Ann Arbor, MI), urinary creatinine being estimated with alkaline picrate solution [22]. MDA was determined in acid-precipitated serum as TBARS [23] or, most sensitively, in whole serum as DETBARS [24].

Antioxidant markers: Total antioxidant capacity was measured in whole and protein-free serum by an enhanced chemiluminescent technique [25]. Selenium was determined using a simple single-tube fluorimetric assay [26]. Vitamin A [27], vitamin C [28] and vitamin E [29] were determined by established laboratory techniques. Total glutathione in
fresh whole blood, and GSH and GSSG in plasma were determined using 5,5′-dithio-bis(2-nitrobenzoic acid) [30].

Other markers: Hepatic fibrogenic activity was measured in serum using the Type III procollagen intact PIIINP radioimmunoassay (Orion Diagnostica, Espoo, Finland). Serum AST, ALT, ALP, γGT, total protein, albumin, bilirubin and urate were determined by standard automated techniques.

Statistics: Since much of the data was not normally distributed, all values are expressed as medians with interquartile ranges; non-parametric statistics were employed. Differences between groups were assessed using the Mann–Whitney U-test. The significance of correlations between parameters was assessed using the Spearman rank test.

3. Results

The concentrations, in AIH and control groups, of all markers determined in this study are shown in Table 1. There is clear evidence of oxidative stress in the group of all AIH patients in that 8-isoprostane was significantly elevated (P<0.001) in both urine and plasma. Although MDA by TBARS was not raised, when MDA was measured by the more sensitive DETBARS method, AIH patients showed a significant elevation (P=0.017). Urinary 8-isoprostane and plasma MDA (by DETBARS) were the two lipid peroxidation marker assays of choice (Fig. 1a and b). Antioxidant status was compromised in AIH patients, with several important components of the antioxidant defence mechanism being significantly decreased. Although the total antioxidant capacity in whole serum was reduced in AIH patients, the decrease was not significant. However, the total antioxidant capacity in protein-free serum did show a significant reduction (P=0.007) (Fig. 1c), suggesting that protein-free components make an important contribution. Total glutathione (GSH + GSSG) in whole blood, a measure of intracellular antioxidant reserve, showed a small, but significant, reduction (P=0.037). A more striking antioxidant depletion occurred with total glutathione in plasma, where levels were significantly reduced (P<0.001) to about 58% of control values (Fig. 1d). Plasma GSSG, expressed as a percentage of total plasma glutathione, was unchanged in AIH patients. Individual antioxidants were also shown to be affected; serum levels of selenium (Fig. 1e), vitamin A (Fig. 1f) and vitamin E were significantly diminished (P=0.007, P<0.001, P=0.025, respectively). In contrast, vitamin C and urate were unchanged. Serum PIIINP was significantly elevated (P<0.001) in AIH patients, demonstrating that hepatic fibrogenesis is a significant component.

Table 1: Oxidant stress in control and AIH groups: markers of lipid peroxidation, antioxidant status, hepatic fibrogenesis, hepatic function and other biochemical parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>AIH, all</th>
<th>AIH, untreated</th>
<th>AIH, treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-Isoprostane (u)</td>
<td>0.31 (0.21–0.45)</td>
<td>1.12 (0.78–2.13)</td>
<td>1.37 (0.79–4.07)</td>
<td>1.11 (0.78–1.34)</td>
</tr>
<tr>
<td>8-Isoprostane (p)</td>
<td>15.71 (11.43–19.22)</td>
<td>84.68 (61.41–127.6)</td>
<td>93.75 (71.82–174.9)</td>
<td>82.65 (58.72–106.4)</td>
</tr>
<tr>
<td>MDA (TBARS)</td>
<td>2.05 (1.77–2.90)</td>
<td>2.06 (1.44–2.64)</td>
<td>2.79 (1.99–5.93)</td>
<td>1.83 (1.29–2.32)</td>
</tr>
<tr>
<td>MDA (DETBARS)</td>
<td>2.50 (2.17–2.96)</td>
<td>2.86 (2.34–5.35)</td>
<td>5.40 (2.51–13.76)</td>
<td>2.76 (2.34–3.75)</td>
</tr>
<tr>
<td>Total antioxidant (ws)</td>
<td>0.96 (0.78–1.30)</td>
<td>0.82 (0.67–1.10)</td>
<td>1.10 (0.73–1.18)</td>
<td>0.79 (0.65–0.89)</td>
</tr>
<tr>
<td>Total antioxidant (psf)</td>
<td>0.45 (0.37–0.50)</td>
<td>0.35 (0.28–0.45)</td>
<td>0.39 (0.28–0.51)</td>
<td>0.35 (0.28–0.42)</td>
</tr>
<tr>
<td>Total glutathione (wb)</td>
<td>1.42 (1.31–1.70)</td>
<td>1.38 (1.08–1.43)</td>
<td>1.37 (0.76–1.41)</td>
<td>1.42 (1.16–1.44)</td>
</tr>
<tr>
<td>Total glutathione (p)</td>
<td>2.67 (1.92–3.11)</td>
<td>1.56 (1.22–1.93)</td>
<td>1.58 (0.86–2.08)</td>
<td>1.53 (1.29–1.84)</td>
</tr>
<tr>
<td>GSSG/(GSH+GSSG)</td>
<td>9.6 (7.5–10.4)</td>
<td>9.7 (6.2–14.3)</td>
<td>14.9 (6.9–21.1)</td>
<td>9.3 (5.0–12.7)</td>
</tr>
<tr>
<td>Selenium</td>
<td>98.97 (91.84–105.2)</td>
<td>93.27 (73.02–98.01)</td>
<td>93.41 (74.01–101.2)</td>
<td>91.18 (73.96–98.01)</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>3.59 (3.10–4.58)</td>
<td>2.75 (1.83–3.18)</td>
<td>2.11 (1.30–2.97)</td>
<td>2.90 (2.36–3.31)</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>17.67 (13.15–21.44)</td>
<td>16.56 (11.61–18.57)</td>
<td>17.01 (9.48–19.31)</td>
<td>16.56 (13.08–18.55)</td>
</tr>
<tr>
<td>PIIINP</td>
<td>2.95 (2.40–3.30)</td>
<td>4.65 (3.20–8.20)</td>
<td>10.00 (4.75–19.80)</td>
<td>3.50 (3.05–5.25)</td>
</tr>
<tr>
<td>AST</td>
<td>21 (19–24)</td>
<td>40 (31–172)</td>
<td>158 (31–818)</td>
<td>37 (30–64)</td>
</tr>
<tr>
<td>ALT</td>
<td>17 (15–22)</td>
<td>44 (20–117)</td>
<td>61 (20–402)</td>
<td>35 (22–86)</td>
</tr>
<tr>
<td>ALP</td>
<td>169 (144–197)</td>
<td>214 (160–323)</td>
<td>289 (211–486)</td>
<td>199 (152–244)</td>
</tr>
<tr>
<td>γGT</td>
<td>16 (12–22)</td>
<td>73 (37–118)</td>
<td>73 (49–115)</td>
<td>73 (30–118)</td>
</tr>
<tr>
<td>Total protein</td>
<td>78 (76–80)</td>
<td>80 (75–83)</td>
<td>80 (72–83)</td>
<td>79 (75–85)</td>
</tr>
<tr>
<td>Albumin</td>
<td>46 (45–48)</td>
<td>42 (38–46)</td>
<td>39 (32–45)</td>
<td>43 (40–46)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>10 (9–15)</td>
<td>15 (10–71)</td>
<td>66 (10–244)</td>
<td>15 (11–30)</td>
</tr>
<tr>
<td>Urate</td>
<td>0.26 (0.20–0.30)</td>
<td>0.22 (0.18–0.30)</td>
<td>0.19 (0.13–0.31)</td>
<td>0.22 (0.21–0.28)</td>
</tr>
</tbody>
</table>

Values shown as medians with interquartile ranges. P values (<0.05) derived by the Mann–Whitney test (vs. control group; b vs. AIH untreated). Units were expressed as follows: urinary 8-isoprostane as ng/g creatinine; plasma 8-isoprostane as ng/l; MDA (TBARS and DETBARS), total glutathione (p), vitamin A and bilirubin as μmol/l; total glutathione (wb) as mM; total antioxidant (ws and psf) as μmol/l trolox equivalents; GSSG/(GSH + GSSG) as %; selenium and PIIINP as μg/l; vitamins C and E as mg/l; total protein and albumin as g/l; AST, ALT, ALP and γGT as U/l; urate as mmol/l. u = urine, p = plasma, ws = whole serum, ps= protein-free serum, wb = whole blood.
prominent feature of this disease. All conventional serum enzyme markers of hepatic injury (AST, ALT, ALP and γGT) were also significantly raised ($P < 0.001$) in the AIH group. Albumin was reduced significantly ($P < 0.001$) in the AIH group whilst total protein remained unchanged. Although serum bilirubin was significantly higher ($P = 0.005$) in the AIH group than in the controls, almost all values were within the normal range.

There is some evidence for a reduction of oxidant stress in treated AIH patients (Table 1) as MDA levels (by DETBARS) and total glutathione (whole blood) in the treated group are no different from control levels. PIIINP, total antioxidant (whole serum) and ALP levels were significantly reduced ($P = 0.008$, $P = 0.002$, respectively) in the treated group compared with the untreated group.

In the combined group of AIH patients, urinary 8-isoprostane correlated positively with total necroinflammatory score $r = 0.54$, $P = 0.024$, but more strongly with interface hepatitis $r = 0.69$, $P = 0.002$ (Fig. 2a). Urinary 8-isoprostane also correlated positively with hepatic fibrogenesis (PIIINP: $r = 0.53$, $P = 0.002$) and with markers of hepatic injury (AST: $r = 0.70$, $P < 0.001$; ALT: $r = 0.58$, $P < 0.001$; ALP: $r = 0.54$, $P = 0.002$; bilirubin: $r = 0.53$, $P = 0.002$; aGT: $r = 0.40$, $P = 0.040$). Urinary 8-isoprostane correlated negatively with antioxidant markers (vitamin A: $r = -0.49$, $P = 0.009$; vitamin E: $r = -0.49$, $P = 0.008$; plasma total glutathione: $r = -0.46$, $P = 0.027$). Other markers of lipid peroxidation (MDA by TBARS: $r = 0.85$, $P < 0.001$; MDA by DETBARS: $r = 0.89$, $P < 0.001$, Fig. 2b) also correlated strongly with interface hepatitis. Interface hepatitis also correlated with hepatic fibrogenesis (PIIINP: $r = 0.79$, $P < 0.001$ Fig. 2c) and with hepatic injury (ALT: $r = 0.74$, $P < 0.001$; AST: $r = 0.77$, $P = 0.004$, bilirubin: $r = 0.82$, $P < 0.001$), and negatively with antioxidant markers (vitamin A: $r = -0.63$, $P = 0.012$; whole blood total glutathione: $r = -0.84$, $P < 0.001$, Fig. 2d). A similar pattern of correlations was found with total necroinflammatory score but with lower $r$ values and less significant $P$ values. Of the 18 patients biopsied, 15 had a fibrotic score between 1 and 3, two patients had a score of 4, and only one was
4. Discussion

Oxidant stress, now recognized in several forms of chronic liver disease, arises from an imbalance between radical-generating and radical-scavenging activity, thereby increasing formation of oxidation products and decreasing or depleting endogenous antioxidant protection mechanisms. The highly reactive free radical can damage the cell by peroxidation of phospholipid membranes and oxidation of proteins and DNA, possibly leading to malignant transformation [31]. Paradis et al. [32] demonstrated histochemically, in a variety of liver diseases, that lipid peroxidation by-products were present. Only one of five cases of AIH showed intracellular cytoplasmic staining of hepatocytes with specific anti-HNE or anti-MDA antibodies. However, HNE adducts were associated with large bundles of collagen fibres in the portal tracts and fibrous septa, whilst MDA labelling displayed a thin network of fibres but did not stain the large collagen bundles. In some cases, the staining was more intense around bile ducts and at the periphery of portal tracts and fibrous septa in areas of fibrogenesis, and was probably localised in hepatic stellate cells. Liver biopsy is, however, unsuitable for regular monitoring of response to treatment and the histochemical techniques currently available are semi-quantitative, at best. For the routine investigation of oxidant stress, the measurement of pro- and antioxidant markers in blood and urine represents the only currently available, noninvasive approach and complements the limited histochemical information available.

Assessment of oxidative damage to lipids traditionally employs the TBARS assay for MDA, a peroxidation product of polyunsaturated fatty acids. However, as previously demonstrated in hepatitis C [14] and confirmed in this study, urinary 8-isoprostane, a free radical mediated product of arachidonic acid peroxidation, is more sensitive. Plasma and urinary 8-isoprostane levels were four- to fivefold higher in the total AIH group than in controls. Although MDA (by DETBARS) was also significantly increased, median levels in AIH were only 14% higher than controls. Urine and plasma levels of 8-isoprostane become elevated under con-
ditions known to produce oxidant stress such as smoking [33] and following carbon tetrachloride-induced liver injury in the rat [34,35].

The total antioxidant assay provides a global assessment of aqueous and lipid phase antioxidant reserves, reflecting a complex array of factors, such as tissue antioxidant turnover rate, ascorbic acid and tocopherol recycling, and bilirubin production [25]. Although no decrease in total antioxidant capacity in whole serum was observed, a significant depletion was found in protein-free serum, suggesting that the factors involved are not protein-bound antioxidants (-SH groups on proteins, vitamin E residing on the lipoprotein moiety and bilirubin bound to albumin).

The antioxidant defence system includes a wide range of enzymatic and nonenzymatic components [36] several of which were reduced in this study. Selenium is an essential cofactor of glutathione peroxidase, the enzyme responsible for catalysing hydroperoxide reduction by glutathione. In our patients, serum selenium showed a small but significant reduction. Selenium levels have been shown to be low in liver disease generally, irrespective of aetiology, suggesting a relationship to overall nutritional status rather than dietary intake [37]. Burk et al. [38] showed that plasma selenium in a variety of liver diseases declined in proportion to the severity of the cirrhotic condition as indicated by the Child’s grade.

Total plasma glutathione mainly reflects the intrahepatic concentration and the balance between efflux from the liver and uptake by the kidney [39]. In our AIH group, both whole blood and plasma glutathione were reduced, suggesting that both intracellular and intrahepatic reserves were compromised. Plasma GSSG arises from both hepatic and extrahepatic sites, and provides a sensitive index of whole-body oxidant stress. However, as no increase in the plasma GSSG/GSH ratio was observed, it must be assumed that the glutathione redox cycle is still working efficiently and that GSSG is being recycled to GSH by NADPH/glutathione reductase.

In our experience, high GSSG/GSH ratios only occur in cirrhotic patients with severe disruption of hepatic metabolism [Pemberton, unpublished results]. Vitamins A and E were significantly reduced in our AIH patients. It could be anticipated that a loss of vitamin A storage capacity might arise when hepatic stellate cells undergo transformation into myofibroblasts. Water-soluble vitamin C levels were unaffected in our AIH group and patients therefore retain their capacity for regeneration of vitamin E by the vitamin C pathway.

As AIH is rare (170 cases per million in Northern Europe [40]), it is difficult to recruit, in one centre, large numbers of treatment-naïve patients and this study therefore also recruited patients receiving immunosuppressive treatment. A few parameters differed significantly between treated and untreated groups (whole serum antioxidant levels, serum alkaline phosphatase and PIIINP) but some parameters exhibited a wide range of values in both groups (such as bilirubin and the transaminases) giving rise to significant overlap and reflecting the diversity in disease activity found in both treated and untreated groups. It is hoped that a future study will use these markers of oxidative stress to monitor the effect of treatment on naïve patients over a prolonged time period.

Our hypothesis that oxidative stress provides a link between necroinflammation and fibrogenesis is strongly supported by the results of this study which shows a significant correlation between multiple markers of oxidant stress and histologically assessed liver damage. The lipid peroxidation marker 8-isoprostane correlated strongly with total necroinflammatory score and, more significantly, with the degree of interface hepatitis. Both 8-isoprostane and total necroinflammatory score also correlated with serum markers of hepatic injury and with PIIINP, a marker of hepatic fibrogenesis, although not with hepatic fibrosis. Although this finding does not necessarily imply a cause and effect relationship, it strongly suggests that in type I AIH, a link exists between oxidant stress and the activity of the disease process leading to fibrogenesis. In addition to oxidant stress, cytokines, mediators released by mononuclear cells, are likely to play a pathogenic role in AIH, a condition characterised by a dense mononuclear cell infiltrate and by altered levels of circulating cytokines [41,42]. Poli and Parola [43] suggested pathogenic mechanisms that may link ROS generation, onset of lipid peroxidation, and increased deposition of collagen and extracellular matrix: he observed that, as lipid peroxidation-derived aldehydes chemoattract and activate inflammatory cells and oxidant stress is known to modulate the expression of genes encoding for inflammatory cytokines, oxidant stress will trigger an increase in inflammatory activity; second, lipid peroxidation is known to up-regulate the expression and synthesis of fibrogenic cytokines, whilst aldehydic end products of lipid peroxidation enhance type I collagen synthesis by fibroblasts and HSC. It has been proposed [44] that progression to fibrosis is primarily governed by the steady-state concentration of oxidant stress-related molecules, i.e. oxidative stress appears to be causally related to an increase in fibrogenesis and suitable antioxidant supplementation may prevent it. Although a significant correlation was found between urinary 8-isoprostane and PIIINP, a marker of hepatic fibrogenic activity, no correlation was found between markers of oxidant stress and histologically assessed fibrosis in our study. This is likely to be a result of the low fibrotic score observed in almost all the AIH patients biopsied and emphasises that the observed oxidative stress is likely to be a consequence of early disease processes rather than just a feature of late stage decompensated liver disease.

In conclusion, our study shows that oxidant stress, assessed for the first time in a large group of AIH patients using a wide range of markers of both oxidant and antioxidant processes in blood and urine, is not just a feature of late stage decompensated liver disease but is a significant feature of early AIH. We have also shown, in this group of
patients with early stage AIH, a clear association between markers of oxidant stress and histologically assessed inflammation and hepatic fibrogenesis. Trials of antioxidant therapy, adequately monitored, are therefore now indicated, especially in patients who fail to respond to prednisone and azathioprine treatment, to determine whether such treatment can prevent or slow progression to cirrhosis and liver failure.

Acknowledgements

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References