SHORT COMMUNICATION

Acid hydrolase activities and lysosomal integrity in liver biopsies from patients with iron overload

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(Received 20 September 1975)

Summary
1. Iron, acid phosphatase and N-acetyl-β-glucosaminidase were assayed in liver biopsies from control subjects and patients with primary and secondary haemochromatosis.
2. The activities of the lysosomal enzymes were significantly higher in liver biopsies from patients with iron overload than in those from other patient groups.
3. Lysosomes from the livers of patients with iron overload were strikingly more fragile than those of control subjects as demonstrated by assays of latent and sedimentable N-acetyl-β-glucosaminidase.
4. Lysosomal integrity was essentially normal in biopsies from patients with a wide variety of chronic liver diseases.
5. It is suggested that iron accumulation damages the lysosomal membrane, releasing acid hydrolases into the cytoplasm and thus initiating cell damage.

Key words: acid hydrolase, haemochromatosis, haemosiderosis, iron overload, liver, lysosomes.

Introduction
It is well recognized that iron overload causes damage to the liver and to other organs. The mechanism of iron toxicity is ill-understood and in this paper evidence is provided which indicates that selective damage to lysosomes occurs in both primary or idiopathic haemochromatosis and in secondary haemochromatosis due to multiple transfusions.

Since lysosomal disruption leads to the release of powerful hydrolytic enzymes into the cytoplasm, initiating cell damage (de Duve & Wattiaux, 1966), this may be a mechanism of tissue damage in iron overload.

Methods

Patients
Specimens of liver were obtained with a Menghini needle from patients with iron overload, from patients with idiopathic cirrhosis and from patients without liver damage. Six patients with untreated primary haemochromatosis were studied. The eight patients with secondary haemochromatosis all had thalassaemia major and were treated with a high blood transfusion regime (Necheles, Chang, Sabbah & Whitten, 1974). Each patient had been given between 100 and 200 units (approximately 40-80 l) of blood but had also received chelation therapy (Barry, Flynn, Letsky & Risdon, 1974). The group of eight patients with idiopathic, alcoholic or post-hepatitic cirrhosis showed no histological evidence of iron overload. Control specimens were obtained from patients with normal serum liver-function tests and normal hepatic histology who had liver biopsies during the investigation of pyrexia of unknown origin or who had open needle biopsies during surgery for duodenal ulcer. Specimens were collected into ice-cold SVE medium, pH 7-4, which contained sucrose (250 mmol/l), disodium EDTA (1 mmol/l) and ethanol (20 mmol/l). Representative portions were taken for routine histological examination. Informed consent for the liver biopsies was obtained from each subject and the project was approved by the local ethical committee.

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**Table 1.** Iron, N-acetyl-β-glucosaminidase, acid phosphatase and latent and sedimentable N-acetyl-β-glucosaminidase in liver biopsy specimens

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>Total iron (nmol/mg of protein)</th>
<th>Total N-acetyl-β-glucosaminidase (munits/mg of protein)</th>
<th>Total acid phosphatase (munits/mg of protein)</th>
<th>Latent N-acetyl-β-glucosaminidase (%)</th>
<th>Sedimentable N-acetyl-β-glucosaminidase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (8)</td>
<td>36.0±9.0</td>
<td>2.1±0.2</td>
<td>11.0±1.4</td>
<td>66.5±3.9</td>
<td>67.8±4.4</td>
</tr>
<tr>
<td>Cirrhosis (8)</td>
<td>21.5±7.0</td>
<td>3.0±0.6</td>
<td>12.1±2.4</td>
<td>59.7±6.0</td>
<td>64.4±4.8</td>
</tr>
<tr>
<td>Primary haemochromatosis</td>
<td>750±120</td>
<td>6.9±2.4</td>
<td>27.5±3.7</td>
<td>29.0±2.9</td>
<td>32.5±2.5</td>
</tr>
<tr>
<td>Secondary haemochromatosis</td>
<td>430±110</td>
<td>5.8±1.2</td>
<td>24.8±2.5</td>
<td>32.1±2.6</td>
<td>33.0±3.3</td>
</tr>
</tbody>
</table>

**Analytical methods**

The biopsy was homogenized in 3 ml of SVE medium with fifteen strokes of a loose-fitting Dounce homogenizer. Aliquots were taken for assays of iron (Barry, 1968), acid phosphatase (EC 3.1.3.2) and N-acetyl-β-glucosaminidase (EC 3.2.1.30) (Peters, Müller & de Duve, 1972) and for protein estimation (Lowry, Rosebrough, Farr & Randall, 1951). The remainder of the homogenate was centrifuged at 600 g for 10 min and the latent and sedimentable N-acetyl-β-glucosaminidase was determined as described by Peters, Heath, Wansbrough-Jones & Doe (1975).

**Results**

Table 1 shows the concentration of iron, acid phosphatase and N-acetyl-β-glucosaminidase and the latent and sedimentable N-acetyl-β-glucosaminidase in the biopsies from the various patient groups. There are no significant differences between the results in the control and the cirrhotic groups. The two groups of patients with haemochromatosis show marked increases in acid hydrolase activities and decreases in latent and sedimentable N-acetyl-β-glucosaminidase activity. The changes tend to be more marked in the primary haemochromatosis group but apart from the iron content the differences from secondary haemochromatosis are not statistically significant.

**Discussion**

Results presented in this paper demonstrate striking abnormalities in the biochemical properties of the lysosomes in liver biopsies from patients with iron overload. There changes appear to be unique to haemochromatosis. The lysosomal properties in biopsies from the patients with other types of cirrhosis are normal, indicating that the lysosomal changes are not due to any fibrous tissue in the biopsy affecting the homogenization procedure. Similar studies in biopsies from patients with different forms of chronic hepatitides including alcoholic liver disease show no lysosomal abnormalities (Seymour, Neale & Peters, 1975, and unpublished results). The increased acid hydrolase activities suggest an accumulation of undegradable material within the lysosomes. Thus in certain congenital lysosomal storage diseases there is an accumulation of the substrate of the missing enzyme with increased activities of the other lysosomal enzymes (van Hoof & Hers, 1968).

It is probable that iron compounds are accumulating within the lysosome. Dense deposits of ferritin and haemosiderin in structures tentatively identified as lysosomes have been noted by several workers in liver biopsies from patients with haemochromatosis (Bessis & Caroli, 1959; Essner & Novikoff, 1960; Scheuer, Williams & Muir, 1962). Moreover Trump and his colleagues have made detailed morphological studies of iron-overloaded rats and have clearly demonstrated ferritin and haemosiderin within liver cell lysosomes (Bradford, Eichlepp, Arstila, Trump & Kinney, 1969; Arstila, Bradford, Kinney & Trump, 1970; Trump, Valigorsky, Arstila, Mergner & Kinney, 1973). Chronic iron administration to experimental animals is associated with elevated liver acid hydrolase activities (Goldberg, Martin & Batchelor, 1960) and subcellular fractionation studies have shown a striking increase in the density of the lysosomes (Arborgh, Ericsson & Glaumann, 1973; Seymour, Budillon & Peters, 1974). It is prob-
able that the iron compounds enter the lysosomes by autophagy, a process in which discrete cytoplasmic structures become surrounded by unit membranes (autophagic vacuole). These fuse with primary lysosomes, forming secondary lysosomes, permitting the acid hydrolases to degrade the contents of the autophagic vacuole (de Duve & Wattiaux, 1966).

The mechanism of increased lysosomal fragility in haemochromatosis is uncertain. Distension of the lysosomes with poorly degradable ferritin (Coffey & de Duve, 1968) may disrupt their membranes, thereby releasing the hydrolases into the cytoplasm. Precipitation of highly insoluble dense deposits of haemosiderin, perhaps derived from incomplete proteolysis of ferritin by the lysosomal cathepsins, may damage the membrane in a similar manner to that described for silica (Allison, Harrington & Birbeck, 1966) and for urate crystals (Shirahama & Cohen, 1974). Alternatively the iron may catalyse free radical formation (Bors, Saran, Lengfelder, Spöttl & Michel, 1974), which readily damage lysosomes and other subcellular organelles by lipid peroxidation (Chio, Reiss, Fletcher & Tappel, 1969; Dillard & Tappel, 1971; Fong, McCay, Pover, Keele & Misra, 1973).

It is not suggested that the lysosomal changes in haemochromatosis represent a primary lesion. Similar changes are demonstrable in both primary and secondary haemochromatosis and preliminary studies have shown a reversion to normal of the lysosomal abnormalities after removal of the excess of iron from the liver (C. A. Seymour and T. J. Peters, unpublished work). Nevertheless haemochromatosis is an example of a secondary lysosomal storage disease and lysosomotropic chelating and stabilizing agents (de Duve, de Barsy, Poole, Trouet, Tulken & van Hoof, 1974) may prove a more useful form of therapy, particularly in secondary haemosiderosis.

Acknowledgments
We thank Professor K. Weinbren for reviewing the liver histology and Professor G. Neale, Dr B. Modell and Professor J. White for allowing us to study their patients. This work is supported by The Wellcome Trust and the Medical Research Council.

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