and toxin production were on the same plasmid and were transferred together. Whether these observations with enterotoxigenic E. coli isolated from animals are applicable to strains isolated from man is unclear.

Enterotoxigenic E. coli isolated from the Far East are frequently resistant to multiple antibiotics and resistance is transferred by R plasmids. Furthermore, genes coding for antibiotic resistance and enterotoxin production can be transferred together in vitro. We do not know if Ent plasmids are transferred with R plasmids in the gastrointestinal tract of man, or whether the use of antibiotics is increasing the prevalence of enterotoxigenic E. coli. Our experience suggests, however, that the widespread use of antibiotics may increase the distribution of toxigenic E. coli by selecting for plasmid-containing strains which carry genes for both enterotoxin production and antibiotic resistance.

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REFERENCES

PERCUTANEOUS LIVER BIOPSY AND CHRONIC LIVER DISEASE IN HEMOPHILIACS

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Summary

Systematic screening of forty-seven haemophiliacs in Sheffield revealed abnormal liver-function tests in thirty-six (77%), with a tendency for these abnormalities to persist. To assess the importance of these abnormalities, percutaneous liver biopsy was carried out on eight symptom-free patients under factor-VIII cover. A wide spectrum of chronic liver disease was demonstrated, including chronic aggressive hepatitis and cirrhosis. The liver pathology bore no relation to clinical history or to biochemical findings. Hepatitis-B virus markers were common, but evidence suggests that this is not the only factor contributing to the development of liver disease. The high incidence of chronic liver disease seems to be a recent development and is probably related to factor-concentrate replacement therapy.

Introduction

Clotting-factor concentrates have been increasingly used for patients with haemophilia and have undoubtedly improved their overall care and management. However, reports indicate that abnormal liver-function tests are common in regularly treated haemophiliacs. These tests do not provide accurate guidance on the nature or severity of the underlying liver disorder. In an attempt to elucidate the importance of these abnormalities we carried out percutaneous liver biopsies in eight haemophiliac patients in whom abnormal liver-function tests had persisted for at least 6 months.

Patients

We assessed the frequency of liver disease in patients under care by testing liver function in forty-seven patients with haemophilia. All patients had received factor-VIII replacement therapy on at least one occasion during the preceding 12 months. Apart from this selection was random. Persistent abnormalities (>6 months) of liver function were noted in twenty-five patients and eight of these were selected for liver biopsy. The selected patients were well known to us and were considered to be intelligent and responsible. The degree of biochemical abnormality was not a factor in selection. All were symptom-free at biopsy. The full nature of the procedure was explained to each patient and all eight gave their written consent.

Liver-biopsy Procedure

Before the biopsy, each patient's 1-stage prothrombin-time and platelet-count were checked and the presence of factor-VIII inhibitors excluded. A calculated dose of factor-VIII concentrate, sufficient to increase the factor-VIII concentration to 1-0 unit/ml, was given immediately before the biopsy. Factor-VIII plasma concentrations were monitored twice daily and further
factor-VIII concentrate was given to maintain the factor-VIII concentration above 0.5 units/ml for the next 72 h. Liver biopsy was carried out by means of a Klatskin needle. Sufficient tissue was obtained from each patient on the first aspiration. All biopsies were performed without incident and the patients were discharged from hospital 72 h after the procedure.

Methods

Liver-function tests, which were used as a screening procedure on all haemophiliacs, included serum-bilirubin, serum-glutamic-oxaloacetic-transaminase (s.g.o.t.), and serum-glutamic-pyruvate-transaminase (s.g.p.t.). These were carried out by standard autoanalyser methods. HBsAg, anti-HBs, and anti-HBc were determined at the Virus Reference Laboratory, Colindale, in coded samples from thirty-three of the forty-seven patients. Twenty-six of these patients had abnormal liver-function tests and seven had normal biochemistry. HBsAg and anti-HBs were determined by radioimmunoassay (R.I.A.) and anti-HBc by immunoelectrophoresis. Liver-biopsy specimens were examined under light and electron microscopy by conventional techniques. The histological features were classified by standard criteria for the diagnosis of chronic hepatitis. Orcine staining and immunoperoxidase methods were used to detect HBsAg in the biopsy tissue.

Results

Liver-function tests were normal in only eleven of the forty-seven patients studied. The thirty-six remaining patients had mild, moderate, or severe haemophilia and half of them had a history of a hepatitis-like illness (table I). All thirty-six had raised s.g.p.t., and although one third of the patients had raised bilirubin concentrations, clinical jaundice was apparent in only one case. 70% had persistently abnormal liver-function tests, arbitrarily defined as persisting for more than 6 months, but this figure is likely to be an underestimate since many of the remaining 30% have been followed up for less than 6 months to date.

Markers of hepatitis B (HBsAg, anti-HBs, and anti-HBc) were studied in twenty-six of the thirty-six patients with abnormal liver-function tests. At the time of testing, only two patients were found to be HBsAg-positive and in both cases this state has persisted for more than 6 months. Anti-HBs was the most commonly detected marker (69%) and five patients had no detectable markers of hepatitis B. Sera from seven patients with normal liver-function tests were also examined for hepatitis B. HBsAg was absent, but anti-HBs was found in three and anti-HBc in five. Only two had no markers of hepatitis B.

Table II shows the biochemical, serological, and histological data on the eight patients selected for liver biopsy.

**Table I**—Data on 36 haemophilic patients with abnormal liver-function tests

<table>
<thead>
<tr>
<th>Clinical and biochemical features</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor-VIII concentration*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-0.01 units/ml (severe)</td>
<td>24</td>
<td>67</td>
</tr>
<tr>
<td>0.01-0.05 units/ml (moderate)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>&gt;0.05 units/ml (mild)</td>
<td>11</td>
<td>30</td>
</tr>
<tr>
<td>History of clinical hepatitis</td>
<td>18</td>
<td>50</td>
</tr>
<tr>
<td>Abnormal liver-function tests:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased s.g.p.t. (&gt;45 units)</td>
<td>36</td>
<td>100</td>
</tr>
<tr>
<td>Increased s.g.o.t. (&gt;45 units)</td>
<td>30</td>
<td>84</td>
</tr>
<tr>
<td>Increased bilirubin (&gt;17 μmol/l)</td>
<td>12</td>
<td>35</td>
</tr>
<tr>
<td>Persistently abnormal</td>
<td>25</td>
<td>70</td>
</tr>
<tr>
<td>liver-function tests (&gt;6 mo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis B:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBsAg</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td>14</td>
<td>44</td>
</tr>
<tr>
<td>Anti-HBc</td>
<td>18</td>
<td>69</td>
</tr>
<tr>
<td>No H.B.V. markers</td>
<td>5</td>
<td>19</td>
</tr>
</tbody>
</table>

* Severity of haemophilia.
† Hepatitis-B markers examined in only 26/36 patients.
‡ An additional 6 sera gave low counts on R.I.A., possibly reflecting passive antibody rather than immunity.
HBsAg = hepatitis-B-surface antigen.
Anti-HBs = hepatitis-B-surface antibody.
Anti-HBc = hepatitis-B-core antibody.

**Table II**—Data on 8 patients undergoing liver biopsy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Factor VIII (units/ml)</th>
<th>Months after clinical hepatitis</th>
<th>Months of biochemical abnormalities</th>
<th>Liver-function tests†</th>
<th>Hepatitis-B markers</th>
<th>Liver histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>0-10</td>
<td>26</td>
<td>24</td>
<td>12</td>
<td>67 100 108 224 — — PLUS</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>0-03</td>
<td>15</td>
<td>15</td>
<td>22</td>
<td>108 224 334 334 334 334 334 334</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>&lt;0-01</td>
<td>22</td>
<td>20</td>
<td>14</td>
<td>117 215 155 224 334 334 334 334</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>&lt;0-01</td>
<td>9</td>
<td>9</td>
<td>17</td>
<td>338 334 334 334 334 334 334 334</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>&lt;0-01</td>
<td>18</td>
<td>18</td>
<td>29</td>
<td>82 190 2 334 334 334 334 334</td>
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<tr>
<td>6</td>
<td>31</td>
<td>&lt;0-01</td>
<td>43</td>
<td>9</td>
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<td>50 67 334 334 334 334 334 334</td>
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<td>7</td>
<td>51</td>
<td>0-07</td>
<td>11</td>
<td>10</td>
<td>20</td>
<td>125 135 2 334 334 334 334 334</td>
</tr>
<tr>
<td>8</td>
<td>53</td>
<td>&lt;0-01</td>
<td>10</td>
<td>10</td>
<td>34</td>
<td>320 245 334 334 334 334 334 334</td>
</tr>
</tbody>
</table>

* Known duration.
† No. of patients with abnormal values at time of liver biopsy.
biopsy. All eight liver-biopsy specimens showed either hepatitis or cirrhosis. HB, Ag was not detected in the biopsy tissue and no specific features were identified on electron microscopy. Unexplained granulomas were present in two patients.

Discussion

77% of our treated hemophiliacs had abnormal liver-function tests and a history of a hepatitis-like illness was elicited in 50%. This contrasts with earlier reports on the frequency of liver-function test abnormalities in treated hemophiliacs of 11.7% in 1970 and 3.8% in 1974. In 1977 Levine et al. reported abnormal liver chemistries in 68% of treated hemophiliacs. This increase in abnormal liver-function tests seems to be associated with the introduction of clotting-factor concentrates in the treatment of hemophilia. Previously, simple joint bleeds were treated by cryoprecipitate. Since each bag of cryoprecipitate is derived from a single blood-donation, the risk of exposure to hepatitis viruses is quite small. This increase in abnormal liver-function tests is probably related to the increased risk of the serum of each vial, since each vial may contain material from as many as 2500 pooled donations. Like others, we found that these abnormalities tend to persist.

We confirmed earlier observations that percutaneous liver biopsy can be carried out safely in hemophiliacs, given adequate factor-VIII cover and appropriate laboratory control. As with any non-hemophiliac patient, there is a risk of hemorrhage with this procedure but our experience supports the statement of Lesesne et al. that the potential risks of complications from liver biopsy in hemophiliacs are outweighed by the therapeutically important histologic information gained from the biopsy. We also found a wide spectrum of chronic liver disease, including benign self-limiting chronic hepatitis, potentially treatable chronic aggressive hepatitis, and established cirrhosis. All our patients were symptom-free at biopsy and it was impossible to differentiate between the different forms of liver disease on the grounds of biochemical abnormalities. Since the patients undergoing biopsy had been arbitrarily selected it is reasonable to conclude that a large proportion of hemophiliacs receiving treatment with factor VIII have chronic liver disease.

Although liver biopsies have been performed before in hemophiliacs this is the first report from the U.K. While the prevalence of hepatitis B is much lower in the U.K. than in other parts of the world, the incidence of liver disease and abnormalities of liver function is much higher in the treated hemophiliacs than in the non-hemophiliac population and the frequency of hepatitis-B markers are comparable. We used simple liver-function tests as a screening test and this may have underestimated the frequency of liver abnormalities, since five of the seven patients with normal liver chemistry had anti-HBc in the serum, which is thought to reflect continuing virus activity. Any hope that the frequency of liver disease may fall as a result of more sophisticated blood-tests for HB Ag may be unduly optimistic. Blood containing HB Ag, dilute to such an extent that the antigen is no longer detectable by R.I.A., may nevertheless induce HB Ag -ve hepatitis in laboratory animals. Cases of type-B post-transfusion hepatitis have been traced to donor blood lacking both HB Ag, and anti-HB (although anti-HB was present), while Spero et al. reported persistent biochemical abnormalities and HB Ag infection in hemophiliacs treated only with concentrates negative for HB Ag by R.I.A.

In addition, non-A non-B hepatitis may well be an important factor and observations in four of our eight patients support this possibility. Patient 5 (chronic active hepatitis) and 1 (micronodular cirrhosis) have no serum markers of hepatitis B. Patient 7 (micronodular cirrhosis) had a well documented bout of acute HB Ag-positive hepatitis and HB Ag had cleared from his serum within 3 months. Liver biopsy only 13 months after the acute hepatitis showed a quiescent well-established cirrhosis. We feel that the time interval and clinical pattern makes it unlikely that the cirrhosis was caused by the hepatitis-B infection, preferring to implicate some earlier non-hepatitis-B agent. Patient 3 (chronic aggressive hepatitis) had an episode of acute hepatitis 18 months before his liver biopsy. At the onset of hepatitis, his serum was negative for HB Ag but positive for anti-HB, and anti-HB. This suggests that he had probably acquired antibodies to at least two separate hepatitis infections, although it is impossible to tell which was responsible for the liver lesion.

Granulomas were identified in two liver-biopsy specimens, an observation not previously recorded in hemophiliacs. In neither case was there clinical evidence of sarcoidosis or tuberculosis and specific pathogens were not identified in the biopsy specimens. Although hepatic granulomas may be seen in many diseases they may be associated with factor-VIII therapy.

We conclude that histological liver disease is common in hemophiliac patients. The nature and severity of these abnormalities can only be assessed by biopsy, which, under suitable control can be carried out without undue risk. It is noteworthy that two patients with cirrhosis (1 and 7) were mildly affected hemophiliacs requiring only occasional factor-VIII transfusion. Such patients may perhaps benefit from the newly developed synthetic vasopressin analogue 1-deamino-8-d-arginine vasopressin.

We thank Dr. E. M. Vanderwelde, Virus Reference Laboratory, Colinda, for performing the hepatitis-B marker tests. Immuno Ltd provided "Kepluran" factor VIII concentrate for giving cover for the liver biopsies.

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REFERENCES