Basement Membrane-Related and Type III Procollagen-Related Antigens in Serum of Patients with Chronic Viral Liver Disease

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To assess the significance of serum basement membrane- and type III procollagen-related antigens in reflecting the degree of liver fibrosis, we measured radioimmunologically the concentrations of 7S collagen, laminin fragment P1, and the aminoterminal propeptide of type III procollagen (P-III-P) in serum from 48 patients with chronic viral liver disease: chronic persistent hepatitis (9), chronic active hepatitis (13), chronic active hepatitis with lobular disorganization (17), and liver cirrhosis (9). Concentrations of 7S collagen, laminin P1, and P-III-P in serum were increased in respectively 92%, 69%, and 77% of the patients with both chronic active hepatitis with lobular disorganization and liver cirrhosis. Concentrations of 7S collagen and laminin P1 in serum correlated well (r = 0.65, P < 0.001, and r = 0.55, P < 0.001, respectively) with the histological grade of liver fibrosis, whereas P-III-P correlated only weakly (r = 0.33, P < 0.05). Evidently, measurement of serum 7S collagen is a reliable noninvasive test for detection of fibrosis in chronic viral liver disease.

Fibrosis develops during chronic and progressive diseases of the human liver. For diagnosis of fibrosis, histological evaluation of a liver biopsy is essential, whereas conventional laboratory tests are of little value. Extracellular matrix is altered in many chronic liver diseases. In the course of fibrosis, matrix proteins (interstitial and basement membrane collagens and associated proteins) are markedly increased (1, 2). Assay of the aminoterminal propeptide of type III procollagen (P-III-P) in serum appears promising as an indicator of the metabolism of interstitial collagens (3–5).² The presence of antigens related to two basement membrane components—the 7S domain of type IV collagen (7S collagen) and P1 fragment of laminin (laminin P1) in the circulation probably reflects the metabolism of basement membrane (6). Concentrations of these antigens in serum increase in alcoholic liver disease (7) and Indian childhood cirrhosis (8). The aim of this study was to determine whether concentrations of these proteins in serum reflect hepatic pathology in patients with chronic viral liver disease.

Patients and Methods

Samples

We studied 48 patients with chronic viral liver disease, 30 men and 18 women (mean age 49 years, range 24–70 years). These patients had no history of habitual drinking or of taking drugs, as verified by cross-checking with the relatives. All patients were negative for antinuclear antibody, lupus erythematosus test, and antimitochondrial antibody.

Blood was obtained for routine liver-function tests and for determination of 7S collagen, laminin P1, and P-III-P. The serum was separated by centrifugation and stored at –20 °C until used. All the patients underwent liver biopsies as part of their medical evaluation. Tissue sections were stained with hematoxylin–eosin and by the Mallory–Azan method and examined by a liver pathologist (T. M.). The patients were classified into four types, according to the histological appearance of the liver: chronic persistent hepatitis (CPH), chronic active hepatitis (CAH), chronic active hepatitis with lobular disorganization (CAH with LD), and liver cirrhosis (LC). Table 1 shows the composition of each group.

Fibroses in the biopsies were graded on a scale of A to D, according to the histological activity index advocated by Knodell et al. (9). In addition, the degree of proliferation of bile ductules was examined and evaluated as one of four grades (1, non; 2, minimal; 3, moderate; 4, severe). Of the 39 patients with chronic hepatitis, 11 were classified as type B on the basis of the presence of HBs antigen, and 28 as type non-A, non-B on the basis of negative results for hepatitis A and B serology.

The control serum samples were obtained from 34 volunteers, ages 33–63 years.

Methods

Concentrations of 7S collagen were determined with a research radioimmunoassay kit (Nippon DPC Corp., Tokyo, Japan) in which ¹²⁵I-labeled 7S domain of type IV collagen isolated from human placenta is used as the tracer, rabbit antiserum against this material is the primary antibody, and goat antirabbit IgG antiserum is the secondary antibody. The pH 7.4 buffer solution used in the RIA contained, per liter, 50 mmol of sodium phosphate, 0.15 mol of sodium chloride, and 1 g of bovine serum albumin.

The assay procedure was as follows: Add 100 µl of primary antibody (diluted 30 000-fold in buffer) to 200 µl of serum and incubate at 37 °C for 2 h. Add 100 µl of competing radiiodinated 7S collagen, and incubate the suspension for 2 h at 37 °C. Then add 1000 µl of the second

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**Table 1. Subjects Investigated in This Study**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No.</th>
<th>Age, y, mean ± SD</th>
<th>Male/female ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>34</td>
<td>47 ± 8</td>
<td>19/15</td>
</tr>
<tr>
<td>CPH</td>
<td>9</td>
<td>51 ± 14</td>
<td>6/3</td>
</tr>
<tr>
<td>CAH</td>
<td>13</td>
<td>42 ± 11</td>
<td>10/3</td>
</tr>
<tr>
<td>CAH with LD</td>
<td>17</td>
<td>53 ± 10</td>
<td>10/7</td>
</tr>
<tr>
<td>LC</td>
<td>9</td>
<td>50 ± 7</td>
<td>5/4</td>
</tr>
</tbody>
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² Nonstandard abbreviations: P-III-P, aminoterminal propeptide of type III procollagen; CPH, chronic persistent hepatitis; CAH, chronic active hepatitis; LD, lobular disorganization; and LC, liver cirrhosis.

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precipitating antibody solution, with polyethylene glycol as sediment retainer. Aspirate the supernate and count the radioactivity of the pellet.

The minimum concentration of 7S collagen detected with the kit, determined as 2 SD of 50 assays of the zero calibrator, was 1.8 \( \mu g/L \). The intra-assay reproducibility (CV) for the low-dose sample (5.8 \( \mu g/L \)) was 4.1\%, for the middle-dose sample (13.3 \( \mu g/L \)) it was 4.4\%, for the high-dose sample (45.7 \( \mu g/L \)) it was 6.0\%, and for pooled serum (12.7 \( \mu g/L \)) it was 6.6\% (n = 10 each). The respective interassay reproducibility results were 7.8\%, 3.3\%, 5.9\%, and 3.2\% (n = 5 each).

Laminin P1 was determined in serum with a commercial RIA kit (Hoechst AB, Frankfurt, F.R.G.). The intra- and interassay CVs were 5.0\% and 6.5\%, respectively. One unit of laminin P1 was defined by the manufacturer as the average concentration of the peptide in pooled sera from a normal German population.

Serum P-III-P was also determined with a commercial RIA kit from Hoechst. The intra- and interassay CVs were 6.2\% and 7.0\%, respectively.

Throughout, results are expressed as the mean \( \pm \) SD. The statistical significance of the differences between the means was assessed by using the least-significant-difference test (\( t \)). Correlations were analyzed by Spearman's rank test.

Results

The concentration of 7S collagen in serum of the control subjects was \( 3.7 \pm 0.6 \) \( \mu g/L \). The corresponding values for laminin P1 and P-III-P were 1.34 \( \pm \) 0.24 kilo-units/L and 7.1 \( \pm \) 1.2 \( \mu g/L \), respectively. Figure 1 summarizes the concentrations of 7S collagen, laminin P1, and P-III-P in the serum of patients with CPH, CAH, CAH with LD, and LC, and it shows that, except for CPH, these values were significantly (\( P < 0.01 \)) higher than those in the control subjects. Concentrations of 7S collagen and laminin P1 in CAH-with-LD patients were also significantly (\( P < 0.01 \)) higher than those for the CAH patients.

The relationship between 7S collagen, laminin P1, and P-III-P concentrations and the degree of hepatic fibrosis are shown in Figure 2A. The concentrations of each showed a significant increase (\( P < 0.001, < 0.001, and < 0.05, \) respectively) with increasing degree of fibrosis. Moreover, the degree of proliferation of bile ductules significantly correlated with the concentrations of 7S collagen (\( P < 0.001 \)), laminin P1 \( (P < 0.001) \), and P-III-P \( (P < 0.01) \) in serum (Figure 2B). In the patients in the four groups—CPH, CAH, CAH with LD, and LC—the correlation between the serum 7S collagen and laminin P1 was significant \( (r = 0.64, P < 0.001) \); we also found correlations between the concentrations of 7S collagen and P-III-P \( (r = 0.61, P < 0.001) \) and between the laminin P1 and P-III-P in serum \( (r = 0.44, P < 0.01) \).

Discussion

The present study indicates that the development of the hepatic lesion in chronic viral hepatic disease is accompanied by increases in the concentrations of matrix proteins in serum. In the extracellular space, procollagen peptides are liberated from procollagen in stoichiometric amounts by means of specific peptidases (11). The propeptides are easily solubilized (12) and evidently are released into the circulation. An increase in P-III-P concentration may thus be an early indicator of accelerated fibrogenetic activity (3–5). However, nonspecific proteolytic degradation of pN-
Collagen during inflammation and tissue remodeling could contribute to the increase in P-III-P and the extent of fibrosis in chronic viral liver disease.

In human liver, basement membranes are found in blood and lymphatic vessels and around bile ducts (1, 2). Lack of typical basement membrane structure in the peri-sinusoidal space in normal liver is considered to allow intimate contact between blood and parenchymal cells. In advanced liver disease, such as cirrhosis, sinusoids may develop a real basement membrane, a process known as capillarization (13, 14). This transformation affects the interchange of material between the blood and the hepatocytes, reducing the effectiveness of the hepatic circulation and aggravating hepatocellular dysfunction.

7S collagen and laminin P1 are components of the basement membrane. In CAH with LD and in LC, the concentrations of 7S collagen and laminin P1 were increased in the serum of 92% and 69% of the patients studied. These data support the existence of a relationship between serum concentration of 7S collagen or laminin P1 and the extent of fibrosis of chronic viral liver disease. Indeed, the changes in these serum markers for connective tissue metabolism were concomitant with changes in liver tissue, as judged by immunostaining with specific antibodies against human 7S collagen and laminin P1 (15), whereas concentrations of both antigens in serum correlated with the degree of proliferation of bile ductules. Because basement mem-

References