Serum type IV collagen in various liver diseases in comparison with serum 7S collagen, laminin, and type III procollagen peptide

Chisato Hirayama, Hirosi Suzuki, Akira Takada, Kiyoshi Fujisawa, Kyuichi Tanikawa, and Shogo Igarashi

1 Saiseikai Gotsu General Hospital, 1551 Gotsu-cho, Gotsu-shi, Shimane 695, Japan
2 Yamanashi Medical College
3 Department of Internal Medicine, Kanazawa Medical University
4 Japan Red Cross Tokyo Metropolitan Blood Center
5 Second Department of Internal Medicine, School of Medicine, Kurume University
6 Department of Internal Medicine, Tokyo Senbai Hospital

Abstract: The clinical significance of the immunoreactive triple helical domain of type IV collagen in serum was evaluated in 73 healthy controls and 161 patients with various biopsy-proven liver diseases. Although serum levels of type III procollagen peptide were increased in all liver diseases, those of type IV collagen, 7S collagen, and laminin were principally increased in chronic liver diseases associated with hepatic fibrogenesis/fibrosis. In both non-alcoholic and alcoholic liver diseases, 7S collagen was increased in serum, while type IV collagen and laminin in serum were particularly increased in alcoholic liver diseases and in hepatocellular carcinoma, in which latter the sensitivity was greater for type IV collagen than for laminin. Gel filtration analysis in Sephacryl S-400 revealed type IV collagen in serum to be a single molecular form with a molecular weight that correspond to type IV collagen, whereas 7S collagen was recognized as several heterogeneous macromolecules. These findings indicate that serum type IV collagen is derived from the type IV procollagen pool, and is a sensitive marker for the fibrogenetic process in hepatic basement membranes.

Key words: type IV collagen, 7S collagen, laminin, type III procollagen peptide, basement membrane, alcoholic liver disease, hepatocellular carcinoma

Introduction

The amount of connective tissue in the liver is determined by synthesis (fibrogenesis) and by degradation (fibroclasia). In hepatic fibrosis, there is a distinct increase of interstitial collagens, principally type I and type III collagens. In the normal liver, the sinusoids grossly lack a basement membrane; however, various basement membrane components have been identified around the vessels and in the portal tract in fibrotic livers.1-3 The major components of the basement membrane are type IV collagen and laminin.4,5 Type IV collagen is a triple helical molecule (Mr, 540 kDa), that forms a supramolecular structure that is stabilized by tetrameric association at the N-terminal domain (7S collagen, Mr, 360 kDa). Laminin is a major non-collagenous high molecular weight glycoprotein (Mr, 800 kDa), that is composed of several subunits, which can be isolated as distinct antigenic fragments, P1 and P2, after treatment with pepsin. Antigens related to 7S collagen and laminin P1 (laminin) are detected by specific radioimmunoassay in serum, and serum levels of 7S collagen and laminin are reported to be increased in various chronic liver diseases, this increase being associated with alterations in hepatic basement membranes.5-15 Non-collagenous binding at the C-terminal domain of type IV procollagen (NC-1) has been detected in serum, and serum levels of NC-1 have been reported to be increased in chronic liver diseases.16,17 Because of the weak immunogenicity of the triple helical domain of collagens, polyclonal antibodies to type IV collagen molecule have not been obtained. However, monoclonal antibodies to pepsin-resistant human type IV collagen have been developed.18,19 Obata et al.20 prepared a sensitive enzyme immunoassay for the major triple helical domain of type IV collagen. The antigen related to type IV collagen has been detected in human serum, and its concentration is increased in the serum of patients with chronic liver diseases associated with hepatic fibrosis.21-24 However, to date the diagnostic significance of serum type IV collagen in various liver diseases has not been elucidated in comparison with other basement membrane-related antigens. In this study, we attempted, first, to elucidate the molecular form of type IV collagen in serum, and
secondly, to determine the diagnostic significance of serum type IV collagen, in comparison with 7S collagen, laminin, and type III procollagen peptide (P III P) in various liver diseases, introducing new analytical statistics to determine the reliability of this protein as a diagnostic test.

**Subjects and methods**

Between April 1988 and December 1990, 73 healthy controls (35 males and 38 females) and 161 patients with various liver diseases were studied. Informed consent was obtained from all subjects. Healthy controls were selected from subjects undergoing voluntary health examinations. The diagnosis of liver disease was principally obtained by routine laboratory tests and imaging criteria. The final diagnosis was confirmed by percutaneous liver biopsy. The mean (±SD) age of the healthy controls was 44.9 ± 13.3 years. Of the 161 patients with liver disease, 15 had acute viral hepatitis (mean age, 43.9 ± 13.5 years; 6 males and 9 females), 28 had chronic persistent hepatitis (46.4 ± 12.3 years; 21 males and 7 females), 32 had chronic active hepatitis (49.5 ± 10.9 years; 21 males and 11 females), 16 had alcoholic liver disease (49.3 ± 11.8 years; all males), 38 had liver cirrhosis (54.3 ± 11.5 years; 25 males and 13 female), and 32 had hepatocellular carcinoma (61.3 ± 7.1 years; 24 males and 8 females). Of the 15 patients with acute viral hepatitis, 2 were A type and 13 were non-A, non-B type, and the serum ALT levels all exceeded 300IU. The alcoholic patients had consumed more than 80 ml of ethanol daily for at least 5 years. The alcoholic liver diseases included alcoholic hepatitis (n = 3), fatty liver (n = 4), and hepatic fibrosis (n = 9), with no viral hepatitis markers. The degree of hepatic fibrosis in liver biopsy specimens was graded as: 0, no fibrosis; I, fibrous portal expansion; II, bridging fibrosis; and III, cirrhosis. The degree of hepatic fibrosis was not determined in patients with hepatocellular carcinoma. Blood was obtained from all patients while they were fasting in the morning, and the serum was stored at −20°C until use within 12 months.

Type IV collagen was determined by a one-step sandwich enzyme immunoassay (EIA) technique, using a commercial assay kit (Fuji Chemical Industries, Toyama, Japan). Briefly, 50 μl of serum was incubated with a polystyrene ball coated with 300 μl of mouse monoclonal antibody against the pepsin-solubilized type IV collagen obtained from human placenta, and with 0.8 mg/l of Fab-peroxidase conjugate in 10 mmol/l phosphate-buffered saline (pH 7.0), containing 0.5% bovine serum albumin, for 60 min at room temperature. The polystyrene ball was washed four times with 3.5 ml of 10 mmol/l phosphate buffered saline, and was then incubated with 300 μl of 0.013% 3,3’,5,5’-tetramethyl benzidine and 100 μl of 0.015% hydrogen peroxide for 30 min. The reaction was stopped by adding 1 ml of 0.67 mol/l sulfuric acid, and the absorbance at 450 nm was measured with a microflow spectrophotometer. A commercial assay kit (Nippon DPC Corp., Tokyo, Japan) was used to determine 7S collagen by radioimmunoassay; laminin and PIIIP were determined by radioimmunoassay, with a commercial assay kit (Hoechst AB, Frankfurt, Germany).

To characterize the molecular form of serum type IV collagen in healthy controls and liver disease patients, 1 ml of serum was fractionated with Sephacryl S-400 (16 × 460 mm) equilibrated in 0.01 mol/l phosphate-buffered saline (pH 7.0) containing 0.05% Tween 20. One-ml aliquots were collected and analyzed for type IV and 7S collagen. The molecular size was calculated with a gel filtration calibration kit (Pharmacia LKB, Uppsala, Sweden).

Statistical analysis was principally done by non-parametric tests, because the majority of measurements in the disease group did not show normal distribution. Values for each group were expressed as medians and ranges, the latter of which was defined as the 25th percentile (Q1) and the 75 percentile (Q3). Significant differences in multiple groups were determined by the Wilcoxon rank sum test, the Kruskal-Wallis test, and Dunnett’s multiple comparison. P values less than 0.05 were considered significant. To evaluate the diagnostic accuracy of serum markers for each liver disease, the odds ratio (relative risk), derived from case-control studies, was calculated with 95% confidence intervals (CI). To evaluate the diagnostic performance of type IV collagen, the receiver-operating characteristic (ROC) curve, an integrated line diagram of the sensitivity and the specificity, was adopted in some selected disease groups.

**Results**

The mean (±SD) level of type IV collagen in the sera of the 73 healthy controls was 101 ± 20 ng/ml (range: 71–173 ng/ml). The intra-assay reproducibility (coefficient of variation; CV) of serum type IV collagen was 4.98%. The mean levels of 7S collagen, laminin, and PIIIP were 4.5 ± 0.8 ng/ml (2.9–6.6 ng/ml), 1.4 ± 0.2 U/ml (1.1–2.0 U/ml), and 0.57 ± 0.07 U/ml (0.40–0.93 U/ml), respectively. No significant difference was observed in serum levels of type IV collagen, 7S collagen, laminin, and PIIIP between men and women. Similarly, no significant difference between any age groups were observed in the serum levels of type IV collagen and PIIIP. However, there was a reduction in 7S collagen and an increase in laminin in the sera of subjects 50–60 years old.