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Increased serum xylosyltransferase activity in patients with liver fibrosis

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ABSTRACT

Background: We investigated the xylosyltransferase (XT) activity in the serum of liver fibrotic patients with hepatitis C virus induced liver fibrosis at different stages as determined according to the scoring system of Desmet and Scheuer.
Methods: Measurement of XT activity was performed by liquid chromatography-tandem mass spectrometry.
Results: We found that serum XT activity in males ($n=59$, median $\pm$ SD, 27.2 ± 2.8 mU/L, $p<0.001$) and females ($n=54$, 23.6 ± 3.0 mU/L, $p<0.01$) with liver fibrosis is significantly elevated in comparison to a corresponding healthy control cohort of males ($n=50$, 23.9 ± 2.8 mU/L) and females ($n=52$, 21.5 ± 3.7 mU/L), respectively. Of note, independent from gender, serum XT activity positively correlated with the stage of fibrosis but declined again in patients with histologically proven cirrhosis.
Conclusions: XT activity is increased in the serum of patients with liver fibrosis at different stages, pointing to a possible pathogenetic role in elevated proteoglycan biosynthesis in fibrotic remodeling of this organ during chronic injury.

1. Introduction

The cells of the vertebrate organism are kept together by a complex architecture of extracellular matrix components providing both, mechanical stability and a high degree of flexibility. The three-dimensional meshwork of connective tissue in normal human liver contains a variety of specialized connective tissue proteins such as several types of collagens, structural glycoproteins, proteoglycans (PGs), and hyaluronan [1,2]. Under normal conditions the amount of extracellular matrix is low, but several clinical conditions such as alcohol abuse, chronic hepatitis B and C infections, non-alcoholic fatty liver diseases, and several inborn errors of metabolism induce an excessive production of connective tissue (fibrogenesis), which leads to a several-fold increase of matrix components in liver (fibrosis). Both the clinical outcome of patients with fibrosing liver diseases and the diagnostic tools for non-invasive monitoring of ongoing fibrogenesis have not significantly improved although several panels of biomarkers have been suggested [3,4]. This dilemma, however, might change in the near future by increasing knowledge of new pathogenetic mechanisms, which complement the "canonical principle" of fibrogenesis [5] based on the activation of hepatic stellate cells and their transdifferentiation to (myo-)fibroblasts. The latter cell type responsible for the excess production of extracellular matrix is induced by hepatocellular injury and consecutive inflammatory reactions mediated by transforming growth factor β [6], connective tissue growth factor (CTGF/CCN2) [7] and its intracellular downstream mediator Smad3 [8]. Stellate cells expressed almost all matrix components laid down in the extracellular compartment of the liver. New pathogenetic mechanisms of fibrosis indicate that the heterogeneous pool of (myo-)fibroblasts derived from hepatic stellate cells can be supplemented by epithelial–mesenchymal transition (EMT) from cholangiocytes [9] and hepatocytes [10,11] to fibroblasts, by influx of bone marrow-derived fibrocytes into the damaged liver tissue [12–14] and possibly by differentiation of a subgroup of monocytes to fibrocytes after homing in the damaged tissue [15]. As a result the deposition of PGs is increased from 3 to 10 fold, their composition is changed, their microstructure is modified, and they are histologically redistributed. As an example, the amount of dermatan sulfate, chondroitin sulfate and hyaluronan is increased strongly, whereas that of heparan sulfate is decreased [2].

Xylosyltransferases 1 and 2 (XT-1, XT-2) initiate the biosynthesis of the carbohydrate moiety of different GAG-side chains attached to the core protein of PGs, such as chondroitin sulfate, heparan sulfate, heparin and dermatan sulfate. PG homeostasis is very important for normal organic function in vertebrates because these polyanionic glycoproteins influence many fundamental biological processes, including biomechanical lubrication, cell–cell interaction, cytokine and growth factor function, tumor cell growth and viral infections [16,17]. On the one hand, liver PGs are influential in hepatocellular

Abbreviations: Bio-BIK-F, Biotin-NH-QEEEGSGGGQKK(5-fluorescein)-$CONH_2$; ECM, extracellular matrix; ESI-MS, electrospray ionization mass spectrometry; GAG, glycosaminoglycan; HCV, hepatitis C virus; LC-MS/MS, liquid chromatography-tandem mass spectrometry; PG, proteoglycan; XT, xylosyltransferase; XT-1, xylosyltransferase 1; XT-2, xylosyltransferase 2.

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differentiation, and XT-2 deficiency drastically reduced hepatocyte PG biosynthesis [18]. XT-2 inactivation causes severe reduction in liver PG content and XT-$2^{-/-}$ mice develop liver biliary epithelial cysts, renal tubule dilation/cysts and decreased renal function [18]. On the other hand, elevated PG biosynthesis was found in fibrotic and sclerotic processes, like liver fibrosis and systemic sclerosis [19–23]. Furthermore, it has been shown that serum XT activity is a marker of fibrotic remodeling processes [14–26].

Here, we investigate for the first time serum XT activities in patients with hepatitis C (HCV) induced liver fibrosis possibly reflecting disturbed PG homeostasis in these patients.

2. Materials and methods

2.1. Materials

UDP-D-xylose was purchased from CarboSource Services (Athens, Georgia). The synthetic peptide Bio-BIK-F (XT acceptor peptide: Biotin-NH-QEEEGSGGGQKK(5-fluorescein)-$CONH_2$) was obtained from Thermo Electron GmbH (Ulm, Germany). HPLC grade water and methanol were from Fisher Scientific GmbH (Schwerte, Germany). All other reagents were of research grade or better and were purchased from commercial sources.

2.2. Patients

Two groups were enrolled in the investigation: group 1 — patients with chronic hepatitis C [thereafter denominated HCV; $n=113$; median age 49 years, range 18–84 years; 59 males (age 20–76 years) and 54 non-pregnant females (age 18–84 years)]. This group also included 4 female patients with autoimmune hepatitis. Results of carbohydrate-deficient transferrin in serum did not indicate current alcohol abuse; group 2 — healthy controls [blood donors, $n=102$; median age 36 years, range 18–65 years; 50 males (age 18–65 years) and 52 non-pregnant females (age 18–61 years)]. Patients with HCV were furthermore separated into subgroups depending on the stage of fibrosis as given in Table 1. All specimens used in our investigations were reserve materials that were not needed for any other diagnostic analysis. No extra material or increased sample volumes were obtained from the patients and all samples were completely anonymized before inclusion in the study.

2.3. Sample collection

After obtaining informed consent, peripheral venous blood samples were obtained. Serum was separated at 4000*g* after clot-retraction and stored at −70 °C for 3 to 4 months before analysis. All patients had a liver biopsy before serum collection. Staging of the fibrotic process was classified according the scoring system of Desmet and Scheuer using a scale of F1–F4 (F1, discrete fibrosis; F4, cirrhosis) [27].

2.4. Measurement of xylosyltransferase activity by HPLC electrospray ionization tandem mass spectrometry

The XT activity measurement is based on the xylosyltransferase-mediated incorporation of xylose into the synthetic peptide Bio-BIK-F. The xylosylated peptide Bio-BIK-F-Xyl was quantified using HPLC electrospray ionization mass spectrometry, as described previously [28].

The enzyme activity was: 1 mU = 1 nmol of incorporated xylose per min, which is equal to the synthesis of 175.5 µg/L Bio-BIK-F-Xyl under the assay conditions (incubation time = 90 min).

2.5. Statistical analysis

For normally distributed XT activity values, a comparison between the groups was performed using an independent samples *t*-test. For non-normally distributed values an independent samples Mann–Whitney test was used. All statistical tests were performed using MedCalc software, version 9.4.2.0.

3. Results

Serum XT activity in the HCV group ($n=113$) was found to be statistically significantly elevated in comparison to controls ($n=102$) ($p<0.001$). The median ± SD of XT activity was 22.7 ± 3.4 mU/L for healthy subjects and 25.4 mU/L ± 3.1 mU/L for HCV patients, respectively (Fig. 1). In agreement with our previous studies [23,25], we found that XT activity in the serum of healthy males ($n=50$, age: 18–65 years) 23.9 ± 2.8 mU/L, was higher than XT activity in the serum of healthy females ($n=52$, age: 18–61 years) 21.5 ± 3.7 mU/L. Serum XT activity in HCV infected males ($n=59$, 27.2 ± 2.8 mU/L, $p<0.001$), as well as serum XT activity in HCV infected females ($n=54$, 23.6 ± 3.0 mU/L, $p<0.01$) were significantly higher in comparison to the corresponding controls (Fig. 2).

Of note, independent from gender, serum XT activity positively correlated with the stage of fibrosis as determined according to Desmet and Scheuer [27] but declined again in patients with histologically proven cirrhosis [F1 ($n=38$), 25.3 ± 3.4 mU/L, $p<0.001$; F2 ($n=29$), 24.9 ± 3.3 mU/L, $p<0.001$; F3 ($n=21$), 26.8 ± 2.4 mU/L, $p<0.001$; and F4 ($n=25$), 23.3 ± 3.7 mU/L, $p=0.02$ (Fig. 3)]. Diagnostic sensitivity and specificity of XT activity as a marker for liver fibrosis subdivided depending on the stage of fibrosis are shown in Table 2.

Furthermore, 4 female patients with autoimmune hepatitis were investigated in the study. The XT activity of these patients was with

Table 1
Distribution of biopsy results according to the classification system for the staging of fibrosis as published by Desmet and Scheuer [27].

	Desmet–Scheuer score for histopathological staging			
	F1	F2	F3	F4
Male (*n*)	17	17	11	14
Female (*n*)	21	12	10	11

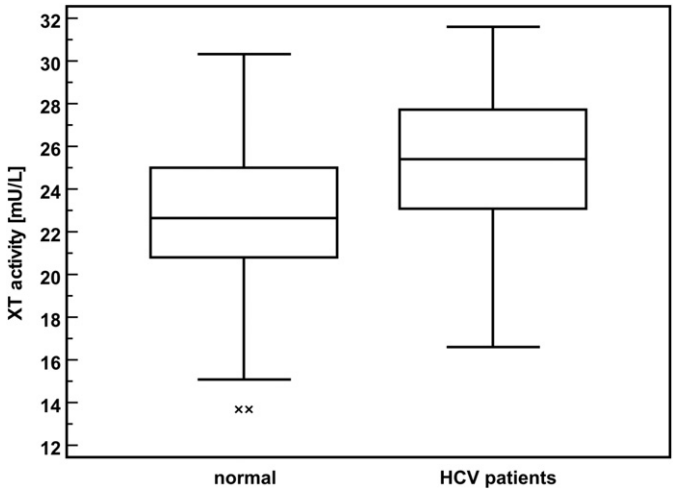

Fig. 1. Serum XT activity of patients with HCV-induced liver fibrosis (Desmet–Scheuer stage F1–F4) in comparison to a healthy control (normal). Each box represents the values from the lower to the upper quartile (25 to 75 percentile), and the middle horizontal line in each box represents the median. The vertical line extends from the minimum to the maximum value, excluding outsides, which are displaced as separate points (x). The left plot represents XT activity in the serum of healthy subjects of both genders, and the right plot represents XT activity in the serum of liver fibrotic patients of both genders.