Changes in the Concentration of Tissue Inhibitor of Type 1 Metalloproteinases in Blood Serum and Liver of Mice with CCl_4-Induced Hepatitis

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The concentration of tissue inhibitor of type 1 metalloproteinases in blood serum from intact CBA mice measured by enzyme immunoassay is similar to that in healthy humans. The concentration of tissue inhibitor of type 1 metalloproteinases in mouse bile was higher than in blood serum, while its concentration in liver homogenate more than 1000-fold exceeded that in the serum, which attests to its primarily intracellular localization in the liver. Loading of liver cell lysosomes with Triton WR-1339 and development of intrahepatic cholestasis did not affect the concentration of tissue inhibitor of type 1 metalloproteinases in liver homogenate and bile. Administration of CCl_4 to mice for 4.5 weeks was accompanied by an increase in the concentration of tissue inhibitor of type 1 metalloproteinases in blood serum, but not in liver homogenate. These changes reflect dysregulation of metalloproteinases, development of inflammation, and progression of the initial stage of connective tissue formation in mouse liver.

Key words: *tissue inhibitor of type 1 metalloproteinases; toxic hepatitis; blood serum; liver; bile*

Activated macrophages (MP) play an important role in the degradation of components of extracellular matrix due to production of metalloproteinases [1-3]. Differentiation of MP is accompanied by intensification of their response to pathogens, which determines the involvement of MP into inflammatory processes and immune reactions. Activated MP regulate activity of matrix metalloproteinases (MMP), in particular MMP-2 and MMP-9 (gelatinases), MMP-12 (metalloelastase), and MMP-7 (matrilysin). Under certain conditions they induce the release of collagenases MMP-1 and MMP-13.

MMP belong to a family of structurally-related endopeptidases cleaving extracellular matrix components and involved in connective tissue remodeling. However, the mechanism for regulation of MMP expression in MP is poorly understood. MMP-2 and MMP-9 cleave denatured collagen, gelatin, and native collagen of types IV, V, and VI in the extracellular matrix. MMP-2 cleaves fibrils of type I and II collagen.

Tissue inhibitors of metalloproteinases (TIMP) of 4 types are the main regulators of MMP function. TIMP-1 and TIMP-2 play a role in the development of liver fibrosis. The development of liver fibrosis and cirrhosis is associated with dysregulation of MMP. An important role in this process is played by TIMP-1, better studied compared to other inhibitors of MMP. However, it remains unclear whether changes in the concentration of

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TIMP-1 in blood serum, bile, and liver tissue can serve as a reliable marker of fibrosis. It was hypothesized that the increase in MMP activity and TIMP-1 concentration is one of the major criteria for liver fibrosis [5-8]. In light of this it is important to detect early signs of MMP dysregulation during hepatitis development.

Here we studied disturbances in MMP regulation associated with changes in TIMP-1 concentration in mouse liver tissue and blood serum during the development of subacute CCl_4-induced hepatitis and in bile during intrahepatic cholestasis.

MATERIALS AND METHODS

Experiments were performed on male CBA mice weighing 25-30 g and obtained from the vivarium of the Institute of Pharmacology (Siberian Division of the Russian Academy of Medical Sciences, Tomsk). Oil solution of CCl_4 (50 mg/kg) was administered through a gastric tube 2 times a week for 4.5 weeks. IFN-α (10,000 U per 20 g body weight) was injected intraperitoneally 24 h before the last treatment with CCl_4. The animals were killed 24 h after the last dose of CCl_4. Intrahepatic cholestasis was induced by intraperitoneal injection of the lysosomotropic preparation Triton WR-1339 in a single dose of 100 mg per 100 g body weight. Triton WR-1339 sharply increases cholesterol synthesis in the liver. The animals were killed 72 h after WR-1339 injection.

Blood serum was obtained by centrifugation in an Eppendorf 5415 R centrifuge at 3000*g* and 4°C for 20 min. Bile was sampled with a microsyringe after sacrifice. Liver homogenates in 0.25 M sucrose were treated with 0.1% Triton X-100 at 0°C for 30 min and centrifuged at 3000*g* for 30 min.

Hepatocyte cytolysis was evaluated by the increase in blood ALT activity using an Abbott biochemical analyzer and Biocon kits.

Cholestasis was verified by measuring serum activities of alkaline phosphatase and γ-glutamyltranspeptidase (Vital Diagnostics SPb kits). The measurements were performed on a LOMO SF-26 spectrophotometer at 405 nm.

β-D-Galactosidase activity in blood serum and bile was estimated by the fluorescence method using 4-methylumbellipheryl-β-D-galactopyranoside as the substrate (Melford Laboratories Ltd.).

TIMP-1 concentration in blood serum, bile, and liver homogenate from mice was measured using commercial ELISA kits for mice (RayBiotech). After application of TIMP-1-containing standards and samples to the well, TIMP-1 binds to immobilized antibodies conjugated with horseradish peroxidase. Addition of tetramethylbenzidine results in staining, whose intensity is proportional to the amount of TIMP-1 bound to the well surface. The intensity of yellow color after addition of 0.2 M H_2SO_4 was measured on a Star 30 plate reader at 450 nm. The results were expressed in pg TIMP-1 per ml blood serum and μg TIMP-1 per gram wet liver tissue (or per gram protein). TIMP-1 concentration in blood serum from donors was measured with commercial ELISA kits for humans (RayBiotech).

The results were analyzed by Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

The development of subacute CCl_4-induced hepatitis was accompanied by hepatocyte cytolysis and sharp increase in serum ALT activity, which did not return to normal after treatment with IFN-α (Table 1).

Serum TIMP-1 concentration in intact CBA mice (Table 1) corresponded to that in healthy donors (285.80±31.40 pg/ml, $n=30$). In the bile from intact mice TIMP-1 concentration was higher than in the serum ($p<0.05$, Table 2). TIMP-1 concentration in the liver homogenate from intact mice was more than 1000 times higher than in blood serum ($p<0.001$, Table 2), which attested to a pronounced gradient of TIMP-1 concentration between the intracellular

TABLE 1. ALT Activity and TIMP-1 Concentration in the Serum and Liver Homogenate of Mice with Subacute Toxic Hepatitis (*M*±*m*)

Group	Serum ALT activity, U/liter/h	Serum TIMP-1 concentration, pg/ml	TIMP-1 concentration in liver homogenate, μg/g tissue weight	TIMP-1 concentration in liver homogenate, μg/g protein
Intact	49.30±5.02 ($n=10$)	425.60±45.64 ($n=9$)	3.70±0.51 ($n=5$)	16.10±3.98 ($n=5$)
CCl_4 administration	406.80±143.30** ($n=10$)	709.30±82.82** ($n=15$)	9.78±2.67 ($n=4$)	74.50±19.74 ($n=4$)
Administration of CCl_4 and IFN-α	325.80±71.55* ($n=9$)	607.30±86.56 ($n=15$)	3.47±0.36 ($n=5$)	28.60±5.88 ($n=5$)

Note. Here and in Table 2: *$p<0.05$ and **$p<0.01$ compared to intact mice.

TABLE 2. β-Galactosidase Activity in Bile and TIMP-1 Concentration in Bile and Liver Homogenate from Mice Receiving Triton WR-1339 (*M*±*m*)

Group	β-Galactosidase activity in bile, μmol MUP/liter/min	TIMP-1 concentration in bile, pg/ml	TIMP-1 concentration in liver homogenate, μg/g tissue	TIMP-1 concentration in liver homogenate, μg/g protein
Intact	15.60±0.36 (*n*=5)	1585±465 (*n*=3)	3.70±0.51 (*n*=5)	16.10±3.98 (*n*=5)
Triton WR-1339	117.20±1.39** (*n*=5)	2065±585 (*n*=3)	3.40±0.52 (*n*=4)	15.80±4.01 (*n*=5)

Note. MUP, 4-methylumbellipheryl-β-D-galactopyranoside. TIMP-1 concentration in bile was measured in pooled samples from 10 mice.

content and extracellular matrix (similarly to ALT) and to primarily intracellular localization of TIMP-1.

Administration of Triton WR-1339 led to the development of intrahepatic cholestasis in mice (increase in alkaline phosphatase and γ-glutamyltranspeptidase activities, Fig. 1) and induced a sharp increase in secretion of the lysosomal enzyme β-galactosidase from hepatocytes to bile (by almost 8 times, $p<0.001$, Table 2), but did not change TIMP-1 concentration in bile and liver homogenate (Table 2).

Serum TIMP-1 concentration increased in mice with subacute toxic hepatitis. Administration of IFN-α had no effect on serum TIMP-1 concentration (compared to intact mice, Table 1). TIMP-1 concentration in the liver of mice significantly exceeded that in blood serum (more than by 1000 times, $p<0.001$, Table 1). The concentration of TIMP-1 per wet tissue weight or protein content tended to increase in animals with toxic hepatitis (Table 1).

MMP play a key role in the proteolytic regulation of proteins involved in inflammation, restoration, metabolism in the extracellular matrix, and pathological destruction of tissue proteins. MMP dysfunction is accompanied by unregulated extracellular activity of proteases and results in destruction of tissue proteins [4]. MMP activity is regulated via various pathways, including the TIMP family. TIMP modulate protein metabolism in the extracellular matrix under the influence of MMP. Published data show that TIMP regulate gp130 cytokines and, primarily, interleukin-6 and oncostatin [7].

Apart from specific inhibitors (TIMP), MMP activity is regulated by nonspecific factors (α_2-macroglobulin, Reck, *etc*.). Previous studies revealed the existence of 4 types of TIMP regulating tissue remodeling [8-14]. TIMP not only bind MMP, but also have important biological function. They stimulate or inhibit cell growth depending on the type of inducing agent. TIMP-1 and TIMP-2 exhibit antiapoptotic activity. Moreover, TIMP-1 promote the growth of some tumors (by MMP-dependent or MMP-independent mechanisms).

MMP are involved in degradation of the extracellular matrix in patients with chronic liver diseases. MMP regulators TIMP-1 and TIMP-2 abolish the fibrinolytic effect of these agents [8-11].

Serum TIMP-1 concentration correlates with histological picture of the liver during chronic hepatitis C, zones of periportal necroses, and portal inflammation [8-11].

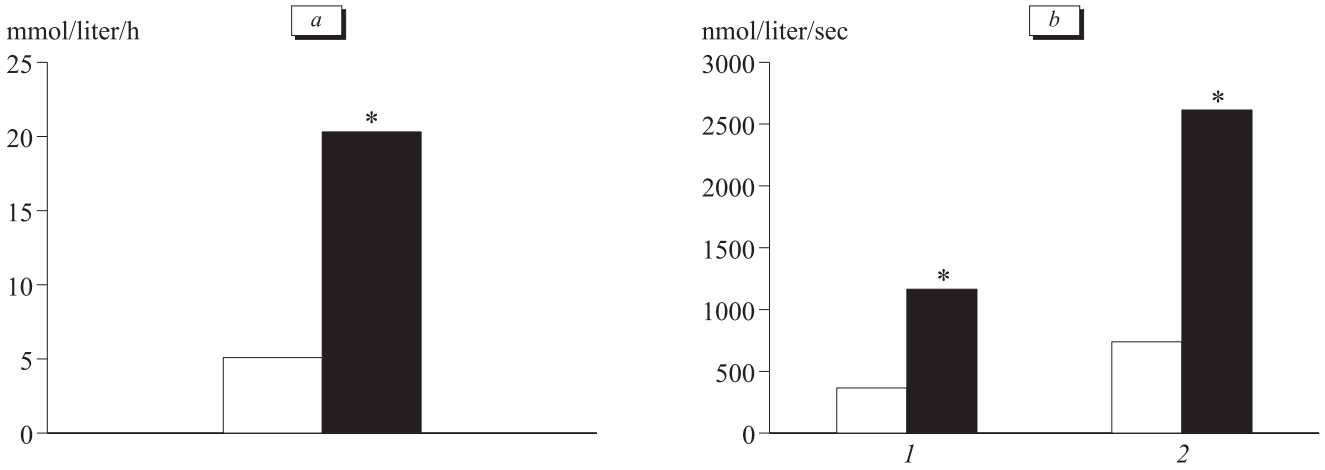

Fig. 1. Effect of Triton WR-1339 on activities of ALT (*a*), alkaline phosphatase, and γ-glutamyltranspeptidase (*b*) in mouse serum (n=10). Light bars, intact animals; dark bars, 72 h after administration of Triton WR-1339. Activities of alkaline phosphatase (*1*) and γ-glutamyltranspeptidase (*2*). *$p<0.001$ compared to intact animals.

We conclude that increased serum concentration of TIMP-1 in mice with CCl_4-induced hepatitis attests to dysregulation of metalloproteinases and reflects the development of inflammation and formation of the connective tissue in mouse liver.

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