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Hepatic stellate cell damage may lead to decreased plasma ADAMTS13 activity in rats

Yukio Kume[a], Hitoshi Ikeda[a,b,*], Morihiro Inoue[a], Kazuaki Tejima[a,b], Tomoaki Tomiya[b], Takako Nishikawa[b], Naoko Watanabe[b], Tatsuya Ichikawa[b], Makoto Kaneko[a], Shigeo Okubo[a], Hiromitsu Yokota[a], Masao Omata[b], Kenji Fujiwara[c], Yutaka Yatomi[a]

[a] *Department of Laboratory Medicine, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan*
[b] *Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan*
[c] *Yokohama Rohsai Hospital, 3211 Kodukue-chou, Koh-hoku-ku, Yokohama, Kanagawa 222-0036, Japan*

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Abstract ADAMTS13 is gaining attention, because its deficiency causes thrombotic thrombocytopenic purpura. Although its regulatory mechanism is not fully understood, we wondered if hepatic stellate cells (HSCs) play a role, because ADAMTS13 mRNA is exclusively expressed in the liver and primarily in HSCs. Plasma ADAMTS13 activity was markedly reduced in dimethylnitrosamine-treated rats, where HSC apoptosis is an essential event, but not in carbon tetrachloride- or thioacetamide-treated rats without HSC apoptosis. Furthermore, plasma ADAMTS13 activity was also reduced in 70% hepatectomized rats, where HSC loss occurs. These results suggest that HSC may be involved in the regulation of plasma ADAMTS13 activity.

Keywords: ADAMTS13; Hepatic stellate cell; Dimethylnitrosamine; Hepatectomy

1. Introduction

A disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13 (ADAMTS13) cleaves the multimers of von Willebrand factor (VWF) into smaller sizes [1]. VWF is synthesized as unusually large multimers of VWF (UL-VWF) in vascular endothelial cells [2]. After being released into plasma, UL-VWFs are rapidly cleaved by ADAMTS13 [1]. ADAMTS 13 is gaining attention, because it is unveiled that a defect in ADAMTS13 activity increases UL-VWFs in the plasma, causes platelet thrombosis under a high sheer stress, and finally results in Upshaw-Schulman syndrome by genetic mutations or thrombotic thrombocytopenic purpura (TTP) via inhibitory autoantibodies [3–5]; TTP is an important hematological illness characterized by thrombocytopenia, microangiopathic hemolytic anemia, fever, renal dysfunction, and central nervous system ischemia resulting from the formation of platelet thrombi within the microvasculature [3]. Various levels of decreasing plasma ADAMTS13 activity were observed in other conditions such as hemolytic uremic syndrome [6], but the regulatory mechanism of this enzyme activity is not yet fully understood.

Recent studies revealed that ADAMTS13 mRNA is exclusively expressed in the liver, which may suggest that the liver is a major site involved in the synthesis of ADAMTS13 [7–9]. Moreover, it has been identified that ADAMTS13 mRNA is primarily expressed in hepatic stellate cells among the liver cells, suggesting that these cells are the major cells to synthesize ADAMTS13 [10,11]. Thus, we wondered whether damage to hepatic stellate cells might be one of the mechanisms of decreasing plasma ADAMTS13 activity.

Many chemical agents cause liver injury. Among them, we previously reported that dimethylnitrosamine (DMN) selectively causes the apoptosis of hepatic stellate cells in rats [12]. With this evidence, we wondered if plasma ADAMTS13 activity would be reduced in DMN-induced acute liver injury in rats, in comparison with carbon tetrachloride (CCl_4)-induced acute liver injury in rats, where the apoptosis of hepatic stellate cells is not an essential event [12].

2. Materials and methods

Animals. Male Sprague–Dawley rats (Shizuoka Laboratory Animal Center, Shizuoka, Japan) were fed a standard pelleted diet and water *ad libitum*, and used in all experiments. They were housed in groups of three or four per cage under normal laboratory conditions. All animals received human care in compliance with the Institution's Guidelines of the University of Tokyo and the National Institutes of Health Guidelines.

Induction of acute liver injury by DMN, CCl_4 or thioacetamide (TAA) and plasma supplementation in rats. Rats (weighing 170–190 g) received an intraperitoneal injection of 10, 25 or 50 mg/kg body weight of DMN as a 2.5% solution in saline and were sacrificed at various times after the injection up to 24 h [12]. In some DMN-treated rats, 2 ml of normal rat plasma was administered at 4 and 8 h after the DMN injection through inguinal vein, and rats were sacrificed at 12 h. To induce acute liver injury by CCl_4 or TAA, rats (weighing 170–190 g) received a subcutaneous injection of 2.0 mg/kg body weight of CCl_4 as a 20% solution in olive oil and were sacrificed at 24 h after the injection [12], or intraperitoneal injections of 300 mg/kg body weight of TAA in saline, twice at 24-h intervals, and were sacrificed at 48 h after the first injection [13].

Blood collection and separation of plasma and serum. Blood was collected through the inferior vena cava with a 21G needle and plastic syringe. Blood at 1.8 ml was mixed with 0.2 ml of 3.2% sodium citrate, and after measuring the platelet count, the rest of the citrated blood

*Corresponding author. Address: Department of Laboratory Medicine, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Fax: +81 3 5689 0495.
E-mail address: ikeda-1im@h.u-tokyo.ac.jp (H. Ikeda).

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was centrifuged to separate the plasma. Serum was separated from non-citrated blood.

Measurement of ADAMTS13 activity. ADAMTS13 activity in the plasma was measured using an ADAMTS13 activity ELISA kit (Japan Clinical Laboratories Inc., Kyoto) [14].

Measurement of platelet count and liver function. The platelet count and serum levels of alanine aminotransferase (ALT) and albumin (Alb) were determined using an automated analyzer (Hitachi 7170; Hitachi Instruments Service Co., Ltd., Tokyo, Japan).

Partial hepatectomy. Partial hepatectomy (70%) was performed in rats according to the methods described by Higgins and Anderson [15]. Control rats underwent sham operations.

Statistical analysis. When indicated, statistical analysis was performed by Student's *t*-test, and $P < 0.05$ was considered significant.

3. Results and discussion

We previously demonstrated apoptosis of hepatic stellate cells occurs in rats with acute liver injury induced by DMN under electron microscopic observation, which is followed by sinusoidal endothelial cell degeneration and, thereafter, hepatocyte necrosis [12]. Fibrin thrombi appear in the sinusoids as well as in the necrotic area after the manifestation of hepatocyte necrosis [12]. In contrast, hepatocyte degeneration is firstly seen without hepatic stellate cell apoptosis and fibrin thrombi in the sinusoids of rats with acute liver injury induced by CCl_4 [12]. The fact that hepatic stellate cell apoptosis is not accompanied in CCl_4-induced acute liver injury in rats was reported elsewhere [16–18]. Thus, DMN-induced acute liver injury is distinct from CCl_4-induced injury in that hepatic stellate cells undergo apoptosis. In this study, we induced acute liver injury in rats with DMN or CCl_4, as we previously described [12], and determined plasma ADAMTS13 activity in those rats. As demonstrated in Fig. 1A, plasma ADAMTS13 activity was markedly reduced in rats treated with 50 mg/kg body weight of DMN compared to untreated rats at 24 h after the injection. In contrast, plasma ADAMTS13 activity was not significantly altered in rats treated with 2.0 mg/kg body weight of CCl_4 compared to untreated rats at 24 h after the injection. On the other hand, ALT and albumin levels were not significantly different between DMN- and CCl_4-treated rats, as shown in Table 1, indicating that there was no significant difference in hepatocyte damage between DMN and CCl_4 intoxication in rats. Furthermore, the reduction of plasma ADAMTS13 activity was dose-dependent in DMN-treated rats as depicted in Fig. 1B. These results suggest that the damage to hepatic stellate cells may explain the reduction of plasma ADAMTS13 activity in rats with acute liver injury induced by DMN.

To explore whether acute liver injury does not always cause reduced ADAMTS13 activity, it was determined in rats with TAA-induced liver injury [13], where apoptosis was reported in hepatocytes, but not in hepatic stellate cells [19]. As shown in Fig. 1A, plasma ADAMTS13 activity was not significantly altered in rats treated with 300 mg/kg body weight of TTA compared to untreated rats at 48 h after the injection. Serum ALT and albumin levels in TAA-treated rats were not significantly different from DMN- or CCl_4-treated rats, as shown in Table 1. Thus, this result suggests that reduced ADAMTS13 activity may not be generally found in acute liver injury and the selective damage to hepatic stellate cells may be a key to the reduction of plasma ADAMTS13 activity in rats with acute liver injury.

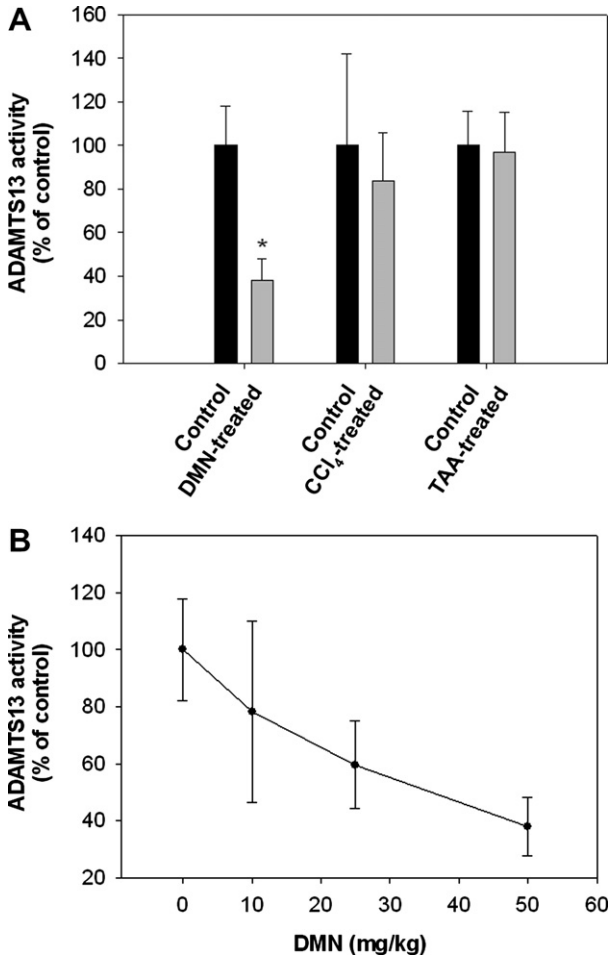

Fig. 1. (A) Plasma ADAMTS13 activity in DMN-, CCl_4- or TAA-treated rats. Rats received an injection of 50 mg/kg body weight of DMN or an injection of 2.0 mg/kg body weight of CCl_4 and were sacrificed at 24 h after the injection, or intraperitoneal injections of 300 mg/kg body weight of TAA in saline, twice at 24-h intervals, and were sacrificed at 48 h after the first injection. ADAMTS13 activity was measured in the plasma using an ADAMTS13 activity ELISA kit. Columns and bars represent the means ± S.D. of eight samples for DMN-terated rats and of four samples for CCl_4- or TAA-treated rats. An asterisk indicates a significant difference between untreated control and DMN-treated rats ($P < 0.05$ or higher degree of significance). (B) Dose response of the effect of DMN on plasma ADAMTS13 activity in rats. Rats received an injection of 10, 25, 50 mg/kg body weight of DMN and were sacrificed at 24 h after the injection. ADAMTS13 activity was measured in the plasma as described above. Bars represent the mean ± S.D. of eight samples.

Table 1
Liver function and platelet count in rats treated with hepatotoxins

	ALT (IU/L)	Alb (g/dL)	Platelets ($\times 10^4$)
Control	39.2 ± 4.2	2.5 ± 0.0	102.2 ± 18.7
DMN-treated	1608.0 ± 993.4	2.5 ± 0.2	13.6 ± 7.5
CCl_4-treated	674.5 ± 414.3	2.4 ± 0.1	85.6 ± 5.4
TAA-treated	1994.5 ± 836.2	2.3 ± 0.2	85.3 ± 18.4

Each value represents the mean ± S.D. of eight samples for control or DMN-treated rats, and of four samples for CCl4-treated or TAA-treated rats.

Because our previous report showed that hepatic stellate cell apoptosis was first detected at 7.5 h and hepatocyte necrosis at