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Crucial Role of ADAMTS13 Related to Endotoxemia and Subsequent Cytokinemia in the Progression of Alcoholic Hepatitis

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1. Introduction

Excessive alcohol consumption causes a variety of liver diseases including alcoholic steatosis, alcoholic hepatitis (AH), liver fibrosis, cirrhosis and hepatocellular carcinoma (Mandayam et al. 2004). Alcoholic steatosis is, generally, a benign lesion if the patient abstains from alcohol intake, whereas AH observed in the approximately 20% of heavy drinkers is much more serious and requires treatment with the development of hepatocellular necrosis and inflammation (Menon et al., 2001). The severe form of AH, severe alcoholic hepatitis (SAH), is characterized by multiorgan failure with manifestations of acute hepatic failure and is associated with high morbidity and mortality (Haber et al., 2003; Ishii et al., 1993; Maddrey et al., 1978; Mookerjee et al., 2003; Sougioultzis, 2005).

Alcohol-induced liver injury (ALD) occurs through the multiple steps involving a range from innate immune cells to the liver parenchymal cells, and out of many factors contributing to the pathogenesis of ALD gut-derived endotoxin plays a central role in the induction of steatosis, inflammation, and fibrosis in the liver (Bode & Bode, 2005; Fukui et al., 1991; Mandrekar & Szabo, 2009; McClain et al., 2005; Nolan, 2010; Vidali et al., 2008). AH is a multifactorial process involving gut-derived endotoxin-induced Kupffer cell activation via hepatic reticuloendothelial dysfunction and increased intestinal permeability, and subsequent cytokine stimulation. Additional factors include ethanol metabolism to toxic products, oxidative stress, acetaldehyde adducts, nutritional impairment and impaired hepatic regeneration (Haber et al., 2003; Ishii et al., 1993; Mookerjee et al., 2003; Nath & Szabo, 2009; Nolan, 2010; Sakaguchi et al., 2011; Sougioultzis et al., 2005; Tsukamoto et al., 2009; Wu & Cederbaum, 2009). In SAH pathogenesis, endotoxemia may trigger enhanced pro-inflammatory cytokine production, potentially causing a systemic inflammatory response syndrome together with microcirculatory disturbances, systemic haemodynamic derangements, and subsequent multiorgan failure (Fukui, 2005; Haber et al., 2003; Ishii et al., 1993; Mookerjee et al., 2003; Sougioultzis et al., 2005).

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ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13) is a metalloproteinase that specifically cleaves multimeric von Willebrand factor (VWF) between Tyr1605 and Met1606 residues in the A2 domain (Moake 2002; Fujimura et al., 2002). In the absence of ADAMTS13 activity (ADAMTS13:AC), unusually large VWF multimers (UL-VWFMs) are released from vascular endothelial cells (ECs) and improperly cleaved, causing them to accumulate and to induce the formation of platelet thrombi in the microvasculature under conditions of high shear stress. Currently, a severe deficiency in ADAMTS13:AC, which results either from genetic mutations in the ADAMTS13 gene (Upshaw-Schulman syndrome, USS) (Fujimura et al., 2002; Kokame et al., 2002; Levy et al., 2001, Moake, 2002) or acquired autoantibodies against ADAMTS13 (Furlan et al., 1998; Tsai & Lian, 1998), is thought to be a specific feature of thrombotic thrombocytopenic purpura (TTP) (Amorosi & Ultmann, 1966; Moschcowitz, 1924).

In 2000, we demonstrated that a decreased plasma ADAMTS13:AC in patients with cirrhotic biliary atresia can be fully restored after liver transplantation, indicating that the liver is the main organ producing ADAMTS13 (Matsumoto et al., 2000). One year later, northern blot analysis showed that the 4.6-kilobase ADAMTS13 mRNA was highly expressed in the liver (Levy et al., 2001; Soejima, et al., 2001; Zheng et al., 2001), and subsequently both in situ hybridization and immunohistochemistry clearly indicated that ADAMTS13 is produced exclusively in hepatic stellate cells (HSCs) (Uemura et al., 2005a). Platelets (Suzuki et al., 2004), vascular ECs (Turner et al., 2006), and kidney podocytes (Manea et al., 2007) also have been implicated as ADAMTS13-producing cells, but the amount produced by these cell types in the liver appears to be far less than that produced by HSCs.

Mannucci et al. (Mannucci et al., 2001) originally reported a reduction of the ADAMTS13:AC in advanced liver cirrhosis (LC). Since HSCs were shown to be the major producing cells of ADAMTS13 in the liver (Uemura et al., 2005a), much attention has been paid to the potential role of ADAMTS13 in the pathophysiology of liver diseases associated with sinusoidal and/or systemic microcirculatory disturbances (Feys et al., 2007; Ishikawa et al., 2010; Ko et al., 2006; Kobayashi et al., 2009; Lisman et al., 2006; Matsuyama et al., 2007; Matsumoto et al., 2007; Okano et al., 2010; Park et al., 2002; Pereboom et al., 2009; Uemura et al., 2005b; Uemura et al., 2008a; Uemura et al., 2008b; Uemura et al., 2010; Yagita et al., 2005). ADAMTS13:AC is significantly decreased in patients with hepatic veno-occlusive disease (VOD) (Matsumoto et al., 2007; Park et al., 2002), AH (Ishikawa et al., 2010; Matsuyama et al., 2007; Uemura et al., 2005b; Uemura et al., 2008b), LC (Feys et al., 2007; Uemura et al., 2008a), and patients undergoing living-donor related liver transplantation (Ko et al., 2006; Kobayashi et al., 2009; Pereboom et al., 2009) and partial hepatectomy (Okano et al., 2010). Furthermore, hepatitis C virus (HCV)-related LC patient with ADAMTS13 inhibitor (ADAMTS13:INH) typically develops TTP (Yagita et al., 2005).

In this review, we will address interesting findings from our previous studies showing that plasma ADAMTS13:AC and its related parameters are potentially involved in the severity of liver disturbances and the development of multiorgan failure in patients with AH (Ishikawa et al., 2010; Matsuyama et al., 2007; Uemura et al., 2005b; Uemura et al., 2008b). We will focus on the importance of ADAMTS13 determination for a better understanding of pathophysiology and/or for possible therapeutic approaches of ADAMTS13 supplementation in this disease.
2. Alcohol-related thrombocytopenia and effects of alcohol on hemostasis

Alcohol-related thrombocytopenia is independent of nutritional state or the presence of liver functional abnormalities, anemia, or leukopenia (Lindenbaum & Hargrove, 1968; Paintal et al., 1975; Post & Desforges, 1968a, 1968b; Rubin & Rand, 1994). Both quantitative and qualitative platelet abnormalities appear during alcohol consumption (Cowan, 1980). A decrease in circulating platelets was first reported in 3 alcoholics who responded to folic acid administration (Sullivan & Herbert, 1964). Subsequently, alcohol has been shown to exert a direct toxic effect on blood platelets in 5 chronic alcoholics with ten episodes of thrombocytopenia (Lindenbaum & Hargrove, 1968) and in 8 patients with acute alcoholism (Post & Desforges, 1968a), who showed no evidence of liver cirrhosis, splenomegaly, folate deficiency, or massive hemorrhage, indicating the presence of alcohol-related thrombocytopenia. Additionally, thrombocytopenia was observed in alcoholics on intravenous injection of ethanol (Post & Desforges, 1968b). The platelet counts in patients with alcohol-related thrombocytopenia return to normal quickly (2-3 days) after ingestion of ethanol is discontinued. Thereafter, the number of platelets increases, and maximum counts occur 5-21 days after cessation of alcohol (Cowan & Hines, 1971), when rebound thrombocytosis after alcohol abuse in some patients may contribute to thromboembolic disease (Haselager & Vreeken, 1977). Potential mechanisms for alcohol-related thrombocytopenia may involve a suppressive effect of alcohol on megakaryocyte maturation and platelet release (Sullivan & Herbert, 1964), reduced platelet survival time (Cowan, 1980), alterations of platelet structure (Cowan, 1980), and abnormalities of platelet metabolism involving adenosine nucleotides and eicosanoids (Cowan, 1980). The spectrum of platelet function defects in alcoholics is complex. In actively drinking alcoholic patients admitted to the hospital for detoxification, platelet aggregation in platelet-rich plasma is considerably reduced in response to agonists (Haut & Cowan, 1974; Neiman et al., 1989; Rubin & Rand, 1994), whereas platelet aggregation is greater in chronic alcoholics compared to normal healthy controls both on admission and 1 week after hospitalization (Arai et al., 1986). It is necessary to clarify the role of platelet function not only in platelet aggregation in vitro, but also in platelet-endothelial interactions under shear stress during alcohol-related thrombocytopenia (Siegel-Axel & Gawaz, 2007).

Alternatively, recent epidemiologic studies have consistently shown that regular light-to-moderate alcohol intake protects against ischemic stroke including coronary heart disease and peripheral arterial disease (Camargo et al., 1997; Lippi et al., 2010; Mukamal et al., 2001; Rimm et al., 1996). Such beneficial effects may be attributable to a number of factors including the inhibition of platelet aggregation and adhesion, the decrease in plasma levels of VWF, fibrinogen, and coagulation factor VII, or the increase in nitric oxide bioavailability and high-density lipoprotein cholesterol (Lippi et al., 2010; Rubin R, 1999). In contrast, soft blood clots are often observed in cadaveric blood in cases of sudden death after excess alcohol consumption, which enhances the procoagulant status via VWF release, and IL-6-related interaction with lipopolysaccharide (LPS) (Kasuda et al., 2009). Additionally, both chronic alcoholism and acute alcohol intoxication have increasingly been recognized as risk factors for circulatory disorders, including thrombotic complications and hemorrhage (Hillbom & Kaste, 1982; Hillbom & Kaste, 1983, Numminen et al., 2000). Patients with chronic alcoholism show a higher incidence of pulmonary infarction, cerebral infarction, and venous thrombosis in their extremities after abstinence (Haselager & Vreeken, 1977; Hillbom
et al., 1985; Walbran et al., 1981), probably due to alterations of haemostatic and fibrinolytic parameters that may be largely influenced by drinking pattern, nutritional balance, and genetic background (Arai et al., 1986; Enomoto et al., 1991; Lippi et al., 2010; Mukamal et al., 2001).

3. Hepatic microcirculation and microcirculatory disturbances in ALD

Hepatic microcirculation comprises a unique system of capillaries, called sinusoids, which are lined by three different cell types: sinusoidal endothelial cells (SECs), HSCs, and Kupffer cells (Kmieć, 2001). The SECs modulate microcirculation between hepatocytes and the sinusoidal space through the sinusoidal endothelial fenestration. The SECs have tremendous endocytic capacity, including for VWF and the extracellular matrix, and secrete many vasoactive substances (Kmieć, 2001). The HSCs are located in the space of Disse adjacent to the SECs, and regulates sinusoidal blood flow by contraction or relaxation induced by vasoactive substances (Rockey, 2001). Kupffer cells are intrasinusoidally-located tissue macrophages, and secrete potent inflammatory mediators during the early phase of liver inflammation (Kmieć, 2001). Intimate cell-to-cell interaction has been found between these sinusoidal cells and hepatocytes (Kmieć, 2001, Rockey, 2001).

Vascular ESs play a pivotal role in hemostasis and thrombosis (Fujimura et al., 2002; Moake, 2002). VWF is a marker of endothelial cell activation (damage), and plays an essential role in hemostasis (Fujimura et al., 2002; Moake, 2002). In the normal state, VWF immunostaining is usually positive in large vessels, but negative in the SECs (Hattori et al., 1991). On the occurrence of liver injury accompanied by a necroinflammatory process, the SECs become positive for VWF, presumably in association with the capillarization of hepatic sinusoids (Schaffner & Popper, 1963). Subsequently, platelets adhere to subendothelial tissue mediated by UL-VWFM. ADAMTS13 then cleaves UL-VWFM into smaller VWF multimers. This interaction of ADAMTS13 and UL-VWFM is the initial step in hemostasis (Fujimura et al., 2002; Moake, 2002).

Hepatic microcirculatory disturbances are considered to play an important pathogenic role in ALD. These disturbances include narrowing of the sinusoidal space due to ballooned hepatocytes and perisinusoidal fibrosis (French et al., 1984), imbalances between endothelin and nitric oxide (Oshita et al., 1993), and contraction of HSCs (Itatsu et al., 1988). After liver injury, fibrogenesis within the Disse’s space and a decrease in the number of sinusoidal endothelial fenestrations together with narrowing of their diameter may lead to neocapillarization of the endothelium (Horn et al., 1987; Mak & Lieber, 1984). Sinusoidal lining cells and the scar-parenchyma interface are stained by anti-VWF antibodies, even at the early stages of ALD (Urashima et al., 1993), indicating capillarization of the SECs. Interestingly, in SAH patients, mild to severe hepatic veno-occlusive disease (VOD) was frequently observed (Goodman & Ishak, 1982; Kishi et al., 2000), and the degree of ascites became more severe as VOD progressed (Kishi et al., 2000), suggesting that the hepatic circulatory disturbance involves not only the sinusoidal microcirculation but also the hepatic terminal veins.

In patients with LC and fulminant hepatitis, VWF plasma levels are remarkably high (Albornoz et al., 1999; Ferro et al., 1996; Langley et al., 1985). In LC, immunostaining for VWF antigen (VWF:Ag) shows positive cells predominantly at the scar-parenchyma
interface, within the septum, and in the sinusoidal lining cells (Knittel, et al., 1995), and many fibrin thrombi are demonstrated in the hepatic sinusoid in patients with fulminant hepatitis (Rake et al., 1971), and in rats with dimethylnitrosamine (DMN) induced acute hepatic failure (Fujiwara et al., 1988). Portal or hepatic vein thrombosis is often observed in advanced LC (Amitrano et al., 2004; Wanless et al., 1995), and microthrombosis formation is seen in one or multiple organs in one-half of autopsied cirrhotic patients (Oka & Tanaka, 1979). Such a hypercoagulable state in liver diseases may be involved in hepatic parenchymal destruction, the acceleration of liver fibrosis and disease progression (Northup et al., 2008; Pluta et al., 2010).

A deficiency of anticoagulant proteins (antithrombin, protein C and protein S) and the high levels of several procoagulant factors (Factor VIII and VWF) may contribute to hypercoagulability in advanced liver diseases (Northup et al., 2008). Locally, the SEC dysfunction could lead to the development of a hypercoagulable state at the hepatic sinusoids corresponding to the site of liver injury, even in the presence of a systemic hypocoagulable state (Northup et al., 2008). Considering that ADAMTS13 is synthesized in HSCs and its substrate, UL-VWF, is produced in transformed SECs during liver injury, decreased plasma ADAMTS13:AC may involve not only sinusoidal microcirculatory disturbances, but also subsequent progression of liver diseases, finally leading to multiorgan failure. Based on these findings, it is of particular interest to evaluate the activity of plasma ADAMTS13:AC in patients with liver diseases including AH and SAH.

4. Cleavage of UL-VWF by ADAMTS13

Although the mechanism by which TTP develops in the absence of ADAMTS13:AC has not been fully elucidated, accumulating evidence has provided a hypothesis as illustrated in Fig. 1. (Fujimura et al., 2008). UL-VWFMs are produced exclusively in vascular ECs and stored in an intracellular organelle termed Weidel-pallade bodies (WPBs) and then released into the circulation upon stimulation. Under physiological conditions, epinephrine acts as an endogenous stimulus, but under non-physiological conditions, DDAVP (1-deamino-8-D-arginine vasopressin), hypoxia, and several cytokines such as interleukin IL-2, IL-6, IL-8 and tumor necrosis factor (TNFα) act as stimuli that up-regulate VWF release. Once ECs are stimulated, UL-VWFMs and P-selectin, both stored in WPBs, move to the membrane surface of ECs, where P-selectin anchors UL-VWFMs on the ECs surface (Padilla et al., 2004). Under these circumstances, high shear stress generated in the microvasculature induces a change in the UL-VWF from a globular to an extended form (Siedlecki et al., 1996). The ADAMTS13 protease efficiently cleaves the active extended form of UL-VWF between the Tyr1605 and Met1606 residues in the A2 domain (Dent et al., 1990). In this context, it has been postulated that multiple exocites within the disintegrin-like/TSP1/cysteine-rich/spacer (DTCS) domains of ADAMTS13 play an important role in the interaction with the unfolded VWF-A2 domain (Akiyama et al., 2009). ADAMTS13 may more efficiently cleave newly released UL-VWFMs that exist as solid-phase enzymes anchored to the vascular EC surface by binding to CD36, because CD36 is a receptor for TSP1, which is a repeated domain within the ADAMTS13 molecule (Davis et al., 2009). When ADAMTS13 activity is reduced, UL-VWF potentially interacts more intensively with platelet GPIb and generates signals that further accelerate platelet activation (Fujimura et al., 2002; Moake, 2002). A series of these reactions leads to platelet microaggregates and thrombocytopenia. However, little information has
been available on the cleavage of the UL-VWFMs by ADAMTS13 in the sinusoidal microcirculation in ALD.

Unusually large von Willebrand factor multimers (UL-VWFMs) are produced in vascular endothelial cells (ECs) and stored in Weidel-pallade bodies (WPBs). UL-VWFMs are released from WPBs into the circulation upon stimulation by cytokines, hypoxia, DDAVP and epinephrine. P-selectin that co-migrates from WPBs anchors UL-VWFMs on the vascular EC surface. Under these circumstances, high shear stress changes the molecular conformation of UL-VWFMs from a globular to an extended form, allowing ADAMTS13 to access this molecule. In the absence of ADAMTS13:AC, UL-VWFMs remain uncleaved, allowing them to excessively interact with platelet glycoprotein (GP)Ibα and activate platelets via intra-platelet signaling, which result in the formation of platelet thrombi. Cytokines, endotoxin, and/or the inhibitor against ADAMTS13 may be candidates to decrease ADAMTS13 activity. (The dotted circle indicates a VWF subunit, which contains a set of binding domains with factor VIII, subendothelial collagen, platelet GPIIbα, and integrin αIIbβ3.) (Partially modified from Fujimura et al, 2008).

Fig. 1. Proposed mechanism of platelet thrombi formation under high shear stress in the absence of ADAMTS13:AC.
5. Assays for plasma ADAMTS13:AC and ADAMTS13:INH

ADAMTS13:AC was determined with a classic VWFM assay in the presence of 1.5 mol/L urea using purified plasma derived VWF as a substrate, according to the method described by Furlan et al. (Furlan et al., 1996); the detection limit of this assay was 3% of the normal control in our laboratory (Kinoshita et al., 2001). In 2005, we developed a novel chromogenic ADAMTS13-act-ELISA using both an N- and C-terminal tagged recombinant VWF substrate (termed GST-VWF73-His). This assay was highly sensitive, and the detection limit was 0.5% of the normal control (Kato et al., 2006). Plasma ADAMTS13:AC levels highly correlated between VWFM assay and ADAMTS13-act-ELISA (Mean±SD, 102±23% vs. 99.1±21.5%, r²=0.72, p<0.01) (Kato et al., 2006). No interference of the ADAMTS13-act-ELISA occurred even in the presence of hemoglobin, bilirubin or chylomicrons in the samples, thus enabling distinction from the FRETS-VWF73 assay (Kokame et., 2005; Meyer et al., 2007). Because of its high sensitivity, easy handling, and lack of interference from plasma components, the ADAMTS13-act-ELISA is recommended for routine laboratory use.

The ADAMTS13:INH has also been evaluated with the chromogenic act-ELISA by means of the Bethesda method (Kasper et al., 1975). Prior to the assay, the test samples were heat-treated at 56°C for 60 min to eliminate endogenous enzyme activity, mixed with an equal volume of intact normal pooled plasma, and incubated for 2 hours at 37°C. The residual enzyme activity is measured after incubation. One Bethesda unit is defined as the amount of inhibitor that reduces activity by 50% of the control value, and values greater than 0.5 U/ml are significant.

6. ADAMTS13 activity and its related parameters in AH

Plasma ADAMTS13:AC was assayed according to the method of Furlan et al. (Furlan et al., 1996) with slight modifications (Mori et al., 2002). ADAMTS13:AC was markedly decreased in fatal SAH cases with multiorgan failure, in contrast to a mild-to-moderate decrease in SAH and AH survivors (Fig. 2a) (Uemura et al., 2005b; Matsuyama et al., 2007). Interestingly, ADAMTS13:AC in fatal SAH cases with multiorgan failure was extremely low, which is consistent with typical TTP. The VWF:AG level was remarkably increased in AH, especially in fatal SAH cases (Fig. 2b). Accordingly, VWF:AG relative to ADAMTS13:AC was extremely high in fatal SAH cases compared to AH and SAH survivors (Fig. 2c).

During recovery, ADAMTS13:AC returned to a normal range, and both VWF:AG levels and levels of VWF:AG relative to ADAMTS13:AC decreased in AH and SAH survivors; however, in fatal SAH cases, the activity remained extremely low while the VWF:AG levels were still high, resulting in an extremely high ratio of VWF:AG to ADAMTS13:AC (Matsuyama et al., 2007; Uemura et al., 2005b). These results suggest that plasma ADAMTS13:AC and its substrate, VWF:AG, are closely correlated with the severity of liver disturbance and may be useful markers for predicting the clinical outcome of AH, especially in SAH with multiorgan failure. Indeed, the prognosis was very poor in three SAH patients with extremely low ADAMTS13:AC and markedly high VWF:Ag. Furthermore, cirrhotic patients with superimposed AH showed higher levels of VWF:Ag (576% vs. 303%, p<0.005) and VWF:Ag relative to ADAMTS13:AC (14.0 vs. 5.3, p<0.01) than those with AH without LC, indicating that cirrhotic patients with superimposed AH may be a high risk for hepatic
Fig. 2. Plasma values of ADAMTS13:AC, VWF:Ag, and the ratio of VWF:Ag to ADAMTS13:AC in the patients with AH, SAH, and alcoholic LC. ADAMTS13:AC was significantly lower in AH, SAH and LC patients than in healthy subjects (a). The activity further decreased in the patients with SAH compared to those with AH and LC. In three fatal SAH cases, ADAMTS13:AC was extremely low. VWF:Ag was significantly higher in AH, SAH and LC patients than in healthy subjects (b). The antigen further increased in SAH patients compared to AH patients. In the three fatal SAH patients, VWF:Ag was extremely high. VWF:Ag relative to ADAMTS13 activity was markedly higher in AH, SAH and LC patients than in healthy subjects (c). It further increased in patients with SAH compared to those with AH and LC. In the three fatal SAH cases, it was extremely high. The dotted lines show the upper limit of the normal range, and dot-dashed lines the lower limit of the normal range. The normal range was 102±23% (mean±2SD, n=60) in ADAMTS13:AC, 100±53% in VWF:Ag, and 1.0 ± 0.4 for the ratio of VWF:Ag to ADAMTS13:AC, respectively. Open and shaded circles indicate survivors and the closed circles indicate nonsurvivors. The shaded circles show alcoholic LC with superimposed AH. AH=alcoholic hepatitis, SAH=severe alcoholic hepatitis, LC=alcoholic liver cirrhosis, ADAMTS13:AC=ADAMTS13 activity, VWF:Ag=von Willebrand factor antigen, VWF:Ag/ADAMTS13:AC=the ratio of VWF antigen to ADAMTS13 activity. *p<0.05, **p<0.01, and ***p<0.005 significantly different between the two groups. (Partially modified from Uemura et al., 2005b and Matsuyama et al., 2007).

In an univariate analysis ADAMTS13:AC was significantly correlated with 10 clinical variables, including functional liver capacity, inflammation signs, renal function, and...
platelet counts (Uemura et al., 2005b, 2008b). VWF:Ag was significantly correlated with nine clinical variables, including functional liver capacity, anemia, inflammation signs, and platelet counts (Matsuyama et al., 2007; Uemura et al., 2008b). The factors associated with decreased ADAMTS13:AC and increased VWF:Ag, reduced functional liver capacity, augmented inflammation, and thrombocytopenia are consistent with the clinical characteristics that frequently appear in AH and SAH (Fujimoto et al., 1999; Haber et al., 2003; Ishii et al., 1993; Maddrey et al., 1978; McClain et al., 1999; Mookerjee et al., 2003). Remarkably, the imbalance between the ADAMTS13:AC and VWF:Ag levels might provide another mechanism for thrombocytopenia that usually occurs in AH even in the absence of signs of apparent disseminated intravascular coagulation (DIC).

On admission, UL-VWFM was detected in 4 (80.0%) of 5 SAH patients and in 5 (55.6%) of 9 AH patients, whose ADAMTS13:AC was less than 50% of normal control plasma levels (Fig. 3a) (Matsuyama et al., 2007). UL-VWFM-positive patients showed lower ADAMTS13:AC, higher plasma VWF:Ag, and a higher ratio of VWF:Ag to ADAMTS13:AC than UL-VWFM-negative patients (mean ADAMTS13:AC = 22% vs. 43%, p<0.02; VWF:Ag = 724% vs. 372%, p<0.05; the ratio of VWF:Ag to ADAMTS13:AC = 66.0 vs. 8.2, p<0.01, respectively). In particular, UL-VWFM was detected in three fatal SAH patients with multiorgan failure (cases 1, 2, and 3, Fig. 3a) (Matsuyama et al., 2007). These findings of enhanced UL-VWFM production with deficient ADAMTS13 activity may, in part, contribute not only to the development of multiorgan failure but also to the progression of liver injury through microcirculatory disturbances in AH. Our results suggest an additional mechanism to explain multiorgan failure with liver disturbance, particularly in SAH patients. In order to confirm a TTP-like phenomenon as described above, it will be necessary to confirm the presence or absence of the platelet thrombi using samples obtained from liver and other organs in SAH patients.

**Fig. 3.** The VWF multimer analysis and the difference in endotoxin level between UL-VWFM positive and negative patients in AH and SAH patients on admission.

UL-VWFM was detected in 4 of 5 SAH patients (80.0%, cases 1, 2, 3, and 5), and 3 of these patients died of hepatic failure with multiorgan failure (cases 1 and 2) (a). In addition, UL-
VWFM was detected in 5 of 9 AH patients (55.6%, cases 6, 7, 8, 10, and 11), who had a moderate ADAMTS13 deficiency together with markedly high VWF:AG values (a). Plasma endotoxin concentrations were higher in UL-VWFM positive patients than UL-VWFM negative ones (b). AH=alcoholic hepatitis, SAH=severe alcoholic hepatitis, ADAMTS13: AC=ADAMTS13 activity, VWF:Ag=von Willebrand factor antigen, UL-VWFM= unusually large VWF multimer, NP shows the normal control plasma. (Partially modified from Matsuyama et al., 2007 and Ishikawa et al., 2010).

Hepatic microcirculatory disturbances are considered to play an important pathogenic role in ALD. VWF immunostaining was positive in sinusoidal lining cells and the scar-parenchyma interface even at the early stages of ALD (Urashima et al., 1993). Deficiencies in plasma ADAMTS13:AC and augmented VWF production in transformed vascular ESC might play an important role in sinusoidal microcirculatory disturbances and subsequent liver injury in AH patients. Furthermore, in SAH patients mild to severe hepatic veno-occlusive disease (VOD) was frequently observed (Goodman & Ishak, 1982; Kishi et al., 2000). After stem cell transplantation (SCT), plasma ADAMTS13:AC was significantly lower in patients with hepatic VOD than those without (Park et al., 2002). Prophylactic infusion of fresh frozen plasma (FFP) as a source of ADAMTS13 may be useful in preventing the development of hepatic VOD after SCT (Matsumoto et al., 2007), indicating a causative role of increased VWF production relative to decreased ADAMTS13:AC. Our present findings of markedly decreased ADAMTS13:AC and markedly increased VWF:AG may be involved in the pathogenesis of VOD in SAH patients.

7. Potential role of decreased ADAMTS13:AC in AH

The reason why ADAMTS13:AC decreases in AH and SAH patients remains to be clarified, but potential mechanisms may include: enhanced consumption due to the degradation of large quantities of UL-VWFM (Mannucci et al., 2001); cytokinemia- and/or endotoxemia-induced deficiency of ADAMTS13 (Ishikawa et al., 2010); the presence of inhibitors as detected in the majority of patients with “idiopathic” pregnancy- or drug-associated TTP (Fujimura & Matsumoto, 2010; Ishikawa et al., 2010); the decreased production of ADAMTS13 in ESCs (Kume et al., 2007); and the direct inhibition of the protease by ethanol and/or its metabolites. It is controversial whether ADAMTS13 deficiency is caused by decreased production in the liver. Kume et al. reported that HSC apoptosis plays an essential role in decreased ADAMTS13:AC using dimethylnitrosamine-treated rats, but not carbon tetrachloride (CCl₄)-treated animals (Kume et al., 2007), whereas Niiya et al. found upregulation of ADAMTS13 antigen and proteolytic activity in liver tissue using rats with CCl₄-induced liver fibrosis (Niiya et al., 2006). In our study, the ADAMTS13:AC gradually decreased (Fig. 4, upper panels), and the VWF:Ag progressively elevated with concomitant increase in concentrations of IL-6, IL-8, and TNFα from normal range to over 100 g/mL on admission (Fig. 4, lower panels) (Ishikawa et al., 2010).

The incidence of UL-VWFM detected in plasma became higher as concentrations of IL-6, IL-8, and TNFα increased (Ishikawa et al., 2010). At the recovery stage in survivors with AH and SAH, the ADAMTS13:AC increased to normal range, the VWF:Ag decreased, and the UL-VWFM disappeared with the decrease in the concentration of IL-6 and IL-8, whereas in a non-survivor with SAH, the ADAMTS13:AC remained at extremely low levels, the VWF:Ag was still high, and the UL-VWFM was persistently present with the increase in
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Fig. 4. Relation of plasma cytokine levels to ADAMTS13:AC and VWF:Ag in AH and SAH patients on admission.

ADAMTS13:AC concomitantly decreased with increasing concentrations of plasma IL-6 and IL-8, and the activity decreased in patients with TNFα concentrations higher than the normal range compared to those without (upper panels). On the other hand, VWF:Ag concomitantly increased with increasing concentrations of plasma IL-6 and IL-8, and the values increased in patients with TNFα concentration higher than the normal range compared to those without (lower panels). Shaded area shows normal ranges. IL-6=interleukin 6, IL-8=interleukin 8, TNFα=tumor necrosis factor α, N=normal range. *p<0.05, **p<0.005, and ***p<0.001 significantly different between the two groups.

ADAMTS13:AC=ADAMTS13 activity, VWF:Ag=von Willebrand factor antigen. (Partially modified from Ishikawa et al., 2010).

concentrations of these cytokines (Ishikawa et al., 2010). These results indicate that the decrease in the ADAMTS13:AC and the increase in VWF:Ag in addition to UL-VWF may be closely associated with increased proinflammatory cytokines including IL-6, IL-8, and TNFα. Recently, it was demonstrated that IL-6 inhibited the action of ADAMTS13 under flow condition, and both IL-8 and TNFα stimulated the release of UL-VWF using human umbilical vein ECs (Bernardo et al., 2004). IFN-γ, IL-4, and TNFα also inhibit ADAMTS13 synthesis and activity in rat primary HSCs (Cao et al., 2008). Additionally, inflammation-associated ADAMTS13 deficiency promotes formation of UL-VWF (Bockmeyer et al.,...
2008; Claus et al., 2010). However, it is unknown whether IL-6 directly hampers the cleavage of UL-VWF or if IL-6 down-regulates gene expression of ADAMTS13 whereby modifying the promoter activity.

Our study shows that plasma endotoxin concentration determined by a chromogenic substrate assay (Obayashi et al., 1984; Obayashi et al., 1985) was higher in patients with SAH and AH than in healthy subjects, and was markedly higher in patients with SAH than in AH (Fig. 5a) (Ishikawa et al., 2010). Our results are consistent with data previously reported (Fujimoto et al., 2000; Fukui et al., 1991; Fukui, 2005) most likely due to hepatic reticuloendothelial dysfunction and increased intestinal permeability caused by the opening of intestinal tight junctions by alcohol and its metabolite (Purohit et al., 2008). Upon admission, the endotoxin concentration correlated inversely with ADAMTS13 activity (Fig. 5b, upper panel), and positively with VWF:Ag (Fig. 5b, lower panel), and was higher in patients with UL-VWF than those without (Fig. 3b). At the recovery stage, the endotoxin concentration decreased with the increase in ADAMTS13:AC and the decrease in VWF:Ag, and the disappearance of UL-VWFM together with the reduction of IL-6 and IL-8 concentrations (Ishikawa et al., 2010). These results indicate that enhanced endotoxemia...

Fig. 5. Plasma endotoxin concentration and relation of endotoxin to ADAMTS13:AC and VWF:Ag in patients with alcoholic hepatitis.

Upon admission, plasma endotoxin concentrations were higher in patients with AH and SAH than in normal subjects, and the values were higher in SAH patients compared to AH (a). In addition, the endotoxin concentration correlated inversely with ADAMTS13:AC (r= - 0.474, p<0.01) (b, upper panel), and positively with VWF:Ag (r= - 0.406, p<0.05) (b, lower panel). N=normal healthy control, AH=alcoholic hepatitis, SAH=severe alcoholic hepatitis, ADAMTS13:AC=ADAMTS13 activity, VWF:Ag=von Willebrand factor antigen. Open circles indicate survivors, and closed circles nonsurvivors. Shaded area shows normal range. (Partially modified from Ishikawa et al., 2010).
may be closely related to the decrease in the ADAMTS13:AC and the appearance of UL-VWF through the enhanced cytokinemia, leading to systemic microcirculatory disturbances and multiorgan failure, particularly in SAH patients. Intravenous infusion of endotoxin to healthy volunteers caused a decrease in plasma ADAMTS13:AC together with the appearance of UL-VWF (Reiter et al., 2005). Severe secondary ADAMTS13 deficiency can be associated with sepsis-induced DIC and may contribute to the development of renal failure (Ono et al., 2006). These observations may support our data and hypothesis.

In response to alcohol intake, innate immune cells initiate and maintain hepatic inflammation via pattern recognition receptors, toll-like receptor 4 (TLR4) (Akira et al., 2006; Szabo, 1999). The TLR4 involves the co-receptors CD14 and myeloid differentiation (MD) protein 2 (MD2), and the LPS binding protein (LBP) (Chow et al., 1999; Visintin et al., 2001), which directly binds LPS and facilitates the association between LPS and CD14 (Wright et al., 1989). The activation of TLR4 in Kupffer cells by LPS is a key pathogenic mediator of ALD through production of inflammatory cytokines (TNF-α and IL-6) and reactive oxygen species (Byun & Jeong, 2010; Soares et al., 2010). More recently, the disruption of interferon regulatory factor 3 in liver parenchymal cells has been shown to increase liver injury due to the deregulated expression of pro- and anti-inflammatory cytokines via Myd88-independent pathways (Hritz et al., 2008; Petrasek et al., 2011). It will be of particular interest to clarify the relation of ADAMTS13 to endotoxin-induced cytokinemia via the TLR4 signaling cascade in ALD.

Alternatively, another mechanism to reduce the activity of ADAMTS13 is the action of the plasma inhibitor against ADAMTS13. In our study, the inhibitor was detected in 80% of SAH patients and 21.4% of AH patients upon admission, and its inhibitory activity averaged 1.5 BU/ml in SAH and 1.0 BU/ml in AH (Ishikawa et al., 2010). Patients with the inhibitor showed lower ADAMTS13:AC and higher VWF:Ag than those without. At the recovery stage, the inhibitor was detected in 5 patients but disappeared with increased ADAMTS13:AC and decreased VWF:Ag, together with the decrease in concentrations of cytokines and endotoxin. Interestingly, patients with AH and SAH who had the inhibitor showed higher levels of serum total bilirubin, polymorphonuclear neutrophil count, plasma C-reactive protein, and plasma endotoxin concentration, and lower serum albumin levels than those with AH who had no inhibitor (Ishikawa et al., 2010). These results indicate that the decrease in the ADAMTS13:AC may be caused by the presence of its inhibitor, which is closely related to lower functional liver capacity, marked inflammation, and enhanced endotoxemia in patients with AH and SAH. Intravenous infusion of endotoxin to healthy volunteers induced the decrease in plasma ADAMTS13:AC together with the increase in VWF:Ag and the appearance of UL-VWF during acute systemic inflammation (Reiter et al., 2005). From our results and previously reported findings (Reiter et al., 2005), endotoxemia itself might be a candidate to reduce plasma ADAMTS13:AC together with inflammatory cytokines in AH patients. It will be necessary to clarify what kinds of inhibitor is involved in association with inflammatory cytokines and endotoxin. Recently, we encountered two patient who developed TTP; one occurred in the course of hepatitis C virus (HCV)-related advanced LC (Yagita et al., 2005) and another occurred a month after pegylated-interferon alpha-2a therapy in a HCV-related case of chronic hepatitis (Kitano et al, 2006). In both cases, plasma ADAMTS13:AC was extremely low, and the inhibitor against ADAMTS13 was detected in the patient’s heated plasma (2.0 BU/ml, 1.6 BU/ml, respectively) and purified IgG (0.19 BU/mg IgG, 0.4 BU/mg IgG, respectively).
Furthermore, we could detect IgG-inhibitor by western blot in 4 patients with advanced LC, who showed extremely lower ADAMTS13 activity (<3% of controls), but had no apparent clinical features of TTP, indicating the existence of “subclinical TTP” (Uemura et al., 2008a). Of 108 patients with idiopathic TTP whose plasma samples were sent to our department of Blood Transfusion Medicine, the inhibitor was detected in 54 (79.4%) of 68 patients analyzed, and its inhibitor activity was 0.5 to 2.0 BU/ml in 33 cases (61.1%), and more than 2.0 BU/ml in the remaining 21 cases (38.9%) (Matsumoto et al., 2004). Taken together, these observations suggest that the inhibitor activity detected in our patients with SAH and AH would be enough to reduce the activity of plasma ADAMTS13.

8. Clinical significance of plasma ADAMTS13:AC determination and future perspective in severe liver diseases

The introduction of ADAMTS13 to the field of hepatology not only enabled us to confirm the diagnosis of TTP early, but also provided novel insight into the pathophysiology of liver diseases. Some diseases were shown to be TTP itself, but others did not show any apparent clinical features of TTP, even in the presence of markedly decreased ADAMTS13:AC and increased UL-VWF that correlate with TTP. Such TTP-like states, but not DIC, may indicate “subclinical TTP” as seen in advanced LC (Uemura et al., 2008a, Uemura et al., 2010) and SAH patients (Matsuyama et al., 2007; Uemura et al., 2005b; Uemura et al., 2008b), or “local TTP” as shown in patients with hepatic VOD after SCT (Matsumoto et al., 2007; Park et al., 2002) and patients with adverse events after living-donor liver transplantation (Ko et al., 2006). One would be unable to detect such TTP-like phenomena without the determination of ADAMTS13:AC, because the interaction of ADAMTS13 and UL-VWF is the initial step in hemostasis, and their abnormalities do occur even in the absence of apparent imbalance in other conventional hemostatic factors. One could, then, notice that the origin of VWF, the substrate of ADAMTS13, is indeed transformed hepatic sinusoidal and/or extrahepatic ECs, but not hepatocytes. The procoagulant and anticoagulant proteins synthesized in hepatocytes decrease as liver disease progresses, whereas VWF markedly increases. Under such circumstances, ADAMTS13 deficiency may lead to microcirculatory disturbances not only in the liver, but also in the systemic circulation. The determination of ADAMTS13 and its related parameters will thus be quite useful for better understanding the pathophysiology and for providing appropriate treatments especially in severe liver disease patients.

Regarding FFP infusion as a unique source of ADAMTS13, we clearly showed that pre-existing UL-VWFMs in the plasma of US patients began to disappear within 1 hour and completely disappeared 24 hours after ADAMTS13 was replenished with infusions of FFP (Yagi et al., 2001), indicating that exogenous ADAMTS13 could efficiently cleave both UL-VWFMs pre-existing in the circulation and the newly produced molecules at the ECs’ surface. Furthermore, prophylactic FFP infusion may be instrumental in preventing the development of hepatic VOD after SCT (Matsumoto et al., 2007). Our five patients with SAH were treated with FFP infusion together with supportive care, and two of them survived, but the remaining three did not (Ishikawa et al., 2010). One of the non-survivors showed a transient increase in ADAMTS13:AC during FFP infusion, which ultimately decreased. The other two patients died of hepatic failure (Ishikawa et al., 2010). The administration of FFP may be useful, in part, as a supplementation of ADAMTS13, but the effects might depend on the severity of the liver
disturbance, or the degree of liver regeneration and multiorgan failure in SAH patients. Additionally, it is extremely important to monitor plasma ADAMTS13:AC in the treatment of thrombocytopenia associated with allograft dysfunction after liver transplantation. This is because the infusions of a platelet concentrate, but not FFP, under an imbalance of decreased ADAMTS13:AC to enhanced UL-VWF production, may further exacerbate the formation of platelet aggregates mediated by uncleaved UL-VWF, leading to graft failure via the “local TTP” mechanism (Ko et al., 2006). FFP infusion as ADAMTS13 replacement therapy may improve both liver dysfunction and thrombocytopenia in liver transplant patients.

From our point of view, it will be indispensable to measure ADAMTS13:AC when patients are encountered with unexplained thrombocytopenia in the course of liver disease. We are particularly interested in the conduct of clinical trials with recombinant ADAMTS13 preparations in severe liver diseases, including SAH, advanced LC, hepatic VOD and liver transplant patients. Further investigations will be necessary to define candidates for ADAMTS13 supplementation therapy, and to evaluate its potential therapeutic efficacy in severe liver diseases.

**Fig. 6. A working hypothesis of ADAMTS13 and its related parameters in the progression of alcoholic hepatitis.**

9. **Conclusion**

Enhanced gut-derived endotoxemia via hepatic reticuloendothelial dysfunction and increased intestinal permeability and subsequent cytokinemia may result in SECs injury and systemic inflammatory response syndrome, leading to microcirculatory disturbance in
hepatic sinusoid and multiple organs, and finally, to the necrosis of hepatocytes and multiorgan failure in patients with AH, and particularly in patients with SAH (Fig. 6.). The imbalance between the enhanced production of UL-VWFM and the deficient activity of ADAMTS13 may, in part, contribute to not only sinusoidal microcirculatory disturbances and subsequent liver injury in AH, but also to the development of multiorgan failure through impaired organ microcirculation in SAH. Decreased ADAMTS13:AC and increased VWF:Ag could be induced by pro-inflammatory cytokinemia and the plasma inhibitor against ADAMTS13, both of which may be closely related to enhanced endotoxemia (Fig. 6.). The determination of ADAMTS13:AC and its substrate will give us new insights into the pathophysiology of acute ALD and help to elucidate additional therapeutic strategies including ADAMTS13 supplementation for this disease.

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Alcoholic liver disease occurs after prolonged heavy drinking. Not everyone who drinks alcohol in excess develops serious forms of alcoholic liver disease. It is likely that genetic factors determine this individual susceptibility, and a family history of chronic liver disease may indicate a higher risk. Other factors include being overweight and iron overload. This book presents state-of-the-art information summarizing the current understanding of a range of alcoholic liver diseases. It is hoped that the target readers - hepatologists, clinicians, researchers and academicians - will be afforded new ideas and exposed to subjects well beyond their own scientific disciplines. Additionally, students and those who wish to increase their knowledge will find this book a valuable source of information.

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