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Brief report

Functional imaging of shear-dependent activity of ADAMTS13 in regulating mural thrombus growth under whole blood flow conditions

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The metalloprotease ADAMTS13 is assumed to regulate the functional levels of von Willebrand factor (VWF) appropriate for normal hemostasis in vivo by reducing VWF multimer size, which directly represents the thrombogenic activity of this factor. Using an in vitro perfusion chamber system, we studied the mechanisms of ADAMTS13 action during platelet thrombus formation on a collagen surface under whole blood flow conditions. Inhibition studies with a function-blocking anti-ADAMTS13 antibody, combined with immunostaining of thrombi with an anti-VWF monoclonal antibody that specifically reflects the VWF-cleaving activity of ADAMTS13, provided visual evidence for a shear rate–dependent action of ADAMTS13 that limits thrombus growth directly at the site of the ongoing thrombus generation process. Our results identify an exquisitely specific regulatory mechanism that prevents arterial occlusion under high shear rate conditions during mural thrombogenesis. (Blood. 2008; 111:1295-1298)

Introduction

The adhesive protein von Willebrand factor (VWF) plays a major role in platelet thrombogenesis, a process crucial for hemostasis. However, the excessive function of VWF is thought to increase the risk of fatal arterial thrombosis.[1,2] The thrombogenic activity of VWF is strictly dependent upon its multimeric structure, which is thought to be regulated in vivo by the metalloprotease ADAMTS13 through its cleavage of the A2 domain of the VWF subunit.[3,4] Indeed, patients with congenital deficiency of ADAMTS13 suffer repeated thrombotic complications attributed to excessive function of the ultra-large VWF (ULVWF) multimer, which is not found in normal blood circulation.[3-6] This concept was recently confirmed by knock-out mouse studies, in which ADAMTS13$^{-/-}$ mice exhibited enhanced thrombogenicity in the ex vivo or in vitro experimental blood flow conditions tested.[7,8]

The mechanisms by which ADAMTS13 regulates VWF remain poorly understood. However, recent studies showing that ADAMTS13 under flow conditions can rapidly cleave ULVWF secreted from and anchored to cultured endothelial cell layers[9,10] have raised the possibility that blood flow is critical in activating ADAMTS13.[11] Indeed, the VWF-cleaving activity of ADAMTS13 cannot be reproduced in vitro under static conditions unless the substrate VWF molecule is somewhat modified (eg, denatured by guanidine-HCl or urea).[3,4] Further, the question arises of whether ADAMTS13, in addition to its known action on ULVWF freshly released from endothelial cells, might also act directly at the local sites of thrombus generation to regulate thrombus growth.

To address these issues, we analyzed the role and mechanisms of ADAMTS13 action in mural platelet thrombogenesis on a collagen-coated glass surface in an in vitro perfusion chamber system. Our visual evidence demonstrates that ADAMTS13 cleaves VWF and down-regulates mural thrombus growth at the site of ongoing thrombus generation in a shear rate–dependent manner under whole blood flow conditions.

Methods

Blood collection

The present work was approved by the institutional review board of Nara Medical University, and informed consent was obtained in accordance with the Declaration of Helsinki. Using 200 μM argatroban as an anticoagulant, blood was collected from 10 nonsmoking healthy volunteers who had not taken any medications in the previous 2 weeks.

Monoclonal antibodies

A function-blocking anti-ADAMTS13 monoclonal antibody (A10), which completely inhibits plasma ADAMTS13 activity at the concentration of 20 μg/mL,[12] was used as a divalent $(ab')_2$ fragment in inhibition studies. An anti-VWF monoclonal antibody (N10) was used that reacts with an epitope within the VWF A2 domain (10-amino acid VWF peptide; D^{1596}REQAPNLVY^{1605}) only after cleavage by ADAMTS13 exposes the epitope; thus, reactivity of antibody N10 specifically reflects the VWF-cleaving activity of ADAMTS13, as described.[13]

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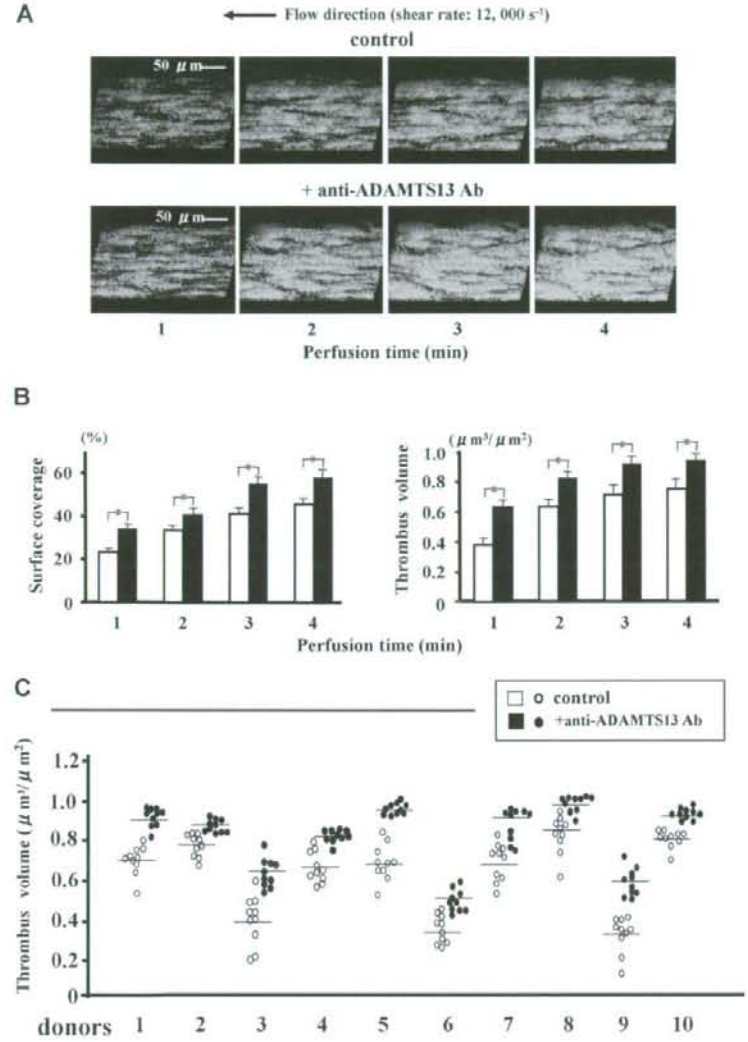

Figure 1. Effects of a function-blocking anti-ADAMTS13 monoclonal antibody (A10) on mural thrombus generation under very high shear rate conditions. Whole blood from healthy volunteers containing DiOC6 (1 μM)-labeled platelets, anticoagulated with argatroban, was perfused over a type I collagen-coated glass surface under very high shear rate (12 000 s^{-1}) with anti-ADAMTS13 antibody A10 or with control mouse IgG (each 20 μg/mL). (A) Time-course changes of 3-dimensional images of thrombi (original magnification, ×600), which were constructed by the image-analyzing system of confocal laser scanning microscopy (CLSM) based on successive horizontal slices at identical portions, are representative of 10-pair flow experiments using blood from 10 independent donors. (B) Statistical analyses corresponding to the above images; bars represent mean (+ SD) surface coverage or total thrombus volume in 10 areas (each 133 × 100 μm) randomly selected in each perfusion using a single donor blood (donor number 1 in panel C). Note that thrombus generation is significantly (*; $P < .01$) accelerated in the presence of the anti-ADAMTS13 antibody. (C) Thrombus volume at 3 minutes' perfusion in 10-pair flow experiments using 10 independent donors; data points represent values of 10 areas randomly selected in each perfusion with (●) or without (○) anti-ADAMTS13 antibody, and transverse lines indicate mean values for each group. Note also that thrombus volumes generated in the presence of anti-ADAMTS13 antibody are significantly ($P < .01$; asterisks not included in the figure) greater than control thrombi in all 10-pair experiments.

In vitro perfusion studies

Thrombus generation on a type I collagen-coated (Sigma-Aldrich, Tokyo, Japan) glass surface was studied under various shear rates in a parallel plate flow chamber system as described.[14-17] Surface coverage and volume of thrombi generated at the indicated time points during whole blood perfusion were evaluated based on images obtained by confocal laser scanning microscopy (CLSM; FV300; Olympus, Tokyo, Japan), as described.[15-17] Immunohistochemical staining of thrombi using anti-VWF antibodies was performed as described.[15-17] Briefly, thrombi on a glass surface were fixed with paraformaldehyde and incubated with a mixture of anti–whole VWF rabbit polyclonal antibody (30 μg/mL; DAKO Cytomation, Kyoto, Japan) and N10 antibody (60 μg/mL) or with the negative control IgG mixture (rabbit; 30 μg/mL, mouse; 60 μg/mL; DAKO Cytomation) for 90 minutes at 37°C. Samples were then stained with a mixture of fluorescein isothiocyanate (FITC)–conjugated anti–rabbit IgG (3.3 μg/mL; BioSource International, Camarillo, CA) and Cy3-conjugated anti–mouse IgG (3.3 μg/mL; Sigma-Aldrich) as secondary fluorescent antibodies for 90 minutes at 37°C, and viewed by CLSM. These conditions were determined in preliminary experiments to confirm the sufficient infiltration of both primary and secondary fluorescent antibodies into thrombi.

Results and discussion

To address the potential role of ADAMTS13 in the ongoing process of mural thrombus generation, we compared the size of thrombi generated in the presence or absence of a function-blocking antibody against ADAMTS13 in a perfusion chamber system, using blood from the same donor. This relatively simple experimental approach is able to precisely evaluate ADAMTS13 function in uniform blood conditions, avoiding the individual heterogeneity of sample blood conditions including VWF and platelets that might otherwise seriously affect the size of thrombi generated in this type of flow experiment.

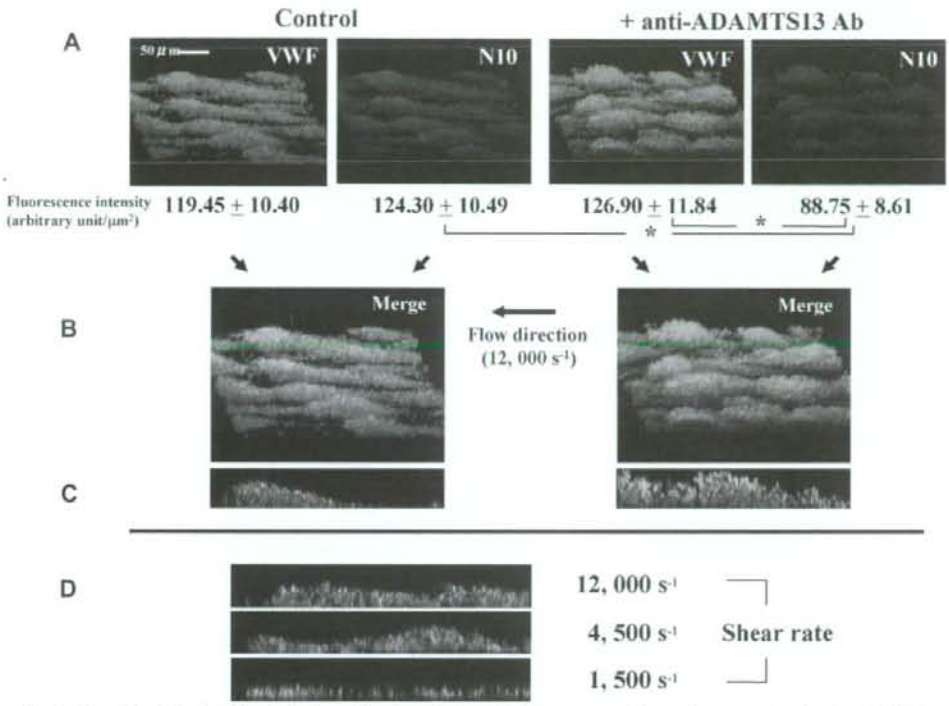

Figure 2. Visual evaluation of ADAMTS13 activity within thrombi generated under high shear rate conditions using a monoclonal antibody (N10) that specifically detects ADAMTS13-cleaved VWF. Experimental conditions were as described in Figure 1, except that platelets were not labeled. Thrombi generated on a collagen-coated glass surface at 3 minutes' perfusion with or without an anti-ADAMTS13 antibody under 12 000 s^{-1} shear were fixed, reacted with both N10 antibody and anti–whole VWF antibody, double-stained with Cy3 (red)- and FITC (green)-fluorescence, and viewed by CLSM. (A) Three-dimensional images of thrombi, representative of 5 independent flow experiments, and the corresponding fluorescence intensity (mean ± SD of 5 areas randomly selected in a single perfusion) corrected for background value (negative control IgGs), indicate that VWF cleavage by ADAMTS13 (red color) within thrombi is significantly (*; $P < .01$) reduced in the presence of anti-ADAMTS13 antibody as compared with control thrombi (original magnification; ×600). (B) Merged 3-dimensional images and (C) the corresponding longitudinal views of thrombi; in the merged images, portions stained with both green and red fluorescence basically show the color of the higher pixel value, whereas a yellowish color is seen when both pixel values are nearly equal. Thus, the predominantly reddish appearance of the surface portions of control thrombi suggests that ADAMTS13 is more active on the surface of thrombi forming under very high shear rate conditions, while this tendency is barely visible in the presence of anti-ADAMTS13 antibody. (D) Longitudinal views of thrombi, generated at 3 minutes' perfusion without an anti-ADAMTS13 antibody under various shear rates and double-stained, are representative of 5 separate experiments. Note the prominent red color, especially at the surface portions of thrombi, indicating higher ADAMTS13 activity under higher shear rates.

Under a very high shear rate of 12 000 s^{-1}, thrombus growth was significantly accelerated by addition of anti-ADAMTS13 antibody (Figure 1). This enhanced thrombogenesis most likely reflects a block in ADAMTS13 activity rather than nonspecific effects of antibody on platelets, since immunostaining of thrombi with N10 antibody, which reacts only with VWF cleaved by ADAMTS13, visually confirmed the reduced VWF cleavage within thrombi by the anti-ADAMTS13 antibody (Figure 2A). Thus, these results clearly point to the regulatory role of ADAMTS13 during thrombus generation.

While the preceding observations were made under a much higher shear rate than the high shear rate typically used in platelet functional studies (ie, 1500 s^{-1} in our laboratory[14-16]), similar observations, although less pronounced, were confirmed under lower shear rates (Figure 2D). In addition, longitudinal views of thrombi revealed the preferential VWF-cleavage activity of ADAMTS13 at the surface portions of forming thrombi during thrombogenesis (Figure 2B,C). The thrombus surface is thought to directly encounter blood flow with the highest shear rate under such flow circumstances, where the wall shear rate can increase as the flow path narrows in parallel with thrombus growth.[15] Together, these observations strongly suggest a shear rate–dependent property of ADAMTS13 function.

Shear forces are thought to transform the globular conformation of the immobilized VWF multimer observed under static conditions to a shape resembling a spreading bird wing, consistent with the shear rate–dependent acceleration of the VWF-glycoprotein Ib interaction under high shear.[18] By analogy, a stretching of the VWF multimeric structure by shear forces may also be critical for the action of ADAMTS13 in exposing the latent reactive site on the VWF molecule. In this regard, increased tensile strength of the VWF multimeric structure on binding to platelets might augment the stretching effects of shearing forces, resulting in up-regulated ADAMTS13 activity.[19,20] This possibility seems compatible with recent findings indicating that even under low shear rate conditions, ADAMTS13 can cleave ULVWF released from endothelial cells,[9,10] because a greater number of platelets can bind spontaneously to ULVWF as compared with normal-sized VWF without shearing forces.

The mechanisms described here represent an exquisite orchestration by platelets, VWF, and ADAMTS13 under high shear to properly regulate the final size of mural thrombi in vivo and

prevent excessive thrombogenesis from occluding the vessel lumen. Because ADAMTS13 activity appears to be triggered in response to the increased local shear rate associated with the development of thrombi, our results may provide a novel avenue toward strategies that prevent arterial thrombosis without bleeding complications.

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Authorship

Contribution: Y.S. performed most of the flow experiments, data analysis, and the manuscript preparation; T.M. and M.H. helped perform the flow experiments and data analysis; S.K., M.M., and Y.F. produced and characterized monoclonal antibodies; A.Y. and K.O. provided direction throughout the work and helped prepare the manuscript; and M.S. and K.N. provided the overall experimental designs and direction of this work, and prepared the manuscript.

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Inherited and *de novo* mutations of *ADAMTS13* in a patient with Upshaw-Schulman syndrome

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Upshaw-Schulman syndrome (USS) is a congenital thrombotic and hemorrhagic diathesis characterized by a deficient activity of plasma von Willebrand factor (VWF)-cleaving protease, ADAMTS13 [1–8]. Some patients with USS develop severe jaundice soon after birth as their first symptom, and some are almost asymptomatic during childhood to adolescence, unless they have precipitating factors such as infection or pregnancy. The pathological condition of USS belongs to thrombotic thrombocytopenic purpura (TTP), which is characterized by thrombocytopenia, hemolytic anemia and microvascular thrombosis. USS, therefore, is also called congenital TTP. Most cases of TTP result from acquired deficient activity of ADAMTS13 caused by the advent of autoantibodies that inhibit the ADAMTS13 activity. In contrast, patients with USS do not carry such inhibitory antibodies in their plasma, and suffer from USS because of the compound heterozygous or homozygous mutations of the *ADAMTS13* gene. More than 80 mutations of *ADAMTS13* have been identified in patients with USS. Here, we report a first case with *de novo* mutations of *ADAMTS13*.

Patient *P* (II-2 in Fig. 1A) is the second child of unrelated Japanese parents (I-1 and I-2). The first child (II-1) was spontaneously aborted from an unknown cause at 6 weeks of pregnancy, while the fourth child (II-4) was aborted by umbilical coiling at 22 weeks. The parents and the third child (II-3) are apparently healthy. The patient showed moderate hyperbilirubinemia soon after birth and received phototherapy without exchange blood transfusion. On the occasion of catching cold at 3 years of age, he developed thrombocytopenia, microangiopathic hemolytic anemia, and renal insufficiency. He was diagnosed as having TTP and treated with fresh frozen plasma (FFP) infusions. Thereafter, he repeated these episodes several times a year, and each time he soon recovered with FFP infusions. At 21 years of age, he developed a hallmark of TTP, consisting of thrombocytopenia, microangiopathic hemolytic anemia, neurological dysfunction, renal failure and fever. He started taking prophylactic infusions of FFP (4–8 ml kg^{-1} body weight) every 2 weeks.

The plasma ADAMTS13 activities of the family members, measured by the method based on VWF-multimer analysis [1,9], are shown under each symbol in Fig. 1A. The ADAMTS13 activity of the patient was less than 3% of that of the control, which was confirmed by measuring his plasma collected after an interval of more than 1 month. The values were consistent with the data obtained by two other methods, a fluorogenic assay [10] and a chromogenic assay [11] (data not shown). As the assay of the ADAMTS13-activity inhibitors [1] showed no detectable inhibitors in the patient plasma (data not shown), the etiology of his TTP symptoms was considered a genetic deficiency of ADAMTS13, that is, USS. To clarify the underlying cause of the TTP crisis, we analyzed the nucleotide sequences of the family's *ADAMTS13* genes.

DNA experiments were carried out with the permission of the ethics committees of the National Cardiovascular Center after obtaining informed consent from the study subjects. The nucleotide sequences of all 29 exons of *ADAMTS13*, including the intron-exon boundaries, were determined by direct sequencing of polymerase chain reaction (PCR) products as described previously [12,13].

The patient was heterozygous for five nucleotide mutations, c.964T > G, c.968C > G, c.969C > A, c.970T > C and c.2723G > A. Of them, c.2723G > A was also detected in the father. The mother and sister were heterozygous for c.2708C > T, which was not found in the patient or his father (Fig. 1B).

The c.2723G > A mutation on exon 21 causing C908Y, heterozygously found in the patient and father, was previously reported by us as a causative mutation in another USS family [14]. This mutation causes the impaired secretion of ADAMTS13 [14]. The moderately decreased ADAMTS13 activity of the father in the present case could be explained by this single mutation. The c.2708C > T mutation on exon 21 causing S903L was heterozygously found in the mother and sister, whose plasma ADAMTS13 activities were normal. This suggested that S903L should not affect the ADAMTS13 activity. In fact, the allele frequency of S903L is 6.0% in the Japanese general population (our unpublished data), making it a common polymorphism.

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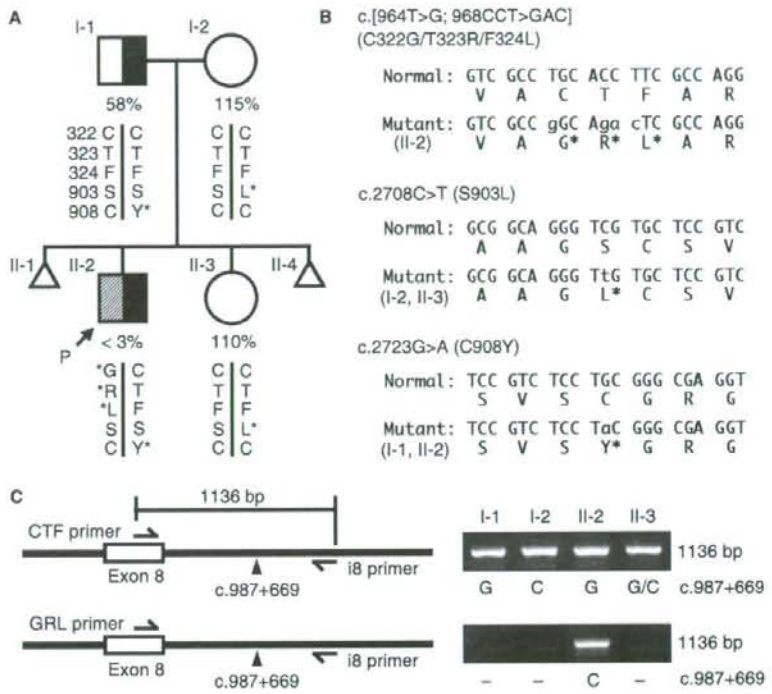

Fig. 1. *ADAMTS13* mutations in the USS patient *P* family. (A) Pedigree of the patient family. Plasma ADAMTS13 activities are shown under each symbol. The haplotype patterns of the five amino-acid residues deduced from each *ADAMTS13* gene are indicated under the ADAMTS13 activities. The arrow indicates the proband. (B) Missense mutations of *ADAMTS13* identified in this family. S903L is a common polymorphism. Asterisks indicate the mutated amino-acid residues. (C) PCR analysis of normal and mutant alleles. The 1,136-bp region was amplified by PCR using either the normal allele-specific CTF primer (5′-CCAAGGCTGTCGCCTGCACCT-3′) or the mutant allele-specific GRL primer (5′-CCAAGGCTGTCGCCGGCAGAC-3′) on exon 8 with the common reverse i8 primer (5′-TGAAGCCAGGAGTCCTAGACA-3′) on intron 8. This region contained a single nucleotide G/C polymorphism at the site of c.987 + 669. Subjects I-1 (father) and I-2 (mother) were homozygotes for G and C, respectively. The normal and mutant alleles of II-2 (patient) carried G and C, respectively.

The four mutations on exon 8, c.964T > G, c.968C > G, c.969C > A and c.970T > C, were detected only in the patient, suggesting that they should be *de novo* mutations in the patient's *ADAMTS13*. All the four mutations were excluded as common polymorphisms by the screening of 346 individuals in the Japanese general population. Cloning and sequencing of the genomic PCR products including exon 8 revealed that all the mutations were located on a single allele. Therefore, they could be described as c.[964T > G; 968CCT > GAC], resulting in three contiguous missense changes C322G/T323R/F324L within the disintegrin-like domain of ADAMTS13. The C322G mutation may disrupt a tertiary structure of the protein because of the defect in disulfide bond formation.

To determine the origin of the freshly mutated allele of the patient, a longer region including exon 8 was amplified by PCR using a combination of either the normal allele-specific CTF primer or the mutant allele-specific GRL primer and the common reverse i8 primer (Fig. 1C). The combinatorial use of CTF and i8 primers produced a 1,136-bp fragment from genomic DNAs of all the family members, whereas the use of GRL and i8 primers produced a 1,136-bp fragment only from the patient, as expected. The region contained a single nucleotide G/C polymorphism at the site of c.987 + 669. Sequencing of the fragments suggested that the father and mother were homozygotes for G and C, respectively, and that the sister was heterozygous. The normal and mutant alleles of the patient carried G and C, respectively. These results suggested that the mutant allele of the patient was derived from one of the maternal alleles. Based on all of the data, we concluded that the patient was a compound heterozygote of paternally transmitted C908Y and freshly mutated C322G/T323R/F324L on the maternal allele.

In conclusion, this is the first report of a case of compound heterozygosity of inherited and *de novo ADAMTS13* mutations resulting in USS.

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Disclosure of Conflict of Interests

The authors have no conflict of interest.

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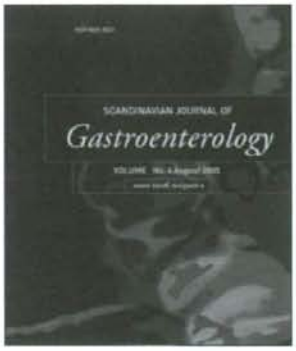

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Plasma ADAMTS13 activity parallels the APACHE II score, reflecting an early prognostic indicator for patients with severe acute pancreatitis

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ORIGINAL ARTICLE

Plasma ADAMTS13 activity parallels the APACHE II score, reflecting an early prognostic indicator for patients with severe acute pancreatitis

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Abstract

Objective. Severe acute pancreatitis (SAP) frequently progresses to pancreatitis-associated multiorgan failure (MOF) with high mortality. Decreased plasma ADAMTS13 activity (ADAMTS13:AC) results in the accumulation of unusually large von Willebrand factor multimers (UL-VWFM) and the formation of platelet thrombi, ultimately leading to MOF. The purpose of the study was to investigate the potential role of ADAMTS13:AC in the severity of SAP. ***Material and methods.*** Plasma ADAMTS13:AC and its related parameters were sequentially determined in 13 SAP patients. ADAMTS13:AC was determined by the chromogenic act-ELISA. ***Results.*** Within 1 or 2 days after admission, ADAMTS13:AC was lower in SAP patients (mean 28%) than in healthy controls (99%), and gradually recovered in the 11 survivors but further decreased in the 2 non-survivors. Patients with higher sepsis-related organ failure assessment (SOFA) scores showed lower ADAMTS13:AC than those without these scores. The inhibitor against ADAMTS13 was undetectable. On day 1, von Willebrand factor antigen (VWF:Ag) was higher (402%, $p < 0.001$) in SAP patients than in controls (100%). VWF:Ag gradually decreased in the survivors, except in the 3 patients needing a necrosectomy, but remained high in the non-survivors. ADAMTS13:AC was inversely correlated with the APACHE II score ($r = -0.750$, $p < 0.005$), and increased plasma concentrations of interleukin 6 (IL-6) and IL-8 at admission. UL-VWFM-positive patients had lower ADAMTS13:AC and decreased serum calcium concentrations, but higher VWF:Ag and IL-8 concentrations than UL-VWFM-negative patients. ***Conclusions.*** Plasma ADAMTS13:AC was closely related to the APACHE II score. This intimate relationship may serve as an early prognostic indicator for SAP patients. The imbalance between decreased ADAMTS13:AC and increased UL-VWFM could contribute to SAP pathogenesis through enhanced thrombogenesis.

Key Words: *ADAMTS13, APACHE II score, multiorgan failure, severe acute pancreatitis, von Willebrand factor*

Introduction

Acute pancreatitis is an inflammatory disease with severity that ranges from a self-limiting inflammation to a rapidly deteriorating condition complicated by multiple organ failure (MOF) [1]. In the majority of patients, acute pancreatitis is mild, but 10–20% of patients develop severe acute pancreatitis (SAP) with high mortality rates of up to 25% [2]. The various pathways that contribute towards increased intrapancreatic and extrapancreatic inflammation result in a systemic inflammatory response syndrome that predisposes patients to MOF and/or pancreatic

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necrosis, ultimately leading to SAP [3]. Upon hospital admission, the risk for acute pancreatitis is determined by a Ranson score [4], the APACHE (acute physiology and chronic health evaluation) II score [5], and the Japanese severity score [6], which includes factors such as older age, obesity, and MOF. These scoring systems have been widely used to predict acute pancreatitis at times earlier than 48 h. Recent studies have indicated that the major cause of death in SAP is pancreatitis-associated MOF followed by systemic microcirculatory disturbance, which can extend beyond the early stage of acute pancreatitis and persist throughout the course of the disease [1,7–9]. These findings indicate the need for a predictive factor associated with the development of systemic microcirculatory disturbance and subsequent MOF.

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 within the VWF A2 domain [10,11]. VWF is synthesized by vascular endothelial cells and secreted into the plasma as an "unusually large" VWF multimer (UL-VWFM). ADAMTS13 rapidly cleaves UL-VWFM to generate smaller VWF multimers [12]. An ADAMTS13 deficiency caused by gene mutations [13] or autoantibodies against ADAMTS13 [14,15] increases plasma UL-VWFM levels, leading to platelet aggregation and/or thrombi formation under conditions of high shear stress and subsequent thrombotic thrombocytopenic purpura (TTP)[12,14–16], as described by Moschowitz's pentad [17]. ADAMTS13 was originally cloned using liver cell libraries [11], and later it was clearly shown that this enzyme is produced exclusively in hepatic stellate cells [18].

In previous studies it has been reported that acute pancreatitis complicates the course of TTP [8], but recent reports indicate that acute pancreatitis may trigger TTP [19–26]. Additional studies have investigated the role of ADAMTS13 in pathological conditions, and Mannucci et al. originally reported a significant reduction in ADAMTS13:AC in advanced cirrhotics [27]. Subsequently, we have shown that ADAMTS13:AC is significantly reduced in patients with hepatic-veno-occlusive disease [28], alcoholic hepatitis [29], and those undergoing living-donor-related liver transplantation [30]. Furthermore, ADAMTS13:AC was reduced in patients with sepsis-induced disseminated intravascular coagulation and renal failure [31,32]. Based on these findings, it is of particular interest to investigate the relationship of plasma ADAMTS13:AC with the progression of SAP.

In this study, we sequentially measured the plasma ADAMTS13:AC, and its substrate VWF and UL-VWFM in patients with SAP, and investigated their potential role in the severity of pancreatitis and development of MOF.

Material and methods

Patients

The study included 13 patients with SAP (10 M, 3 F, mean age 53.0 ± 14.6 years) (Table I). All patients were originally admitted to the Department of Emergency and Critical Care Medicine of our hospital between October 2004 and August 2006. Patients with a known history of coagulopathies, sepsis, or platelet disorders were excluded from the study. Eleven patients were survivors (cases 1–11), and the remaining 2 patients were non-survivors (cases 12 and 13). The etiology of SAP was alcohol abuse in 7 patients, idiopathic in 3 patients, common bile-duct stones in 2 patients, and post endoscopic retrograde cholangiopancreatography (ERCP) in 1 patient. The severity of pancreatitis was scored according to the APACHE II system [5], and an imaging grade was made by computed tomography (CT) scans, as described previously [33]. Disseminated intravascular coagulation was diagnosed on the basis of the scoring system [34]. MOF was evaluated according to the SOFA (sepsis-related organ failure assessment) score [35]. Specific treatments included continuous infusion of antibiotics and protease inhibitor via the superior mesenteric artery and splenic artery ($n=9$), partial necrosectomy ($n=4$), oral administration of lactulose ($n=4$), and continuous hemodiafiltration ($n=1$). Nine patients had underlying diseases including hypertension ($n=3$), cholecystectomy due to gallbladder stones ($n=3$), diabetes mellitus ($n=2$), cerebral vascular attack ($n=2$), chronic pancreatitis ($n=2$), liver cirrhosis ($n=2$), and others. All subjects gave informed consent to participate in the study. The study protocol was approved by the Nara Medical University Hospital Ethics Committee.

Assays of ADAMTS13:AC, VWF antigen, and UL-VWFM

Blood was obtained from patients at the time of admission or during hospitalization. For the 11 SAP survivors, plasma samples were taken serially every 2 to 7 days until the initial recovery phase. Among two non-survivors, one patient was taken twice, and the other was taken once on admission. Samples were stored in plastic tubes containing 1/10th volume of 3.8% sodium citrate. Platelet-poor plasma was prepared by centrifugation at 3000*g* at 4°C for 15

Table 1. Clinical characteristics of severe acute pancreatitis patients on admission.

No.	Age/ Gender	Etiology	CT grade[a]	APACHE II score[b]	SOFA score[c]	DIC score[d]	Hb (g/dl)	WBC (/mm^3)	Plt ($\times 10^3$/ mm^3)	CRP[e] (mg/dl)	ADAMTS13 activity (%)	VWF:Ag (%)	UL-VWFM	Specific treatment
1	60/M	Alcoholic	D	10	9	2	18.5	19900	107	22.9	17	334	+	Arterial infusion[i] CHDF Necrosectomy
2	53/F	CBD stone	D	5	2	0	14.7	10000	238	4.2	30	318	–	–
3	41/M	Alcoholic	D	8	1	0	12.7	10100	200	22.1	45	277	+	Necrosectomy
4[f]	51/M	Alcoholic	D	2	4	1	16.3	10400	87	0.7	39	489	–	Arterial infusion
5	36/M	CBD stone	C	2	4	1	15.6	10400	116	16.3	42	295	–	Arterial infusion
6	40/M	ERCP	D	8	2	2	14.7	17700	295	23.8	37	312	–	Arterial infusion
7	57/M	Alcoholic	D	7	5	2	17.2	21500	151	10.1	28	369	–	Arterial infusion Necrosectomy
8	60/M	Idiopathic	D	8	4	2	16.6	12800	156	20.6	22	462	+	Arterial infusion
9[g]	64/F	Idiopathic	D	7	4	3	11.2	9600	61	5.5	65	374	+	Arterial infusion
10	51/M	Alcoholic	D	3	1	2	11.6	15600	432	2.0	75	208	–	–
11[h]	48/M	Alcoholic	D	7	3	2	21.4	7000	82	10.1	37	699	+	Arterial infusion
12[j]	37/M	Alcoholic	D	10	8	2	11.5	13200	162	16.4	23	645	+	Arterial infusion necrosectomy
13[j]	91/F	Idiopathic	D	12	6	3	12.7	16900	165	17.1	15	443	+	–

Abbreviations: CT = computed tomography; APACHE = Acute Physiology and Chronic Health Evaluation; SOFA = Sepsis-related Organ Failure Assessment; DIC = disseminated intravascular coagulation; Hb = hemoglobin; WBC = white blood cell count; Plt = platelet count; CRP = C-reactive protein; ADAMTS13 = a disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13; VWF:Ag = von Willebrand factor antigen; UL-VWFM = unusually large von Willebrand factor multimer; CBD = common bile duct; ERCP = endoscopic retrograde cholangiopancreatography; CHDF = continuous hemodiafiltration.

[a]According to the scoring system as described by Balthazar et al. [33]; from A to E, with E being worst; [b]according to the scoring system as described by Knaus et al. [5]; from 0 to 71, with 71 being worst. [c]according to the scoring system as described by Vincent et al. [35]; from 0 to 24, with 24 being worst; [d]according to the scoring system as described by Taylor et al [34]: from 0 to 8, with 8 being worst; and the score 5 or more is compatible with overt disseminated intravascular coagulation; [e]the normal range is less tham 0.2 mg/dl; [f]alcoholic liver cirrhosis; [g]cryptogenic cirrhosis; [h]hepatitis C virus-related chronic hepatitis; [j]non-survivors; [i]continuous infusion of antibiotics and protease inhibitor via the superior mesenteric artery and splenic artery.

min and stored in aliquots at −80°C until analysis. The plasma ADAMTS13:AC was determined by a sensitive chromogenic ELISA (ADAMTS13-act-ELISA: Kainos Inc., Tokyo, Japan) [36]. The normal value was 99±22% (n=56; 30 F, 26 M, age range 20–39 years), and the detection limit was 0.5% by ELISA. We therefore considered the activity as low when it was less than 50% of that in healthy subjects (mean −2 SD). Plasma UL-VWFM was analyzed by a vertical SDS-1.0% agarose gel electrophoresis system [37], and evaluated using NIH Image J software. The inhibitor against ADAMTS13 was evaluated using plasma that was heat-inactivated at 56°C for 30 min [36]. One Bethesda unit of inhibitor was defined as the amount of plasma that reduced ADAMTS13:AC to 50% of the control, and its titer was defined to be significant at >0.5 Bethesda U/ml [38]. The plasma VWF:Ag was measured with a sandwich enzyme immunoassay using a rabbit anti-human VWF polyclonal antibody (Dako, Kyoto, Japan). The value in the healthy subjects at our laboratory (n=54; 30 M, 24 F, age range 20–39 years) was 100±53% (mean±SD).

Measurements of cytokines

Plasma concentrations of tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), and interleukin 8 (IL-8) were determined by Immunoassay kits (Bio-Source International, Camarillo, CA, USA).

Statistical analysis

Differences between paired and unpaired groups were analyzed using the Mann-Whitney U-test. Correlations were calculated with the Spearman rank test. The analyses were done using Statview statistical software (version 5.0; SAS Institute, Cary, N.C., USA). The data are expressed as the means± SD. A two-tailed p-value of less than 0.05 was considered significant.

Results

Clinical characteristics of SAP patients

Patients' clinical features and laboratory data are summarized in Table I. The APACHE II score at admission ranged from 2 to 12 (mean 6.6±2.7), and the CT grade was D in all the cases with the exception of case 5, which was grade C. The SOFA score describing MOF ranged from 1 to 9 (mean 4.2±2.5). The two non-survivors whose APACHE II scores were 10 and 12 died of MOF within 2 months and 10 days, respectively. The disseminated intravascular coagulation score was estimated as 0–3, and none of the patients was diagnosed as having overt disseminated intravascular coagulation. All patients had signs of inflammation, including leukocytosis and elevated levels of C-reactive protein. Thrombocytopenia of less than $100 \times 10^3/mm^3$ was detected in 3 patients: case 4 who had alcoholic liver cirrhosis, case 9 with cryptogenic cirrhosis, and case 11 with hepatitis C virus-related chronic hepatitis. Severe necrotizing pancreatitis requiring a necrosectomy developed in 4 patients (cases 1, 3, 7, and 12).

ADAMTS13:AC

Among patients with SAP, plasma ADAMTS13:AC was significantly decreased on day 1 (on admission) (37±17%, p<0.001), and further decreased at day 2 (32±18%, p<0.001) compared with healthy controls (99±22%) (Figure 1). Thereafter, the ADAMTS13:AC gradually recovered (mean 49% on day 5, 58% on day 7, 70% on day 10, 72% on day 17) in survivors, whereas in the two non-survivors, ADAMTS13:AC decreased from 23% on day 1 to 10% on day 2 in case 12 and was 15% on day 1 in case 13 (Figure 1). The lowest plasma ADAMTS13:AC activity within 1 or 2 days after admission was also decreased compared with that in the controls (28±16% versus 99±22%, p<0.001) (Figure 2A), and the APACHE II score at that time showed 6.8±3.1. Regarding the relationship of ADAMTS13:AC with clinical features and laboratory findings, the lowest ADAMTS13 activity within 1 or 2 days after admission was markedly lower in patients with higher SOFA scores (5–9 in cases 1, 7, 12, and 13) than in those with lower SOFA scores (1–4 in others) (17±8% versus 40±15%, p<0.02) (Table I). The ADAMTS13:AC was correlated

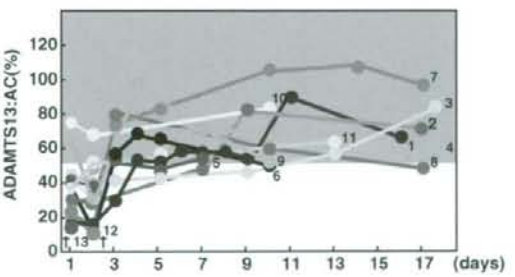

Figure 1. Sequential analysis of plasma ADAMTS13:AC in patients with SAP. Numbers correspond to each case listed in Table I. The shaded area shows the normal range, and the crosses indicate non-survivors. Plasma ADAMTS13:AC significantly decreased on day 1, and further decreased on day 2 compared with in healthy controls. Thereafter, the activity gradually recovered in survivors, but further decreased in case 12 and was the lowest in case 13, both of whom were non-survivors. Abbreviations: ADAMTS13:AC = ADAMTS13 activity; SAP = severe acute pancreatitis.

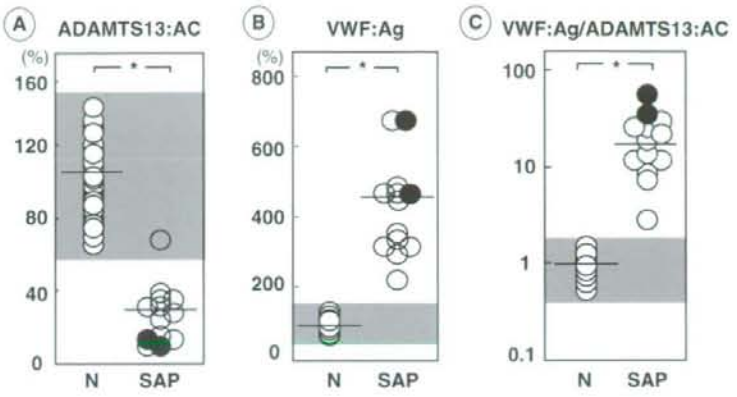

Figure 2. The lowest plasma ADAMTS13:AC, highest VWF:Ag, and the ratio of VWF:Ag to ADAMTS13:AC within 1 or 2 days after admission in patients with SAP. Open circles indicate survivors, and closed circles denote non-survivors. The shaded area shows the normal range. In SAP patients, plasma ADAMTS13:AC was significantly lower (A), and VWF:Ag was markedly higher (B), resulting in a higher VWF:Ag to ADAMTS13:AC ratio (C), compared with in normal healthy controls. Non-survivors showed the lowest ADAMTS13:AC and highest VWF:Ag, resulting in the highest VWF:Ag to ADAMTS13:AC ratio. Abbreviations: ADAMTS13:AC = ADAMTS13 activity; VWF:Ag = von Willebrand factor antigen; N = normal healthy controls; SAP = severe acute pancreatitis. *$p < 0.001$ significantly different between the two groups.

negatively with the APACHE II score ($r = -0.750$, $p < 0.005$) (Figure 3), and positively with platelet counts ($r = 0.615$, $p < 0.03$). There was no correlation between ADAMTS13:AC and other clinical parameters, including prothrombin time, fibrinogen degradation products, leukocytes, C-reactive protein, hemoglobin, and pancreatic enzymes such as serum amylase, elastase 1, trypsin, lipase, and phospholipase A_2 activity. The inhibitor against ADAMTS13 was not detected.

VWF:Ag

Plasma VWF:Ag was significantly higher on day 1 ($402 \pm 143\%$, $p < 0.001$) in SAP patients than in healthy controls (100%). Thereafter, plasma VWF:Ag gradually decreased to $247 \pm 69\%$ in the 7 surviving patients within 17 days. However, in the 3 patients needing a necrosectomy, plasma VWF:Ag transiently increased during severe necrotizing pancreatitis and then decreased after the necrosectomy (334% on day 1 → 708% on day 16 → 305% on day 22 in case 1, 277% on day 1 → 467% on day 3 → 134% on day 22 in case 3, 369% on day 1 → 949% on day 14 → 319% on day 17 in case 7). In one patient (case 4) with alcoholic liver cirrhosis, VWF:Ag decreased but thereafter remained high (489% on day 1 → 374% on day 3 → 396% on day 18). In the two non-survivors, VWF:Ag was markedly high on day 1 (645%) and day 2 (680%) in case 12, and on day 1 (443%) in case 13. The highest VWF:Ag within 1 or 2 days after admission was significantly increased in SAP patients compared with in the controls ($423 \pm 143\%$ versus $100 \pm 50\%$, $p < 0.001$) (Figure 2B), resulting in a significantly higher ratio of VWF:Ag to ADAMTS13:AC (20.8 ± 16.2 versus 1.0 ± 0.5, $p < 0.001$) (Figure 2C).

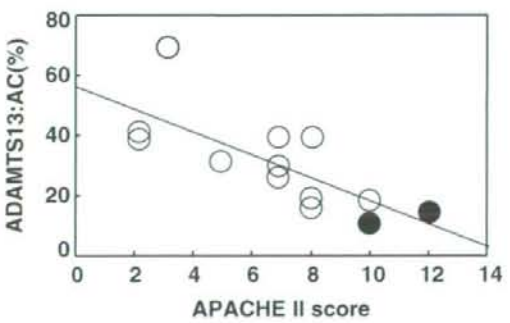

Figure 3. Correlation between plasma ADAMTS13:AC and APACHE II score on admission. Plasma ADAMTS13:AC was inversely correlated with the APACHE II score ($r = -0.750$, $p < 0.005$). Abbreviations: ADAMTS13:AC = ADAMTS13 activity.

UL-VWFM

UL-VWFM was detected in 7 cases between day 1 and day 17 after admission (Table I). Within 1 or 2 days after admission, ADAMTS13:AC was significantly lower while VWF:Ag was higher in patients with UL-VWFM than in those without (ADAMTS13:AC: $25 \pm 13\%$ versus $42 \pm 17\%$, $p < 0.05$; VWF:Ag: $481 \pm 137\%$ versus $332 \pm 93\%$, $p < 0.05$) (Figure 4A, B), resulting in a higher ratio of VWF:Ag to ADAMTS13:AC in UL-VWFM-positive patients compared with negative patients (25.2 ± 18.7 versus 9.1 ± 3.8, $p < 0.02$) (Figure 4C).

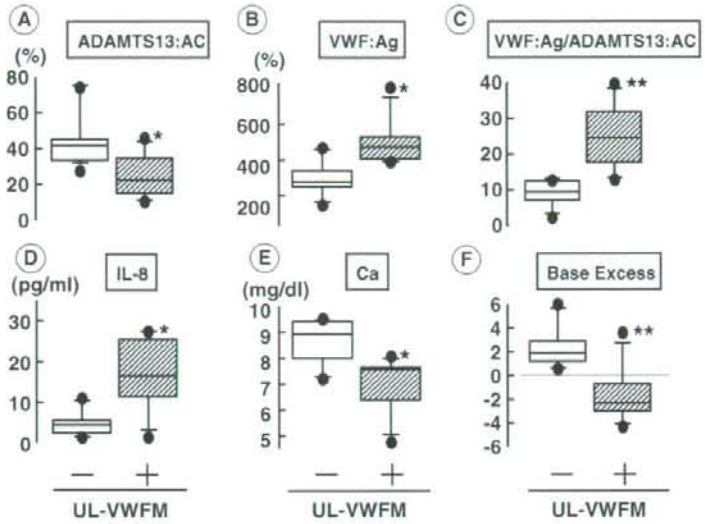

Figure 4. Clinical parameters of SAP patients with or without unusually large von Willebrand factor multimers. Within 1 or 2 days after admission, ADAMTS13:AC was significantly lower (A), and VWF:Ag was higher (B) in patients with UL-VWFM than those without, resulting in a higher VWF:Ag to ADAMTS13:AC ratio in UL-VWFM-positive patients compared with negative patients (C). IL-8 concentrations were higher (D), and the values of serum calcium concentrations (E) and base excess (F) were lower in patients with UL-VWFM than in those without. Abbreviations: SAP = severe acute pancreatitis; UL-VWFM = unusually large von Willebrand factor multimer; ADAMTS13:AC = ADAMTS13 activity; VWF:Ag = von Willebrand factor antigen; IL-8 = interleukin 8 concentration; Ca = serum calcium concentration. $*p<0.05$, and $**p<0.02$ significantly different between the two groups.

IL-8 concentrations were higher, and the values of serum calcium concentrations and base excess were lower in patients with UL-VWFM than in those without (IL-8: $15\pm9\%$ versus $3\pm3\%$, $p<0.05$; Ca: 7.2 ± 1.1 mg/dl versus 8.7 ± 0.9 mg/dl, $p<0.05$; base excess: -1.99 ± 2.56 versus 2.02 ± 1.96, $p<0.02$) (Figure 4D–F).

Figure 5 shows representative cases with and without UL-VWFM. Cases 12 and 13 were non-survivors with higher SOFA scores describing MOF

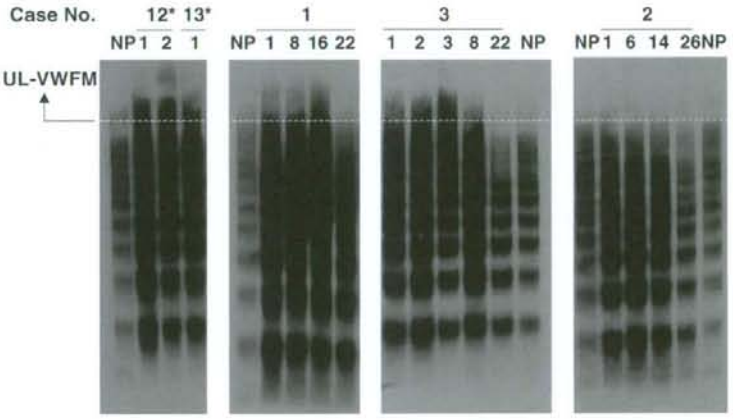

Figure 5. Representative cases with and without unusually large von Willebrand factor multimers (UL-VWFM) in patients with severe acute pancreatitis (SAP). In the two non-survivors, UL-VWFM was detected on days 1 and 2 in case 12 and on day 1 in case 13. Among survivors, case 1, who had SAP and an APACHE II score of 10 on day 1, had detectable UL-VWFM on days 1, 8, and 16 that became undetectable on day 22 after a necrosectomy on day 7. In case 3, whose APACHE II score was 8 on day 1, UL-VWFM was detected on days 1, 2, 3, and 8, but became negative on day 22 after necrosectomy was performed on day 2. In contrast, in case 2, whose APACHE II score was 5 on day 1, the UL-VWFM was undetectable from day 1 to day 26. *Cases 12 and 13 were non-survivors. Abbreviation: NP = normal control plasma.

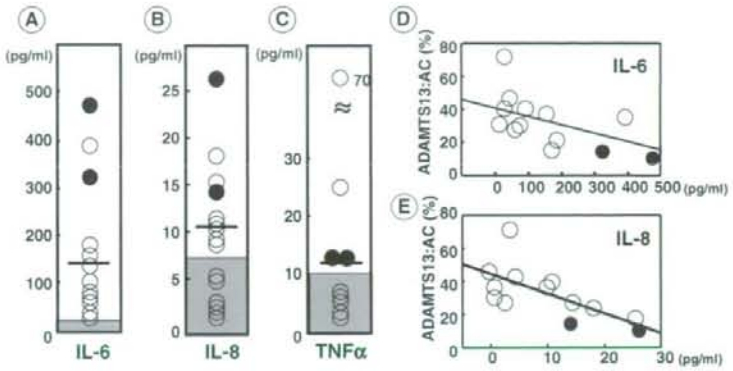

Figure 6. Plasma cytokine concentrations and their correlation with plasma ADAMTS13 activity in patients with severe acute pancreatitis (SAP). Open circles indicate survivors, and closed circles denote non-survivors. The shaded area shows the normal range. Plasma concentrations of interleukin 6 (IL-6) and interleukin 8 (IL-8) within 1 or 2 days after admission were significantly higher in patients with SAP than in healthy controls (A and B), and these values tended to be much higher in the two non-survivors than in the survivors. Plasma tumor necrosis factor-α (TNF-α) concentrations were higher in the four patients with SAP (C). Plasma ADAMTS13:AC was inversely correlated with plasma concentrations of IL-6 ($r = -0.556$, $p < 0.05$)(D) and IL-8 ($r = -0.659$, $p < 0.02$)(E). Abbreviations: ADAMTS13:AC = ADAMTS13 activity.

whose APACHE II scores on day 1 were 10 and 12, respectively. UL-VWFM was detected on days 1 and 2 in case 12 and on day 1 in case 13. In case 1 with MOF and an APACHE II score of 10 on day 1, UL-VWFM was detected on days 1, 8, and 16, but became undetectable on day 22 after a necrosectomy was performed on day 7. In case 3 with an APACHE II score of 8 on day 1, UL-VWFM was detected on days 1, 2, 3, and 8, but became negative on day 22 after a necrosectomy was performed on day 2. In contrast, in case 2 with an APACHE II score of 5 on day 1, UL-VWFM was undetectable between days 1 and 26.

Plasma cytokine concentrations

Plasma concentrations of IL-6 and IL-8 within 1 or 2 days after admission were significantly higher in SAP patients than in healthy controls (IL-6: 159 ± 167 pg/ml versus <7.8 pg/ml, $p < 0.001$; IL-8: 11.7 ± 9.0 pg/ml versus <7.8 pg/ml, $p < 0.01$) (Figure 6A, B), and these values tended to be much higher in the two non-survivors than in survivors. Plasma TNF-α concentrations were higher in the four patients with SAP (Figure 6C). Plasma ADAMTS13:AC was negatively correlated with plasma concentrations of IL-6 ($r = -0.556$, $p < 0.05$) (Figure 6D) and IL-8 ($r = -0.659$, $p < 0.02$) (Figure 6E).

Discussion

Several markers, including biochemical data, scoring systems, and imaging techniques, have been recommended in order accurately to identify patients who are likely to develop SAP. However, these markers are not always useful in evaluating the severity of acute pancreatitis in the early stage [4–6,39]. In the present study, within 1 or 2 days after admission, plasma ADAMTS13:AC decreased significantly (Figure 1 and 2) while VWF:Ag increased markedly, resulting in 20-fold higher VWF:Ag to ADAMTS13:AC ratios in patients with SAP compared with those in healthy controls (Figure 2). In particular, in the two non-survivors, ADAMTS13:AC was the lowest, and VWF:Ag was the highest, resulting in the highest VWF:Ag to ADAMTS13:AC ratio (Figure 2). Additionally, ADAMTS13:AC was lower in patients with higher SOFA scores describing MOF than in those with lower SOFA scores, and was inversely correlated with the APACHE II score (Figure 3), an early prognostic marker for SAP. These results suggest that decreased ADAMTS13:AC together with increased VWF:Ag is closely associated with the severity of acute pancreatitis and the development of MOF, and may be an additional early prognostic indicator in patients with early SAP.

It is known that acute pancreatitis impairs pancreatic and systemic microcirculation, which is a key pathological process in SAP development [9]. The initial insult in acute pancreatitis is the intracellular activation of proteases, but most studies have focused on changes in blood coagulation and the fibrinolytic system in SAP pathogenesis [7]. Our SAP patients, however, had no apparent signs of disseminated intravascular coagulation, although mild thrombocytopenia was observed in some patients (Table I). Deficiencies in ADAMTS13 increase plasma UL-VWFM levels and lead to platelet aggregation and/or thrombi formation under

conditions of high shear stress, resulting in TTP [12,14–16]. In earlier studies it has been reported that acute pancreatitis complicates the course of TTP [8], but recent reports indicate that some acute pancreatitis may precede acute episodes of TTP [19–26]. We showed that decreased ADAMTS13:AC during the early phase gradually increased in the recovery phase of survivors, but further decreased in non-survivors (Figure 1). With the exception of patients needing a necrosectomy, VWF:Ag gradually decreased in the survivors but remained high in the non-survivors. In addition, we could detect UL-VWFM during the early period in half of the SAP patients whose ADAMTS13:AC was remarkably low and VWF:Ag was high, resulting in a significantly higher ratio of VWF:Ag to ADAMTS13:AC than in UL-VWFM-negative patients (Figure 4A–C). Furthermore, UL-VWFM-positive patients had high concentrations of plasma IL-8 and low values of serum calcium concentrations and base excess compared to UL-VWFM-negative patients (Figure 4D–F). These results indicate that the appearance of UL-VWFM is closely related to decreased ADAMTS13:AC and the severity of SAP. Pathological conditions including cytokinemia, lower calcium concentrations, and acidosis seen in UL-VWFM-positive patients are considered to be factors that correlate with the severity of SAP [4–6]. Remarkably, our representative cases showed apparent UL-VWFM in the early phase in two non-survivors with higher SOFA scores describing MOF (cases 12 and 13) (Figure 5). Similarly, in a survivor with a high APACHE II score and MOF (case 1), UL-VWFM was detected at admission but became undetectable in the recovery phase (Figure 5). These results suggest that the presence or absence of UL-VWFM correlated with the changes in SAP severity together with the progression to MOF during the course of the disease. Therefore, the imbalance between reduced ADAMTS13:AC and increased VWF:Ag not only might be a prognostic marker for SAP but might also be a causative factor by enhancing thrombogenesis, leading to microcirculatory disturbances in the intrapancreatic and systemic circulation in patients with SAP. Olsen demonstrated that platelet thrombi are deposited in pancreatic arterioles in patients with acute pancreatitis [8], and in experimental studies using a rat model of severe acute pancreatitis, microcirculatory disorders are not confined to the pancreas but can also be found in multiple organs including the colon, liver, and lung from the early stage to 48 h onward [7], which may support our hypothesis. However, it will be necessary to demonstrate the presence of platelet microthrombi in SAP patients whose ADAMTS13:AC is extremely low.

It is still unclear why ADAMTS13 decreases in SAP. We could not detect the inhibitor against ADAMTS13. One possible explanation involves the consumption of ADAMTS13 to degrade a large amount of UL-VWFM. UL-VWFM may be overproduced from damaged endothelial cells after pancreatic injury, enhanced leukocyte-endothelial interactions [40], and/or inflammatory cytokines including IL-8 and TNF-α, which may stimulate UL-VWFM release from endothelial cells [41]. Interestingly, we found a negative correlation between decreased ADAMTS13:AC and increased concentrations of IL-8 and IL-6, which inhibit the action of ADAMTS13 *in vitro* [41]. Recently, it was reported that inflammation-associated ADAMTS13 deficiency promotes the formation of UL-VWFM [42]. Therefore, inflammatory cytokines, which are thought to be prognostic factors for SAP [43], may cause the extremely low activity of plasma ADAMTS13 together with the increased production of UL-VWFM in SAP. Another explanation might be the decreased production of ADAMTS13 in pancreatic tissue, as reduced ADAMTS13 production was reported in experimental acute hepatic failure [44]. We demonstrated that ADAMTS13 was produced exclusively in hepatic stellate cells [18], and might also be produced in stellate cells in pancreatic tissue.

In summary, the imbalance of markedly decreased ADAMTS13:AC and increased UL-VWFM might contribute to SAP pathogenesis and the development of SAP through enhanced thrombogenesis. ADAMTS13:AC and its related parameters may be one of the most promising markers to assess major complications and to determine disease prognosis in the early stages of SAP. In addition, these predictive factors might lead to new strategies for the treatment of this disease.

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Ticlopidine- and clopidogrel-associated thrombotic thrombocytopenic purpura (TTP): review of clinical, laboratory, epidemiological, and pharmacovigilance findings (1989–2008)

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Thrombotic thrombocytopenic purpura (TTP) is a fulminant disease characterized by platelet aggregates, thrombocytopenia, renal insufficiency, neurologic changes, and mechanical injury to erythrocytes. Most idiopathic cases of TTP are characterized by a deficiency of ADAMTS13 (a disintegrin and metalloprotease, with thrombospondin-1-like domains) metalloprotease activity. Ironically, use of anti-platelet agents, the thienopyridine derivates clopidogrel and ticlopidine, is associated with drug induced TTP. Data were abstracted from a systematic review of English-language literature for thienopyridine-associated TTP identified in MEDLINE, EMBASE, the public website of the Food and Drug Administration, and abstracts from national scientific conferences from 1991 to April 2008. Ticlopidine and clopidogrel are the two most common drugs associated with TTP in FDA safety databases. Epidemiological studies identify recent initiation of anti-platelet agents as the most common risk factor associated with risks of developing TTP. Laboratory studies indicate that most cases of thienopyridine-associated TTP involve an antibody to ADAMTS13 metalloprotease, present with severe thrombocytopenia, and respond to therapeutic plasma exchange (TPE); a minority of thienopyridine-associated TTP presents with severe renal insufficiency, involves direct endothelial cell damage, and is less responsive to TPE. The evaluation of this potentially fatal drug toxicity can serve as a template for future efforts to comprehensively characterize other severe adverse drug reactions.

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KEYWORDS: drug-associated TTP; epidemiology; ADAMTS13

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Thrombotic thrombocytopenic purpura (TTP) is a microvascular occlusive disorder characterized by systemic or intrarenal aggregation of platelets, leading to thrombocytopenia and mechanical injury to erythrocytes.[1] Conditions and factors associated with TTP include organ transplantation, infectious diseases, and drugs.[2] The most common drugs reported to the Food and Drug Administration (FDA) in association with TTP are the thienopyridine-derivative anti-platelet agents, ticlopidine and clopidogrel.[3,4] Before 1999, ticlopidine was widely used for prevention of cerebrovascular, cardiovascular, and peripheral vascular complications and following coronary artery stent procedures.[5] Since 2000, owing to concerns over ticlopidine-associated agranulocytosis, clinicians switched to clopidogrel in these settings.[5–9] Herein, we summarize the clinical, laboratory, and epidemiological information on thienopyridine-associated TTP.

PHARMACOLOGY

Ticlopidine and clopidogrel, thienopyridine derivatives that inhibit platelet aggregation,[5] differ structurally by a carboxymethyl side group. Animal studies and *in vitro* laboratory studies indicate that ticlopidine, but not clopidogrel, is associated with bone marrow toxicity.[5] As all clopidogrel metabolites contain the carboxymethyl side group, the two drugs have no common metabolites.[10] Ticlopidine and clopidogrel are administered orally, requiring hepatic breakdown to an active metabolite to achieve *in vivo* activity. The major therapeutic target of the thienopyridines is one of the adenosine diphosphate receptor types on human platelets, $P2Y_{12}$. Blockade of this receptor impairs adenosine diphosphate-induced platelet aggregation and decreases the propensity for arterial thrombosis.