

Antibody Inhibitors to von Willebrand Factor Metalloproteinase and Increased Binding of von Willebrand Factor to Platelets in Ticlopidine-Associated Thrombotic Thrombocytopenic Purpura

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Background: Thrombotic thrombocytopenic purpura (TTP) affects 1 in 1600 to 1 in 5000 patients who receive ticlopidine, but little is known about the pathogenesis of this complication.

Objective: To investigate whether von Willebrand factor (vWF), which has been associated with idiopathic TTP, is involved in the pathogenesis of ticlopidine-associated TTP.

Design: Case series.

Setting: Three tertiary care, university-affiliated medical centers.

Patients: Seven patients who developed TTP 2 to 7 weeks after initiation of ticlopidine therapy. Controls were 7 consecutive patients without thrombocytopenia who had been receiving ticlopidine for 3 to 5 weeks and 10 randomly selected hospitalized patients.

Measurements: Platelet-bound vWF in patients' EDTA-anticoagulated whole blood samples; vWF proteinase activity in patients' plasma samples; inhibitory activity of IgG isolated from patients' plasma samples against the proteinase from the controls' plasma samples; and vWF multimeric patterns in patients' EDTA-anticoagulated plasma samples.

Results: Binding of vWF to single platelets was increased in the three patients tested during the most thrombocytopenic phase of TTP episodes. Initial plasma samples from all seven patients lacked the largest vWF multimers and were severely deficient in vWF metalloproteinase. IgG molecules, isolated from plasma samples of five patients, inhibited metalloproteinase in plasma samples from the controls. In patients examined, these abnormalities resolved upon the remission that accompanied plasma exchange and discontinuation of ticlopidine therapy.

Conclusion: In the patients who developed ticlopidine-associated TTP, autoantibodies to the vWF metalloproteinase were formed; this led to the same type of vWF abnormalities observed in patients with idiopathic acute TTP. The findings suggest that failure to process large and unusually large vWF multimers in vivo caused binding of vWF to platelets, systemic platelet thrombosis, and TTP.

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Ticlopidine, a potent antiplatelet agent used to maintain patency after coronary artery stenting and to prevent strokes in high-risk persons (1), has been associated with thrombotic thrombocytopenic purpura (TTP) (2-5).

Thrombotic thrombocytopenic purpura, first described by Moschcowitz (6), is characterized by extensive platelet thrombi in the arterioles and capillaries. Abnormalities in von Willebrand factor multimers, including the presence of unusually large multimers and disappearance of the large multimers found in normal plasma, have been detected in many cases of the disease (7, 8). Furthermore, von Willebrand factor is abundant in the thrombi of patients with TTP (9), and flow cytometric studies have demonstrated that the factor is bound to platelets in the circulation of these patients during the most thrombocytopenic phase of the disease (10).

The von Willebrand factor, a glycoprotein critical in mediating platelet deposition at sites of vessel injury, is synthesized and secreted by endothelial cells as a disulfide-linked polymer composed of a 2050-amino acid monomer (11). Upon release into the circulation, it is cleaved by a plasma metalloproteinase in a shear-dependent manner (11) at the peptide bond between tyrosine-842 and methionine-843 (12). This cleavage decreases the size of the von Willebrand factor polymer, generates a series of multimers found in normal plasma, and produces dimers of 176-kD and 140-kD fragments (11). In the absence of the proteinase, large and unusually large von Willebrand factor multimers accumulate in the plasma. When unfolded by shear stress (13), these multimers exhibit an increased capacity to support platelet aggregation (14). Indeed, a deficiency of the proteinase has been reported in idiopathic TTP (15, 16).

We investigated whether von Willebrand factor is involved in ticlopidine-associated TTP.

Methods

Patients

Seven consecutive patients who developed TTP after initiation of ticlopidine therapy and were treated at the participating institutions from 1 January

1996 to 31 December 1998 were investigated. The criteria for the diagnosis of TTP were those described elsewhere (10, 16).

We also determined proteinase activity in 17 controls: 7 consecutive, unselected patients without thrombocytopenia (age range, 62 to 81 years; 5 men and 2 women) who donated blood samples at routine follow-up examinations after 3 to 5 weeks of ticlopidine therapy prescribed for cardiac stents, and 10 randomly selected hospitalized patients not taking ticlopidine.

Blood samples were obtained by venipuncture or at the time of plasmapheresis. The investigational protocol was approved by the institutional review boards of the participating centers.

von Willebrand Factor Studies

Platelet-bound von Willebrand factor, von Willebrand factor multimers, von Willebrand factor-cleaving metalloproteinase activity, and the inhibitory activity of IgG to the von Willebrand factor-cleaving metalloproteinase were measured as described elsewhere (10, 16). The von Willebrand factor bound to single platelets in EDTA-anticoagulated whole-blood samples was quantified by flow cytometry. Proteinase activity was expressed as a percentage of that in the pooled normal plasma control.

Results

The initial clinical and laboratory findings of the patients are summarized in the **Table**. The duration of ticlopidine therapy before diagnosis of TTP ranged from 2 to 7 weeks (median, 3 weeks). None of the patients had a history of autoimmune disorders, and none were receiving penicillins, antineoplastic chemotherapy, or oral contraceptives before onset of the disease. In all patients, remission occurred after ticlopidine therapy was discontinued and daily plasma exchange was instituted. The median number of plasma exchanges received by the patients was 10 (range, 5 to 30). None of the patients had relapse after plasma exchange was discontinued.

von Willebrand Factor Binding to Single Platelets

Binding of von Willebrand factor to platelets was studied in patients 1 to 3; the test was not available for the other 4 patients. Platelet-bound von Willebrand factor was 7.5, 4.5, and 4.5 arbitrary units, respectively (normal value < 2.1 arbitrary units) in the initial blood samples; these values returned to normal when patients received plasma exchange and platelet counts increased.

von Willebrand Factor Multimers

In all seven patients, the largest multimers, which are found in normal plasma, were missing in the

initial plasma samples. For patient 1, von Willebrand factor multimeric patterns in three of seven subsequent plasma samples were abnormal; one sample lacked the largest von Willebrand factor multimers, and two contained unusually large multimers.

The initial plasma sample (obtained on day 1) for patient 3 was missing the largest multimers. The next two samples (obtained on days 2 and 4) contained unusually large multimers. The multimers were normal in the subsequent two samples (obtained on days 8 and 9). For patients 1, 2, and 6, plasma samples obtained upon remission were available for investigation; all samples showed normal multimeric patterns.

von Willebrand Factor Metalloproteinase Activity

For patient 1, only plasma samples obtained on days 4 to 6 after admission (when his platelet counts were $77 \times 10^9/L$, $70 \times 10^9/L$, and $76 \times 10^9/L$) were available for the study. These samples contained 28%, 17%, and 14%, respectively, of the proteinase activity found in plasma from normal controls.

Proteinase activity was 0% in the initial plasma samples of patients 2 to 4 and 7%, 12%, and 4%, respectively, in patients 5 to 7. However, the plasma samples of these three patients were obtained from the plasmapheresis bags in amounts of 200 to 250 mL during the initial plasma exchanges. For patient 2, the protease activity increased to 100% on day 3, when the platelet count was $260 \times 10^9/L$. For patient 3, protease activity increased to 6%, 10%, 81%, and 77%, respectively, on days 2, 4, 8, and 9, when platelet counts were $25 \times 10^9/L$, $130 \times 10^9/L$, $280 \times 10^9/L$, and $325 \times 10^8/L$. Plasma proteinase levels in patient 5 increased to 23% and 55% on days 4 and 6, respectively, when platelet counts were $140 \times 10^9/L$ and $180 \times 10^9/L$. A plasma sample obtained from patient 6 on day 5, when the platelet count had increased to $277 \times 10^9/L$, contained 94% proteinase activity.

The mean (\pm SD) plasma proteinase activity in the 7 controls receiving ticlopidine for 3 to 5 weeks was $114\% \pm 36\%$, which did not differ from the activity in the 10 randomly selected controls who were not treated with ticlopidine ($97\% \pm 17\%$). In a previous study (16), 74 randomly selected patients without TTP had proteinase activity of $103\% \pm 23\%$.

Inhibitors of von Willebrand Factor Proteinase

To explore the causes of proteinase deficiency, the initial plasma sample of patient 2 was mixed with normal control plasma after treatment at 56 °C for 30 minutes. The von Willebrand factor metalloproteinase activity in the mixture was suppressed to 23% of the activity found in a control mixture of heated normal pooled plasma and normal control plasma.

Plasma samples from patients 3 to 7 were suffi-

Table. Clinical and Laboratory Findings*

Patient	Ethnicity	Sex, Age	Indication for Ticlopidine	Duration of Ticlopidine Therapy	Neurologic or Other Manifestations	Fever	Hematuria	Hemoglobin Level	Microangiopathic Hemolysis	Platelet Count
1	African-American	M, 56	Stent†	2	Confusion Hemiparesis	No	Yes	92	Yes	9
2	White	F, 57	Stent§	7	Headache Syncope	No	Yes	97	Yes	6
3	White	F, 49	Stent§	3	Abdominal pain Nausea, vomiting	Yes	Yes	100	Yes	9
4	African-American	M, 42	Stent§	3	Lethargy	No	Yes	140	Yes	8
5	White	F, 89	Transient ischemic attack	4	Confusion	Yes	Yes	75	Yes	13
6	White	F, 65	Myocardial infarction	2	Confusion Aphasia Hemiparesis	Yes	Yes	84	Yes	6
7	White	F, 79	Retinal embolism	4	None	No	Yes	89	Yes	4

* CAD = coronary artery disease; F = female; M = male; NA = not available; vWf = von Willebrand factor.

† Renal arterial stent.

‡ Plasma sample obtained on day 6.

§ Coronary arterial stent.

|| Baseline creatinine level was 185.6 $\mu\text{mol/L}$.

cient in volume for studies to determine whether their immunoglobulins inhibited the proteinase. The IgG isolated from patient 3 on day 1 exhibited a concentration-dependent inhibition of proteinase activity in normal control plasma. The concentration of the IgG molecules required to inhibit 50% of the protease activity in the mixture (IC_{50}) was 2.2 mg/mL. The IgG isolated from the same patient on day 9 was not inhibitory.

The IC_{50} of the IgG isolated from initial plasma samples of patients 4 to 7 was 5.5, 2.2, 4.4, and 2.2 mg/mL, respectively. In tests comparing susceptibility to inhibition, von Willebrand factor metalloproteinase in plasma from the normal controls and that in the plasma samples from controls who received ticlopidine were equally sensitive to inhibition by IgG isolated from patients with ticlopidine-associated TTP (data not shown). The inhibitory activity of IgG was abolished when it was incubated with antibodies to IgG Fab (data not shown).

Discussion

In two series of single-episode and intermittent idiopathic TTP (15, 16), inhibition of plasma von Willebrand factor proteinase by IgG autoantibodies was found to be characteristic. In support of a role of von Willebrand factor proteinase deficiency in the

pathogenesis of platelet thrombosis, the deficiency was not observed in persons who did not have the disease. Furthermore, shear stress was found to increase the capacity of von Willebrand factor to support platelet aggregation (16).

We now describe seven patients with ticlopidine-associated TTP who also had severely decreased levels of von Willebrand factor proteinase 2 to 7 weeks after initiation of ticlopidine therapy. The durations of ticlopidine therapy before the diagnosis of TTP are similar to the 2 to 12 weeks observed in 98 cases of TTP in a recently described series (17). The deficiency in our patients resolved after ticlopidine therapy was discontinued and plasmapheresis was instituted. The deficiency was not observed in randomly selected patients who had been receiving ticlopidine for similar durations but did not develop the disease.

The absence or severe reduction of von Willebrand factor metalloproteinase was accompanied by binding of von Willebrand factor to platelets. Concurrently, the large von Willebrand factor multimers were missing. The level of von Willebrand factor proteinase activity required to prevent or decrease binding of von Willebrand factor to platelets and thrombosis was low (approximately 10% to 15%). Thus, even a slight increase in the proteinase activity was sufficient to suppress the values of platelet-bound von Willebrand factor. At this low level of

Table—Continued

Lactate Dehydrogenase Level	Creatinine Level	Plasma-phereses Performed	Pred-nisone	Comorbid Conditions	Other Medications	Increased Platelet-Bound vWf	Decreased Large vWf Multimers	vWf Proteinase Deficiency	Inhibitors of vWf Proteinase
IU/L	$\mu\text{mol/L}$	<i>n</i>							
5370	79.6	30	Yes	CAD Hypertension	Aspirin Lisinopril Metoprolol succinate	Yes	Yes	Yes‡	No‡
1863	88.4	13	Yes	CAD	Metoprolol succinate Lovastatin Isosorbide dinitrate Alprazolam Metoprolol tartrate	Yes	Yes	Yes	Yes
2436	238.7	11	Yes	CAD	Aspirin Atenolol	Yes	Yes	Yes	Yes
2862	318.2	10	No	Hypertension Diabetes	Aspirin Conjugated estrogen Metoprolol tartrate Isosorbide mononitrate Atorvastatin	NA	Yes	Yes	Yes
700	106.1	6	No	Atrial fibrillation Congestive heart failure	Warfarin Furosemide Digoxin	NA	Yes	Yes	Yes
6123	61.9	5	No	CAD	Aspirin Propranolol hydrochloride Omeprazole Amoxicillin	NA	Yes	Yes	Yes
314	565.8	9	Yes	CAD Chronic renal failure	Lorazepam Omeprazole Metoprolol tartrate	NA	Yes	Yes	Yes

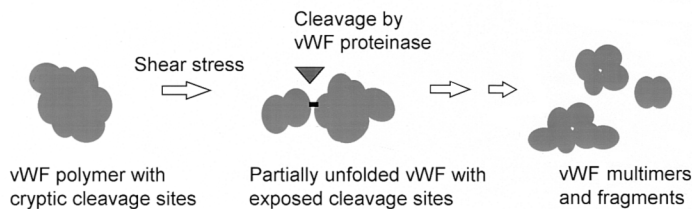
proteinase activity, von Willebrand factor proteolysis remained defective. This explained the presence of unusually large von Willebrand factor multimers in the plasma. The decrease in von Willebrand factor platelet binding in response to an increasing proteinase level was also reflected in the elevated platelet counts. When von Willebrand factor metalloproteinase activity normalized, the multimers also returned to a normal pattern.

Among the seven patients, six had initial samples available for mixing studies and all six were positive for inhibitors to the von Willebrand factor metalloproteinase. Immunoglobulin G was isolated from five patients and inhibited proteinase activity in all samples. The presence of IgG inhibitors suggests that in susceptible patients, ticlopidine or its metabolites may induce the formation of autoantibodies against von Willebrand factor metalloproteinase. This is analogous to the emergence of warm-reactive autoantibodies against red-cell Rh components in patients treated with the antihypertensive agent α -methyldopa (18). It is also plausible that ticlopidine or its metabolites may interact with the metalloproteinase to form a neoantigen that elicits antibody formation. As evidence against this latter possibility, we did not detect a difference between the plasma of normal controls and the plasma of the controls who received ticlopidine with regard to their susceptibility to inhibition by IgG isolated from patients with

ticlopidine-associated TTP. Given the similarity in chemical structures between ticlopidine and clopidogrel, another thienopyridine derivative that has been approved for use as an antiplatelet agent (19), it will be important to determine whether clopidogrel is also associated with an immune response to the von Willebrand factor proteinase in susceptible patients.

Six of the patients recovered within 2 weeks after discontinuation of ticlopidine therapy. The other patient required 30 plasma exchanges before remission occurred. We speculate that when stimuli for antibody production are removed, antibody production diminishes or ceases. Furthermore, any antibodies that remain in the circulation would be removed during plasma exchange or neutralized by newly released proteinase molecules or the proteinase molecules present in the fresh frozen plasma given to the patients. This may explain why most patients recover quickly upon discontinuation of ticlopidine therapy plus initiation of plasma exchange. None of the patients had a relapse after achieving remission. However, because clinical disease occurs only when the proteinase activity is severely decreased, serial measurements of von Willebrand factor proteinase activity during follow-up examinations are needed to determine how long the immune-mediated proteinase deficiency persists.

Normal Circulation



TTP Circulation

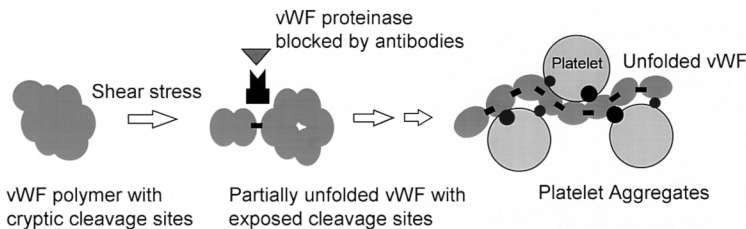


Figure. Proposed pathophysiology of ticlopidine-associated thrombotic thrombocytopenic purpura (TTP). In normal circulation (*top*), large and unusually large forms of von Willebrand factor (vWF), with cleavage sites exposed by shear stress, undergo proteolysis by von Willebrand factor proteinase. Proteolysis reduces the size of the von Willebrand factor multimers and prevents binding of von Willebrand factor to platelets. In the circulation of patients with TTP (*bottom*), proteolysis is blocked by antibodies to the proteinase. As a result, unfolded large and unusually large multimers of von Willebrand factor accumulate in the circulation and bind to platelet glycoproteins Ib/IX/V and IIb/IIIa. This causes platelet thrombi in capillaries and arterioles. Further increase in shear stress caused by microvascular thrombi results in additional unfolding of von Willebrand factor. This sets off a cycle of systemic arteriolar thrombosis characteristic of the disorder.

In summary, all seven patients with ticlopidine-associated TTP demonstrated the same type of von Willebrand factor abnormalities observed in patients with idiopathic TTP. The correlation between von Willebrand factor proteinase inhibition and the binding of von Willebrand factor to platelets *in vivo* further supports a role of the von Willebrand factor proteinase in preventing von Willebrand factor-platelet interaction in the circulation (**Figure**).

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References

1. Sharis PJ, Cannon CP, Loscalzo J. The antiplatelet effects of ticlopidine and clopidogrel. *Ann Intern Med.* 1998;129:394-405.
2. Page Y, Tardy B, Zeni F, Comtet C, Terrana R, Bertrand JC. Thrombotic thrombocytopenic purpura related to ticlopidine. *Lancet.* 1991;337:774-6.
3. Bennett CL, Weinberg PD, Rozenberg-Ben-Dror K, Yarnold PR, Kwaan HC, Green D. Thrombotic thrombocytopenic purpura associated with ticlopidine. A review of 60 cases. *Ann Intern Med.* 1998;128:541-4.
4. Bennett CL, Kiss JE, Weinbert PD, Pinevich AJ, Green D, Kwaan HC, et al. Thrombotic thrombocytopenic purpura after stenting and ticlopidine. *Lancet.* 1998;352:1036-7.
5. Steinhubl SR, Tan WA, Foody JM, Topol EJ. Incidence and clinical course of thrombotic thrombocytopenic purpura due to ticlopidine following coronary stenting. EPISTENT Investigators. Evaluation of Platelet IIb/IIIa Inhibitor for Stenting. *JAMA.* 1999;281:806-10.
6. Moschowitz E. Hyalin thrombosis of the terminal arterioles and capillaries: A hitherto undescribed disease. *Proceedings of the New York Pathology Society.* 1924;24:21-4.
7. Moake JL, Rudy CK, Troll JH, Weinstein MJ, Colannino NM, Azocar J, et al. Unusually large plasma factor VIII: von Willebrand factor multimers in chronic relapsing thrombotic thrombocytopenic purpura. *N Engl J Med.* 1982;307:1432-5.
8. Moake JL, McPherson PD. Abnormalities of von Willebrand factor multimers in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. *Am J Med.* 1989;87:9N-15N.
9. Asada Y, Sumiyoshi A, Hayashi T, Suzumiya J, Kaketani K. Immunohistochemistry of vascular lesions in thrombotic thrombocytopenic purpura, with special reference to factor VIII related antigen. *Thromb Res.* 1985;38:469-79.
10. Chow TW, Turner NA, Chintagumpala M, McPherson PD, Nolasco LH, Rice L, et al. Increased von Willebrand factor binding to platelets in single episode and recurrent types of thrombotic thrombocytopenic purpura. *Am J Hematol.* 1998;57:293-302.
11. Tsai HM. Physiologic cleavage of von Willebrand factor by a plasma protease is dependent on its conformation and requires calcium ion. *Blood.* 1996;87:4235-44.
12. Dent JA, Berkowitz SD, Ware J, Kasper CK, Ruggeri ZM. Identification of a cleavage site directing the immunochemical detection of molecular abnormalities in type IIa von Willebrand factor. *Proc Natl Acad Sci U S A.* 1990;87:6306-10.
13. Siedlecki CA, Lestini BJ, Kottke-Marchant KK, Eppell SJ, Wilson DL, Marchant RE. Shear-dependent changes in the three-dimensional structure of human von Willebrand factor. *Blood.* 1996;88:2939-50.
14. Tsai HM. Shear stress enhances the adhesive activity of large von Willebrand

- factor multimers [Abstract]. *Blood*. 1996;88:326a.
15. **Furlan M, Robles R, Galbusera M, Remuzzi G, Kyrle PA, Brenner B, et al.** von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. *N Engl J Med*. 1998;339:1578-84.
 16. **Tsai HM, Lian EC.** Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *N Engl J Med*. 1998;339:1585-94.
 17. **Bennett CL, Davidons CJ, Raisch DW, Weinberg PD, Bennett RH, Feldman MD.** Thrombotic thrombocytopenic purpura associated with ticlopidine in the setting of coronary artery stents and stroke prevention. *Arch Intern Med*. 1999;159:2524-8.
 18. **Carstairs KC, Breckenridge A, Dollery CT, Worledge SM.** Incidence of a positive direct Coombs test in patients on alpha-methyldopa. *Lancet*. 1966; 2:133-5.
 19. A randomized, blinded trial of clopidogrel versus aspirin in patients at high risk of ischaemic events (CAPRIE). CAPRIE Steering Committee. *Lancet*. 1996; 348:1329-39.

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