Binding of von Willebrand Factor to Collagen and Glycoprotein Ibα, But Not to Glycoprotein IIb/IIIa, Contributes to Ischemic Stroke in Mice—Brief Report

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Objective—To unravel crucial von Willebrand factor (VWF) interactions that are detrimental in stroke development.

Methods and Results—VWF<sup>−/−</sup> mice received gene transfer to express mutants of VWF defective either in binding to fibrillar collagen, glycoprotein (GP)Ibα or GPIIb/IIIa, and underwent 60 minutes of transient middle cerebral artery occlusion. In VWF<sup>−/−</sup> mice reconstituted with VWF mutants defective in binding to collagen or GPIbα, protection against stroke was sustained, whereas VWF lacking the GPIIb/IIIa binding site restored full susceptibility similar to normal VWF.

Conclusion—VWF-collagen and VWF-GPIbα (but not VWF-GPIIb/IIIa) interactions are instrumental in thrombus formation after transient middle cerebral artery occlusion, and their inhibition could be a promising target for stroke treatment.

Key Words: ischemic stroke ■ von Willebrand factor ■ gene transfer

Ischemic stroke is a leading cause of death and disability worldwide. A key event during cerebral ischemia and reperfusion is thrombus formation at the site of the injured endothelium. We and others have recently identified an important pathophysiologic role of von Willebrand factor (VWF) during ischemic stroke. VWF contains separate binding sites for collagen, platelet glycoprotein (GP)Ibα and GPIIb/IIIa, which participate in platelet adhesion and aggregation at vascular lesions. By binding to collagen via its A3 domain and to GP Ibα via its A1 domain, VWF recruits circulating platelets to the exposed subendothelium. In addition, together with fibrinogen, VWF supports subsequent platelet aggregation through interaction of its RGD sequence with platelet integrin GPIIb/IIIa. Because exact insights on the specific involvement of the different VWF interactions in stroke remained elusive, we addressed this question using VWF-deficient mice that were reconstituted with variants of VWF defective in either binding to collagen, GPIbα or GPIIb/IIIa.

Materials and Methods
VWF<sup>−/−</sup> and wild-type (WT) mice on a C57BL/6 background (8 to 10 weeks old) were used throughout the study. Cerebral ischemia was induced by inserting and advancing a standardized silicon rubber-coated 6.0 nylon monofilament via the right internal carotid artery to occlude the origin of the right middle cerebral artery (MCA) for 60 minutes. Twenty-four hours before transient MCA occlusion (tMCAO), VWF<sup>−/−</sup> mice were reconstituted with mutants of VWF defective in binding to either fibrillar collagen, GPIbα or GPIIb/IIIa, by hydrodynamic gene transfer. After 23 hours of reperfusion, infarct volumes, intracerebral thrombosis, and functional outcomes were determined. Detailed methods can be found in supplemental materials, available online at http://atvb.ahajournals.org.

Results
After gene transfer, expression levels of the different transgene-encoded VWF variants showed no significant differences (supplemental Figure IA). In accordance with extensive previous studies, all transgenic VWF mutants showed a multimer pattern comparable with transgene-encoded WT VWF (supplemental Figure IB) and had the expected binding characteristics to collagen and GPIb (supplemental Figure II).

At 23 hours after reperfusion, stroke sizes were determined. Infarct volumes in VWF<sup>−/−</sup> animals treated with vehicle were significantly reduced compared with WT animals treated with vehicle (46.0±28.8 mm<sup>3</sup> versus 93.0±25.2 mm<sup>3</sup>; P<0.05) (Figure 1A). Reconstitution of the plasma compartment of...
VWF−/− mice with WT VWF totally restored the susceptibility of VWF−/− animals to cerebral ischemia (mean infarct volume of 94.5±20.3 mm³; Figure 1A), reaffirming the critical role of plasma VWF in ischemic brain damage.1 Importantly, VWF−/− mice supplemented with mutant VWF deficient in binding to either collagen (A3-mutant) or GPIbα (A1-mutant) were still protected from stroke (infarct volumes of 42.8±31.8 mm³ and 43.1±26.3 mm³, respectively; P<0.05) (Figure 1A). Notably, VWF−/− mice reconstituted with VWF defective in binding GPIIb/IIIa (RGD-mutant) developed infarctions similar to controls (infarct volume of 102.2±30.9 mm³), indicating that the RGD-mutant could fully replace normal VWF. The differences in infarct volumes were also functionally relevant, because the Bederson score assessing global neurological function and the grip test, which specifically measures motor function and coordination, were significantly better in VWF−/− mice treated with vehicle and VWF−/− mice reconstituted with VWF defective in binding to fibrillar collagen or GPIbα (Figure 1B). These data strongly suggest a crucial role of the VWF-collagen and the VWF-GPlbα interaction but not the VWF-GPllb/Illa interaction in the pathophysiology of ischemic stroke.

To confirm that VWF deficiency reduces thrombus formation after tMCAO, we measured the amount of fibrin(ogen) in the infarcted hemispheres by immunoblot analysis and found considerably reduced fibrin(ogen) accumulation in the ischemic hemisphere of VWF−/− mice treated with vehicle and VWF−/− mice reconstituted with VWF defective in binding to fibrillar collagen or GPIbα compared with controls (Figure 2).

Discussion

The molecular mechanisms involved in ischemic brain injury are still unclear.2 Protection of VWF−/− mice from ischemic brain injury,1 together with larger stroke infarctions in AD-AMTS13−/− mice,2,8,9 suggested a decisive role of VWF. We here show that binding of VWF to both collagen and GPIb are mandatory steps in stroke development. This is not the case

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**Figure 1.** Brain infarct volumes and functional outcomes 24 hours after tMCAO. A, Representative 2,3,5-triphenyl tetrazolium chloride stains of 3 corresponding coronal brain sections at day 1 after 60 minutes tMCAO. Ischemic infarctions appear white (top). Brain infarct volumes as measured by planimetry at day 1 after tMCAO (bottom). B, Neurological Bederson score (left) and grip test score (right) as assessed at day 1 after tMCAO. Control groups include VWF−/− mice injected with vehicle (n=7), VWF−/− mice injected with vehicle (n=9), and VWF−/− mice injected with a vector encoding WT VWF (n=6). VWF−/− mice expressing VWF mutants include animals injected with a vector encoding VWF having a mutated A1 domain (n=8), A3 domain (n=8), or RGD sequence (n=7) with defective binding to GPIbα, collagen, and GPIIb/IIa, respectively. Data are expressed as mean±SD; *P<0.05 compared with VWF−/− mice injected with vehicle.

**Figure 2.** Fibrin(ogen) deposition 24 hours after transient middle cerebral artery occlusion. Accumulation of fibrin(ogen) in the infarcted (+) and contralateral (−) hemispheres 24 hours after tMCAO as analyzed by immunoblotting of the mouse groups indicated above.
for VWF-GPIIb/IIIa interactions, which is in line with the ineffectiveness of GPIIb/IIIa blockade in experimental and clinical stroke.\textsuperscript{10,11} In further support of our results, GPIb and downstream signaling via phospholipase D1 were indeed shown to be involved in experimental stroke.\textsuperscript{10,12}

A limitation of this study is the defective upregulation of endothelial P-selectin due to the lack of Weibel–Palade bodies in VWF\textsuperscript{−/−} mice. Evidence that P-selectin may mediate infarct severity is, however, limited,\textsuperscript{13} and a recent study even demonstrates no reduction of stroke volume in P-selectin\textsuperscript{−/−} mice.\textsuperscript{14} In accordance, our data also argue against an important role of endothelial P-selectin, because restoration of VWF deficiency in plasma is sufficient to reestablish full susceptibility of VWF\textsuperscript{−/−} mice to stroke.

In summary, in this study, we could identify the binding sites of VWF functionally relevant for stroke development. These insights provide compelling evidence that inhibition of VWF-mediated platelet adhesion may provide a novel therapeutic option in stroke management. It is encouraging that several antithrombotic compounds blocking VWF-collagen or VWF-GPIIb\textsuperscript{α} interactions are currently being developed,\textsuperscript{15,16} which could have beneficial effects in treatment of stroke. Further studies using ultrahigh-field MRI are necessary to disclose whether and in what time window inhibition of VWF-mediated platelet adhesion results in better cortical reperfusion, as one would expect. This has recently been shown for FXII\textsuperscript{−/−} mice after tMCAO, which are similarly protected.\textsuperscript{17}

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**Disclosures**

None.

**References**