Endothelial Cell–Derived von Willebrand Factor Is the Major Determinant That Mediates von Willebrand Factor–Dependent Acute Ischemic Stroke by Promoting Postischemic Thrombo-Inflammation

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Objective—von Willebrand factor (VWF), which is synthesized in endothelial cells and megakaryocytes, is known to worsen stroke outcome. In vitro studies suggest that platelet-derived VWF (Plt-VWF) is biochemically different from the endothelial cell–derived VWF (EC-VWF). However, little is known about relative contribution of different pools of VWF in stroke.

Approach and Results—Using bone marrow transplantation, we generated chimeric Plt-VWF mice, Plt-VWF mice that lack ADAMTS13 in platelets and plasma (Plt-VWF/*Adamts*13$^{-/-}$), and EC-VWF mice to determine relative contribution of different pools of VWF in stroke. In brain ischemia/reperfusion injury model, we found that infarct size and postischemic intracerebral thrombo-inflammation (fibrin(ogen) deposition, neutrophil infiltration, interleukin-1β, and tumor necrosis factor-α levels) within lesions were comparable between EC-VWF and wild-type mice. Infarct size and postischemic thrombo-inflammation were comparable between Plt-VWF and Plt-VWF/*Adamts*13$^{-/-}$ mice, but decreased compared with EC-VWF and wild-type mice ($P<0.05$) and increased compared with *Vwf*$^{-/-}$ mice ($P<0.05$). Susceptibility to $FeCl_3$ injury–induced carotid artery thrombosis was comparable between wild-type and EC-VWF mice, whereas Plt-VWF and Plt-VWF/*Adamts*13$^{-/-}$ mice exhibited defective thrombosis. Although most of the injured vessels did not occlude, slope over time showed that thrombus growth rate was increased in both Plt-VWF and Plt-VWF/*Adamts*13$^{-/-}$ mice compared with *Vwf*$^{-/-}$ mice ($P<0.05$), but decreased compared with wild-type or EC-VWF mice.

Conclusions—Plt-VWF, either in presence or absence of ADAMTS13, partially contributes to VWF-dependent injury and postischemic thrombo-inflammation after stroke. EC-VWF is the major determinant that mediates VWF-dependent ischemic stroke by promoting postischemic thrombo-inflammation. **(*Arterioscler Thromb Vasc Biol*. 2016;36:1829-1837. DOI: 10.1161/ATVBAHA.116.307660.)**

Key Words: brain ischemia/reperfusion injury ■ platelets ■ thrombosis ■ von Willebrand factor

Von Willebrand factor (VWF) is a large multimeric protein that provides the initial adhesive link between circulating platelets by binding to platelet GPIbα and sites of vascular injury by binding to components of extracellular matrix. VWF also serves as a carrier for coagulation Factor VIII in circulation, significantly prolonging its half-life.[1] VWF synthesis is limited to endothelial cells and megakaryocytes. It is stored as ultra large VWF (ULVWF) multimers or high molecular weight multimers in endothelial Weibel–Palade bodies and platelet α-granules.[2] On secretion in blood and under shear conditions, ULVWF multimers are cleaved by the protease ADAMTS13 (A disintegrin and metalloprotease with thrombospondin type I repeats-13) into the less active VWF multimers that support normal hemostasis and thrombosis.[3,4] A majority of VWF present in plasma is derived from endothelial cells through a constitutive secretory pathway or regulated secretion in response to secretagogues, such as histamine, thrombin, and tumor necrosis factor-α,[5] whereas ≈15% to 20% of VWF measured in plasma is released from activated platelets.[6]

VWF is implicated in hemostatic and thrombotic processes,[7,8] including deep vein thrombosis,[9] nonhemostatic processes, including tumor metastasis and angiogenesis,[10,11] and in thrombo-inflammatory processes, including myocardial infarction,[12,13] atherosclerosis,[14,15] and acute ischemic stroke.[16–18] However, little is known about the relative

Received on: April 7, 2016; final version accepted on: July 11, 2016.

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The online-only Data Supplement is available with this article at http://atvb.ahajournals.org/lookup/suppl/doi:10.1161/ATVBAHA.116.307660/-/DC1.

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Nonstandard Abbreviations and Acronyms	
ADAMTS13	a disintegrin and metalloprotease with thrombospondin type I repeats-13
EC-VWF	endothelial cell–derived VWF
Plt-VWF	platelet-derived VWF
ULVWF	ultra large von Willebrand factor
VWF	von Willebrand factor
WT	wild-type

contributions of platelet-derived VWF (Plt-VWF) versus endothelial cell–derived VWF (EC-VWF) in these conditions. Several in vitro studies suggest that there are biochemical and functional differences between Plt-VWF from megakaryocytic origin and plasma VWF from endothelial cell origin: (1) unlike ECVWF, Plt-VWF does not bind to factor VIII[19]; (2) a recent in vitro study suggested that Plt-VWF differs in glycosylation profile, particularly with a reduction in N-linked sialic acid expression when compared with EC-VWF.[20] Although platelets contain ADAMTS13, this study suggested that because of different glycosylation profile, Plt-VWF is resistant to ADAMTS13 cleavage and, therefore, enriched in ULVWF multimers.[20] In contrast, other in vitro studies have shown that Plt-derived ULVWF multimers are only observed when platelets are activated or lysed in presence of EDTA.[21,22] (3) Despite ULVWF, Plt-VWF was demonstrated to be less capable of binding to GPIbα.[23] Other studies suggested that Plt-VWF binds efficiently to activated αIIbβ3 and unfractionated heparin.[23,24] Although ≈15% to 20% of VWF measured in plasma is released from activated platelets,[6] aforementioned studies suggest that Plt-VWF may alone be sufficient to mediate thrombosis and stroke.

Herein, using reciprocal bone marrow (BM) transplantation, we generated the following strains of mice: (1) chimeric Plt-VWF mice that express VWF only in megakaryocytes, (2) chimeric Plt-VWF/*Adamts*13$^{-/-}$ mice that express VWF in megakaryocytes and are deficient for ADAMTS13 both in plasma and platelet, and (3) EC-VWF mice that express VWF only in endothelial cells. We found that Plt-VWF partially contributes to VWF-dependent cerebral injury and postischemic inflammation after acute ischemic stroke, but this alone is not sufficient to maintain hemostasis and thrombosis. Furthermore, we show that EC-VWF is the major determinant that mediates VWF-dependent acute ischemic stroke by promoting postischemic thrombo-inflammation.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Characterization of Chimeric Mice Expressing Plt-VWF and EC-Derived VWF

In the remainder of the article, (1) irradiated wild-type (WT) mice reconstituted with BM from WT donors (WT-BM→WT mice) are controls and are referred as WT mice (source of VWF: endothelial cells and platelets), (2) irradiated *Vwf*$^{-/-}$ mice reconstituted with BM from *Vwf*$^{-/-}$ donors (*Vwf*$^{-/-}$-BM→*Vwf*$^{-/-}$ mice) are referred as VWF-KO mice, (3) irradiated WT mice reconstituted with BM from *Vwf*$^{-/-}$ donors (*Vwf*$^{-/-}$-BM→WT mice) are referred as EC-VWF mice (express VWF in endothelial cells, but lack VWF in platelet), (4) irradiated *Vwf*$^{-/-}$ mice reconstituted with BM from WT donors (WT-BM→*Vwf*$^{-/-}$ mice) are referred as Plt-VWF mice (have platelet-derived VWF, but lack VWF in endothelial cells), and (5) irradiated *Vwf*$^{-/-}$*Adamts13*$^{-/-}$ mice reconstituted with BM from *Adamts13*$^{-/-}$ donors (*Adamts13*$^{-/-}$-BM→*Vwf*$^{-/-}$*Adamts13*$^{-/-}$ mice) are referred as Plt-VWF/*Adamts*13$^{-/-}$ mice (deficient for ADAMTS13 in plasma and platelet and lack VWF in endothelial cells; Figure 1A). Complete blood counts were comparable, suggesting that BM transplantation did not affect the number of BM-derived blood cells (Table I in the online-only Data Supplement). To confirm successful engraftment of BM, the relative content of VWF in plasma and platelets was quantified by ELISA. Plasma VWF levels were comparable between WT mice (103.3±4.4%, n=22) and EC-VWF mice (96.6±3.6%, n=22; Figure 1B). No detectable levels of plasma VWF were found in VWF-KO mice (n=18), whereas a minor amount of plasma VWF, although nonsignificant (Figure 1B), was found in Plt-VWF (11.3±1.8%, n=12) and Plt-VWF/*Adamts*13$^{-/-}$ mice (6.4±1.1%, n=20). Plt-VWF levels were comparable between WT, Plt-VWF mice, and Plt-VWF/*Adamts*13$^{-/-}$ mice (Figure 1C). No detectable level of Plt-VWF was found in VWF-KO or EC-VWF mice (Figure 1C).

EC-Derived VWF Is the Major Determinant That Contributes to VWF-Dependent Cerebral Ischemia/Reperfusion Injury

To define the relative importance of Plt-VWF versus EC-VWF pool in the pathophysiology of acute ischemic stroke, EC-VWF mice and Plt-VWF mice with controls (WT and VWF-KO) were subjected to 60 minutes of ischemia followed by 23 hours of reperfusion. We found that infarct volume and neurological outcome were comparable in WT mice (24.7±1.1%) and EC-VWF mice (21.7±1.6%; Figure 2A and 2B). Similar to previous reports,[16,17] VWF-KO mice exhibited decreased infarct volume (12.2±0.7%; $P<0.05$) and better neurological outcome when compared with either WT mice or EC-VWF mice (Figure 2A and 2B). Plt-VWF mice exhibited significantly decreased infarct volume (17.6±0.8%; $P<0.05$) and better neurological outcome when compared with either WT mice or EC-VWF mice, but increased infarct volume and worse neurological outcome when compared with VWF-KO mice (Figure 2A and 2B). These results suggest that Plt-VWF by itself partially contributes to cerebral ischemia/reperfusion injury, whereas EC-VWF is the major determinant.

Next, we determined whether Plt-VWF, in the absence of ADAMTS13 in plasma and platelets, is able to induce stroke outcome comparable to EC-VWF. Infarct volume and neurological outcome were comparable in Plt-VWF mice (17.6±0.8) and Plt-VWF/*Adamts*13$^{-/-}$ mice (18.6±1.4%), but significantly decreased when compared with WT mice (24.7±1.1%) or EC-VWF mice (21.7±1.6%; $P<0.05$; Figure 2A and B). This result suggests that Plt-VWF even in the absence of

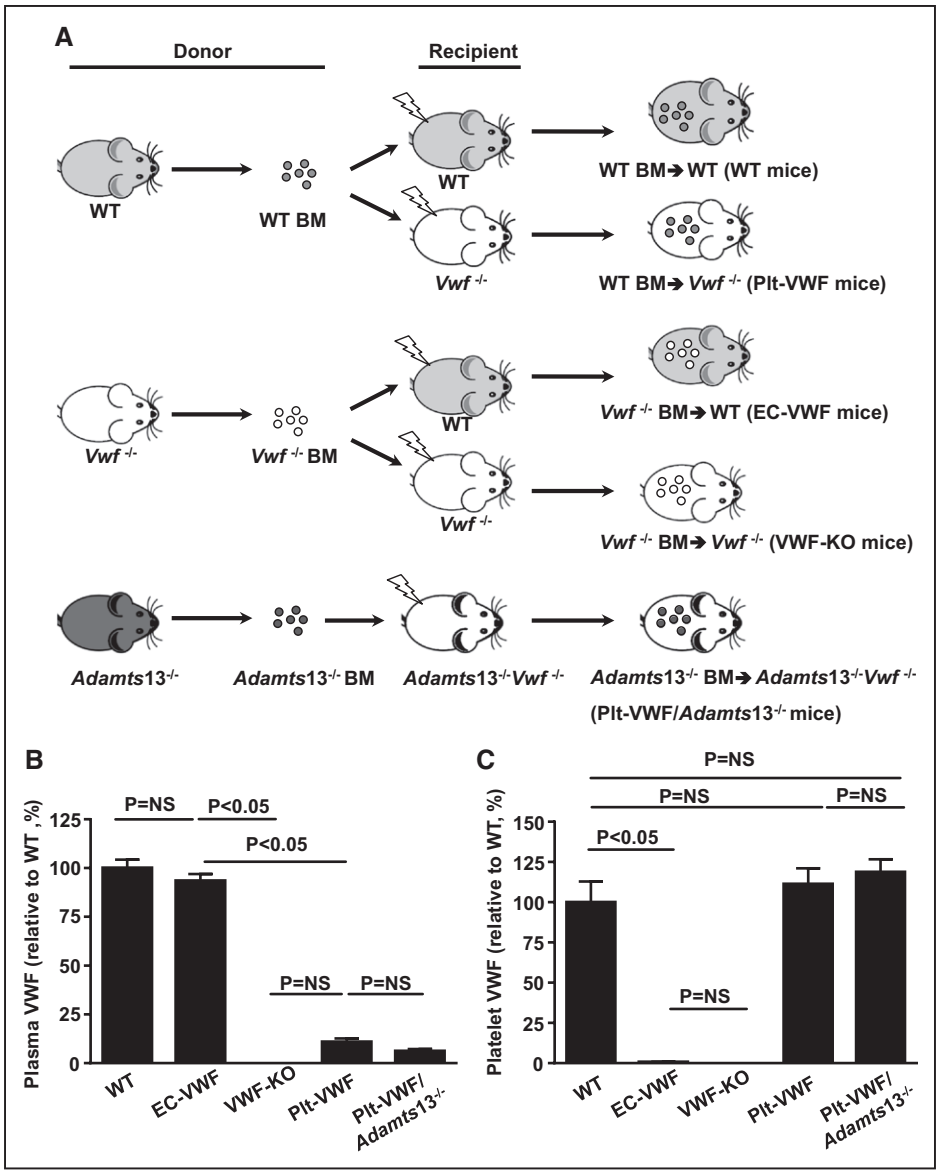

Figure 1. Generation and characterization of chimeric mice, which express von Willebrand factor (VWF) either in megakaryocytes or endothelial cells. **A,** Schematic representation of bone marrow (BM) transplantation protocol. Reciprocal BM from wild-type (WT) or *Vwf*$^{-/-}$ mice were transplanted into either WT or *Vwf*$^{-/-}$ recipient mice to generate 4 experimental groups of mice: (1) WT mice: source of VWF is platelets and endothelial cells, control; (2) platelet-derived VWF (Plt-VWF) mice: source of VWF is platelets; (3) endothelial cell–derived VWF (EC-VWF) mice: source of VWF is endothelial cells; (4) VWF-KO mice: lack VWF in platelets and endothelial cells. Additionally, BM from *Adamts*13$^{-/-}$ mice was transplanted into *Adamts*13$^{-/-}$*Vwf*$^{-/-}$ mice to generate Plt-VWF/*Adamts13*$^{-/-}$ mice that contain VWF in platelets, but are deficient for ADAMTS13 in both platelets and plasma. **B**, Plasma VWF levels. N=12 to 20 mice/group. **C**, Platelet VWF levels. N=8 to 12 mice/group. Data are presented as mean±SEM.

ADAMTS13 by itself was not sufficient to promote cerebral ischemia/reperfusion injury similar to EC-VWF. Laser Doppler flow measurements (Table II in the online-only Data Supplement) were similar among groups before, during, and after ischemia. To determine whether worse stroke outcome in WT mice was concomitant with reduced local cerebral blood flow (CBF), laser Doppler flowmetry was performed at different time points (0.5–4 hours). We found that local CBF was significantly decreased at 0.5, 1, 2, and 4 hours after reperfusion in EC-VWF mice (P<0.05 versus VWF-KO or Plt-VWF or Plt-VWF/*Adamts*13$^{-/-}$ mice; Figure 2C). Local CBF was significantly decreased at 1 and 2 hours after reperfusion in Plt-VWF and Plt-VWF/*Adamts*13$^{-/-}$ mice when compared with VWF-KO mice but significantly increased when compared with EC-VWF mice (Figure 2C).

Transfusion of High Number of WT Platelets Into Plt-VWF or *Adamts*13$^{-/-}$ Platelets Into Plt-VWF/*Adamts*13$^{-/-}$ Mice Does Not Aggravate Stroke Outcome

Next, we determined whether transfusion of high number of WT platelets (≈3.2×10^8) into Plt-VWF mice and *Adamts*13$^{-/-}$ platelets (≈3.2×10^8) into Plt-VWF/*Adamts*13$^{-/-}$ mice would result in stroke outcome similar to EC-VWF mice.

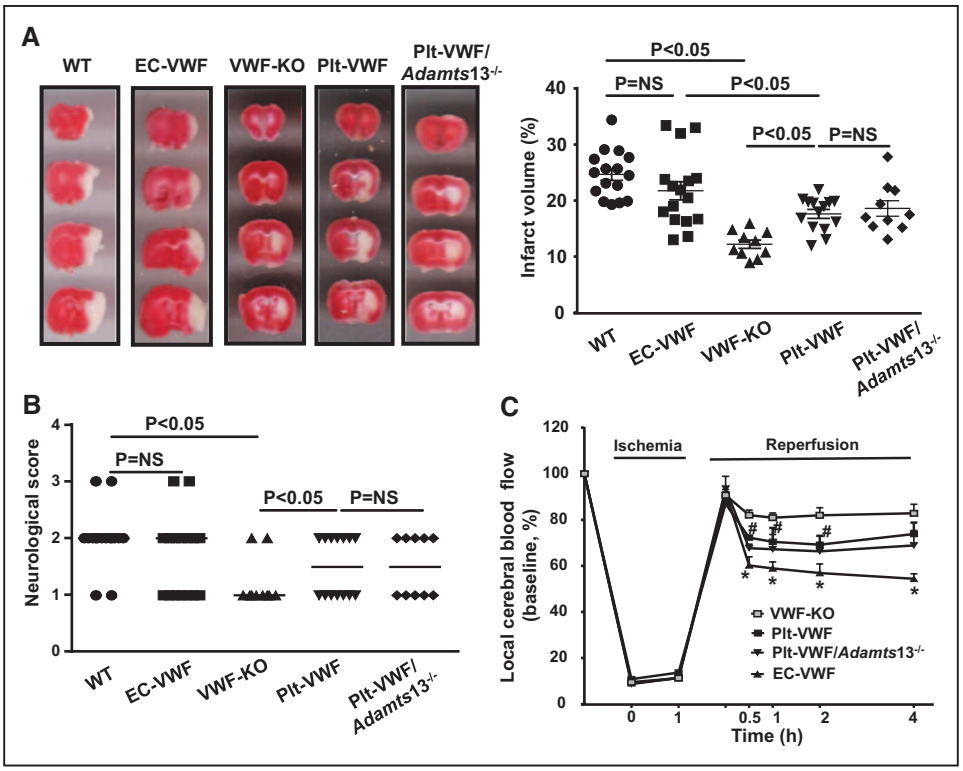

Figure 2. Stroke outcome in EC-VWF mice, but not in Plt-VWF mice, is comparable to WT mice. **A**, **Left**, Rrepresentative 2,3,5-triphenyltetrazolium chloride–stained serial coronal brain sections from one mouse after transient middle cerebral artery occlusion. Viable tissue is stained red, whereas infarcted area is unstained (white). **Right**, Corrected mean infarct volumes of each group as mean±SEM (n=10–16 mice/group). Number of mice is a pool of 2 independent experiments. **B**, Neurological score. Horizontal line depicts the median. Analysis of variance on ranks was applied to test for significant differences in the neurological score. **C**, Doppler flow measurements of local cerebral blood flow in the territory of the right middle cerebral artery at 0.5, 1, 2, and 4 hours of reperfusion (#*P*<0.05 versus VWF-KO mice, **P* versus VWF-KO mice, repeated measures analysis of variance [ANOVA]; N=8–9 mice/group). EC-VWF indicates endothelial cell–derived von Willebrand factor; Plt-VWF, platelet-derived von Willebrand factor; VWF, von Willebrand factor; and WT, wild-type.

Compared with EC-VWF mice, Plt-VWF mice and Plt-VWF/Adamts13$^{-/-}$ mice transfused with additional high number of platelets did not aggravate stroke outcome (Figure 3A and 3B). Quantification of VWF in the brain homogenates from the infarcted and surrounding regions revealed a marked decrease in local VWF antigen levels in both Plt-VWF and Plt-VWF/*Adamts*13$^{-/-}$ mice transfused with additional VWF+ platelets when compared with EC-VWF mice (Figure 3C). Together, these results suggest that although Plt-VWF contributes to partial brain injury after stroke, it is the EC-VWF that drives VWF-dependent ischemic stroke.

Postischemic Thrombo-Inflammation Was Comparable Between EC-VWF and WT Mice

Both thrombosis and inflammatory processes contribute to acute stroke. To quantify thrombosis in cerebral microvasculature, we measured intracerebral fibrin(ogen) deposition, whereas to quantify inflammation, we determined neutrophil infiltration and inflammatory cytokines (tumor necrosis factor-α and interleukin-1β levels) within the infarct and periinfarct region of the perfused brain after 60 minutes of tMCAO (transient middle cerebral artery occlusion) followed by 23 hours of reperfusion. We found that intracerebral fibrin(ogen) deposition, as determined by Western blot, was comparable between EC-VWF and WT mice but significantly increased when compared with either Plt-VWF mice or Plt-VWF/*Adamts*13$^{-/-}$ mice (Figure 4A). Fibrin(ogen) deposition was similar in Plt-VWF and Plt-VWF/*Adamts*13$^{-/-}$ mice but significantly increased when compared with VWF-KO mice (Figure 4A). Immunostained sections of the ischemic region revealed that neutrophil infiltration was similar in EC-VWF and WT mice but significantly increased when compared with either Plt-VWF or Plt-VWF/*Adamts*13$^{-/-}$ mice (P<0.05; Figure 4B). On the contrary, neutrophil infiltration within the ischemic region was comparable in Plt-VWF and Plt-VWF/*Adamts*13$^{-/-}$ mice and significantly increased when compared with VWF-KO mice (Figure 4B). Total leukocyte counts were similar among genotypes (Table I in the online-only Data Supplement). In line with these findings, ELISA experiments showed a significant increase in tumor necrosis factor-α and interleukin-1β levels in both WT and EC-VWF mice (P<0.05 versus VWF-KO or Plt-VWF or Plt-VWF/*Adamts*13$^{-/-}$ mice; Figure 4C and 4D). Together, these findings suggest that although Plt-VWF partially contributes to postischemic thrombo-inflammation, it is the EC-VWF that is the major determinant.

EC-VWF Is a Major Determinant for Occlusive Thrombus Formation

To test the hypothesis that EC-VWF is the major determinant that promotes cerebral thrombosis and, thereby contributes to

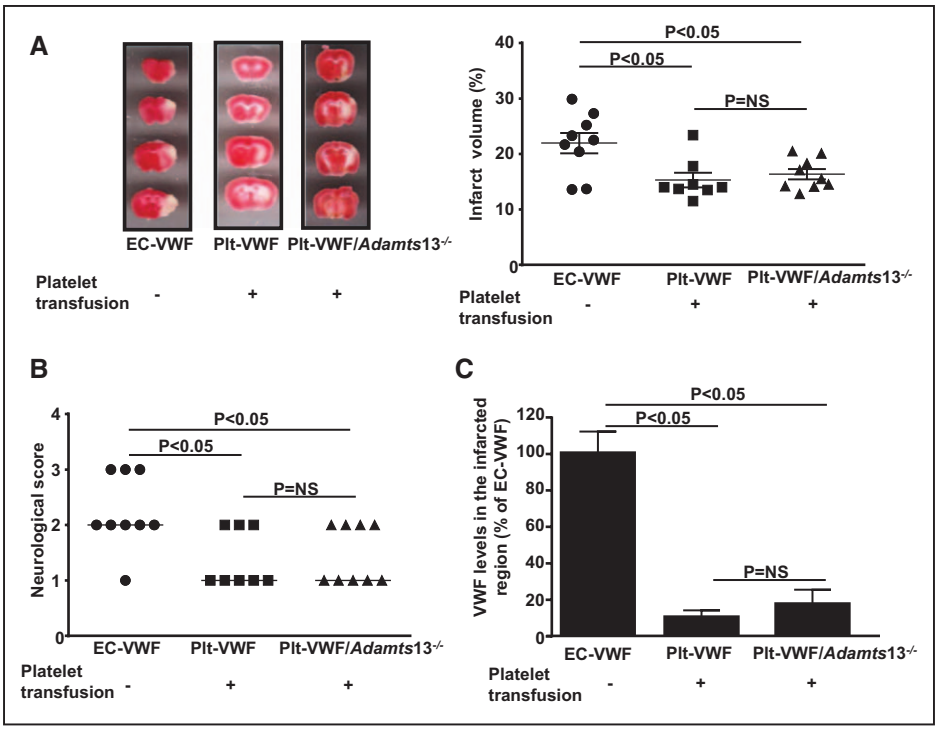

Figure 3. Transfusion of high number of WT platelets into Plt-VWF mice or *Adamts*13$^{-/-}$ platelets into Plt-VWF/*Adamts*13$^{-/-}$ mice does not aggravate stroke outcome. **A**, **Left**, Representative 2,3,5-triphenyl-tetrazolium chloride–stained serial coronal brain sections from one mouse after transient middle cerebral artery occlusion. **Right**, Corrected mean infarct volumes of each group as mean±SEM (n= 8–9 mice/group). **B,** Neurological score. Horizontal line depicts the median. **C**, Quantification of VWF in the tissue homogenates of infarcted and surrounding region by ELISA. Data are presented as mean±SEM (n=8–9 mice/group). EC-VWF indicates endothelial cell–derived von Willebrand factor; Plt-VWF, platelet-derived von Willebrand factor; VWF, von Willebrand factor; and WT, wild-type.

brain injury after acute stroke, we determined relative contribution of Plt-VWF versus EC-VWF to susceptibility to $FeCl_3$ injury–induced carotid artery thrombosis. All mice used were similar in age and weight to those used for stroke experiments. Using in vivo imaging, we found that the mean time to form first thrombus (≥100 μm) and the mean time to occlusion were similar in EC-VWF mice when compared with WT mice (Figure 5). The mean time to form the first thrombus was similar in Plt-VWF and Plt-VWF/*Adamts*13$^{-/-}$ mice when compared with VWF-KO mice but significantly prolonged when compared with EC-VWF or WT mice (Figure 5A). Similar to VWF-KO mice, none of the injured vessels occluded in Plt-VWF mice (Figure 5B). Interestingly, 23% of Plt-VWF/*Adamts*13$^{-/-}$ mice (4 out of 17) had occlusive thrombus. Although the mean time to occlusion was shortened, it was not significant when compared with Plt-VWF or VWF-KO mice (Figure 5B). In tail-transection bleeding assay, EC-VWF mice exhibited normal hemostasis, whereas Plt-VWF and Plt-VWF/*Adamts*13$^{-/-}$ mice exhibited defective hemostasis (Figure I in the online-only Data Supplement). Together, these results suggest that EC-VWF, but not Plt-VWF, is the major determinant for occlusive thrombus formation.

Both EC-VWF and Plt-VWF Modulate Thrombus Growth

Next, we determined whether Plt-VWF alone is able to support thrombus growth to a certain extent in the absence of EC-VWF. Using intravital microscopy, we measured thrombus growth kinetics and percentage occlusion (relative to vessel lumen) in injured vessels. Slope over time showed that the rate of thrombus growth was comparable in EC-VWF and WT mice and significantly increased when compared with VWF-KO mice (Figure 6A). Interestingly, rate of thrombus growth was also significantly increased in Plt-VWF mice when compared with VWF-KO mice (Figure 6A), but decreased when compared with EC-VWF or WT mice, suggesting that Plt-VWF partially contributes to thrombus growth. To our surprise, rate of thrombus growth was comparable between Plt-VWF and Plt-VWF/*Adamts*13$^{-/-}$ mice (Figure 6A). Consistent with these results, Plt-VWF mice or Plt-VWF/*Adamts*13$^{-/-}$ mice developed larger thrombi (≈85%–90% occlusion) when compared with VWF-KO mice (≈45%–50% occlusion) within 40 minutes after $FeCl_3$ injury (Figure 6B). Next, we determined whether Plt-VWF is able to support thrombus growth in another experimental model of thrombosis (laser injury–induced mesenteric artery thrombosis). We transfused 6×10^8 (≈50% platelets) from either WT or VWF-KO mice into VWF-KO mice. After laser injury, we quantified thrombus growth kinetics and % occlusion. Slope over time showed that rate of thrombus growth was increased in VWF-KO mice transfused with WT platelets when compared with VWF-KO mice transfused with VWF-KO platelets (Figure IIA in the online-only Data Supplement). Consistent with these results, VWF-KO mice transfused with WT platelets developed larger thrombi (≈60%–70% occlusion) when compared with VWF-KO mice

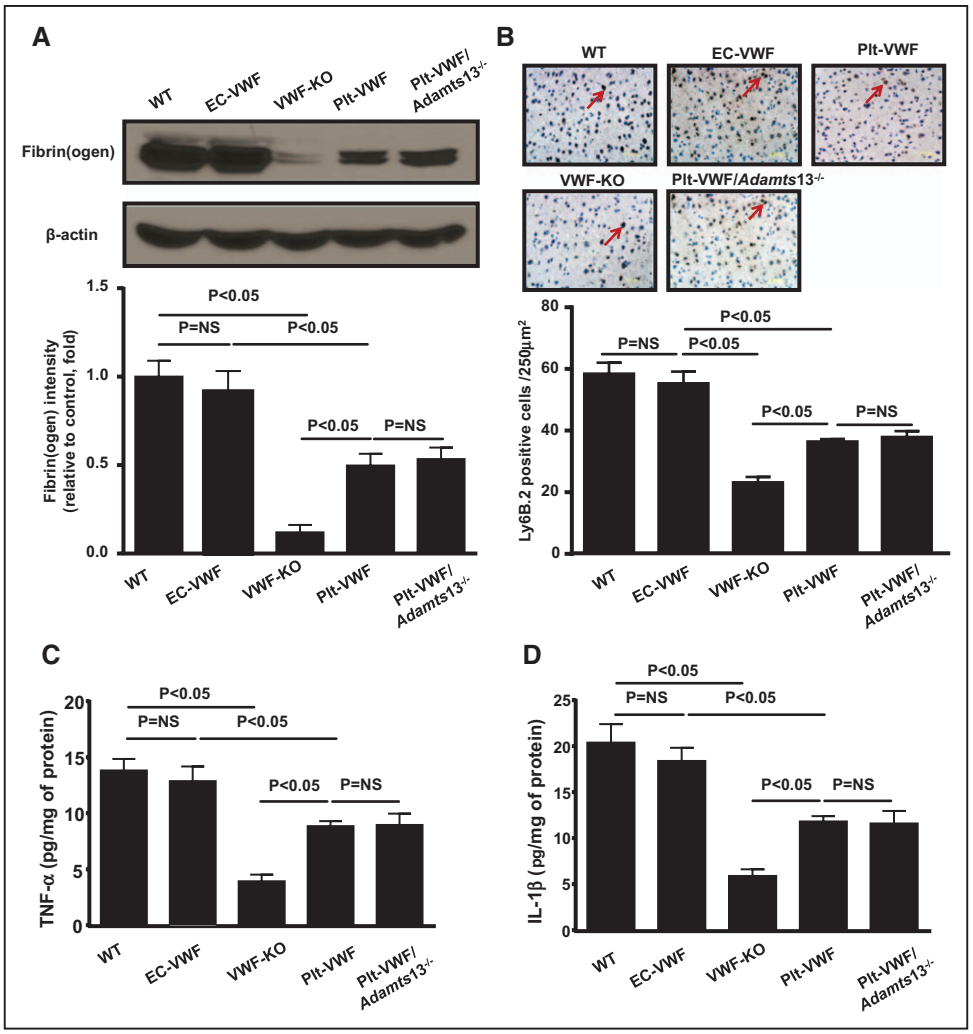

Figure 4. EC-VWF mice, but not Plt-VWF mice, exhibit thrombo-inflammation similar to WT mice after cerebral ischemia/reperfusion injury. **A, Top**, Representative immunoblots showing fibrin(ogen) accumulation in the tissue homogenates of infarcted and surrounding region. **Bottom**, Densitometric quantification (top bands) normalized to corresponding β-actin (loading control). Data are presented as mean±SEM (N=4–5 mice/group). **B, Top**, Representative coronal brain sections from each group stained for neutrophils (Ly6 B.2-positive cells stained as brown are indicated by arrows) and counterstained with hematoxylin (blue). The scale bar =50 μm. **Bottom**, Quantification. Mean for individual mouse was calculated from 4 coronal sections/mouse (separated by 100 μm). Data are presented as mean±SEM (n=5 mice/group). **C** and **D**, Quantification of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) levels by ELISA in brain homogenates prepared from the infarcted and surrounding areas after tMCAO (transient middle cerebral artery occlusion). Data are presented as mean±SEM (n=6 mice/group). EC-VWF indicates endothelial cell–derived von Willebrand factor; Plt-VWF, platelet-derived von Willebrand factor; VWF, von Willebrand factor; and WT, wild-type.

transfused with VWF-KO platelets (≈30%–40% occlusion; Figure IIB in the online-only Data Supplement). Together, these results suggest that Plt-VWF by itself partially contributes to transient thrombus growth, whereas EC-VWF is the major determinant.

Discussion

The results presented here shed mechanistic insights on the relative importance of different pools of VWF in the pathophysiology of acute ischemic stroke. The key findings are the following: (1) EC-VWF is the major determinant that mediates VWF-dependent cerebral ischemia/reperfusion injury by promoting thrombo-inflammation. (2) Plt-VWF, in the presence or absence of ADAMTS13, promotes transient nonocclusive thrombus growth and contributes partially to brain injury and postischemic thrombo-inflammation after cerebral ischemia/reperfusion injury.

Herein, we found that chimeric EC-VWF mice express normal VWF levels in the plasma and no detectable VWF in platelets. On the contrary, chimeric Plt-VWF mice or chimeric Plt-VWF/*Adamts*13$^{-/-}$ mice express normal VWF levels in platelets and only trace amount of VWF in the plasma. Most likely, the source of trace amount of VWF in the plasma of chimeric Plt-VWF mice or chimeric Plt-VWF/*Adamts*13$^{-/-}$ mice may be because of an artifact of platelet activation during the blood draw. Together, these results imply that origin of plasma VWF is endothelium and not quiescent circulating platelets.

Recently, Verhenne et al showed that chimeric Plt-VWF mice and chimeric EC-VWF mice had infarct size similar to WT mice in brain ischemia/reperfusion model, suggesting

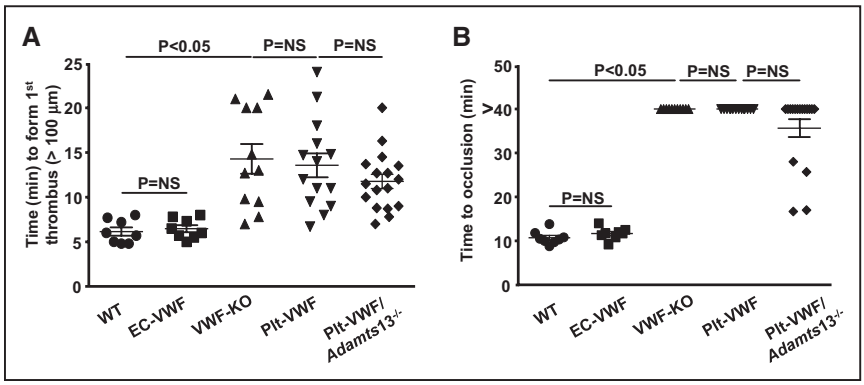

Figure 5. EC-derived VWF, but not Plt-derived VWF, alone is sufficient for formation of occlusive thrombus in $FeCl_3$ injury–induced carotid thrombosis model. **A,** Time to first thrombus formation. **B,** Mean time to complete occlusion of $FeCl_3$-injured carotid artery. Each dot represents single mouse. Occlusion time was compared by Fisher exact test. Data are presented as mean±SEM (N= 8–17 mice/group). EC indicates endothelial cell; EC-VWF, endothelial cell–derived von Willebrand factor; Plt, platelet; Plt-VWF, platelet-derived von Willebrand factor; VWF, von Willebrand factor; and WT, wild-type.

that VWF by itself, independent of its origin and overall local levels, is sufficient to mediate VWF-dependent stroke exacerbation.[25] Of note, this study did not quantify the overall local levels of VWF in the infarcted and surrounding region after stroke. Similar to the Verhenne et al study, we found that chimeric Plt-VWF mice exhibited significantly bigger infarcts compared with VWF-KO mice. However, in contrast to their study, we found that chimeric Plt-VWF mice infarcts were significantly smaller when compared either to chimeric EC-VWF mice or WT mice after cerebral ischemia/reperfusion injury. The discrepancy between these studies remains unclear but could be related to different experimental set-up used to induce cerebral ischemia/reperfusion injury. Of note, our studies were in progress when Verhenne et al published their findings.[25] We speculated that the transfusion of additional WT platelets (≈30%) in Plt-VWF mice might worsen stroke outcome similar to EC-VWF mice because of overall increased local levels of VWF released by activated platelets after injury. Surprisingly, we found that transfusion of additional high number of VWF+ platelets did not rescue the phenotype in either Plt-VWF or Plt-VWF/*Adamts*13$^{-/-}$ mice. Based on our findings, we suggest that although Plt-VWF could partially contribute to stroke exacerbation, it is the EC-VWF that drives VWF-dependent ischemic stroke. Additional studies will be required to determine whether increasing plasma VWF levels in the *Vwf*$^{-/-}$ mice similar to WT, independent of the source of VWF, will worsen stroke outcome comparable to WT mice.

It is well established that cerebral ischemia/reperfusion injury elicits a strong post-thrombo-inflammatory response that promotes brain tissue damage in the ischemic penumbra.[26] VWF is known to promote intracerebral thrombosis and postinflammatory response after acute stroke.[17,27] Herein, we found that chimeric EC-VWF mice exhibited increased intracerebral fibrin(ogen) deposition when compared with chimeric Plt-VWF mice, which was concomitant with lower local CBF as estimated by noninvasive laser Doppler flowmetry. This result suggests that EC-VWF present in the plasma most likely promotes cerebral thrombosis and, thereby, decreases local CBF at several isolated points in the cortical area. Additionally, compared with Plt-VWF, we found that EC-VWF significantly increases postischemic inflammatory response characterized by an increase in neutrophil infiltration and inflammatory cytokines, such as tumor necrosis factor-α and interleukin-1β within ischemic region.

The relative in vivo role of different pools of VWF in arterial thrombosis has not been studied in detail yet. We found that EC-VWF alone, but not Plt-VWF, was sufficient to form occlusive thrombus formation. Our findings are in accordance with previous studies done in chimeric mice[25] and pig models,[28] which demonstrate that EC-VWF present in the plasma is essential for development of arterial thrombosis. It is important to note that these studies did not determine whether Plt-VWF by itself contributes to thrombus growth. We found that although Plt-VWF is not able to support occlusive thrombus formation, it partially contributes to transient thrombus growth both in $FeCl_3$-induced carotid thrombosis and laser injury–induced mesenteric thrombosis models. Our findings are in agreement with previous in vitro studies, which suggested that Plt-VWF might contribute to platelet aggregation under flow conditions.[29,30]

Platelets contain ADAMTS13, and few studies have suggested that Plt-derived ULVWF multimers are only observed if platelets are activated and lysed in presence of EDTA.[21,22] In contrast, another in vitro study by Mcgrath et al suggested that Plt-VWF has different glycosylation profile and is, therefore, resistant to ADAMTS13 cleavage.[20] ULVWF multimers are considered hyperactive because they bind avidly to the extracellular matrix[31] and form high strength bonds with platelet glycoprotein GPIbα.[32] We speculated that presence of ULVWF multimers in platelets in absence of ADAMTS13 might be sufficient to support hemostasis and thrombosis. To answer this question, we transplanted irradiated *Vwf*$^{-/-}$*Adamts13*$^{-/-}$ mice with BM from *Adamts13*$^{-/-}$ donors, so that ULVWF released after platelet activation is not cleaved by ADAMTS13. We found that majority (75%) of chimeric Plt-VWF/Adamts13$^{-/-}$ mice exhibited defective thrombosis. In ≈25% chimeric Plt-VWF/*Adamts*13$^{-/-}$ mice, injured vessels had occlusive thrombus; however, the time to mean occlusion was significantly prolonged compared with WT or EC-VWF mice. We speculate that this could be because of a significant heterogeneity in platelet VWF levels, which has been observed in clinical studies with type 1 von Willebrand disease patients.[33] Finally, cerebral ischemia/reperfusion injury in chimeric Plt-VWF/*Adamts*13$^{-/-}$ mice was comparable to Plt-VWF mice, but significantly less when compared with EC-VWF mice. Results from these murine studies clearly suggest that under physiological conditions, it is the EC-VWF that drives brain injury after acute stroke most likely through platelet GPIbα.[18]

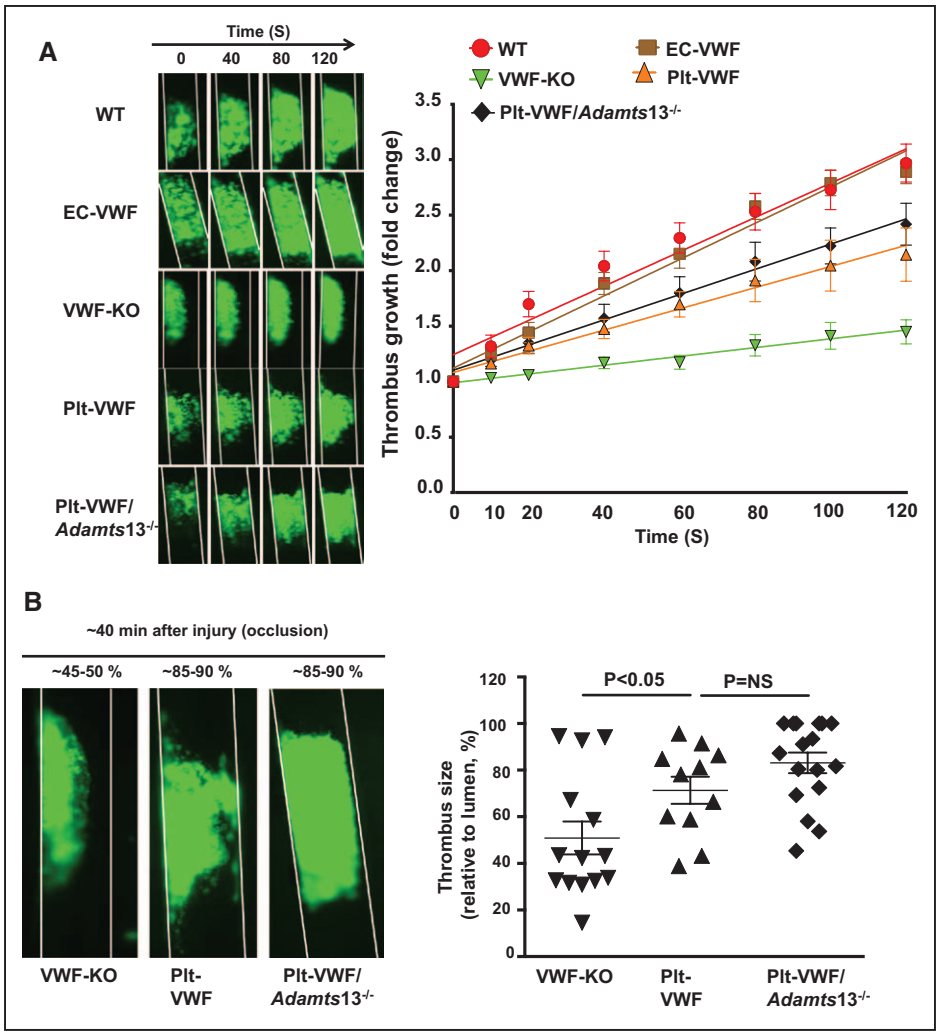

Figure 6. Although Plt-derived VWF partially contributes to thrombus growth, EC-derived VWF present in plasma is the driving factor that contributes to occlusive thrombus formation in injured vessel. **A**, **Left**, Representative microphotographs of thrombus growth in $FeCl_3$-injured carotid arteries as visualized by upright intravital microscopy. **Right**, Thrombus growth kinetics. The fold increase in diameter was calculated by dividing the diameter of the thrombus at time (*n*) by the diameter of the same thrombus at time (0) (defined as the time point at which the thrombus diameter first reached 100 μm). Slopes over time showed that the rate of thrombus growth were comparable in EC-VWF mice and WT mice. Rate of thrombus growth was significantly increased in Plt-VWF or Plt-VWF/*Adamts*13$^{-/-}$ mice when compared with VWF-KO mice, but decreased when compared with EC-VWF mice or WT mice. Data are presented as mean±SEM (N=8–17 mice/group). **B**, **Left**, Representative microphotographs depicting percentage occlusion ≈40 minutes after $FeCl_3$-induced injury. **Right**, Quantification. Platelets were labeled with calcein green. White lines delineate the arteries. Data are presented as mean±SEM (N=11–17 mice/group). EC indicates endothelial cell; EC-VWF, endothelial cell–derived von Willebrand factor; Plt, platelet; Plt-VWF, platelet-derived von Willebrand factor; VWF, von Willebrand factor; and WT, wild-type.

In conclusion, our results provide insights on the role of different pools of VWF in pathophysiology of acute stroke. This study further supports the notion that therapeutically targeting VWF may have the potential to reduce brain injury and improve outcomes in patients at high risk for stroke.

Sources of Funding

A.K. Chauhan lab is supported by grants from the National Heart, Lung and Blood Institute of the National Institutes of Health grants (R01 HL118246 and R01 HL118742) and by innovative grant 16IRG27490003 from American Heart Association.

Disclosures

None.

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Highlights

- von Willebrand factor (VWF) is synthesized in endothelial cells and megakaryocytes.
- Platelet-derived VWF partially contributes to VWF-dependent cerebral brain injury and postischemic inflammation after acute ischemic stroke, but alone is not sufficient to maintain hemostasis and thrombosis.
- Endothelial cell-derived VWF is the major determinant that mediates VWF-dependent ischemic stroke, hemostasis, and thrombosis in mice.