The role of glycoprotein Ibalpha and von Willebrand factor interaction in stroke development

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Summary
Ischaemic stroke is a devastating disease with limited treatment options due to numerous uncertainties regarding the underlying pathophysiology. The contribution of glycoprotein (GP)Ibα and von Willebrand factor (VWF) in stroke development has only recently been established in mice. Complete blockade of GP Ibα led to a significant reduction of infarct volumes in mice undergoing one hour of transient middle cerebral artery occlusion (tMCAO). High shear-induced changes in VWF confirmation are a prerequisite for VWF binding to collagen and GP Ibα expressed on platelets. Importantly, transgenic VWF⁻/⁻ mice were similarly protected against ischemic stroke after tMCAO, and hydrodynamic injection of a VWF-encoding plasmid restored VWF serum levels and the susceptibility towards stroke. Secreted VWF is rapidly cleaved by ADAMTS13. Accordingly, ADAMTS13 deficient mice developed larger infarction after tMCAO, while infusion of recombinant ADAMTS13 into wild-type mice was stroke-proective. In conclusion, there is compelling evidence that GP Ibα/VWF interactions and downstream signaling via phospholipase D1 (PLD1) provide new therapeutic targets in ischemic stroke.

Schlüsselwörter
Von-Willebrand-Faktor, Thrombusbildung, Schlaganfall

Zusammenfassung

Stroke is the second leading cause of death worldwide (21). Cerebral ischaemia due to
● arterial occlusion accounts for about 80% of strokes,
● the remaining 20% are caused by intracerebral haemorrhages (14).

Atrial fibrillation and high-grade carotid artery stenoses represent the major sources of cerebral thromboembolism (18,31). Thromboembolic occlusion of major or multiple smaller intracerebral arteries leads to focal impairment or cessation of the downstream blood flow which triggers a plethora of consecutive pathophysiological events in the brain (12, 30). Although the beneficial role of platelet aggregation inhibitors such as acetylsalicylic acid, dipyridamole, and the platelet P2Y12 receptor inhibitor clopidogrel in stroke prevention is well established (36), the role of platelets during the acute phase of ischaemic lesion development is less clear. It is still uncertain whether commonly used anti-platelet agents in acute stroke patients help to prevent thrombus growth within the brain microvasculature or whether their effect is restricted to diminishing recurrence of secondary thromboembolism (1).

In this review we summarize recent evidence suggesting that VWF and its interaction with the platelet receptor GP Ibα play a central role in initiating thrombus formation in the ischaemic brain.

Platelet GPIb-V-IX and ischaemic stroke

The initial tethering of platelets at sites of vascular injury is mediated by GPIb-V-IX, a structurally unique receptor complex exclusively expressed in platelets and megakaryocytes (35). GPIbα is indispensable for
platelet adhesion under conditions of high shear, found e.g. in stenosed arteries. Inhibition of the VWF-binding site of GPIbα with Fab fragments of the antibody p0p/B in wild-type mice abrogated platelet tethering and adhesion in a model of mechanically induced arterial thrombosis (24). Although such mice have prolonged tail bleeding times they do not suffer from spontaneous haemorrhage (20, 24). The central role of GPIbα in arterial thrombus formation was later confirmed and extended in a study showing that transgenic mice expressing GPIbα in which the extracellular domain was replaced by that of the human interleukin-4 receptor (GPIb-TG) are completely unable to produce intravascular thrombi (3). In the model of transient middle cerebral artery occlusion (tMCAO) in mice complete blockade of the VWF binding site of GPIbα by i.v. injection of 100 μg Fab fragments of p0p/B led to a significant reduction in ischaemic lesion volume (20). Importantly, delayed application of anti-GPIbα Fab 1 h after MCAO was still effective. Although tail bleeding times were elevated in anti-GPIbα Fab treated mice, no increase in intracerebral haemorrhages occurred as revealed by serial magnetic resonance imaging (MRI). Our study indicates that GPIbα is critically involved in the pathogenesis of focal ischaemic stroke, but not required to prevent bleeding at sites of ischaemia/reperfusion damage in the brain. In further support of a pathophysiological role of GPIbα allelic variants causing enhanced VWF/ GPIbα interactions were associated with an increased risk of ischaemic stroke in humans (22). The binding of GPIbα to the A1 domain of VWF is the principal interaction necessary for tethering platelets to the vessel wall at high shear flow conditions (>500 s⁻¹) (9), whereas this interaction may not be relevant at lower shear rates (28). GPIbα also binds thrombin, high molecular weight kininogen, coagulation factor XII (FXII), Mac-1 (an integrin expressed on neutrophils and monocytes (CD11b/CD18), and P-selectin (4).

Thus, multiple molecular GPIbα interactions could be involved in stroke development. Downstream of GPIbα phospholipase D1 (PLD1) plays a decisive role in the formation of stable thrombi (13). Platelets from Pld1⁻/⁻ mice displayed impaired αIibβ3 integrin activation in response to major agonists and defective GPIbα-dependent aggregate formation. Importantly, formation and stabilisation of platelet thrombi required PLD1 only under high shear conditions, but not under low or intermediate shear as in the venous system. The fact that Pld1⁻/⁻ mice were protected against focal cerebral ischaemia (13) further supports the notion that GPIbα/VWF interactions and downstream signalling via PLD1 are instrumental in ischaemic stroke.

**VWF-deficiency protects mice from ischaemic stroke**

To further proof that GPIbα-VWF interactions are critical for stroke development we also subjected VWF⁻/⁻ mice to tMCAO. 24 h after reperfusion, the infarct volumes in the mutant mice were reduced to ~60% of the infarct volumes in wild-type controls (19). Similar results were obtained in an independent study by Zhao and colleagues (37). The reduction in infarct size was functionally relevant, as established neurological scores assessing e.g. motor function and coordination were significantly better in VWF⁻/⁻ mice compared to controls. Reconstitution of plasma VWF by hydrodynamic gene transfer (23) restored the susceptibility of VWF⁻/⁻ mice to cerebral ischaemia (19).

These rescue experiments provide strong evidence that the protection conferred by VWF deficiency is specifically caused by the absence of plasma VWF. Since VWF⁻/⁻ mice completely lack Weibel-Palade bodies it appears that co-secretion of P-selectin is less important in ischaemic brain damage. It has already been well established that lack of VWF is associated with profound anti-thrombotic effects: In the ferric chloride injury model, thrombus formation in mesenterial vessels was significantly reduced in VWF⁻/⁻ mice (10, 11) and antibodies against VWF reversed cyclical flow reductions after experimental femoral or coronary artery stenosis (5, 34). VWF contains multiple binding sites allowing complex interactions, e.g. with collagen, GPIbα and GPIIb/IIIa (9). The availability of different VWF mutants lacking these specific binding epitopes (9, 23) will allow to further decipher stroke-relevant VWF signaling pathways. Importantly, increased VWF serum levels have been recognized as an independent stroke risk factor in patients (5).

"Disintegrin-like and metalloprotease with thrombospondin repeats' 13 (ADAMTS13) was discovered as a VWF-cleaving protease in human plasma in the mid 1990ties (17,33). ADAMTS13 rapidly cleaves ultralarge VWF after its release from Weibel-Palade bodies (reviewed in 26). By this mechanism thrombus formation is restricted to the site of vessel injury and surplus VWF is removed from the circulation to avoid uncontrolled clotting. Patients with thrombotic thrombocytopenic purpura (TTP) express ultralarge circulating VWF multimers in their plasma which are absent in healthy persons. Patients with TTP are prone to ischaemic stroke. TTP is mainly caused by autoantibodies against ADAMTS13. Experimental studies revealed an important role for ADAMTS13 in preventing excessive Weibel-Palade body secretion and regulating leukocyte adhesion and extravasation during inflammation (7).

The concept that VWF and ADAMTS13 play central roles in stroke development has recently been reinforced by observations in transgenic Adamts13⁻/⁻ mice. These mice developed significantly larger infarcts after 30 min of tMCAO than wild-type controls indicating that ADAMTS13 deficiency aggravates ischaemic brain damage (16,25). In full support of this notion intravenous application of ADAMTS13 to naïve mice provided partial protection from cerebral ischaemia after tMCAO (37).

**Conclusion**

There is now compelling evidence that endothelial cell/collagen/platelet interactions employing GPIbα and VWF are instrumental in reperfusion injury during focal cerebral ischaemia. Thus, GPIbα, VWF and PLD1 represent promising new targets for stroke prevention and treatment in the future.
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Conflict of interest
The authors declare no conflict of interest.

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