Platelets and thromboxane receptors: pivotal players in arteriogenesis

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This editorial refers to ‘Thromboxane A₂ induces blood flow recovery via platelet adhesion to ischaemic regions’ by H. Amano et al., pp. 509–521.

The reconstitution of oxygen and nutrient supply in cases of disturbed blood flow caused by tissue damage or vascular occlusion is driven by sprouting angiogenesis, arteriogenesis as an adaptation of pre-collateral vessels into collateral conduit vessels, and vasculogenesis characterized by invasion of circulating monocyctic and endothelial progenitor cells. To study these mechanisms, a mouse model is often used, in which ligation of the femoral artery causes interruption of blood flow in the hind leg and hypoxia in the distal tissue. To save the hind leg tissue, the body responds immediately by the stimulation of a collateral circulation that starts proximal to the ligation. In a healthy C57/BL6 mouse, the transformation of pre-existing narrow collaterals into arterial conduit vessels with a many-fold wider diameter restores the blood supply and tissue perfusion to the hind leg within 7–10 days. In the occluded hind leg model, arteriogenesis is usually accompanied by angiogenesis in the ischaemic muscle, which results in an increase in local microvascular density. This further facilitates blood distribution and oxygen diffusion to meet the increased oxygen demand of the injured tissue.

Haemostasis, as a defence system against tissue injury, prevents an excessive blood loss upon damage. However, the actors in haemostasis also provide substrate and many factors for a subsequent healing process that follows after injury. Fibrin acts as a temporary repair matrix, while the adherent and activated platelets release many factors, such as VEGF, SDF-1, PDGF, and TGF-β that stimulate formation of new blood vessels (angiogenesis), and differentiation and growth of tissue-resident stem and progenitor cells. Despite extensive proof for involvement of many coagulation factors and platelet-derived factors in angiogenesis during development and post-natal live, the evaluation of the contribution of the haemostasis system in arteriogenesis occurred only in few studies. However, the role of platelets in arteriogenesis regained attention very recently.

One of the factors that received attention was thromboxane A₂ (TXA₂) generated by platelets, which, although platelets usually showed an angiogenesis stimulatory effect, also can induce release of angiogenesis-inhibiting factors. In man, two isoforms of thromboxane receptors (TPs) are encountered, while in mouse only one isoform (TPα) is present. TXA₂ enhances contraction and platelet activation, while prostacyclin generated by the endothelium has an opposite effect. A recent study suggested a pro-regenerative role of TXA₂ signalling in mice.

In this issue, Amano and colleagues used a genetic approach to further investigate the effect of TXA₂/TP signalling on collateral formation and the angiogenic response in leg muscle after femoral occlusion. To this end, they used TP-deficient (TP−/−) mice in a C57/BL6 background and compared their response to arterial occlusion with that of WT and prostacyclin receptor-deficient (IP−/−) mice. The authors reported that after ligation of the femoral artery of TP−/− mice, arteriogenesis was delayed and angiogenesis in the muscle distal of the occlusion was reduced in comparison with WT animals, while IP−/− mice showed the opposite effect. This shows that TXA₂/TP signalling is required for optimal restoration of perfusion in ischaemic murine hind leg (Figure 1).

Using intravital microscopy, these authors observed that P-selectin was abundantly present on the surface of platelets that adhered along ischaemic vessels in WT and IP−/− mice. In contrast, this occurred hardly in TP−/− mice. Thus, TP signalling stimulates the expression of P-selectin. P-selectin binds to PSGL-1 on the endothelial cells, but a causal involvement of PSGL-1 in arteriogenesis has still to be proved. Anti-P-selectin IgG reduced platelet binding to endothelial cells and limited arteriogenesis in WT and IP−/− to the level as obtained in TP−/− mice. This underpins the importance of TP, but this does not exclude an indirect effect involving leucocytes. One would anticipate that the adherence and activation of platelets by TXA₂ might cause thrombosis, but apparently the platelets did cover the endothelium rather than that they aggregated in vessel-occluding thrombi. This is in line with the study by Chandraratne et al., who observed that transient platelet adhesion to the endothelium of collateral vessels was an important event during arteriogenesis. It depended on GPIbα, which probably interacted with endothelial von Willebrand factor. The importance of this interaction was underscored by the observation that platelet depletion and loss of GPIbα function resulted in poor reperfusion recovery of the hind leg.

Activated platelets can facilitate the endothelial binding and diapedesis of leucocytes, including monocytes and neutrophils.
Chandraratne et al. observed increased interaction between platelets and both monocytes and neutrophils, and an enhanced accumulation of leucocytes at one day after vascular occlusion. Amano et al. pointed to the pivotal role of bone marrow-derived platelets and CXCR4+/VEGFR1+ cells in participating in the TP-dependent stimulation of arteriogenesis, as transplantation of WT bone marrow into TP−/− mice restored the neovascularization of the hind leg. Although in vivo visualization studies of Amano et al. did not study platelet–leucocyte interactions in detail, they provided evidence pointing to the TP-dependent facilitation of the recruitment and accumulation of CXCR4+/VEGFR1+ cells into the affected area. This was further underpinned by the finding that anti-VEGF-A IgG and CXCR4 (SDF-1 receptor) IgG limited arteriogenic flow recovery in WT and IP−/− mice to the level of TP−/− mice. It has still to be established whether these cells regard monocytes or other progenitor cells, but in the context of present knowledge, a monocytic contribution seems likely.

The reduced release of VEGF and SDF-1 from platelets in freshly isolated platelet-rich plasma of TP−/− animals fits with stabilization of the platelet by the altered TP/IP balance towards IP signalling in TP−/− mice.

Figure 1 Involvement of platelets and TPs in arteriogenesis in mice. After femoral occlusion, altered shear forces and oxygen availability modulate endothelial functioning, after which platelets start adhering the endothelium of pre-existing collaterals and vessels that became ischaemic distal of the occlusion. These platelets expose P-selectin and can release many factors, including VEGF-A and SDF-1, but do not form occluding thrombi. In animals deficient of TP-α, platelet interaction and subsequent arteriogenesis is inhibited. Platelet binding requires GPIbα which interacts with endothelial vWF under high shear forces, and P-selectin, which can bind to PSGL1 on leucocytes and activated endothelium. Subsequently, these platelets interact and facilitate the binding and influx of VEGFR1- and CXCR4-bearing monocytes into the vessel wall. The invaded cells subsequently contribute in orchestrating the arteriogenic process.
The stimulation of the arteriogenesis and angiogenesis by TXA2b/TP signalling suggests that low-dose aspirin, which inhibits TXA2 generation, also may reduce neovascularization. In mice, excessive retinal neovascularization was indeed inhibited after administration of aspirin. After femoral ligation in rabbits, aspirin significantly inhibited collateral artery growth, while clopidogrel had a neutral effect. Do these findings have clinical consequences on the induction of new collaterals in man? The abundant reports on the effects of low-dose aspirin indicate an overall beneficial effect in cases of cardiovascular disease. But this does not exclude a negative effect on a subpopulation of patients with poor arteriogenesis. However, further interpretation in a clinical setting should take into account that two types of TP exist in man, as Amano et al. did acknowledge. TPα and TPβ display identical effects on IP3/PLCγ/Ca2++ signalling, but opposite effects on cAMP generation. In human endothelial cells, TPβ but not TPα expression is required for inhibition of VEGF-induced migration and angiogenesis.

Data on arteriogenesis are not available. Therefore, the study by Amano et al. may not give a conclusion about the effects of TXA2b/TP signalling in human arteriogenesis. But they do provide clarity about the contribution of TXA2b/TPα, which pinpoints—together with the study by Chandraratne et al.—the importance of platelets in stimulating arteriogenesis.

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**References**