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Reperfusion failure despite recanalization in stroke: New translational evidence

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Abstract

Current treatment for acute ischemic stroke aims at recanalizing the occluded blood vessel to reperfuse ischemic brain tissue. Clot removal can be achieved pharmacologically with a thrombolytic drug, such as recombinant tissue plasminogen activator, or with mechanical thrombectomy. However, reopening the occluded vessel does not guarantee full tissue reperfusion, which has been referred to as reperfusion failure. When it occurs, reperfusion failure significantly attenuates the beneficial effect of recanalization therapy and severely affects functional recovery of stroke patients. The mechanisms of reperfusion failure are somewhat complex and not fully understood. Briefly, after stroke, capillaries show stalls, constriction and luminal narrowing, being crowded with neutrophils, and fibrin–platelet deposits. Furthermore, after recanalization in stroke patients, a primary clot can break, dislodge, and occlude distal arterial branches further downstream. In this review, we highlight a rodent model that allows studying the pathophysiological mechanisms underlying reperfusion failure after stroke. We also describe the vascular and intravascular changes involved in reperfusion, which may provide relevant therapeutic targets for improving treatment of stroke patients.

Keywords

Stroke, ischemia, thrombin model, reperfusion, capillary stalls

Introduction

Stroke is a major global health problem in our society, particularly due to many people affected from lifelong disabilities.[1] In the majority of cases, stroke is ischemic, caused by a clot occluding an artery supplying the brain. Because of the occlusion, blood flow to the brain suddenly stops or severely diminishes in parts of the vascular system. In the infarct "core," flow reduction is most severe, while the "penumbra" surrounding the core receives residual blood supply, mainly via vascular collaterals. In the core, oxygen and glucose deprivation lead to irreversible necrotic cell death within minutes while in the penumbra, tissue remains viable for several hours.[2,3] Various endogenous mechanisms, including excitotoxicity, oxidative damage, and inflammation contribute to the progression of neuronal death.[4] Stroke research focused on developing neuroprotective drugs to reduce the extent of neuronal damage in the penumbra, but apart from recanalization therapies, no therapeutic agent has clearly demonstrated neuroprotection and reliable preservation of neurological functions in the clinical setting.[5] One reason for this problem could be that after stroke, damage is not restricted to neurons only, but affects vascular cells as well, which so far was largely neglected.[6] Recovery of vascular function and full restoration of blood supply after stroke are likely to be critical prerequisites for neuroplasticity and favorable clinical outcome.[7]

The mainstay of contemporary acute ischemic stroke treatment is timely removal of the occlusive clot with intravenous thrombolysis and endovascular thrombectomy.[8,9]

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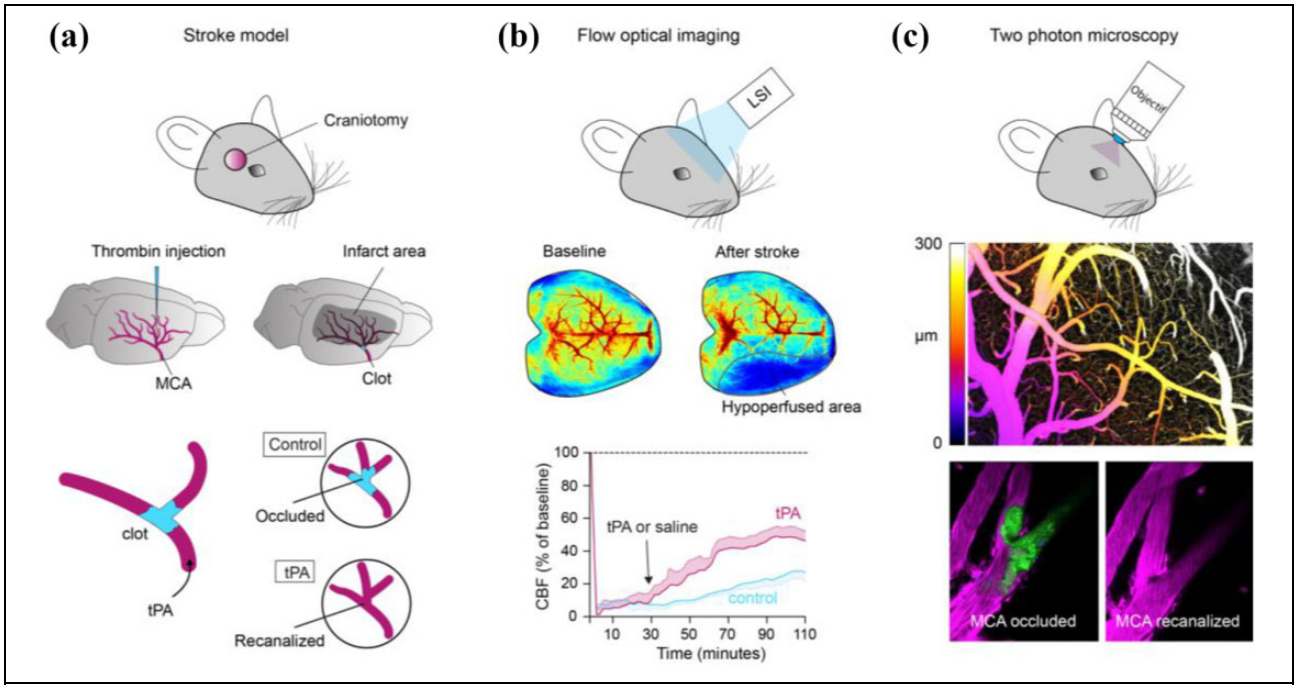

Figure 1. Thrombin model of stroke to study reperfusion failure: (a) schematic drawing of craniotomy (magenta oval) and thrombin injection site leading to clot formation and cortical infarction. On the lower part, schematic drawing of the MCA occluded after control treatment and recanalized after tPA thrombolysis. (b) Cortical blood flow during stroke and reperfusion are analyzed through the intact skull using LSI. Middle: Representative imaging of brain perfusion at baseline and after stroke. Lower: LSI analysis of cerebral blood flow within the MCA region of interest (gray, dashed line). At 30 min, rt-PA or saline control is infused ("treatment"). Reperfusion is significantly better with tPA; however, reperfusion levels are only about 60% of baseline even with complete recanalization of the MCA. (c) Two-photon microscopy through a cortical window (maximum Z-projection) reveals the cortical vascular network in the affected area, after intravenous (i.v.) injection of Texas red to label plasma. Lower: MCA branch showing the clot stained after i.v. injection of rhodamine 6G, Hoechst, and Texas red. On the right, the MCA recanalized after t-PA thrombolysis.[12] LSI: laser speckle imaging; rt-PA: recombinant tissue plasminogen activator; tPA: tissue plasminogen activator.

Many patients with acute ischemic stroke receive recanalization therapies; however, more than 50% of patients with large vessel occlusion stroke show no improvement or even worsening of clinical status despite successful thrombectomy.[10] Such futile recanalization (or reperfusion failure) is attributable to a failure of regaining normal macro- and/or microvascular function despite recanalization of the occluded vessel. Today, increasing evidence suggests that—similar to ischemic heart disease—the vascular bed during and after stroke becomes dysfunctional, and removing the occlusive clot is not enough to recover a proper vascular function.[11,12] Here we will summarize the mechanisms suggested to result in reperfusion failure after stroke.

Studying reperfusion failure: The thrombin stroke model

There is a paucity of rodent models to study ischemic stroke in the middle cerebral artery territory, which can be categorized into models of permanent versus transient vessel occlusion.[13] More recently, experimental research has adopted rodent models to incorporate vascular risk factors and comorbidities, including age, hypertension, or small vessel disease.[14,15] To study reperfusion failure, transient middle cerebral artery occlusion (MCAO) by a filament for 30 or 60 min is a widely applied technique, which is easily performed and well characterized in mice and rats.[16] However, direct visualization of the occlusion site is not possible, which results in considerable lesion variability due to slight differences in vascular anatomy.[17] Furthermore, with the silicone-covered microfilament, the real interaction of a clot with vessel endothelium, and more importantly, clot resolution by thrombolysis is not reproduced. In addition, since the filament is inserted mechanically through a cut into the common carotid or external carotid artery, large vessels have to be permanently sutured after filament retraction to prevent bleeding, altering the natural collateral network.

The thrombin model of MCAO overcomes these drawbacks and creates the unique opportunity to mimic clot formation and intravenous thrombolysis in rodents.[18] Albeit technically more challenging than filament occlusion models, it is very reproducible in different laboratories.[19] In brief, thrombin is injected into the distal middle cerebral artery (MCA)-M2 segment using a micropipette, so that a clot is formed in situ, which can be directly visualized (Figure 1). At any desired time,

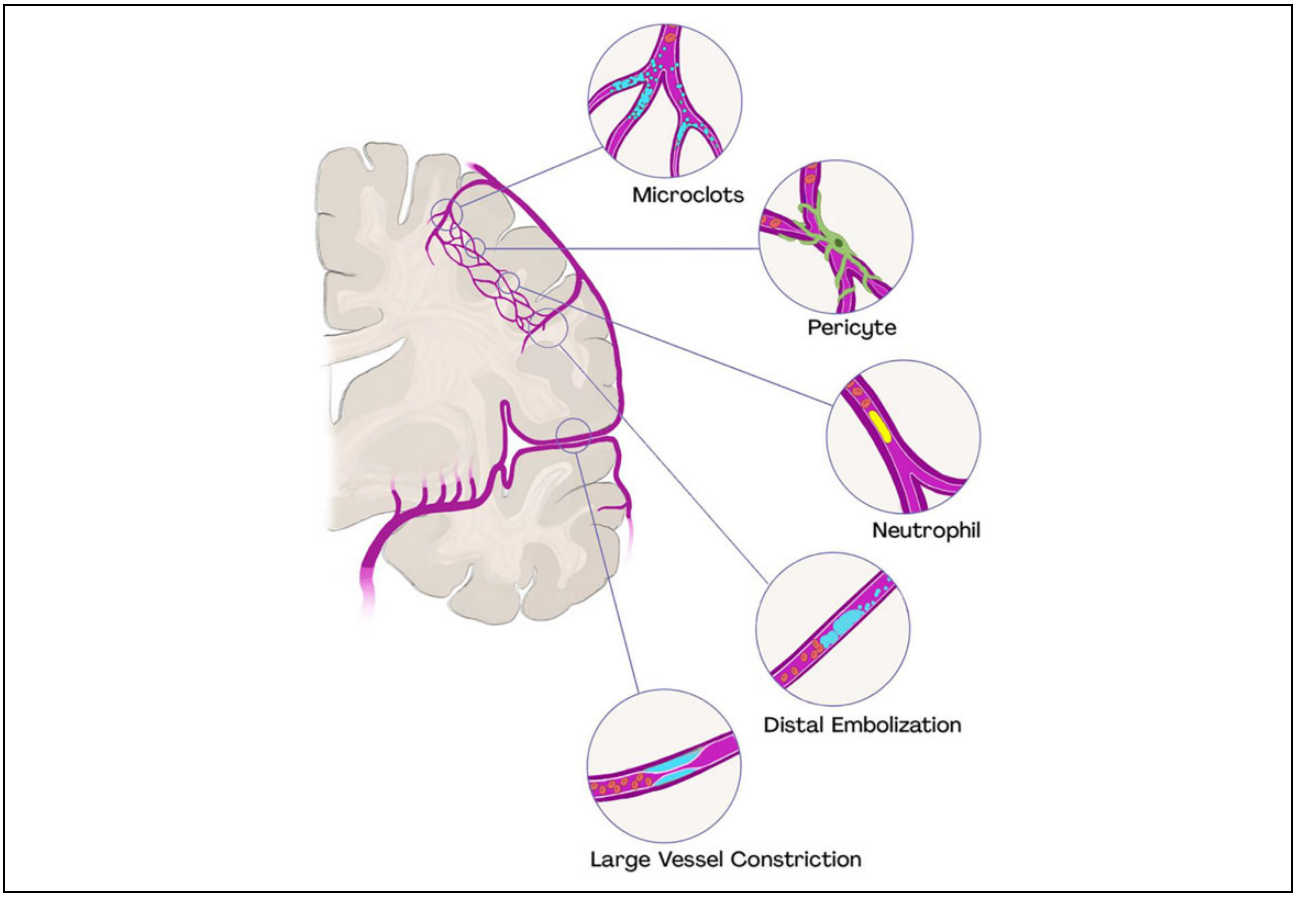

Figure 2. Reperfusion failure after recanalization in stroke. Several mechanisms have been postulated, including microthrombosis, distal capillary constriction by pericytes, capillary stalls with neutrophils, large vessel constriction, and distal clot embolization.

thrombolysis is initiated by infusion of tissue plasminogen activator (tPA) through the tail vein. If early treatment time windows are targeted, tPA infusion is started at around 30 min, which usually achieves partial or complete clot resolution.[20] If effects of late thrombolysis are the focus of the study, tPA treatment is started at around 4 h. The model has provided highly valuable insights into efficacy of early/late thrombolysis and has revealed that despite complete clot resolution, cortical reperfusion of the middle cerebral artery (MCA) territory is far from complete, thereby confirming reperfusion failure (see Figure 1(b) and the literature[12]). *In vivo* imaging allows observation of the clot dissolution and different pathophysiological processes involved (Figure 1(c)). Specific staining techniques inform about the composition of cells and particles within the imaged vessels, as well as type of vascular or perivascular structures.

Mechanisms of reperfusion failure after stroke

The structural integrity of the brain vasculature is a major determinant of the local metabolic supply. The brain vasculature is anatomically and functionally compartmentalized into arteries, capillaries, and veins. The cortex receives its blood supply via pial arteries, which diverge into arterioles and eventually end in the capillary network.[21] Proper blood flow in capillaries is crucial for the brain due to their role in maintaining the exchange of oxygen, nutrients, and waste between tissue and blood. Adequate reperfusion requires the coordination between all vascular compartments. Blood flow is regulated by specialized cell types located within vessels: smooth muscle cells and endothelial cells (ECs) in large vessels and ECs and pericytes in capillaries. In fact, there is accumulating clinical and experimental evidence that post-stroke vascular and hemodynamic impairments are due to alterations in different components: intravascular components (distal embolization, microclots, and capillary stalls with neutrophils) and vascular components (large vessels or capillary constriction; see Figure 2).[12,22–24]

Microvascular occlusion with microclots (local thrombosis and distal embolism)

Microvascular occlusion by clot material is regarded as one of the key mechanisms contributing to microcirculatory

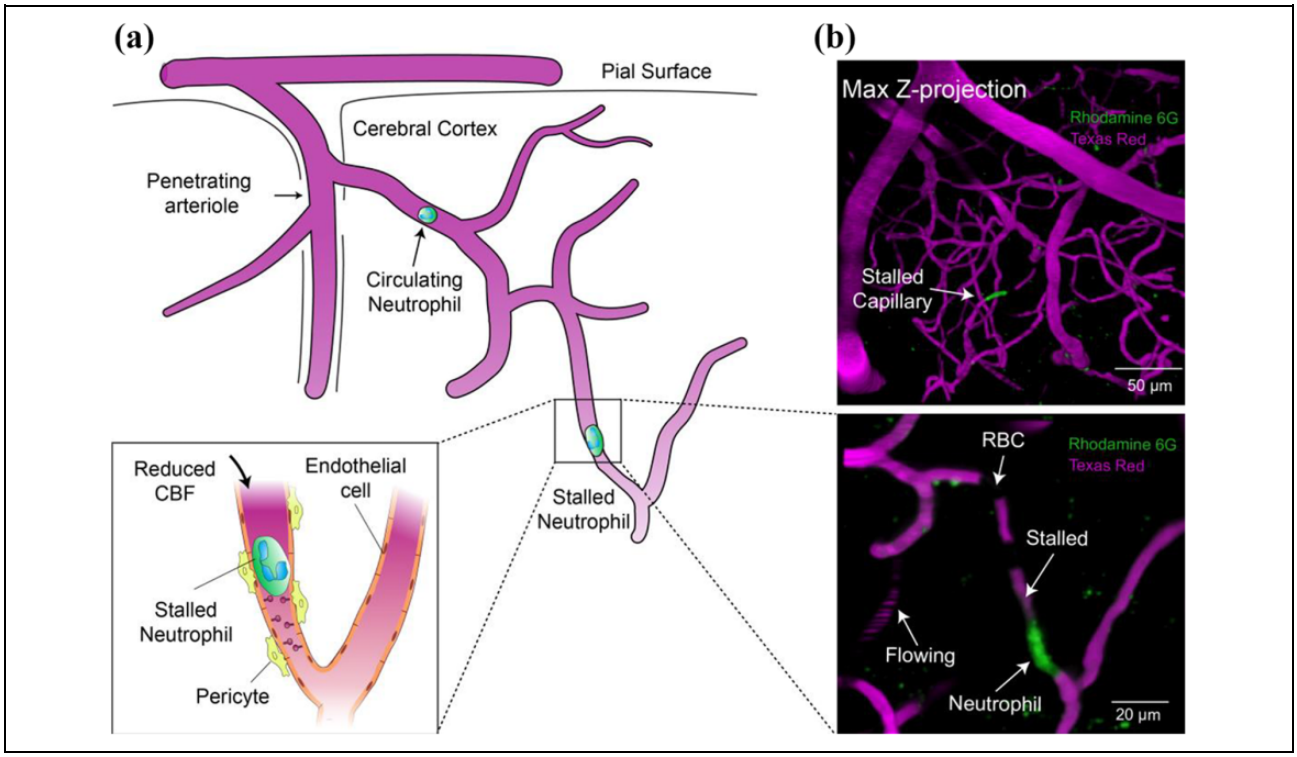

Figure 3. Neutrophils arrest in a capillary after recanalization in stroke and the possible vascular changes that may increase stalls: (a) Several factors such as reduced CBF and shear forces, changes in the mural cells (pericytes and endothelial cells) properties, vasoconstriction, and increased adhesion molecules could lead to neutrophils stall in stroke. (b) Representative two-photon images of vascular network including capillary segments either stalled or patent. Scale bar = 50 μm. Lower: Representative images of a stall caused by neutrophil distinguished by fluorescence labels (Rhodamine 6G, green) and morphology. Flow in capillaries was identified by streaks generated by passing red blood cells (RBCs). Scale bar = 20 μm. CBF: cerebral blood flow.

failure after stroke. Microvascular occlusion may be triggered by embolic fragments from the original proximal thrombus[25] or by local (micro)-thrombosis formed in situ by local platelet activation.[26] The length, density, and composition of a large artery clot depends on stroke etiology.[27] In fact, during clot retrieval, there is a risk of generating small clot debris that may dislodge and migrate, causing downstream circulatory arrest. Clot migration occurs more often in erythrocyte-rich clots and less often in fibrin-rich clots. However, the occurrence, dynamics, and effects of clot migration need further experimental and clinical investigation.

Microclots could also result from the endovascular clot retrieval procedure per se, when endothelial erosion activates the clotting cascade.[28] Microclots have been found in brain microvessels of stroke patients who died within a month after the stroke onset.[29] Cerebral microthrombi usually consist of aggregated platelets and fibrin, sometimes mixed with leukocytes and may even extent into the venous system.[30] Based on these findings, it was suggested that reducing microvascular clogging by antiplatelet drugs or fibrin inhibitors restores microcirculation, reduces no-reflow, and improves stroke outcome.[31] For example, cilostazol, a phosphodiesterase inhibitor acting as an antiplatelet agent, reduced microvascular dysfunction in a model of stroke.[32] Administration of a direct thrombin inhibitor, argatroban, was shown to enhance recanalization rates induced by t-PA.[33] Some of these strategies are currently in clinical trials, such as the combination of argatroban with thrombolysis.[34]

Capillary stalls with neutrophils

Previous studies in patients with stroke demonstrated that the number of circulating neutrophils rises within the first hours and that this increase is proportional to stroke severity and functional impairment.[35,36] Within the healthy capillary network, neutrophils appear to flow smoothly without rotation and without margination.[37] However, in diseases such as stroke and Alzheimer's disease, neutrophils may get stuck in capillaries and disturb flow, enhancing tissue injury.[38] In stroke, despite reopening of the occluded MCA, capillary stalls occur in the core of the infarction as well as the penumbral region.[24] By imaging flow in cerebral capillaries using two-photon imaging, we recently observed that capillary stalls after stroke were caused by neutrophils getting stuck in the distal capillary segments (Figure 3).[12] Remarkably, depletion of circulating neutrophils using an anti-Ly6G antibody led to a release of capillary stalls and restoration of microvascular

perfusion. This resulted in an improved neurological outcome and did not aggravate adverse outcomes such as haemorrhages.[12] What causes neutrophils to perturb capillary flow? Hemodynamic factors (slow flow), ischemia-induced alterations in the mural cells (ECs and pericytes), and their interaction with the blood cells (adhesion molecules) might be involved. While neutrophil depletion is not a feasible stroke treatment, knowledge about the factors inducing neutrophil clogging of capillaries may reveal new promising therapeutic strategies.

Capillary constriction and the role of pericytes

Pericytes are specialized mural cell types located within capillaries that play a crucial role in regulation of the vascular tone.[39] Despite some controversy regarding the contribution of pericytes compared to arteriolar and venular smooth muscle cells in regulation of cerebral blood flow during ischemia,[40] pericytes seem to be particularly sensitive to ischemia, and their death causes irreversible capillary constriction "rigor."[22] This leads to breakdown of the blood brain barrier, which likely contributes to the ingress of peripheral inflammatory cells to the brain parenchyma and increased risk of haemorrhage[41] when spontaneous or therapeutic reperfusion occurs. Furthermore, damage to the endothelium may impair release of vasoactive substances such as nitric oxide and endothelium-derived relaxing factor, further aggravating pericyte contraction. Pericyte proliferation and recruitment are tightly regulated by ECs, but the mechanisms of interaction between these mural cells are still an area of active investigation.[42] Astrocytes also contribute to the generation of vascular tone and mediate neurovascular signaling to capillary pericytes.[43] Several mechanisms of astrocytic synthesis of vasodilators such as prostaglandin E2, epoxyeicosatrienoic acids, and K+ have been described.[44,45] However, the precise role of astrocytes in regulating blood flow under pathological conditions, including stroke and Alzheimer's disease, remains an open question.

Large vessel constriction

Stroke is associated with structural and functional alterations in large vessels just downstream of the vessel occlusion, which can present as vascular wall thickening and endothelial dysfunction. Constrictions of cerebral arterioles, resulting in a significant decrease in cerebral blood flow, have been reported in MCAO models[46] and are likely to contribute to incomplete reperfusion after stroke. Mechanisms contributing to such pathological constriction and vascular dysfunction include an increase in arterial stiffness, impaired vascular reactivity, and reduced endothelial nitric oxide synthase (eNOS) phosphorylation.[41] Stroke affects the whole neurovascular unit, and thus astrocytes, microglia, and neurons in addition to vascular ECs and pericytes. Therefore, multiple regulatory levels exist, and their interplay is complex.[41] However, own preliminary data and observations from other groups indicate that vascular collaterals are key players in disseminating pressure gradients and enabling nutrient supply to facilitate pathological constriction of distal arteriolar vessel segments in stroke.[47] Therefore, support of collateral flow and/or vascular protection through enhancement of endothelial function or prolongation of EC survival could be a very efficient approach for stroke protection.

Conclusion

There is ample evidence for the detrimental effects of reperfusion failure after recanalization in stroke patients. With more aggressive recanalization approaches, including patients with symptom onset >12 h and large infarct core, reperfusion failure will become even more prevalent. To study this important obstacle to clinical recovery from stroke, sophisticated rodent models are now available. Combined with *in vivo* imaging, these models have revealed key components that could be therapeutic targets to counteract reperfusion failure. Importantly, these targets go beyond earlier neuroprotection-centered approaches toward a more integrated view that incorporates vascular structures and their interaction with the neurovascular unit. These models offer the unique chance for a successful translation of knowledge into clinical practice.

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