Angiophagy
Mechanism of Microvascular Recanalization Independent of the Fibrinolytic System
Jaime Grutzendler, MD

Cell viability in the brain is critically dependent on uninterrupted blood flow through the microvasculature. However, microvessels, because of their small diameter and low-flow velocity, are prone to occlusion by blood clots, fragments of atheromatous plaques, or other circulating debris. Furthermore, microvascular occlusions can occur after large-vessel thrombosis because of blood stasis or due to clot fragmentation and distal microembolization. Microvascular occlusion could thus be a frequent phenomenon throughout life that may play important roles in the pathogenesis of cerebral ischemia and reperfusion, age-related vascular cognitive impairment, and cognitive decline after surgical procedures, such as coronary artery bypass graft.

Given the propensity of microvessels to occlusion, it is likely that robust mechanisms have evolved to ensure microvascular patency. The main mechanism that prevents microvascular occlusion is constituted by the combined effects of the fibrinolytic system and hemodynamic pressure, which lead to embolus dissolution and washout. However, the fibrinolytic system is limited to breaking down fibrin-based blood clots and is unable to disrupt other materials, such as calcium and cholesterol crystals and cell debris, which are present in complex thrombi and may occlude microvessels throughout life.

Using an experimental mouse model of internal carotid embolization with fluorescently labeled microemboli (10–60 µm), we were able to study the outcome of individual microvascular occlusions in the mouse brain. We tracked individual microvessels using high-resolution confocal imaging in histological brain preparations and by time-lapse 2 photon microscopy of the meninges and superficial cortex in living mice. Using these methods, we recently found a novel mechanism of microvascular recanalization, which we termed “angiophagy.” This mechanism involves the engulfment of emboli by the endothelium followed by their translocation through the vessel wall into the perivascular space.

Angiophagy is a robust mechanism that leads to blood flow reestablishment within hours of the embolus extrusion.1 Embolus extravasation occurs 1 to 6 days after occlusion in >80% of occluded vessels ranging from terminal capillaries (≈7 µm) to arterioles (≈60 µm) in various organs, including the brain, retina, heart, muscle, lung, and kidney. The speed of translocation seems to be heterogeneous and may depend on variables such as vessel location, size of embolus, and type of embolic material (fibrin clots extravasate somewhat faster than cholesterol crystals). The vast majority of vessels that undergo successful emboli extrusion do not display any evidence of endothelial or mural cell death, as evidenced by normal nuclear and cellular morphology and the lack of cell death markers caspase-3 and Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). Furthermore, using longitudinal 2 photon imaging of individual microvessels over weeks to months, we found that recanalized vessels were spared in the long term. Thus, angiophagy seems to provide a net angioprotective effect.

To determine whether angiophagy also has a protective effect on the perivascular neural tissue, we examined various cellular markers around occluded microvessels. Using the hypoxia marker, Pimonidazole, we found that occlusion of vessels 10 to 20 µm in diameter induced a substantial area of hypoxia downstream of the occlusion site. However, the degree of hypoxia was not severe enough to induce cell death as determined by nuclear morphology, TUNEL, and caspase-3 staining. Instead, we observed a focal decrease in the density of dendritic spines, which suggests that the threshold for synaptic loss is lower than that for cell death. Interestingly, the density of dendritic spines in the area supplied by the occluded microvessels recovered over days to weeks after angiophagy had occurred. Although this does not prove a cause and effect relationship, it suggests that recanalization by angiophagy could have an overall protective effect on the neurovascular unit.

This protective effect may be impaired in aging because we found the rate of angiophagy to be severely reduced in mice >20 months old. Furthermore, at this age, occluded microvessels frequently displayed apoptotic markers in endothelial and perivascular cells, suggesting that in the aging brain, microvascular occlusions are much more damaging. This phenomenon may be attributable to a combination of factors, including a reduced rate of vessel recanalization by angiophagy as well as an overall increased cellular susceptibility to hypoxia/ischemia.
Potential Molecular Mechanisms Involved in Angiophagy

The precise molecular mechanisms involved in angiophagy are likely to be complex. One potential trigger for the process of endothelial engulfment could be local hypoxia. Although most of the observed hypoxia (as detected by pimonidazole) occurs downstream of the micro-occlusion site, we observed some hypoxia within the endothelium immediately adjacent to the embolus. Hypoxia is a strong stimulus for vascular endothelial growth factor (such as \( \beta V \alpha \)) expression, and this molecule is known to mediate the local protrusion and migration of endothelial cell processes and could thus be involved in endothelial embolus engulfment.

In addition to local hypoxia, direct mechanical pressure on the endothelium by the occluding embolus or reduced shear stress attributable to restricted blood flow could trigger mechanosensitive responses that could induce endothelial plasticity.\(^5\) \(^6\) Engulfment could also be mediated by molecular interactions between the endothelial protrusions/lamellipodia and the embolus itself. This may occur via integrins (such as \( \alpha V \beta 3 \) on endothelial cells), which are adhesion molecules capable of binding molecules, such as fibrinogen, within a clot.\(^5\) Given that the engulfment process also occurs with emboli composed of other materials such as cholesterol crystals, it is possible that these emboli become coated with fibrinogen within the circulation, allowing them to interact with endothelial integrins.

The engulfment process itself is likely to involve extensive cytoskeletal remodeling. A large number of molecules have been implicated in cytoskeletal dynamics during cell motility and lamellipodia formation. Among them are Rho GTPases, which are key regulators of the actin cytoskeleton, and myosin light chain kinase, which governs the interaction between actin and myosin in endothelial cells and has been implicated in neutrophil transmigration, endothelial contractility, permeability, and motility.\(^5\) These pathways are likely to be important in the initiation and completion of the engulfment process.

The precise mechanism by which emboli are extruded out of the vessel into the perivascular parenchyma remains unclear. On the basis of confocal and in vivo 2 photon images, we have observed that after engulfment, the original endothelial wall undergoes a retraction process, opening a path for embolus translocation. One possibility is that extrusion occurs through a large transcellular pore.\(^6\) Alternatively, a paracellular path could be involved by disruption of interendothelial junctions similar to leukocyte diapedesis.\(^7\)

Finally, for emboli to cross the endothelial barrier into the perivascular space, it is likely that substantial remodeling of the perivascular extracellular matrix is required. We have observed that in most cases, extravasated emboli are surrounded by collagen IV immunoreactivity.\(^1\) This suggests that either the embolus pushes out the existing microvascular basal lamina or, alternatively, additional collagen IV is secreted and deposited around extravasated clots. The proteolytic enzymes matrix metalloproteinases (MMPs) have been shown to be capable of degrading the microvascular extracellular matrix and have been implicated in vascular remodeling.\(^8\) Indeed, when we administered the systemic MMP inhibitor (SB3CT), we observed a significant reduction in the rate of angiophagy. This suggests that MMPs play an important role in this cellular process.

Implications for Stroke Pathogenesis

Microvascular occlusion is likely to be an important factor in determining the extent of ischemia after occlusion of larger cerebral vessels, as well as the degree of reflow after spontaneous or tissue-like plasminogen activator–induced dissolution of the primary clot. To determine the relative importance of angiophagy in microvascular recanalization, we asked how effective hemodynamic forces and the fibrinolytic system were in clearing microvessels early after occlusion and before angiophagy. We implemented our embolization model with fluorescent fibrin clots. We then measured clot washout by quantifying the number of clots that remained in the microvasculature after various time intervals. Surprisingly, we found that <50% of infused emboli (20–30 \( \mu \)m in diameter) were lysed and washed out within 48 hours. In fact, most washout occurred within the first 6 hours after occlusion, with almost no further washout beyond this time point. Given this high failure rate for embolus washout/fibrinolysis, angiophagy could represent an important backup mechanism for microvascular clearance after stroke.

Even though effective angiophagy seems to be limited to smaller vessels (up to <60 \( \mu \)m), the majority of brain vessels fall within this diameter range. Occlusion of these microvessels can occur spontaneously or following large vessel thrombosis. Furthermore, after tissue-like plasminogen activator administration, breakdown of the primary clot could produce many small fragments that end up occluding the microvasculature. Angiophagy could thus play an important role in reestablishing terminal perfusion of the capillary bed.

In the future, it might be possible to find drugs that accelerate the angiophagy process as potential therapeutic agents for microvascular recanalization in the postischemic period. It would also be important to determine whether future drugs aimed at treating stroke have unsuspected negative effects on the angiophagy process. For example, MMP inhibitors have been proposed as potential therapeutic agents in brain ischemia to reduce blood–brain barrier disruption. However, we have shown that inhibition of MMPs could also lead to reduced angiophagy, which, in theory, would be counterproductive. This phenomenon might explain why MMP inhibitors have failed to improve long-term recovery after stroke.\(^9\)

Implications in Vascular Cognitive Impairment

Sporadic late-onset dementia is a pathologically heterogeneous disorder that, in addition to amyloid plaques and neurofibrillary tangles, can be characterized by substantial microvascular pathology in the form of microischemic areas and amyloid angiopathy. Furthermore, there is substantial evidence that microvascular pathology in hypertension and diabetes mellitus increases the risk of cognitive decline,\(^10\) whereas microembolic events may cause brain dysfunction even in the absence of overt ischemia\(^11\) in individuals with
atrial fibrillation and after vascular surgical procedures, such as coronary artery bypass grafts and carotid endarterectomy/ stenting.

Microvessels could be occluded by a variety of materials, including spontaneously occurring microclots, fragments of atheromatous plaques, cell debris, inflammatory cells, or even exogenous particles reaching the microcirculation. Transient or permanent occlusions could occur in normal individuals but might become more prevalent because of diminished microemboli washout in individuals with structural and functional microvascular abnormalities, such as in hypertension and diabetes mellitus, increased vascular tortuosity in aging, or decreased hemispheric perfusion resulting from critical carotid stenosis. Given the heterogeneity of potential occluding materials, angiophagy could play a critical role in maintaining microvascular patency throughout life.

We have shown that aging is associated with a reduction in the efficiency of angiophagy. This may be attributable to age-associated functional or structural changes in the endothelium or extracellular matrix. In addition, a variety of conditions affecting the microvasculature, such as hypertension, diabetes mellitus, and amyloid angiopathy, could, in theory, reduce the efficiency of angiophagy. A detrimental effect of microvascular amyloid on angiophagy would provide an interesting mechanistic link between vascular factors and Alzheimer’s pathology. Given the virtually inexistent angiogenesis in the adult brain, an impairment in the efficiency of angiophagy could lead to gradual microvascular loss, and result in a progressively increased propensity to neurovascular damage as a result of new occlusions. The cumulative effect of microocclusions over the long-term could eventually lead to a microvascular form of cognitive impairment, further reducing the capacity to compensate new occlusions.

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None.

References

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