In Vivo Imaging of Neurovascular Remodeling After Stroke

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Stroke is one of the leading causes of adult disability throughout the world and, even though neural mechanisms of loss of function have been extensively studied, many aspects of poststroke cerebral responses remain poorly understood. Of particular interest is the mounting evidence of the capacity of the adult brain to reorganize after injury, which is believed to contribute to limitation of the extent of neural dysfunction and to restoration of affected neural functions. Processes such as neuronal plasticity, glial proliferation, and neovascularization may be essential for preservation or recovery of function after stroke and may conceivably go hand-in-hand at nearby and remote sites of active tissue reorganization. Specifically, the acute initiation of neurovascular remodeling, stimulating the proliferation and growth of existing arteries/arterioles (ie, arteriogenesis) or new capillaries (ie, angiogenesis), may be critical to facilitate the survival and restructuring of neural tissue, which ultimately may contribute to functional recovery at later stages. A variety of vascular imaging strategies is available for in vivo detection, characterization, and quantification of these processes, which can significantly aid in elucidation of the role of neurovascular remodeling after stroke, as discussed in this review. Because most of the described imaging modalities are present in experimental models, but imaging modalities are also available in the clinic, and preliminary studies in patients are currently emerging.

Arteriogenesis and Angiogenesis

Growth and remodeling of existing and nascent vascular networks in health and disease involve a number of critical steps that have been comprehensively described by Risau and Carmeliet. Arteriogenesis involves adaptive growth and proliferation of preexisting (collateral) arteries and arterioles in response to increase in intravascular shear forces. The increased shear stress leads to upregulation of cell adhesion molecules, followed by accumulation of monocytes and other leukocytes that release cytokines and growth factors around the proliferating and maturing arteries. Although arteriogenic growth of collateral arteries is oxygen-dependent, angiogenesis is triggered by hypoxia, in which oxygen shortage tips the balance of expression of antiangiogenic and proangiogenic factors in favor of the latter. This first leads to upregulation of hypoxia-inducible factors, followed by angiopoietin,

erythropoietin, nitric oxide synthase, and vascular endothelial growth factors and their receptors, many of which also promote neuronal survival or regeneration. Vasculogenesis, ie, the development of blood vessels from endothelial precursor cells, also may occur in adult brain, but this process remains largely unexplored.

Increased capillary density in peri-infarct areas in post-mortem brains of stroke patients has been shown to be associated with longer survival time. Endothelial cell proliferation may occur within 24 hours after cerebral ischemia, followed by formation of new capillary buds in subsequent days, which, after pruning and restructuring, may lead to a stable newly formed vasculature in a few weeks. The formation of an extended vascular network would improve the supply of energy substrates, necessary for tissue survival, but in addition paves the way for other restorative processes, such as neuronal sprouting, neurogenesis, and glial proliferation.

The ability to locate and follow the precise course of neurovascular remodeling can provide valuable information on the endogenous repair mechanisms of the brain and outlook for functional recovery, which may be used for therapeutic enhancement of neurorestorative processes after stroke. In vivo imaging techniques offer attractive means to assess the spatiotemporal profile of neurovascular alterations in the brain after cerebrovascular injury. In this review, we introduce methods that have been applied for in vivo imaging of changes in structure, permeability, density, markers, and perfusion of the vasculature in poststroke reorganizing brain. Most of the presented work refers to studies in animal–especially rat and mouse–models, but imaging modalities are also available in the clinic, and preliminary studies in patients are currently emerging.

Optical Imaging

Optical microscopy techniques, such as laser-scanning confocal microscopy, have been widely used for detailed post-mortem assessment of changes in neurovascular anatomy after ischemic injury. For in vivo optical imaging studies in rats and mice, brain tissue can be directly exposed through a cranial window, or the skull can be thinned. Subsequently, video microscopy with intravascular dyes enables imaging of cerebral vessel size, morphology, and flow. In a study by

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Wei et al., fluoresein isothiocyanate angiograms and transits were recorded through a cranial window over rat barrel cortex before, immediately after, and 30 days after ligation of branches of the middle cerebral artery (MCA). Larger and more tortuous collateral arterioles, ie, arteriogenesis, were observed in the ischemic border zone after 30 days, in combination with restored arteriocapillary dye transit latencies. These morphological changes in surface vessels and perfusion also correlated with angiogenesis, as identified from endothelial bromodeoxyuridine labeling and αβ3 integrin expression on postmortem brain sections. In a similar fashion, capillary and venous remodeling has been detected by repeated in vivo laser-scanning confocal fluorescence microscopy of fluorescent erythrocytes and dextran through a closed cranial window in mice during a 1-month period after focal ischemia.

Multiphoton fluorescence microscopy is an advanced combination of laser-scanning microscopy with pulsed long-wavelength multiphoton fluorescence excitation, which offers high-resolution 3-dimensional images of fluorophore-labeled living tissue up to a depth of \(1 \text{ mm}\). Multiphoton microscopy has been successfully applied for intravital assessment of tumor angiogenesis at \(<1 \text{ μm}\) resolution. Studies in experimental stroke models are still sparse, but recent publications have demonstrated the possibility to reveal vascular adaptations in response to ischemic injury. In an in vivo transcranial study with 2-photon microscopy—a variant of multiphoton fluorescence microscopy that uses red-shifted excitation light—Brown et al. scanned neurons expressing yellow fluorescent protein as well as blood plasma labeled with fluorescent dextran in a mouse photothermbotic stroke model. Progressive joint reorganization of dendrites and vessels in the infarct border zone was visualized in a longitudinal fashion (Figure I). Dendritic and vascular segments radiated in parallel outward from the edge of the infarct border, which started 2 weeks after stroke and became more apparent in subsequent weeks. Furthermore, blood vessel density increased over time, accompanied by preserved blood flow velocity. These findings support the concept of a close relationship between vascular and neuronal remodeling in peri-infarct tissue.

A previously unknown mechanism of microvascular plasticity was recently revealed with transcranial 2-photon laser-scanning microscopy in living mice after cerebral embolization. Envelopment of emboli by endothelial membrane parenchyma was detected after a few days. This process may be a critical endogenous mechanism through which reestablishment of perfusion occurs after occlusion of cerebral microvessels.

In general, multiphoton microscopy allows imaging of fluorescently labeled vessels at a cellular and subcellular level, yet imaging speed, penetration depth, and field-of-view remain limiting factors. Optical coherence tomography and, in particular, Doppler optical coherence tomography provide alternative means using near-infrared light for in vivo imaging of tissue and flow, with capacity of real-time data acquisition with microscopic resolution. Because of the relatively long optical wavelength, light penetrates deeper in the optical

Figure. Optical imaging (I), magnetic resonance imaging (MRI) (II), and positron emission tomography (PET) (III) of neurovascular remodeling after experimental stroke. IA and ID, Low-magnification brightfield images showing the surface of mouse brain and vessels through a cranial window. IB, IC, IE, IF, Axial and coronal PET images of rat brain ((2007). Copyright by the Society for Neuroscience, 2007. Courtesy of Drs C. E. Brown and T. H. Murphy. II, \(\Delta R_2 (s^{-1}), \Delta R_2^* (s^{-1})\), Q (\(\Delta R_2/(\Delta R_2^*+\Delta R_2^*) s^{-1/3}\)) and vessel size index (VSI) (μm) maps of consecutive coronal rat brain slices, calculated from steady-state contrast enhanced (ssCE) MRI at 21 days after 60 minutes middle cerebral artery (MCA) occlusion. Increased \(\Delta R_2^*\) (ie, microvascular cerebral blood volume [CBV]), \(\Delta R_2 (\text{total CBV})\) and Q (ie, vascular density values), and decreased VSI reflect significant vascular reorganization in cortical and subcortical perilesional areas (arrowheads) (Seevinck PR, Yanev P, Dijkhuizen RM, unpublished data, 2012). III, Axial and coronal PET images of rat brain at 2 hours postinjection of \(1 \text{ mCi}\) of \(^{64}\text{Cu-DOTA-VEGF}_{242}\) at 2, 9, 16, and 23 days after MCA occlusion. Uptake was increased in the stroke area (arrowheads). The publisher of this copyrighted material is Wolters Kluwer Health. Courtesy of Dr W. Cai.
scattering media as compared with confocal microscopy. The ability of Doppler optical coherence tomography to quantify microcirculatory changes after stroke recently has been demonstrated in a mouse model.18

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a versatile imaging modality with outstanding abilities to depict in vivo soft tissue properties up to 50 to 100 μm resolution, which has been extensively used in experimental and clinical stroke studies.19,20 Different methods, like anatomic MRI, magnetic resonance angiography, perfusion MRI, functional MRI, and diffusion tensor imaging, may be combined in a single scanning session, enabling concurrent collection of information on tissue status, hemodynamics, and connectivity in poststroke brain of small (eg, mice and rats) and large animals (eg, pigs and monkeys), as well as those of humans. With regard to vascular MRI, there has been significant progress in recent years. Advances in scanning protocols, contrast agents, and data analyses have allowed opportunities for original studies on poststroke neurovascular modulations, as described in the next paragraphs.

The large variety of available MRI techniques enables measurement of different characteristics of poststroke neurovascular remodeling, starting from the first phase of endothelial adaptations to the final stage of establishment of a functional vascular network. Initially, hypoxia/ischemia–induced factors activate receptors on endothelial cells that trigger a cascade of events leading to disruption of adherent endothelial junctions and increased vessel permeability, which would allow plasma proteins to extravasate and build a temporary scaffold for migrating endothelial cells to initiate the formation of a new vascular network.4,5 Therefore, extravasation of intravascularly injected contrast media may provide a marker of newly forming vasculature with an undeveloped blood–brain barrier (BBB). The integrity of the BBB can be evaluated with dynamic contrast-enhanced MRI.21 Dynamic contrast-enhanced MRI is sensitized to changes in the longitudinal relaxation time T1, induced by local tissue accumulation of an intravascularly injected paramagnetic contrast agent (usually a gadolinium chelate) that leaks through a permeable BBB. The degree of poststroke BBB permeability, which is time-dependent and region-dependent, can be further assessed based on the size of the contrast agent.22,23 With tracer kinetic models, a blood-to-brain transfer constant, Ki (or Ktrans), can be calculated from the time course of T1 changes. Elevated Ki in the first weeks after stroke in rats has been observed in ischemic border zone areas where vascular density was chronically increased.24,25 However, it is important to note that BBB leakage may not solely reflect an early state of effective angiogenesis. BBB breakdown, particularly at early stages, also is a direct feature of ischemia-induced endothelial injury.26 Furthermore, after the initial burst of angiogenesis, some immature capillaries may remain leaky and eventually regress.27

After proliferation of endothelial cells, newly formed vessels appear during the first few days in the ischemic border zone,9 resulting in alterations in vascular density, cerebral blood volume (CBV), and cerebral blood flow (CBF). Hemodynamic parameters, eg, CBV and CBF, can be measured with perfusion MRI techniques, such as dynamic susceptibility contrast-enhanced MRI and arterial spin labeling, whereas information on vascular fractions can be derived from steady-state contrast-enhanced (ssCE) MRI (for an overview of these methods, see Zaharchuk28 and Seevinck et al29). Dynamic susceptibility contrast-enhanced MRI involves dynamic measurement of the first passage of an intravascularly injected paramagnetic contrast agent, typically a gadolinium chelate (eg, Gd-DTPA), from which different hemodynamic indices, including CBV and CBF, can be estimated by tracer kinetic modeling of the contrast concentration time curve. Alternatively, CBF can be calculated without the use of an exogenous contrast agent by using endogenous contrast from magnetically labeled arterial water, a method known as arterial spin labeling. Another perfusion MRI method, ssCE MRI, is based on detection of magnetic resonance relaxation shifts induced by a (super)paramagnetic blood pool agent with a long blood half-life, such as certain ultrasmall superparamagnetic particles of iron oxide. Resultant changes in the transverse relaxation rates, R1* and R2*, can be measured with gradient-echo and spin-echo MRI, respectively. Differences in precontrast and postcontrast R1* (ΔR1*) and R2* (ΔR2*) values depend on morphological properties of the vascular network, such as the density and diameter of the vessels. Indices of the vascular architecture, such as the blood volume fraction, microvessel density, and vessel size index, subsequently can be measured by means of mathematical modeling. This approach has been originally applied to characterize tumor angiogenesis,30 but has recently found its way in a number of studies on angiogenesis in experimental stroke models (Figure II).29

In a serial ssCE MRI study, Lin et al32 observed an increase in intravascular contrast-induced ΔR1*, a measure of total CBV, in outer cortical layers and leptomeninges from day 3 to day 21 after transient MCA occlusion in rats. Such an increase in total CBV may be associated with vasodilation, arteriogenesis, and angiogenesis. Intravascular contrast-induced ΔR1*, a measure of microvascular CBV, declined during the first days, suggestive of regression of existing capillaries. At later stages microvascular CBV was elevated in reperfused cortical tissue, which was accompanied by prolonged vascular permeability, as measured with dynamic contrast-enhanced MRI. At the same time the parameter Q—a ssCE MRI-based index of vascular density—increased, which corresponded with higher immunohistochemical vessel counts. The pattern of these vascular modifications in this study suggests that angiogenic formation of capillaries originated from the leptomeninges.

In a comparable study, ssCE MRI-based microvessel density values at 2 weeks after embolic stroke in rats were 313±32 mm−2, 209±60 mm−2, and 26±14 mm−2 in the contralateral hemisphere, the ischemic recovery region, and the ischemic core, respectively, which corresponded with postmortem measurements based on immunoreactive staining of an endothelial marker.31 The same authors have calculated vessel size index and mean microvessel segment length based on ssCE MRI in rats at 6 weeks after embolic stroke.32 Mean segment length was decreased in the ischemic
recovery zones as compared with contralateral, whereas the opposite was found for vessel size index. An increased vessel size index also has been observed in reperfused cortex after transient MCA occlusion in rats. However, this was only significant at days 1 and 3, followed by normalization at later stages. Further investigations are warranted to firmly establish the significance and reliability of the various vascular indices that can be calculated with ssCE MRI, because the calculation depends on several theoretical assumptions and basic mathematical models that may not uniformly hold under different (patho)physiological conditions.

The initial stages of angiogenesis are followed by pruning and growth of new sprouts, which eventually yield perfused microvessels and macrovessels. Large vessels can be imaged with magnetic resonance angiography, but the capacity of magnetic resonance angiography to visualize small vessels is limited. Recently introduced methods such as CBV-based microscopic magnetic resonance angiography, a version of ssCE MRI that enables 3-dimensional visualization of the architecture of small vessels in rodent brain provides promising opportunities for improved in vivo assessment of the cerebral microvasculature over time.

Newly generated venous structures may be identified with $T_2^*\text{-weighted or susceptibility-weighted MRI.}$ Local magnetic field inhomogeneities caused by the relatively high levels of paramagnetic deoxyhemoglobin in venules and veins result in a lower signal intensity on $T_2^*\text{-weighted and susceptibility-weighted images, which have been used to identify maturing angiogenesis in sildenafil-treated rats after embolic stroke.}$ A characteristic pattern of significant $T_2^*$ shortening in ipsilesional brain regions was associated with angiogenesis. It should be noted, however, that local tissue $T_2^*$ is also influenced by other factors associated with cerebrovascular disease, such as parenchymal hemorrhage and iron accumulation in macrophages.

Perfusion of the newly formed vascular network can be established with perfusion MRI techniques such as dynamic susceptibility contrast-enhanced MRI and arterial spin labeling. A gradual increase in CBV and CBF in outer cortical layers as measured with dynamic susceptibility contrast-enhanced MRI and arterial spin labeling, respectively, from days 1 to 7 after transient MCA occlusion in rats has been shown to correlate with increase in vascular density on the brain surface. Similarly, in an embolic stroke model, MRI-based measurements of elevated CBF and CBV were evident in ischemic boundary regions with neural progenitor cell-induced angiogenesis.

Although most studies report poststroke neovascularization in (cortical) perilesional areas, there are also reports of neurovascular remodeling in remote regions, such as the thalamus. In a recent arterial spin labeling study, it was shown that moderate hyperperfusion in the thalamus during the first days after transient MCA occlusion in rats was followed by long-term hyperperfusion at chronic time points in association with increased vessel density, as determined with immunohistochemistry.

Other Imaging Modalities: Molecular Imaging

In comparison with optical imaging and MRI, other imaging modalities such as computed tomography (CT), single-photon emission CT, positron emission tomography (PET), and ultrasound (US) have been much less frequently applied for in vivo studies of neovascularization in poststroke brain. CT, single-photon emission CT, and PET use ionizing radiation, which is not ideal for repeated measurements. Nevertheless, these modalities, as well as US, have been successfully used for experimental and clinical assessments of angiogenesis in tumors and myocardium. With current advancements in imaging technology and design of molecular imaging probes, similar studies of poststroke neurovascular remodeling can be expected to emerge in the near future.

Contrast-enhanced CT approaches such as micro-CT and volumetric CT, which benefit from fast and sensitive X-ray detector systems, allow relatively rapid and straightforward visualization of cerebral microvessels. Micro-CT devices are well-suited for 3-dimensional imaging of small structures, and the anatomy of cerebrovasculature with vessel sizes as low as 50 $\mu$m can be visualized with iodinated contrast agents. CT methods are increasingly applied in studies on vascularization in rat and mouse tumor models but still largely await application in stroke models.

PET and single-photon emission CT are hampered by poor resolution (0.5–2 mm)—particularly for studies in mice—but benefit from high tracer sensitivity, which allows detection of various molecular markers, including angiogenesis markers such as vascular endothelial growth factor receptors and $\alpha_\beta$ integrins. The potential for detection of angiogenesis after stroke has been demonstrated by Cai et al., who used a specific PET radiopharmaceutical to measure expression of vascular endothelial growth factor receptors in rats after MCA occlusion. Tracer uptake in the lesion was elevated at day 2, peaked at day 9, and normalized at day 23, and corresponded spatially with areas where angiogenesis was detected with postmortem histology (Figure III).

Another approach for in vivo detection of vascular molecular markers is offered by targeted contrast-enhanced US imaging. US allows noninvasive real-time imaging at relatively high spatial resolution (50–100 $\mu$m), but tissue penetration and tracer sensitivity are limited. The method is based on the reception, analysis, and display of acoustic signals produced by reflection or backscatter of sound with use of intravascular contrast agents, usually microbubbles with a diameter of 1 to 4 $\mu$m. US imaging allows assessment of the expression of angiogenesis markers, whereas the functionality of newly formed vessels may be evaluated by measurement of microvascular perfusion with nontargeted microbubbles. To our knowledge, studies that have used US imaging to assess poststroke neurovascular remodeling have not yet been published.

The recent developments in design of targeted superparamagnetic (and fluorescent) contrast agents that can be functionalized with specific ligands also has enabled the detection of molecular vascular markers with relatively insensitive MRI (and optical imaging) methods. A few pioneering studies have demonstrated the feasibility of molecular MRI to detect upregulation of inflammatory markers in rat and mouse cerebrovasculature after cerebral ischemia (reviewed
in Deddens et al5), and this approach also may find its way in characterization of poststroke neurovascular remodeling in the near future.

Conclusions
Changes that occur in the neurovascular environment after stroke suggest an organized multitude of complex adaptive responses that may significantly contribute to preservation or restoration of affected tissue. Although it is impossible to obtain complete and quantitative estimation of different stages in vessel remodeling within a single experimental setting, a variety of in vivo imaging methods offers a diverse set of approaches that can be applied to identify, depict, and measure specific facets and interactions in the process of neurovascular reorganization. The ultimate goal is to better-understand how the endogenous repair mechanisms of the brain may be tweaked to limit negative effects and to stimulate positive effects of the neurovascular responses to ischemia. This could provide a critical basis for development of recovery-enhancing therapeutic strategies that may be effectively monitored with noninvasive multiparametric in vivo imaging methods in experimental as well as (pre)clinical settings.

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References

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