Rodent Models of Cerebral Microinfarct and Microhemorrhage

Andy Y. Shih, PhD; Hyacinth I. Hyacinth, MD, PhD, MPH; David A. Hartmann, BA; Susanne J. van Veluw, PhD

Microinfarcts are prevalent but tiny ischemic lesions that may contribute to vascular cognitive impairment and dementia (Figure 1A and 1B). They are defined as areas of tissue infarction, often with gliosis or cavitation, visible only by examination of the autopsied brain at a microscopic level. Numerous autopsy studies have now shown that a greater microinfarct burden is correlated with increased likelihood of cognitive impairment. Cerebral microinfarcts are observed postmortem in the brains of ≈43% of patients with Alzheimer’s disease, 62% of patients with vascular dementia, and 24% of nondemented elderly subjects. However, reported microinfarct numbers are a significant underestimation of total burden, as only a small portion of the brain is examined at routine autopsy. Indeed, they can number in the hundreds to thousands within a single brain. Microinfarcts can arise from a variety of etiologies, including cerebral small vessel disease, large vessel disease, cerebral hypoperfusion, and cardiac disease, but their role in the pathogenesis of vascular cognitive impairment and dementia remains poorly understood.

Microhemorrhages are microscopic bleeds caused by rupture of cerebral microvessels, generating lesions on a similar scale as microinfarcts (Figure 1C and 1D). Pathologically, old microhemorrhages are defined as focal depositions of iron-positive hemosiderin-containing macrophages. Unlike microinfarcts, microhemorrhages easily escape detection on neuropathological examination, suggesting that they are not as widespread as microinfarcts. However, they can be detected with high sensitivity using in vivo magnetic resonance imaging (MRI). The 2 most common etiologies of age-related microhemorrhages are hypertensive arteriopathy and cerebral amyloid angiopathy (CAA). Microhemorrhages are associated with higher likelihood of dementia, and like microinfarcts, their role in vascular cognitive impairment and dementia remains incompletely understood.

Clinical studies have emphasized the need to better understand microinfarcts and microhemorrhages (microlesions) because their widespread nature and far-reaching effects could contribute to broad disruption of brain function in dementia. However, it is challenging to measure their functional impact in the human brain because their onset times and locations are unpredictable. Further, microlesions often coexist with other disease processes, making it difficult to isolate their specific contribution to brain dysfunction. Animal models that allow microlesions to be recreated in a more controlled environment are, therefore, valuable for understanding their impact on the brain. The purpose of this review is to collate existing rodent models of both microinfarcts and microhemorrhages that can be used to study microlesions at a preclinical level.

Lesion Size Criterion

Microinfarcts and microhemorrhages are thought to arise from the occlusion or rupture of small parenchymal arterioles, such as penetrating arterioles and their smaller branches. We define microinfarcts in rodent models as lesions with sizes that could only arise from the occlusion of single penetrating arterioles or their downstream branches. Microinfarcts are typically no larger than 1 mm in diameter in the mouse and rat cortex. We also apply this size criteria to microinfarcts in deeper brain structures, though the relationship between vascular architecture and microinfarcts beyond cortex remains understudied. Microhemorrhages induced in cortex, and occurring spontaneously in rodent models, seem to be ≈200 to 300 μm or smaller at histopathology. We have adhered to this size range in our review of the literature. We further note that the term microhemorrhage is used for histologically verified bleeds and microbleeds for the MRI-visible correlate of microhemorrhages. Supplemental information for defining microinfarcts and microhemorrhages in rodent tissues is provided in the online-only Data Supplement.
Microinfarct

260 μm

10 mm

Microhemorrhage

100 μm

250 μm

Figure 1. Human microinfarcts and microhemorrhages in cerebral amyloid angiopathy (CAA) cases. A, A cortical microinfarct on a hematoxylin & eosin–stained section. B, Microinfarct (black arrow) on a T2-weighted ex vivo magnetic resonance imaging (MRI) scan. C, A cortical microhemorrhage on a hematoxylin & eosin–stained section. D, Multiple lobar microbleeds (white arrows) on a gradient-repeat echo ex vivo MRI scan.

Models of Induced Microinfarcts

Intracarotid Injection of Microemboli

One method of generating cerebral microinfarcts involves the injection of microemboli into the blood circulation, such as occlusive microbeads or cholesterol crystals (Figure 2, left). Injections are typically made through the internal carotid artery. This produces broadly distributed microinfarcts, with cortex, hippocampus, and thalamus being the major sites of accrual. A spectrum of microinfarct types are seen, including wedge or column-shaped lesions, in the cerebral cortex that are continuous with the pial surface (Figure 3A), as well as smaller circumscribed microinfarcts contained within the parenchyma. The choice of microembolus size, type, or number injected is important to achieve consistency of microinfarct formation.

Laser-Induced Occlusion of Penetrating Arterioles

A second method allows reproducible targeting of microinfarct location and size in rodent cortex (Figure 2, right). Typically coupled with in vivo two-photon imaging, single penetrating arterioles (or small pial arterioles that support flow to penetrating arterioles) are selectively occluded by inducing clots with precise laser irradiation. This is achieved most commonly by activating circulating photosensitizing agents with focused green lasers, that is, focal photothermolysis. Occlusions can also be made without a photosensitizer by using amplified femtosecond laser ablation or targeted irradiation with higher laser powers from conventional two-photon imaging lasers. Targeted penetrating arteriole occlusions primarily generate wedge or column-shaped cortical microinfarcts because clots are made in vessels at or near the pial surface (Figure 3B).

Models With Spontaneous Microinfarcts

Bilateral Carotid Artery Stenosis

Bilateral carotid artery stenosis (BCAS) is a common manipulation to induce chronic cerebral hypoperfusion in rodents. Normal C57Bl/6 mice develop subcortical microinfarcts after long periods of BCAS (6 months) but not after shorter periods (2–3 months). Two groups recently examined the effects of BCAS in the Tg-SwDI mouse model, which develops early and pronounced CAA. Interestingly, only 2 to 3 months of BCAS was necessary to induce microinfarcts in these mice. Microinfarcts were observed in the cerebral cortex and hippocampus and were related to CAA severity, whereas no microinfarcts were seen in age-matched sham-operated Tg-SwDI mice. Further, a recent study showed that atherosclerotic Apoe knockout mice develop microinfarcts within 1.5 months after BCAS. Thus, prolonged cerebral hypoperfusion itself can cause microinfarcts, but this effect is exacerbated in transgenic mice with existing cerebrovascular disease.

Obesity and Diabetes Mellitus

Spontaneous microinfarcts were observed in a mouse model that crossed the Aβ overexpressing mouse line, APP/PS1, with the db/db mouse model for diabetes mellitus. The db/db line harbors a mutation of the diabetes mellitus (db) gene that leads to a leptin signaling defect, causing severe obesity, hypertension, and type 2 diabetes mellitus with hyperglycemia. The progeny of the APP/PS1-db/db cross-retained features of parental lines, but the combined risk factors led to cortical microinfaracts that were not observed in either parental strain. Microinfarcts appeared as small cystic cavities in various layers of cortex. The authors suspected that aberrant angiogenesis, unique to the crossed mice, led to immature and leaky microvessels that were prone to occlusion.

Endothelial NOS-Deficient Mice

Endothelial nitric oxide synthase is critical for regulation of vascular tone and blood pressure. A recent study showed that mice with partial deletion of endothelial nitric oxide synthase develop microinfarcts in cortex and, to a lesser extent, in the hippocampus and thalamus (Figure 3C). Cortical microinfarcts accrued in watershed regions between the perfusion territories of major cerebral arteries. They were noticeable by 6 months of age but were most prevalent at 12 to 18 months. This was accompanied by microvascular pathology, including intravascular clots, diffuse CAA, neuroinflammation, and blood–brain barrier disruption. Microinfarcts in these mice were postulated to result from small vessel thrombosis because of endothelial and platelet dysfunction.

Notch3 Mutant Mice

Microinfarcts (and microhemorrhages) have been reported in a mouse model of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy...
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(CADASIL). CADASIL is a hereditary form of vascular dementia caused by mutations on the gene for Notch3, a transmembrane receptor critical for mural cell–endothelial communication and vascular development. Mice with an Arg170Cys (R170C) mutation knocked into the endogenous Notch3 gene (a prevalent substitution mutation seen in human CADASIL) developed cerebrovascular pathology akin to that seen in human CADASIL. The authors reported microinfarcts in the motor cortex of 20-month-old mice, which appeared as small cystic cavities in deeper cortical layers. In contrast, another CADASIL mouse line (PAC-Notch3R169C) carrying rat Notch3 with an Arg169Cys mutation exhibited cerebral hypoperfusion and isolated white matter lesions but no microinfarcts. For reasons still unclear, other mouse lines with various Notch3 mutations develop arteriopathy but do not exhibit ischemic or hemorrhagic lesions.

Sickle Cell Mice

Cerebrovascular disease is a well-established complication of sickle cell disease (SCD). About 40% of children with SCD develop small “silent” cerebral infarcts, with some falling in the size range of microinfarcts. Recently, spontaneous cortical microinfarcts were reported in the Townes model of SCD (Figure 3D), a model that involves replacement of murine β-globin gene with the human sickle β-globin and human γ-globin genes. Aged sickle cell mice (13 months old) exhibited faster capillary flow velocities and altered microvascular topology, akin to that described in humans with SCD who were at high risk for stroke. Spontaneous cortical microinfarcts in SCD mice were larger and more frequent than in controls, and were associated with blood–brain barrier leakage and local tissue hypoxia, suggesting vascular pathology as an origin.

Models of Induced Microhemorrhage

Laser-Induced Rupture of Parenchymal Vessels

Microhemorrhages can be induced with high spatiotemporal precision in rodent cortex by directly rupturing cortical microvessels using focused lasers (Figure 4A). Much like optically induced microinfarcts, this model requires implantation of a cranial window and a two-photon microscope to visualize and ablate the desired microvessel. An amplified femtosecond laser is used to damage the wall of target vessels, such as penetrating arterioles or capillaries. In vivo two-photon imaging of laser-induced microhemorrhages shows rapid extravasation of blood cells forming a lesion core roughly 100 μm in diameter and broad dissipation of blood plasma over a region 5 times larger than the core.

Models With Spontaneous Microhemorrhages

CAA Models

A variety of APP overexpressing mouse lines develop CAA. These models include the PDAPP, Tg2576, double transgenic APP/PS1, triple transgenic Tg-SwDI, and APP23 mouse lines. In these mice, Aβ plaque load, as well as CAA, increases gradually in an age-dependent fashion, with some model-specific variation. However, reports of spontaneous microhemorrhages (or microinfarcts) have been sparse and only described in lines that develop severe CAA during old age or after a second hit.

The most commonly described observations of spontaneous microhemorrhages occur in APP23 mice. Cerebral microbleeds could be observed with in vivo T2*-weighted MRI in APP23 mice starting around 16 months of age. Microbleed number and volume increased with animal age. Postmortem analyses revealed that these MRI-observed microbleeds were true microhemorrhages on corresponding histopathologic Prussian blue–stained sections (Figure 4B). Parenchymal microvessels in these mice show severe vascular pathology, including smooth muscle cell degeneration and aneurysm-like vasodilation.

The APPDutch mouse model bears the mutation (E22Q-mutated Aβ) that causes hereditary cerebral hemorrhage with amyloidosis-Dutch type, a rare autosomal dominant disorder in humans characterized by early-onset of severe CAA and multiple recurrent lobar hemorrhages. Interestingly, when APP23 mice are crossed with APPDutch mice, twice as many...
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Microhemorrhages arise compared with the APP23 genotype alone. Further exacerbation of CAA was observed in crossed mice, which may explain the increased incidence of microhemorrhage.

**Hypertension Models**

A widely used model of severe hypertension is the inbred strain stroke-prone spontaneously hypertensive rat. Early characterization studies showed that these rats developed spontaneous ischemic lesions and hemorrhages around 9 to 12 months of age. Microhemorrhages (and some microinfarcts) coexisted with larger ischemic or hemorrhagic strokes. Fibrinoid necrosis and thickening of the vascular walls were regularly observed with cerebral penetrating arterioles, which likely contributes to vascular occlusions and ruptures in these animals. Abnormal vascular remodeling may also generate weakened microvessels, leading to microhemorrhage.

Hypertension-induced microhemorrhages in mice require combining transgenic lines with treatments to chronically increase vascular tone. One study used a transgenic mouse line expressing both the human renin and angiotensinogen genes (R+/A+), which develop chronic hypertension but are otherwise normal. Challenging these hypertensive mice with a high salt diet and L-NAME (an inhibitor of neuronal and endothelial NOS) led to formation of microhemorrhages in multiple brain regions, including brain stem, cerebellum, and basal ganglia. Another study administered chronic angiotensin II and L-NAME to aged Tg2576 mice and reported the development of more microhemorrhages compared with mice receiving vehicle.

**Hyperhomocysteinemia Model**

Hyperhomocysteinemia is a risk factor for stroke and Alzheimer’s disease. In diet-induced hyperhomocysteinemia, mice are placed on a diet deficient in folate, vitamin B6, and
Table. Summary of Animal Models.

<table>
<thead>
<tr>
<th>Model</th>
<th>Model Type</th>
<th>Brain location</th>
<th>Lesion size reported/depicted</th>
<th>Lesion number reported; Consistency</th>
<th>Animal age</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microinfarcts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injected microemboli</td>
<td>Induced</td>
<td>Cortex, hippocampus, striatum, thalamus, corpus callosum</td>
<td>∼100–500 µm diameter (≈0.3 mm² area)</td>
<td>Numerous microinfarcts per injection; Very consistent</td>
<td>Any age</td>
<td>Silasi et al; Wang et al</td>
</tr>
<tr>
<td>Laser-induced</td>
<td>Induced</td>
<td>Cortex</td>
<td>∼500 µm diameter (≈0.2 mm³ volume)</td>
<td>One microinfarct per microvessel occlusion; Very consistent</td>
<td>Any age</td>
<td>Shih et al; Taylor et al</td>
</tr>
<tr>
<td>BCAS (C57Bl/6)</td>
<td>Spontaneous</td>
<td>Cortex and thalamus</td>
<td>Wide range of sizes (≈0.5 mm³ volume)</td>
<td>&lt;10 microinfarcts per brain; 50% of mice examined</td>
<td>3 mo start plus 6 mo of BCAS</td>
<td>Holland et al</td>
</tr>
<tr>
<td>BCAS (TgSwDI)</td>
<td>Spontaneous</td>
<td>Cortex and hippocampus</td>
<td>∼100–300 µm diameter (0.01–0.03 mm² area)</td>
<td>1–10 microinfarcts per brain; 25%–40% of mice examined</td>
<td>4–8 mo start plus 2–3 mo of BCAS</td>
<td>Salvadores et al; Okamoto et al</td>
</tr>
<tr>
<td>BCAS (Apoe−/−)</td>
<td>Spontaneous</td>
<td>Hippocampus</td>
<td>∼200 µm diameter</td>
<td>Multiple microinfarcts per brain; 75% of mice examined</td>
<td>3 mo start plus 1.5 mo of BCAS</td>
<td>Lee et al</td>
</tr>
<tr>
<td>APP/PS1 × db</td>
<td>Spontaneous</td>
<td>Cortex</td>
<td>∼500 µm diameter</td>
<td>Multiple microinfarcts per brain; 75% of mice examined</td>
<td>7 mo</td>
<td>Niedowicz et al</td>
</tr>
<tr>
<td>eNOS−/−</td>
<td>Spontaneous</td>
<td>Cortex, hippocampus, thalamus</td>
<td>∼100–500 µm diameter</td>
<td>Multiple microinfarcts per brain; Consistency unspecified</td>
<td>12, 18 mo</td>
<td>Tan et al</td>
</tr>
<tr>
<td>Notch3 mutant (R170C)</td>
<td>Spontaneous</td>
<td>Cortex (motor)</td>
<td>∼50–100 µm in diameter</td>
<td>Number per brain not specified; 12% of mice examined</td>
<td>20–22 mo</td>
<td>Wallays et al</td>
</tr>
<tr>
<td>Townes sickle cell mouse</td>
<td>Spontaneous</td>
<td>Cortex</td>
<td>∼100–500 µm diameter (≈0.05 mm² area)</td>
<td>=5 microinfarcts over 20 sections per brain; 100% of mice examined</td>
<td>13 mo</td>
<td>Hyacinth et al</td>
</tr>
<tr>
<td><strong>Microhemorrhages</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laser-induced</td>
<td>Induced</td>
<td>Cortex</td>
<td>∼100 µm diameter (hemorrhage core)</td>
<td>1 microhemorrhage per rupture; Very consistent</td>
<td>Any age</td>
<td>Nishimura et al; Rosidi et al</td>
</tr>
<tr>
<td>APP23</td>
<td>Spontaneous</td>
<td>Cortex, thalamus</td>
<td>∼50–300 µm diameter</td>
<td>15–30 microbleeds per brain; Very consistent</td>
<td>20–28 mo</td>
<td>Reuter et al; Winkler et al; Beckmann et al; Marinescu et al; Dudefrant et al</td>
</tr>
<tr>
<td>BCAS (TgSwDI)</td>
<td>Spontaneous</td>
<td>Thalamus</td>
<td>Not depicted</td>
<td>Very few microhemorrhages per brain; 30% of mice examined</td>
<td>4–8 mo</td>
<td>Salvadores et al; Okamoto et al</td>
</tr>
<tr>
<td>BCAS (C57Bl/6)</td>
<td>Spontaneous</td>
<td>Thalamus</td>
<td>∼150 µm diameter, but wide range reported</td>
<td>Multiple microhemorrhages per brain; 75% of mice examined</td>
<td>6 mo</td>
<td>Holland et al</td>
</tr>
<tr>
<td>eNOS−/−</td>
<td>Spontaneous</td>
<td>Cortex</td>
<td>∼25 µm diameter</td>
<td>∼8 microhemorrhages over 9–10 sections per brain; Consistency unspecified</td>
<td>18 mo</td>
<td>Tan et al</td>
</tr>
<tr>
<td>Notch3 mutant (R170C)</td>
<td>Spontaneous</td>
<td>Cortex</td>
<td>∼25 µm diameter</td>
<td>Number per brain not specified; 12% of mice examined</td>
<td>20–22 mo</td>
<td>Wallays et al</td>
</tr>
<tr>
<td>SPSHR</td>
<td>Spontaneous</td>
<td>Cortex, hippocampus, basal ganglia, corpus callosum</td>
<td>∼50–150 µm diameter</td>
<td>Multiple microhemorrhages per brain; 60% of rats examined</td>
<td>8–12 mo</td>
<td>Schreiber et al</td>
</tr>
<tr>
<td>R+/A+ (high salt and L-NAME)</td>
<td>Spontaneous</td>
<td>Brain stem, cerebellum, basal ganglia</td>
<td>∼50–150 µm diameter</td>
<td>Multiple microhemorrhages per brain; Very consistent</td>
<td>≈11 mo start plus 6 mo treatment</td>
<td>Iida et al</td>
</tr>
<tr>
<td>Tg2576 (Angl and L-NAME)</td>
<td>Spontaneous</td>
<td>Cortex and hippocampus</td>
<td>&lt;100 µm in diameter</td>
<td>Multiple microhemorrhages per brain; Very consistent</td>
<td>≈15 mo start plus 1 wk treatment</td>
<td>Passos et al</td>
</tr>
<tr>
<td>HHcy (C57Bl/6)</td>
<td>Spontaneous</td>
<td>Cortex and hippocampus</td>
<td>∼100 µm diameter</td>
<td>2–3 microhemorrhages per section; Consistency unspecified</td>
<td>7 mo start plus 4 mo diet</td>
<td>Sudduth et al</td>
</tr>
<tr>
<td>HHcy (APP/PS1)</td>
<td>Spontaneous</td>
<td>Cortex and hippocampus</td>
<td>∼100 µm diameter</td>
<td>4 microhemorrhages per section; Consistency unspecified</td>
<td>12 mo start plus 6 mo diet</td>
<td>Sudduth et al</td>
</tr>
</tbody>
</table>

BCAS indicates bilateral carotid artery stenosis; eNOS, Endothelial nitric oxide synthase; and HHcy, hyperhomocysteinemia.
B12 and supplemented with excess methionine. Treated mice developed microhemorrhages, visualized by in vivo MRI and Prussian blue staining of brain sections postmortem (Figure 4C). When hyperhomocysteinemia was induced in APP/PS1 mice, significantly more microhemorrhages were observed in transgenics compared with their wild-type littermates. This increase was believed to be mediated by a heightened CAA and activation of matrix metalloproteinase-9 at the cerebrovascular wall.

Model Selection: Advantages and Disadvantages

The models discussed in this review have been collated in the Table. Inducible models allow one to ask how a microlesion affects local brain activity or structure, independent of other disease factors. Laser-induced microlesions are limited to cortex but provide exquisite control over the location and timing of their onset. In a complementary fashion, intracarotid injection of microemboli provides less control over lesion location but produced distributed microinfarcts that cause measurable deficits in common tests of cognitive function.

Models developing spontaneous microinfarct or microhemorrhage provide an opportunity to understand the vascular deficiencies that lead to lesion formation and to identify potential targets for prevention. A wide variety of model types develop spontaneous microinfarcts and microhemorrhages, and this supports the idea that diverse disease processes can be contributing factors, including CAA, mural cell or endothelial cell dysfunction, cerebral hypoperfusion, and vascular inflammation.

Physiological Impact of Induced Microinfarcts and Microhemorrhages

In vivo optical imaging studies have revealed that microinfarcts produce lasting neural and hemodynamic deficits in surrounding tissues. When microinfarcts were photothrombotically induced in the cortices of APP/PS1 or Tg2576 mice, increase in Aβ plaque formation was seen in surrounding tissues. This effect was attributed to impaired drainage of interstitial fluid. Indeed, 2 recent studies reported that distributed microinfarcts produced bi-hemispheric disruption of the brain’s glymphatic system. Further, consistent with having a large effect on brain function, distributed microinfarcts also lead to deficits in cognitive tasks, despite total microinfarct volume being small compared with overall brain volume.

Surrounding the microinfarct core, neurons are viable, but there is atrophy of neuronal dendrites, reduced dendritic spine density, axonal damage, and myelin loss. This is consistent with recent histopathologic findings from human microinfarcts. Extensive peri-lesional astrogliosis, mislocalization of aquaporin 4, and blood–brain barrier disruption are also observed, indicating neuroinflammation and altered neuronal–glial signaling. Collectively, these findings support the idea that microinfarcts impair the function of tissues well beyond their restricted lesion cores.

The existing data on experimentally induced microhemorrhages suggest that these lesions also produce neural deficits in surrounding tissues but more transiently than microinfarcts. In vivo calcium imaging revealed impaired neuronal responses ≤150 μm from the lesion core, but tissue function recovered within 1 day. However, microhemorrhages induced persistent peri-lesional microgliosis and astrogliosis. Penetrating arterioles often remained flowing even after rupture, which suggests that local ischemia (as seen with microinfarcts) is necessary to induce neural deficits distant to the lesion core.

Summary and Future Directions

Clinical efforts are now focused on understanding the causes, risk factors, and functional effects of microinfarcts and microhemorrhages in vascular cognitive impairment and dementia. However, these microlesions can be difficult to study in humans because of their small size, unpredictable onset, and coexistence with other disease factors. Preclinical studies can complement these clinical efforts by providing insight into the impact and pathogenesis of microlesions. This review has shown that microlesions similar to those seen in humans can be reliably induced through microvascular occlusion and manipulation. Further, microlesions develop spontaneously in a variety of genetic and dietary-induced models of cerebrovascular disease. Future studies could use high-field MRI and white matter tractography to understand how microlesion accrual affects white matter integrity and brain connectivity. This addresses the possibility that individually small but broadly distributed microinfarcts can impair brain function on a global scale. Mechanistic studies can also be performed to understand the cellular/molecular changes occurring beyond the lesion core. Technologies such as in vivo two-photon imaging allow direct visualization of local neuronal, glial, vascular, and glymphatic changes in tissues affected by ischemic injury. Further, longitudinal imaging of models that develop spontaneous microlesions can shed light on disease pathogenesis by identifying changes to the neurovascular unit that could account for narrowing or weakening brain microvessels. Finally, animal models serve as test beds for therapeutics. A small number of past studies have shown that microinfarct volume can be extensively reduced by neuroprotectants, suggesting a relatively large penumbra and window for therapeutic intervention. Thus neuroprotective strategies previously considered for large ischemic stroke may be worth re-evaluating as preventative therapies for smaller ischemic events.

Addendum

After the manuscript was accepted for publication, Dönmez-Demir et al published an article describing occlusion of single cortical penetrating arterioles using focal application of FeCl₃ from a micropipette.

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Disclosures

None.

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SUPPLEMENTAL MATERIALS

Criterion for defining microinfarcts and microhemorrhages in rodent studies.

Recent reviews have established guidelines for identifying human cerebral microinfarcts and microhemorrhages. However, no criteria yet exist for defining spontaneously occurring microlesions in rodent models. Microinfarct size is the primary means to distinguish these lesions from larger ischemic strokes caused by blockage or rupture of major cerebral arterioles. We suggest that an upper size threshold of 1 mm lesion diameter be used for microinfarcts. This would encompass most microinfarcts observed across the preclinical studies reviewed here, and is also consistent with the size of penetrating arteriole perfusion domains observed in rats and mice. A caveat, however, is that microinfarct shapes can be irregular and individual tissue slices may not transect the widest region of the lesion. It is therefore best to report microinfarct volume, if possible, by measuring lesion area over multiple adjacent brain slices. In addition to defining a region of infarction with loss of NeuN or Hematoxylin & Eosin pallor, it is recommended to also confirm the presence of neuroinflammation by staining for CD68, Iba1 or GFAP, which should be upregulated following tissue ischemia. For old microhemorrhages, stains such as Perl’s Prussian blue should be used to detect the presence of iron. We suggest that lesions should consist of at least 5 iron-positive depositions as a lower threshold and the overall region of staining be less than approximately 0.5 mm in diameter. We noticed that regions with only a single iron deposit were counted as microhemorrhages in some studies, and these may be too small to represent true microhemorrhages. By standardizing the reporting of microinfarcts and microhemorrhages with lesion size criterion, and data on lesion size range, brain location, and prevalence, consistency among research groups and comparisons between different models can be evaluated more rigorously.

References