Potential Animal Models of Lacunar Stroke
A Systematic Review

Emma L. Bailey, BSc; James McCulloch, PhD; Cathie Sudlow, MRCP, PhD; Joanna M. Wardlaw, FRCR, FMedSci

Background and Purpose—Lacunar ischemic stroke accounts for 25% of all ischemic strokes, but the exact etiology is unknown. Numerous pathophysiologies have been proposed, including atheroma and endothelial dysfunction. Models of any of these pathological features would aid understanding of the etiology and help develop treatments for lacunar stroke. We therefore aimed to assess the relevance of all available potential animal models of lacunar stroke.

Methods—We systematically reviewed the published literature for animal models that could represent lacunar stroke using validated search strategies. We included studies that could represent an aspect of lacunar stroke as well as those aiming to model conditions with potentially similar pathology and extracted data on species, induction method, and resulting brain and vessel lesions.

Results—From 5670 papers, 41 studies (46 papers) met inclusion criteria representing over 10 different classes of stroke induction. Important data like infarct size and animal numbers were often missing. Many models’ infarcts were too large or affected the cortex. Emboli mostly caused cortical but not small subcortical lesions. Most models focused on creating ischemic lesions in brain tissue. Only one (spontaneous lesions in spontaneously hypertensive stroke-prone rats) also mimicked small vessel pathology. Here, the precursor to small vessel and brain damage was blood–brain barrier failure.

Conclusion—Some animal models produce small subcortical infarcts, but few mimic the human small vessel pathology. Models of small vessel disease could help improve understanding of human lacunar disease, particularly to clarify factors associated with the small vessel morphological changes preceding brain damage. Much lacunar stroke may arise after blood–brain barrier disruption. (Stroke. 2009;40:e451-e458.)

Key Words: emboli ■ experimental model ■ fibrinoid necrosis ■ lacunar stroke ■ stroke ■ subcortical

Twenty-five percent of ischemic strokes are of the lacunar subtype.1 These have distinct clinical features and are due to small lesions in the subcortical gray or white matter or brainstem.2,3 The causes and prognosis of lacunar stroke probably differ from large artery stroke. For example, carotid stenosis and atrial fibrillation are less common and the risk of early recurrence is lower in lacunar than in large artery ischemic stroke.4,5 Most lacunar strokes are thought to arise secondary to an abnormality in the walls of the small, deep perforating (lenticulostriate) arteries. The arterial wall changes have been referred to as “segmental arteriolar disorganization” or “lipohyalinosis” or “fibrinoid necrosis,” but the exact initiating cause of these small vessel changes is unknown.6 There are, however, several suggestions: embolic or thrombotic occlusion; atheromatous stenosis of the intracranial large arteries occluding the lenticulostriate artery origin; microatheroma in the lenticulostriate arteries; vasospasm; endothelial dysfunction; or other forms of endovascular damage (eg, inflammation).7,10,11 The relative importance and frequency of these alternative pathologies is unknown. The brain parenchymal lesions that result are generally considered to be “ischemic.” However, some pathological observations suggest that lacunar infarction could result from an alternative initial process such as perivascular edema after blood–brain barrier (BBB) derangement.12

Animal models are useful in cerebral ischemia research to replicate ischemic damage encountered in humans. In contrast to large artery occlusive ischemic stroke, the lack of clarity regarding the pathophysiological events in lacunar stroke makes this stroke subtype particularly difficult to model. However, a model that can mimic at least some features of human lacunar stroke would undoubtedly be valuable to improve understanding of the disease in humans and to test treatments. We therefore undertook a systematic review to assess the suitability of all available animal models that could be pertinent to lacunar stroke. We also assessed their likely pathological mechanism and the lesions produced to determine their relevance to the human condition.

Methods

Study Identification
We used Cochrane Collaboration methodology for systematic reviews modified for observational studies (www.equator-net.org).
network.org).13 We also applied the Collaborative Approach to Meta Analysis and Review of Animal Data from Experimental Stroke (CAMARADES) checklist for systematic reviews of animal models in stroke.14 We sought to include a broad range of studies that claimed to (or could be taken to) mimic the vascular pathology and/or the brain damage associated with lacunar stroke (ie, that produced small lesions secondary to a disorder of a perforating artery of a size and distribution consistent with human lacunar lesions). We also sought models of conditions associated with similar pathology (eg, generalized white matter disease or vascular dementia).

**Search Strategy**

We identified studies with a search strategy developed with advice from the Cochrane Collaboration Stroke Group across 3 English language databases (Medline, Embase, and Biosis Previews) adjusted to accommodate different MeSH terms or subcategories available on each database (Appendix). We searched from January 1, 1950, to June 30, 2007, and included English and non-English language publications. We also hand-searched 2 journals that had published a large proportion of relevant material (Stroke and Journal of Cerebral Blood Flow and Metabolism) and crosschecked reference lists of primary papers, review articles, and books on subcortical stroke.

**Inclusion and Exclusion Criteria**

We sought animal models that could produce small subcortical lesions in white or deep gray matter by ischemic or other mechanisms. We excluded studies published only as an abstract (due to insufficient information). We were careful to include each study only once and to identify the original description and validation of the model, not its subsequent use. In the case of multiple publications from the same study, all available nonduplicate relevant data from each study were included. We specifically excluded (Figure 1): nonstroke literature; human studies; primary cerebral hemorrhage, global ischemia, and distal middle cerebral artery occlusions (MCAO, because only cortical infarcts are produced); models that only produced lesions over 100 cm³ (too large); and the COL4A1 genetic mouse15 (develops primary intracerebral hemorrhage at birth). We included the cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADA-SIL) transgenic mouse16 because it displays small vessel pathology pertinent to human CADA-SIL.

**Data Extraction**

From each study, we extracted the following data: (1) the purpose of the model and the aspect of pathology being modeled; (2) the characteristics of animals used (species, strain, weight, sex, age, any comorbidity) and number of animals studied; (3) the method of stroke induction, anesthetic used (in particular any neuroprotective properties) during the induction of ischemia, and control of physiologic variables during the procedure; (4) the use of randomization (if appropriate); (5) the time (after induction of lesion) to, and method of, euthanasia plus time interval between induction and lesion assessment; (6) the outcomes assessed (histology, imaging, neurobehavioral tests, and so on) and the blinding of outcome assessment to key variables and/or observer reliability of outcome assessments; (7) the mortality rate; (8) the lesion size, measurement method (lesion area or volume), and reproducibility of measurement; the brain territory affected and definition, if given, of “infarct” or “lesion”; (9) in studies using histology, number and size of sections, stains used, and whether tissue parenchyma or vessels were assessed or affected; and (10) in studies using in vivo whole animal imaging, type of imaging, slice thickness, lesion location, and distribution, and (11) in studies using neurobehavioral assessments, whether the lesion(s) resulted in functional deficit(s) consistent with stroke.

We assessed study quality using the CAMARADES database.14 We focused on 7 of the 10 CAMARADES items relevant to observational studies: blood pressure and blood gas monitoring, publication in peer-reviewed journal, temperature control, avoidance of anesthetics with marked intrinsic neuroprotective properties, sample size calculation before start of experiment, statement of compliance with regulatory requirements, and a statement regarding possible conflict of interest. We categorized studies by induction method and analyzed the type and distribution of tissue and vessel lesions produced in each model and their relevance to human lacunar stroke.

**Results**

We identified 17,821 references (Figure 1) of which 5670 appeared relevant according to the title and were examined in more detail. Review of the abstracts of these 5670 papers left
Table. Summary of Included Studies by Induction Method

<table>
<thead>
<tr>
<th>Author/Reference</th>
<th>Year</th>
<th>Induction Method</th>
<th>Species</th>
<th>n</th>
<th>Age and Comorbidity?</th>
<th>Infarct Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molinari</td>
<td>1970</td>
<td>Silicone rubber emboli</td>
<td>Dog</td>
<td>25</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Futrell</td>
<td>1991</td>
<td>Photocoagulation of ICA</td>
<td>Rat</td>
<td>33</td>
<td>Unknown</td>
<td>200–1200 µm diameter</td>
</tr>
<tr>
<td>Miyake</td>
<td>1993</td>
<td>Microsphere emboli</td>
<td>Rabbit</td>
<td>18</td>
<td>Unknown</td>
<td>0.5–3 mm diameter</td>
</tr>
<tr>
<td>MacDonald</td>
<td>1995</td>
<td>Microsphere emboli</td>
<td>Monkey</td>
<td>9</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Roos</td>
<td>1996</td>
<td>Black bead emboli</td>
<td>Rabbit</td>
<td>12</td>
<td>Unknown</td>
<td>77% of ipsilateral hemisphere</td>
</tr>
<tr>
<td>Chen</td>
<td>2001</td>
<td>Preformed fibrin clot into right ICA</td>
<td>Rat</td>
<td>27</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Atochin</td>
<td>2004</td>
<td>Fibrin microemboli</td>
<td>Mouse</td>
<td>44</td>
<td>Adult</td>
<td>&lt;40 mm³ UK</td>
</tr>
<tr>
<td>Lozano</td>
<td>2007</td>
<td>Photocoagulation of ICA</td>
<td>Mouse</td>
<td>35</td>
<td>Range</td>
<td>7–15% brain volume</td>
</tr>
<tr>
<td>MCAO</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crowell</td>
<td>1970</td>
<td>Scoville clip on MCA origin</td>
<td>Monkey</td>
<td>65</td>
<td>Unknown</td>
<td>3 mm diameter average</td>
</tr>
<tr>
<td>DeGirolami</td>
<td>1984</td>
<td>Transorbital snare ligation of MCA</td>
<td>Monkey</td>
<td>87</td>
<td>Unknown</td>
<td>&lt;2 cm diameter</td>
</tr>
<tr>
<td>Memezawa</td>
<td>1992</td>
<td>Intraluminal thread MCAO</td>
<td>Rat</td>
<td>42</td>
<td>Unknown</td>
<td>40% of CAO</td>
</tr>
<tr>
<td>Belayev</td>
<td>1999</td>
<td>Intraluminal thread MCAO</td>
<td>Mouse</td>
<td>49</td>
<td>Unknown</td>
<td>4.5–9 mm²</td>
</tr>
<tr>
<td>He</td>
<td>1999</td>
<td>Intraluminal occlusion of AChA and HTA</td>
<td>Rat</td>
<td>80</td>
<td>Adolescent</td>
<td>6–48 mm³</td>
</tr>
<tr>
<td>Lindner</td>
<td>2003</td>
<td>Intraluminal thread MCAO</td>
<td>Rat</td>
<td>50</td>
<td>Adult</td>
<td>4–27% hemisphere</td>
</tr>
<tr>
<td>Gharbawie</td>
<td>2006</td>
<td>Intraluminal thread MCAO</td>
<td>Rat</td>
<td>12</td>
<td>Adolescent</td>
<td>Percent of 3 structures damaged</td>
</tr>
</tbody>
</table>

Perforating artery occlusion

<table>
<thead>
<tr>
<th>Author/Reference</th>
<th>Year</th>
<th>Induction Method</th>
<th>Species</th>
<th>n</th>
<th>Age and Comorbidity?</th>
<th>Infarct Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yonas</td>
<td>1981</td>
<td>Retro-orbital occlusion of lateral lenticulostriates</td>
<td>Monkey</td>
<td>9</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Vajda</td>
<td>1985</td>
<td>Posterior thalamic arteries occluded</td>
<td>Monkey</td>
<td>10</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Del Zoppo</td>
<td>1986</td>
<td>Balloon cuff around MCA</td>
<td>Monkey</td>
<td>22</td>
<td>Adolescent</td>
<td>3.9 cm³</td>
</tr>
<tr>
<td>Kuwabara</td>
<td>1988</td>
<td>Occlusion of posterior cerebral perforators</td>
<td>Dog</td>
<td>24</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Bronowitz</td>
<td>1990</td>
<td>Occlusion of anterior cerebral artery branches</td>
<td>Dog</td>
<td>42</td>
<td>Unknown</td>
<td>0.73 cm³</td>
</tr>
<tr>
<td>Pevsner</td>
<td>2001</td>
<td>Cortical photocoagulation</td>
<td>Rat</td>
<td>12</td>
<td>Adult</td>
<td>1.7– to 4.2-mm depth</td>
</tr>
<tr>
<td>Wang</td>
<td>2003</td>
<td>Cortical pial vessel crush</td>
<td>Rat</td>
<td>9</td>
<td>Adult</td>
<td>1629 ± 261 µm diameter</td>
</tr>
<tr>
<td>Hua</td>
<td>2006</td>
<td>Cortical pial vessel crush</td>
<td>Rat</td>
<td>9</td>
<td>Adult</td>
<td>1.09 mm³</td>
</tr>
</tbody>
</table>

ET-1 Injection

<table>
<thead>
<tr>
<th>Author/Reference</th>
<th>Year</th>
<th>Induction Method</th>
<th>Species</th>
<th>n</th>
<th>Age and Comorbidity?</th>
<th>Infarct Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuxe</td>
<td>1992</td>
<td>ET-1 injection (intrastratial)</td>
<td>Rat</td>
<td>11</td>
<td>Unknown</td>
<td>1–6 mm³</td>
</tr>
<tr>
<td>Hughes</td>
<td>2003</td>
<td>ET-1 injection (striatum and subcortical WM)</td>
<td>Rat</td>
<td>38</td>
<td>Adult</td>
<td>17.5 µL/20 µm²</td>
</tr>
<tr>
<td>Frost</td>
<td>2005</td>
<td>ET-1 injection (striatum)</td>
<td>Rat</td>
<td>40</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Whitehead</td>
<td>2006</td>
<td>ET-1 injection (internal capsule)</td>
<td>Rat</td>
<td>25</td>
<td>Unknown</td>
<td>−500 µM diameter</td>
</tr>
</tbody>
</table>

Spontaneous stroke

<table>
<thead>
<tr>
<th>Author/Reference</th>
<th>Year</th>
<th>Induction Method</th>
<th>Species</th>
<th>n</th>
<th>Age and Comorbidity?</th>
<th>Infarct Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knox</td>
<td>1980</td>
<td>Naturally occurring strokes in SHRS</td>
<td>Rat</td>
<td>43</td>
<td>Range + hypertension</td>
<td>Unknown</td>
</tr>
<tr>
<td>Ogata</td>
<td>1981</td>
<td>Naturally occurring strokes in SHRS</td>
<td>Rat</td>
<td>88</td>
<td>Range + hypertension</td>
<td>150 µm – 1.5 mm diameter</td>
</tr>
<tr>
<td>Fredriksson</td>
<td>1985</td>
<td>Naturally occurring strokes in SHRS</td>
<td>Rat</td>
<td>55</td>
<td>Range + hypertension</td>
<td>Unknown</td>
</tr>
<tr>
<td>Tagami</td>
<td>1987</td>
<td>Naturally occurring strokes in SHRS</td>
<td>Rat</td>
<td>84</td>
<td>Range + hypertension</td>
<td>Unknown</td>
</tr>
<tr>
<td>Su</td>
<td>1998</td>
<td>Naturally occurring strokes</td>
<td>Dogs</td>
<td>18</td>
<td>Range</td>
<td>Unknown</td>
</tr>
<tr>
<td>Lin</td>
<td>2001</td>
<td>Naturally occurring strokes in SHRS</td>
<td>Rat</td>
<td>38</td>
<td>Range + hypertension</td>
<td>Unknown</td>
</tr>
<tr>
<td>Sironi</td>
<td>2004</td>
<td>Naturally occurring strokes in SHRS</td>
<td>Rat</td>
<td>96</td>
<td>Unknown + hypertension</td>
<td>Unknown</td>
</tr>
<tr>
<td>Garosi</td>
<td>2006</td>
<td>Naturally occurring strokes</td>
<td>Dog</td>
<td>38</td>
<td>Range</td>
<td>Unknown</td>
</tr>
<tr>
<td>Rossmeisl</td>
<td>2007</td>
<td>Naturally occurring strokes</td>
<td>Dog</td>
<td>6</td>
<td>Range</td>
<td>9–21 mm diameter</td>
</tr>
</tbody>
</table>

(Continued)
primary papers for examination in full after exclusion of abstract-only publication and review articles. After discussion among coauthors, 46 papers describing 41 studies were included (Table).

### Methodological Characteristics of Included Studies

Of the 41 studies, 8 stated that their model represented lacunar stroke and 12 models clearly attempted to recreate an aspect of lacunar stroke pathology (eg, vasoconstriction through endothelin-1 injection). The rest were not stated by their authors to represent lacunar stroke but of these, 3 models were cited by others as representing lacunar stroke, and 18 appeared pertinent to us because they produced small deep lesions in relevant areas of the brain.

Most models used rodents, but dogs, primates, and rabbits were also used (Table). The mean number of animals per study was 42 (range, 6 to 242) with considerable variation in number between induction techniques. The sex and age of animals were particularly difficult to extract: 42% of studies used male animals only, 5% female only, 30% both, and 23% did not specify the sex. At least 18% used adults (age not given), 10% used adolescents, and 25% used a range of ages; 47% did not specify the age and only one study specifically used older animals. Few animals had comorbidities like hypertension.

Infarct size was poorly described and standardized. Thirty-nine percent did not give infarct measurements at all. Of those that did, there were 10 different measurement methods (Figure 2). Standardized comparisons of infarct size between or within models were therefore limited to descriptive analyses. From descriptions and figures provided, infarcts from many studies were diversely spread around the brain in cortical gray and white matter. Many subcortical lesions better resembled a human striatocapsular than a lacunar infarct. Figure 3 demonstrates the size of a lacunar lesion relative to each species’ brain assuming a similar lesion:brain size ratio to humans. These small deep lesions are not only extremely difficult to create, but also difficult to measure accurately with current techniques. Physiological variables during surgery were poorly reported; 28 of 41 studies stated that body temperature was controlled, but blood pressure and respiration were only stated to be controlled in 8 (20%) and 5 (12%), respectively. Most models euthanized animals within 1 week of surgery: 9 of 41 (22%) within 24 hours and 23 of 41 (56%) between 1 and 7 days. Long-term survival experiments were conducted mainly in transgenic animals or spontaneously hypertensive stroke-prone rats (SHRSP). There were few neurological assessments; 10 of 41 (24%) publications used a validated neurological test and 12 of 41 others (29%) used a self-devised scale usually described as “mild,” “moderate,” and “severe.” The median score on the CAMARADES study quality checklist was 3 of 7 (range, 2 to 6/7) reflecting rather poor reporting of key methodological criteria.

### Table. Continued

<table>
<thead>
<tr>
<th>Author/Reference</th>
<th>Year</th>
<th>Induction Method</th>
<th>Species</th>
<th>n</th>
<th>Age and Comorbidity?</th>
<th>Infarct Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoshimoto69</td>
<td>1978</td>
<td>Simultaneous occlusion of ICA, ACA, MCA, and PCA</td>
<td>Dogs</td>
<td>43</td>
<td>Adult</td>
<td>Amount of thalamus damaged</td>
</tr>
<tr>
<td>Matsumoto67</td>
<td>1990</td>
<td>Unilateral carotid occlusion</td>
<td>Gerbil</td>
<td>20</td>
<td>Unknown</td>
<td>&lt;/-/0.2 mm</td>
</tr>
<tr>
<td>Toshima68</td>
<td>2000</td>
<td>2 injections of sodium laurate into ICA</td>
<td>Rat</td>
<td>56</td>
<td>Adult</td>
<td>8% of slice area?</td>
</tr>
<tr>
<td>Ruchoux16</td>
<td>2003</td>
<td>Genetic model of CADASIL</td>
<td>Mice</td>
<td>15</td>
<td>Range</td>
<td>0.2–0.6 µm?</td>
</tr>
<tr>
<td>Shibata70</td>
<td>2004</td>
<td>Bilateral carotid artery occlusion</td>
<td>Mice</td>
<td>60</td>
<td>Adolescent</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

n indicates no. of animals; ICA, internal carotid artery; MCA, middle cerebral artery; ACA, anterior choroidal artery; HTA, hypothalamic artery; WM, white matter; ACA, anterior cerebral artery; PCA, posterior cerebral artery.

Techniques for Infarct Size Quantification

![Figure 2. Methods of infarct quantification.](https://example.com/figure2.png)

![Figure 3. Approximate brain volume (cm³) and gray:white matter ratio in healthy (young) animals shown relative to the human brain.](https://example.com/figure3.png)

Red dots indicate the size of lacunar lesions relative to the animal species’ brain assuming a similar size ratio to that in humans. The red dots also illustrate the difficulty in targeting a small lacunar-like lesion to white matter, particularly in smaller rodents, due to the paucity of white matter.
Lesion Induction Methods

There were 6 categories of primary stroke induction technique: embolism, MCAO, individual perforating vessel occlusion, parenchymal endothelin-1 (ET-1) injection, spontaneous lesions, and miscellaneous (Table).

**Emboli**

Eight studies,20–28 including 433 animals, mostly rodents, were included. Injection of different amounts and sizes of emboli (microspheres, black beads, silicone rubber cylinders, preformed clots) into the internal carotid artery produced unpredictable infarcts.20,21,24–27,29 Although small emboli in sparse numbers could produce subcortical infarcts, most emboli produced cortical infarcts.24 Some models irradiated the internal/common carotid arteries in the presence of a photosensitive dye to produce an embolic source said to mimic thrombus generation on an atherosclerotic carotid artery plaque.22 This produced multiple infarcts, mostly cortical with a few in the basal ganglia and the caudate nucleus, but the subcortical lesions were poorly documented making their relevance to lacunar infarction uncertain.

**Middle Cerebral Artery Occlusion**

Seven studies,30–37 including 385 animals consisting of primates, pigs, and rodents, were included. Two approaches were described: (1) the intraluminal middle cerebral artery thread occlusion; and (2) selective anterior choroidal or hypothalamic artery occlusion. The intraluminal thread occlusion model is a widely used large artery ischemic stroke model, which first damages subcortical and later cortical structures.31 Thirty minutes of MCAO produced purely subcortical lesions <1 cm diameter in primates.32 Occlusion for up to 4 hours created infarcts in subcortical structures in primates32 that mimicked human striatocapsular infarcts38 in terms of size and structures affected. Striatocapsular infarcts affect the majority of the basal ganglia or adjacent white matter and are accounted for by either transient middle cerebral artery mainstream occlusion with early reperfusion or, if the occlusion persists, then with very good collateral flow from the anterior or posterior arteries to the cortical middle cerebral artery territory. Selective occlusion of the anterior choroidal and hypothalamic artery origins by precise placing of the intraluminal thread in the artery mouth34,35 produced subcortical infarcts. The anterior choroidal lesions were generally of striatocapsular dimensions; the hypothalamic lesions seemed particularly pertinent to lacunar stroke but were poorly described.

**Perforating Artery Occlusion**

Eight studies,39–46 including 119 animals consisting of rat, monkey, and dog, were included. Small cortical infarcts, produced in rats by occluding a cortical pial artery on the brain surface using forceps or photochemical irradiation,43,45 were described as mimicking lacunar infarction because they showed “cavitation caused specifically by ischemia of smaller vessels” (however, the lesions were cortical).41 In primates, a balloon cuff placed around the proximal middle cerebral artery to occlude the lenticulostriate arteries40 leaving the middle cerebral artery patent, resulted in lenticulostriate artery occlusion by eosinophilic thrombus with brain tissue softening, necrosis, and cyst formation of 3.9 cm³ average volume. Others coagulated or crushed many arterioles (thalamoperforators, lenticulostriaties, and so on). These lenticulostriate occlusion models all produced striatocapsular-sized rather than lacunar-sized lesions.

**ET-1 Injection**

Four studies,47–50 including 114 rats, were included. ET-1, a powerful vasoconstrictor, when injected into the subcortical tissues in a dose-dependent manner produces ischemic lesions.51 The powerful vasoconstrictive action may affect several microvessels. ET-1 injected into deep gray matter caused a small lesion with neuronal and astrocyte loss and delayed macrophage/microglia response48; into white matter, it caused axonal and oligodendrocyte disruption followed by myelin damage and increased astrocyte reactivity.50 The histological changes in the vasconstricted vessels were not reported.

**Spontaneous Lesions**

Nine studies,52–61 including 466 animals comprising rats and dogs, were included. The SHRSP60 develops hypertension followed by stroke-like neurological symptoms with increasing age. Studies in rats from 4 to 52 weeks of age showed that a combination of malignant hypertension, a genetic predisposition to stroke, and a high-salt diet were all risk factors for spontaneous stroke.52,53,55–58,60 Pathology, all in the perforating arteriolar territories, included microinfarcts, small hemorrhages, and multiple white matter cysts. The primary event preceding these tissue lesions was endothelial disruption with BBB breakdown (shown using multiple techniques) being a major contributor to the parenchymal lesions.52,53,60,62 Arteriolar wall thickening (similar to lipohyalinosis) and in late stages to fibrinoid necrosis, luminal narrowing or dilatation, and thrombotic occlusion appeared to be late secondary features in this model, long after extensive subcortical damage was already apparent.48,50–53,52,62 Inflammatory changes in the vessel walls proceed hypertension and vessel wall damage and at least some components of the “stroke prone-ness” appear to be blood pressure-independent. For example, SHRSP have impaired endothelium-dependent large vessel relaxation compared with spontaneously hypertensive rats without stroke proneness.64 Angiotensin II type 1 receptor blockers protect against stroke and reduce vessel wall, BBB, and tissue changes beyond any effect attributable to blood pressure reduction alone.65 Similarly, angiotensin-converting enzyme inhibitors reduce stroke-related morbidity despite the animals developing hypertension.66 Elderly dogs also sustain spontaneous strokes with neurological deficits (hemiparesis, facial hypalgesia, and hemianopia) but are less researched.54,59,61

**Miscellaneous**

Five studies,16,67–70 including 194 animals of various species, were included. Simultaneous occlusion of 4 main cerebral arteries for 60 to 120 minutes in dogs produced thalamic infarction alone and was considered to be a low-perfusion model.69 Sodium laurate injected into a cortical cerebral artery in rats induced endothelial necrosis, intravascular thrombus formation, and occlusive stroke but caused damage that was predominantly cortical and too extensive for lacunar stroke.
stroke.68 Unilateral carotid occlusion from 5 to 30 minutes in the gerbil produced a large artery territorial infarct.67 A transgenic notch3 mouse modeling CADASIL pathology produced smooth muscle proliferation in the small arteriolar walls similar to human CADASIL (mostly in the tail, not the brain) and few stroke lesions.16 Bilateral carotid artery stenosis, created by placing microcoils around the carotid arteries in mice, produced hypoperfusion accompanied 14 days later by white matter lesions70 judged to be a cumulative consequence of hypoperfusion. However, these white matter lesions share common features with those seen in subcortical vascular dementia and were considered primarily to reflect BBB disruption.70

Discussion

We found models that were potentially relevant to the brain lesions, the microvessel pathology, or both in lacunar stroke. However, most models produced infarcts that were too large or affected the wrong brain territory to be pertinent to lacunar disease. Some models could be useful for evaluating novel treatments for lacunar-sized ischemic subcortical lesions, but few attempted to explain the small vessel changes associated with most lacunar stroke in humans. Only one established model, the SHRSP, produced lesions that mimicked the small vessel and brain parenchymal pathology. In this model, the primary event is BBB disruption with vessel occlusion and brain ischemia occurring well after tissue damage associated with extravasation of edema fluid is visible. The widespread and primarily cortical infarct distribution encountered in models using emboli indicated that emboli or microthrombi were an unlikely cause of most human lacunar stroke. This finding is consistent with human epidemiological data.4

This review has limitations. The methodology for systematic reviews of observational studies in animals is still under development. Identifying the relevant literature is difficult because key words that would help to identify relevant papers are inconsistent and suboptimal. We used multiple overlapping study ascertainment methods but may still have missed some key models. Any full assessment of the relevance of experimental models of subcortical stroke is hampered by the incomplete knowledge and confusing terminology of lacunar stroke pathology in humans. The location and small lesion size makes modeling of lacunar stroke difficult in small mammals. Reproducing a 1-cm³ human infarct relative in size to a mouse or rat’s brain requires skill and advanced measuring techniques (Figure 3).17–19 A further limitation is the substantially lower white:gray matter ratio in rodents (approximately 14:86%) compared with primates (40:60%).19 Reporting of studies could be improved because the average score for key methodological factors was only 3 of 7. Few studies used older animals or ones with pre-existing vascular problems, although most patients with lacunar stroke are older and have vascular risk factors.71 Few studies used appropriate neurological/behavioral tests to assess physiological/cognitive impairments. Clinical examination for “lacunar syndromes”738 is the first assessment of a potential patient with human lacunar stroke. Behavioral tests, sensitive enough to detect the effects of lacunar-like syndromes in animals, are important to ascertain whether the lesion created by a model is relevant to lacunar stroke in humans. However, neurological testing may be limited in some models in which damage incurred during induction of the model (eg, visual impairment) can restrict neurological assessment (eg, blindness).70 The short time from induction to euthanasia did not allow the lesions to mature, further limiting determination of lesion size and relevance.

All of the models were limited as models of lacunar disease. In general, the embolic models produced mostly cortical infarcts, not subcortical infarcts. The poor documentation of any subcortical lesions produced precluded reliable assessment of whether the lesions were large subcortical (ie, striatocapsular equivalent in size) or true small subcortical single perforator lacunar infarcts. Similarly, there is evidence in humans that few lacunar infarcts are likely to be embolic.72

Human lacunar stroke may be caused by middle cerebral artery atheroma occluding the lenticulostriate artery origin.9 The most promising model equivalent was selective occlusion of the anterior choroidal or hypothalamic arteries, which produced some small infarcts.84 The hypothalamic infarcts in particular appeared comparable with lacunar infarcts (author’s comment) but were overlooked in favor of the anterior choroidal artery lesions.35 Other models that occluded lenticulostriate trunks or multiple ostia produced striatocapsular-like, not lacunar, lesions.39 ET-1 injection produced small subcortical lesions but caused several microvessels to be constricted simultaneously so was inconsistent with the “single vessel” theory of lacunar stroke. The CADASIL transgenic mouse mimics some aspects of human CADASIL,46 but the vessel changes are mainly encountered in the tail with minimal effects on the cerebral vessels and few, if any, cerebral lesions. A lacunar stroke model in the miniature pig was published after the June 2007 cutoff.73

The only model that mimics both microvessel and parenchymal changes is the spontaneous stroke in SHRSPs.60,74 This species is highly inbred and may therefore be of limited relevance, but the microvascular and brain morphological features are so similar to those described in humans that it is hard to ignore. In this model, the initiating step appears to be BBB disruption,52,60,75 which leads to parenchymal perivascular edema-related damage in white matter60 and arteriolar damage.53,57 The arteriolar damage includes arteriolar wall thickening and fibrinoid necrosis, which could lead to luminal narrowing and thrombosis, but these features seem to occur late after much brain damage associated with perivascular edema fluid has already occurred.53 The microvascular changes and brain parenchymal damage may not simply be the result of hypertension, but relate to impaired angiotensin production or sensitivity.65,66 Endothelium-dependent vasorelaxation is also impaired84 and inflammation may play a role.50 Thus, although late events may be “ischemic,” the early changes that lead to established disease are related to BBB failure. Therefore, if the SHRSP truly does mimic human lacunar disease, a move away from traditional occlusive/ischemic mechanisms toward endothelial/BBB disruption mechanisms will be required to advance understanding of the development of lacunar stroke and associated features of small vessel disease in humans.76 Further studies to characterize the small vessel morphological changes in SHRSP in more detail would be worthwhile.
Appendix

Search Strategy Used in Embase

A similar strategy using the closest available terms was used in Medline and Biosis Previews.

1. exp Models, Biological/ or exp Disease Models, Animal/ or exp Models, Animal/
2. (experimental adj5 stroke),tw
3. Animals/
4. exp mammals/ or exp primates/ or exp mice/ or exp rats/ or exp rats, inbred strains/ or exp rats, mutant strains/
5. 1 or 2 or 3 or 4
6. exp cerebrovascular disorders/ or exp basal ganglia cerebrovascular disease/ or exp brain ischemia/ or exp cerebrovascular accident/ or exp brain infarction/ or exp hypoxia-ischemia, brain/
7. brain edema/ or cerebrovascular accident/ or exp dementia, vascular/ or exp intracranial arterial diseases/ or exp “intracranial embolism and thrombosis”/ or exp vaso- spasms, intracranial/ 
8. (Stroke$ or cerebrovasc$ or cerebral $ or cerebral$).tw
9. 6 or 7 or 8
10. 5 and 9
11. limit 10 to animals
12. ((micro or small or perforat$) adj3 vessel) or arteriole).tw
13. (lacunar stroke or lacunar infarct).tw
14. (focal or multifocal or subcortical).tw
15. (emboli or microemboli).tw
16. (Blood brain barrier or BBB or plasma proteins).tw
17. ((small or micro) adj5 (stroke$ or occlusion$ or disease$)).tw
18. ((lacun$ or small or subcortical or deep or silent) adj5 (infarct$ or lesion$ or stroke$)).tw
19. (microgli$ or astrocyte$).tw
20. 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19
21. 11 and 20
22. limit 21 to humans
23. 21 not 22
24. (heart or bone or eye or lung or kidney or liver or renal or intestin$ or spinal or pulmonary or hepatic or global).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer name]
25. 23 not 24
26. (AD or PD or Alzheimer$ or Parkinson$ or epilepsy or MS or Multiple Sclerosis).tw
27. 25 not 26

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Disclosures

None.

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