Systematic Review and Meta-Analysis of Interventions Tested in Animal Models of Lacunar Stroke

Hugo Pedder, BSc; Hanna M. Vesterinen, PhD; Malcolm R. Macleod, PhD, FRCP(Edin); Joanna M. Wardlaw, MD

Background and Purpose—A total of 25% of strokes are lacunar, and these are pathophysiologically different from large artery strokes. Despite emerging evidence of a substantial impact on physical disability and dementia, little attention has been paid to the development of specific treatments. The optimal use of the animal models of lacunar stroke used to test candidate interventions is not known.

Methods—We conducted a systematic review and meta-analysis of studies testing candidate interventions in animal models of lacunar stroke. We used random-effects meta-analysis to assess the impact of study characteristics and trim and fill to seek evidence of publication bias.

Results—The efficacy of 43 distinct interventions was described in 57 publications. The median number of quality checklist items scored was 3 of 8 (interquartile range, 2–4). Many models reflected mechanisms of limited relevance to lacunar stroke. Meta-analysis of results from 27 studies showed that on average, infarct size and neurobehavioral outcome were improved by 34.2% (24.1–44.2) and 0.82 standardized mean difference (0.51–1.14), respectively. Four interventions improved both infarct size and neurobehavioral outcome but there were insufficient data for this finding to be considered robust. For infarct size, efficacy was lower in studies reporting blinding and higher in studies reporting randomization. For neurobehavior, efficacy was lower in randomized studies. For infarct size there was evidence of publication bias.

Conclusions—No intervention has yet been tested in sufficient range and depth to support translation to clinical trial. There is limited reporting of measures to reduce the risk of bias and evidence for a substantial publications bias.

Key Words: meta-analysis □ review, systematic □ stroke, lacunar

Lacunar strokes are small subcortical strokes caused by occlusion of single penetrating arteries. Although thromboembolism is a common cause of large artery stroke, the lacunar hypothesis, much debated, proposes that key mechanisms in lacunar pathology are microatheroma and lipohyalinosis, and that thromboembolic lacunar stroke is uncommon. Microatheroma are lipid-containing plaques, which are thought to accumulate in the parent main intracranial artery, such as the middle cerebral artery, and affect the origin of penetrating arteries, or develop in the proximal-penetrating arterioles themselves. Lipohyalinosis is a small-vessel pathology characterized by abnormal endothelial architecture and fibrosis, leading to thickening of the vessel wall and irregular luminal diameter. Despite associations with inflammatory endothelial dysfunction and blood–brain barrier disruption, the causes of these small-vessel changes are poorly understood.

Around 25% of all ischemic strokes are lacunar, and although they have an apparently good functional prognosis compared with cortical ischemic stroke, similar proportions of patients have poststroke cognitive impairment and long-term studies suggest that they identify cerebral small vessel disease (SVD), which puts patients at high risk of recurrent ischemic strokes, and of cognitive decline.

However, there is incomplete understanding of the precise mechanisms that lead to the pathology described above, and current therapeutic strategies are limited. This occurs against a background of translational failure, where many interventions reported to improve outcome in animal models of ischemic stroke more generally are not effective in human clinical trial.

It may be that animal models do not model human disease with sufficient validity to guide drug development; or that they do have this external validity but their conduct and reporting make them a poor guide, in practice, to support clinical drug development and trial design. For several animal models of neurological disease, systematic review and meta-analysis...
have given useful insights to the impact of study design and quality\textsuperscript{13–16} within the limitations inherent in combining data from different studies. Here, we assess the evidence supporting the efficacy of different treatments tested in animal models of lacunar stroke on behavioral and structural outcomes, with particular focus on the reporting and impact of measures to reduce bias and on the likelihood of publication bias.

Methods

The study protocol is available at www.camarades.info/index_files/protocols.htm, and further details of the methodological approach are given in Vesterinen et al.\textsuperscript{17}

Search Strategy

We searched Medline (from 1950), ISI Web of Science (from 1969), and EMBASE (from 1980) on March 6, 2012, with a strategy to identify animal experiments modeling lacunar stroke (including but not limited to intervention studies) modified from a previously reported search strategy\textsuperscript{18} and described in detail in the online-only Data Supplement. In addition, we searched (March 23, 2012) for publications citing original studies identified in the previous systematic review of the animal modeling of lacunar stroke.\textsuperscript{14} There were no language restrictions.

Inclusion and Exclusion Criteria

We included experiments where animals had been exposed to a lesion modeling lacunar stroke (defined as those caused by occlusion or stenosis or other disease of perforating blood vessels and leading to presumed ischemic lesions in focal subcortical areas)\textsuperscript{18,19} and where outcome in a cohort of animals subject to an intervention was compared with that in a control group. We included studies that quantified structural (lesion size) or functional (behavioral) outcomes. We excluded studies where single lesions were >1 of 140th of total brain volume, where lesions were not induced by single-vessel mechanisms (eg, traumatic brain injury), hemorrhages, and those involving partial or complete occlusion of the middle cerebral or common carotid artery. We excluded transgenic studies and those modeling neonatal hypoxia/ischemia. We excluded studies where the intervention was given with the expressed intention of worsening rather than improving outcome.

Data Extraction

We recorded the author, year of publication, intervention used and dose, type of animal (including species, strain, and sex), type of intervention, time of administration and of outcome assessment, outcome measure used, mean outcome, SD or SE, number of animals per group, presence of salt loading, method of stroke induction, anesthetic used, study quality assessment parameters, and reporting of measures to avoid bias (see below).

We defined treatment comparisons as those which compared the outcome in control and treated animals. For studies using spontaneously hypertensive stroke–prone rats (SHRSPs), we did not record time of administration or outcome assessment, as there was no clear time point at which stroke was induced. Where >1 intervention was given, we considered this combination to be a separate, unique intervention. Where treatment was administered in multiple doses, we considered treatment to occur at the time of the first dose and the dose to be the sum of all doses administered in the first 24 hours. Where data from multiple brain slices were reported, we included only the infarct size from the slice with the largest corresponding infarct in control animals. Where neurobehavioral outcomes were reported for >1 time point, we included only the latest time of assessment as the most clinically relevant end point. Where data were presented graphically, we contacted authors seeking further information, and if necessary we measured values from graphs (Universal Desktop Ruler, version 2.9).

Quality Assessment

Risk of bias was assessed using 8 of 10 Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES) checklist items.\textsuperscript{13} We did not include statement of control of temperature or avoidance of anesthetics with marked intrinsic properties as we considered these less relevant in models of lacunar stroke. We recorded whether the study was (1) published in a peer-reviewed journal, and whether it reported (2) randomized allocation to experimental group; (3) the blinding of the group allocation during the conduct of the experiment; (4) the blinded assessment of outcome; (5) the use of animals with relevant comorbidities; (6) a statement of sample size calculation; (7) a statement of compliance with regulatory requirements; and (8) a statement on possible conflicts of interest.

Data Analysis

We anticipated substantial heterogeneity between studies so used DerSimonian and Laird random-effects meta-analysis. For infarct size we assumed that unlesioned animals would have no infarct, and therefore we used a normalized mean difference approach and report percentage improvement in infarct volume. For behavioral outcomes it was not always possible to infer, for the test reported, how an unlesioned animal would perform, so we used a standardized mean difference (SMD) approach and report SMDs in units of SD. Where >1 functional outcome was reported for the same cohort of animals at the same time point, we combined these in a fixed-effects meta-analysis to give a summary estimate of efficacy at that time point, and the last time point was used for meta-analysis. We used stratified analysis with partitioning of heterogeneity to test the extent to which 8 study characteristics explained differences in reported efficacy, with a critical threshold of $P<0.057$ determined using Bonferroni correction to account for multiple comparisons. This approach tests whether studies are drawn from the same population, but not whether the point estimates in strata are significantly different. Therefore, for randomization and blinding, we calculated the impact of not reporting these measures as a relative change in efficacy compared with studies which did, along with a 95% confidence interval (CI).

SHRSPs were excluded from analyses comparing delay from stroke to drug administration and to outcome assessment as there was no clear time point at which stroke occurred in these studies. Continuous data are reported as means±95% CI and discrete data are reported as median with interquartile range.

We assessed publication bias using funnel plot and Egger regression\textsuperscript{20} and used trim and fill (STATA, version 10) to estimate the number of missing publications and to calculate adjusted global efficacy.\textsuperscript{21} Because the process of pooling data from different behavioral outcomes measured at the same time point would confound the analysis of publication bias, we used all data rather than pooled data, resulting in a different global estimate of efficacy in the publication bias analysis.

Results

Study Characteristics

Our electronic search identified 4379 publications of which 4322 were excluded, leaving 57 for inclusion in the systematic review (see online-only Data Supplement).

Lacunar stroke was introduced by microthrombi injection into the internal carotid artery in 16 of 57 studies (28%), microsphere injection into the internal carotid artery in 15 (25%), and endothelin injection into deep gray matter in 12 (21%). Salt loading to accelerate the occurrence of spontaneous stroke in SHRSPs, and spontaneous strokes without salt loading in SHRSPs, was each used in 5 studies (9%). A total of 41 (72%) used rats, with others using rabbits or mice (see online-only Data Supplement). Only 47 studies reported using an anesthetic during stroke induction.
The median number of study quality checklist items scored was 3 of 8 (interquartile range, 2–4). All studies had been published in peer-reviewed journals. Twenty-six of 57 (46%) studies reported randomized allocation to treatment group, 10 (18%) reported blinding to group allocation during the experiment, 26 (46%) reported blinded assessment of outcome, 12 (21%) used animals with relevant comorbidities (hypertension), 7 (12%) stated possible conflicts of interest, 5 (9%), all

Figure 1. Timber plots of the improvement in neurobehavioral scores for 18 interventions calculated using standardized mean differences (SMDs; A) and infarct size for 18 different interventions calculated using normalized mean differences (B). Horizontal error bars represent 95% confidence interval (CI). Vertical gray bars represent the 95% CI of the global estimate of efficacy. Symbol sizes represent the relative number of animals tested for each intervention. NDP-a-MSH indicates [Nle(4), D-Phe(7)]-α-Melanocyte-stimulating hormone; and PPP, (3-(3-hydroxyphenyl)-N-n-propylpiperidine).

Figure 2. A significant impact of reporting randomizing was found on neurobehavioral calculated using standardized mean differences (SMDs; A) and infarct size calculated using normalized mean differences (B). A significant impact of reporting blinding was found on infarct size (B) but not on neurobehavioral score (A). Vertical error bars represent 95% confidence interval (CI). Horizontal gray bars represent the 95% CI of the global estimate of efficacy. Bar widths represent the relative number of animals tested for each intervention. The relative change in reported efficacy for neurobehavioral scores and infarct size in nonrandomized and unblinded studies are shown in C.
from the same laboratory,25–27 reported a sample size calculation, and 44 (77%) stated compliance with animal welfare regulatory requirements (see online-only Data Supplement).

Meta-Analysis
Data from 16 publications could not be included in the meta-analysis because key information such as variance or, in the case of lesion size, data for unlesioned animals were not reported or could not be inferred. Thirteen publications reported the quantity of microclots producing neurological dysfunction in 50% of animals. Although an entirely valid model of lacunar stroke, these do not provide data suitable for meta-analysis and so were excluded. Twenty-seven remaining publications described 67 experiments involving 1099 animals reporting the efficacy of 22 drugs; of these, 37 experiments using 736 animals reported changes in neurobehavior, and 30 experiments using 422 animals reported infarct size (see online-only Data Supplement).

Efficacy
Overall, neurobehavioral score was improved by 0.82 SMD (95% CI, 0.51–1.14; 37 comparisons; 736 animals) in experiments testing 18 interventions, with substantial heterogeneity between studies ($\chi^2=170.3; F=79%$; $df=36; P<0.0057$). Stratification by intervention showed significant improvement in outcome for 8 of 18 interventions ($\chi^2=128.4; df=17; P<0.0057$; Figure 1A).

Infarct size was improved by 34.3% (95% CI, 24.2%–44.4%; 30 comparisons; 422 animals) in experiments testing 18 interventions, and again there was substantial heterogeneity ($\chi^2=91.0; F=68%; df=29; P<0.0057$). Stratified analysis by intervention showed significant improvement in outcome for 10 of 18 interventions ($\chi^2=83.1; df=17; P<0.0057$; Figure 1B).

Four interventions improved both neurobehavioral outcome and infarct size: preclamol (1 publication, 68 animals, 4 quality checklist items scored), fasudil (1 publication, 44 animals, 2 quality checklist items scored), nicotiflorin (1 publication, 48 animals, 4 quality checklist items scored), and hepatocyte growth factor (1 publications, 21 animals, 2 quality checklist items scored). Other compounds improved either neurobehavior (DY9760e, atorvastatin, hydroxyfasudil, ozagrel) or infarct size (dihydralazine, neural progenitor cells, $\gamma$-hydroxybutyrate, cilostazol, modafinil, and minocycline) but not both, although for 4 of these drugs only neurobehavior (atorvastatin, ozagrel) or infarct size (dihydralazine, cilostazol) was reported.

Study Quality
For neurobehavioral score, stratifying studies by randomization status, but not by blinding status, explained a significant proportion of the observed heterogeneity (Figure 2A). The impact of nonrandomization was a relative increase in reported efficacy of +282% (95% CI, +17% to +546%; Figure 2C). There was no apparent effect of blinding (relative reduction in efficacy of –8% [95% CI, –110% to +94%]) or of the use of animals with relevant comorbidities (Figure 2C).

For infarct size, stratifying studies by either randomization or blinding status explained a significant proportion of the observed heterogeneity (Figure 2B). The impact of nonrandomization was a relative reduction in efficacy of –25.5% (95% CI, –84.3% to +33.1), and of nonblinding a relative increase in reported efficacy of +28.4% (95% CI, –60% to +116%; Figure 2C). There was no apparent difference in animals with comorbidities. Overall, the number of study quality checklist items scored explained a significant proportion of the observed heterogeneity for both neurobehavioral score ($\chi^2=21.0; df=4; P<0.0057$; Figure 3A) and infarct size ($\chi^2=44.3; df=5; P<0.0057$; Figure 3B), with highest quality studies giving the lowest estimates of efficacy.

Publication Bias
Funnel plotting showed obvious asymmetry for neurobehavioral outcome (Figure 4A) but not infarct size (Figure 4B), whereas Egger regression suggested publication bias for both...
Using trim and fill analysis, we estimate 20 unpublished neurobehavioral outcomes (Figure 4A) giving an adjusted overall effect of 0.13 SMD (95% CI, –0.30 to 0.55; compared with 1.04 SMD [95% CI, 0.67–1.40]). For infarct size, we estimate 2 unpublished studies (Figure 4B) with a small reduction in the global efficacy from 33.9% (95% CI, 24.4–43.3) to 33.0% (95% CI, 24.4–42.4).

### Study Characteristics

For neurobehavioral score, interventions administered intraperitoneally were the most effective (1.62 SMD; 95% CI, 0.90–2.34) and those administered intrastriatally the least (–0.15 SMD; 95% CI, –0.66 to 0.37; χ²=31.3; df=6; \( P<0.0057 \); Figure 5A). Highest efficacy was reported in studies using spontaneous stroke in SHRSPs (1.56 SMD; 95% CI, 0.60–2.52) and lowest efficacy in those using microspheres (0.50 SMD; 95% CI, –0.02 to 1.02; χ²=23.3; df=4; \( P<0.0057 \); Figure 5B). Studies assessing outcome within 1 week after stroke reported highest efficacy (1.12 SMD; 95% CI, 0.58–1.65). This was significantly lower for later times of assessment—those assessing outcome >1 month after stroke did not report significant improvement (–0.06 SMD; 95% CI, –0.60 to 0.48; χ²=18.7; df=4; \( P<0.0057 \); Figure 5C). We found no significant impact of the timing of treatment.

For infarct size, interventions administered >3 hours after stroke onset were substantially less effective (20.8%; 95% CI, 6.8–34.7) than those given within 3 hours (37.4%; 95% CI, 20.0–54.9) or before stroke onset (43.6%; 95% CI, 17.1–70.1; χ²=22.1; df=3; \( P<0.0057 \); Figure 6A). Where outcome was measured 1 to 3 weeks after stroke, efficacy was significantly lower than at other times (14.1%; 95% CI, –4.7 to 32.8), whereas the highest efficacy was found in studies assessing outcome in the first week (43.3%; 95% CI, 20.9–65.8; χ²=42.4; df=2; \( P<0.0057 \); Figure 6B). We found no significant impact of the route of administration or method of stroke induction.

### Discussion

We report the meta-analysis of 22 interventions tested in 7 distinct animal models of lacunar stroke. Fourteen interventions improved either infarct size or neurobehavioral outcomes, of which 10 already have Food and Drug Administration approval for other indications.28 However, the low prevalence of measures to reduce bias compromises the internal validity of the data and the likelihood of publication bias compromises
their external validity; even for apparently promising interventions these concerns suggest the need for further high quality in vivo data in relevant models before embarking on clinical trials, particularly of novel agents.

**Potential Weaknesses**

This study is observational, analyzing previously collected data, and our findings are only hypothesis-generating; it may be that observed differences are because of some other factor that cosegregates with the variable of interest, and the data set is too small to allow multivariate analysis. We were unable to extract required data from 11 publications. Because low-quality studies overstate efficacy, and we included all studies, we will have overestimated treatment effects. Many of the experimental models reflect mechanisms that are not relevant to most lacunar stroke in humans. Some data from SHRSPs, the most relevant current model, could not be included through lack of time about stroke onset.

**Efficacy**

Four interventions improved both neurobehavioral outcome and infarct size. Of the four, only fasudil has been tested clinically, with promising initial results. Several other available agents improved the one outcome on which they were tested. In focal ischemia, stable estimates of efficacy emerge with data from ≈1000 animals, and we think that, even for these promising treatments, more and better evidence of efficacy in animals is required to help plan clinical trials.

**Risk of Bias**

The prevalence of reporting of measures to avoid bias, while modest, compares well with systematic reviews in stroke.
and other neurological conditions. Studies reporting fewest measures to avoid bias gave highest measures of treatment effect. The overstatement of efficacy in nonrandomized studies reporting neurobehavioral outcome is consistent with previous findings. Although stratification of infarct volume by randomization or blinding status explained a significant proportion of the observed heterogeneity, the strata were different in other respects; for instance, the median time to treatment was substantially shorter in randomized studies. With the exception of randomization status and neurobehavioral score, the 95% CIs for the difference in efficacy in studies at risk of bias include zero. The difference in impact of nonrandomization between studies reporting neurobehavioral outcome and those reporting infarct size may be because of the confounding effect of other variables, or to a true difference perhaps because of baseline differences in neurobehavioral performance biasing group allocation in nonrandomized studies. We think the evidence for small study effects, particularly for neurobehavioral outcomes, reflects publication bias; this provides further support for the development of systems to address this issue.

Diverse lesions are used to model lacunar stroke, and it is not clear whether there is 1 best model, or whether different models are suited to different research questions. Thromboembolism is an unusual cause of human lacunar stroke but more than half of the included studies used an embolic model. This substantially limits the relevance of the data reported here and highlights the need for use of more relevant existing models and the development of better models of lacunar stroke. Spontaneous strokes in the SHRSPs may be more similar to human lacunar stroke, so focus on this model may be relevant. Future trials’ design should account for assessing when stroke occurs in spontaneous lacunar models. Brain damage in patients with lacunar stroke is generally not confined to the tissue affected by the stroke but is much more diverse because of the diffuse nature of SVD. Therefore, a diffuse model, such as the SHRSPs, seems more relevant for drug testing as it models both recovery from the index stroke and these other cerebral effects of SVD.

Even a small improvement in outcome, if in an appropriate model, at an appropriate time and in an experiment at low risk of bias, might provide substantial evidence on which to embark on clinical trial. However, to have adequate power such studies would have to be substantially larger those identified here, and it may be that this can only be achieved in multicenter animal studies.

Evidence-Based Clinical Trial Design
Lacunar stroke is part of the spectrum of SVD. Patients who present with an acute lacunar stroke syndrome might start treatment to prevent recurrent lacunar stroke immediately. However, other SVD features typically accumulate silently, and patients might not present for treatment until they develop cognitive or physical decline, at which stage efficacy may have declined. Treatments preventing further lacunar strokes would be useful, yet only 4 studies tested treatments administered before stroke induction. An ideal treatment would have long-term efficacy preventing both the recurrence of clinically apparent acute lacunar stroke syndromes and the build up of silent features of SVD. It is therefore reassuring that over time the effect on neurobehavioral outcome did not seem to decline, and if anything the impact on structural outcome increased.

Conclusions
Our findings provide some guidance for future laboratory and clinical research. First, it is unclear which is the most appropriate animal model of lacunar stroke in which to test interventions; the most relevant current models raise trial design questions concerning how to detect and time new strokes. Second, improvements in internal (study quality) and external (publication bias) validity might provide a firmer foundation for the translation to clinical trials, and more robust exploration of the limits to efficacy in such studies could inform the inclusion and exclusion criteria for such trials.

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Disclosures
None.

References
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A systematic review and meta-analysis of interventions tested in animal models of lacunar stroke

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Supplement I: Search strategy

Search strategy used for EMBASE.

1. exp Models, Biological/ or exp Disease Models, Animal/ or exp Models, Animal/
2. Animals/
3. exp mammals/ or exp primates/ or exp mice/ or exp rats/
4. 1 or 2 or 3
5. 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/
6. brain edema/ or cerebrovascular accident/ or exp dementia, vascular/ or exp intracranial arterial diseases/ or exp vasospasm, intracranial/
7. 5 or 6
8. (Stroke$ or cerebrovasc$ or cerebral vasc$ or cerebral$).tw
9. 4 and 7 and 8
10. limit 9 to animals
11. (((micro or small or perforat$) adj3 vessel) or arteriole).tw
12. (lacunar stroke or lacunar infarct).tw
13. ((small or micro) adj5 (stroke$ or occlusion$ or disease$)).tw
14. ((lacun$ or small or subcortical or deep or silent) adj5 (infarct$ or lesion$ or stroke$)).tw
15. 11 or 12 or 13 or 14
16. 10 and 15
17. (heart or bone or eye or lung or kidney or liver or renal or intestine$ or spinal or pulmonary or hepatic or global).mp
18. 16 not 17
19. (AD or PD or Alzheimer$ or Parkinson$ or epilepsy or MS or Multiple Sclerosis).tw
20. 18 not 19
Search strategy used for Medline.

1. Biological Models or Animal Disease Models or Animal Models
2. Animals
3. mammals or primates or mice or rats
4. 1 or 2 or 3
5. cerebrovascular disorders or basal ganglia cerebrovascular disease or brain ischemia or cerebrovascular accident or brain infarction or brain hypoxia ischemia
6. brain edema or cerebrovascular accident or vascular dementia or intracranial arterial diseases or intracranial vasospasm
7. 5 or 6
8. (Stroke* [TW] or cerebrovasc* [TW] or cerebral vasc* [TW] or cerebral* [TW])
9. 4 and 7 and 8
10. limit 9 to animals
11. micro vessel [tw] OR small vessel [tw] OR perforat* vessel [tw] OR arteriole [tw]
12. lacunar stroke [tw] OR lacunar infarct [tw]
13. small stroke* [tw] or small occlusion* [tw] or small disease* [tw] or micro stroke* [tw] or micro occlusion* [tw] or micro disease* [tw]
14. lacun* infarct* [tw] or lacun* lesion* [tw] or lacun* stroke* [tw] or small infarct* [tw] or small lesion* [tw] or small stroke* [tw] or subcortical infarct* [tw] or subcortical lesion* [tw] or subcortical stroke* [tw] or deep infarct* [tw] or deep lesion* [tw] or deep stroke* [tw] or silent infarct* [tw] or silent lesion* [tw] or silent stroke* [tw]
15. 11 or 12 or 13 or 14
16. 10 and 15
17. heart [tiab] or bone [tiab] or eye [tiab] or lung [tiab] or kidney [tiab] or liver [tiab] or renal [tiab] or intestine* [tiab] or spinal [tiab] or pulmonary [tiab] or hepatic [tiab] or global [tiab]
18. 16 not 17
19. AD [tw] or PD [tw] or Alzheimer* [tw] or Parkinson* [tw] or epilepsy [tw] or MS [tw] or Multiple Sclerosis [tw]
20. 18 not 19
Search strategy used for ISI Web of Science.

1. Biological Models OR Animal Disease Models OR Animal Models
2. Animals
3. mammals OR primates OR mice OR rats
4. 1 OR 2 OR 3
5. cerebrovascular disorders OR basal ganglia cerebrovascular disease OR brain ischemia OR cerebrovascular accident OR brain infarction OR brain hypoxia ischemia
6. brain edema OR cerebrovascular accident OR vascular dementia OR intracranial arterial diseases OR intracranial vasospasm
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15. 10 AND 15
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17. 16 not 17
18. (AD OR PD OR Alzheimer* OR Parkinson* OR epilepsy OR MS OR Multiple Sclerosis)
19. 18 not 19
Supplement II: Quorum diagram showing summary of study selection procedure

**Identification**

Hand search: 2113 citations

Electronic database searches: 2868 citations

**Duplicate Removal**

Citations after duplicate removal: 4379 citations

600 duplicates

**Screening**

4277 excluded based on title and abstract

4277 included based on title and abstract

102 studies

**Eligibility Assessment**

Inclusion based on full text assessment of eligibility criteria

45 full text studies excluded

57 studies

16 studies missing key information

13 studies reporting data not suitable for meta-analysis

**Meta-Analysis**

27 studies

22 drugs

67 experiments

**Neurobehavioural**

21 studies

18 drugs

37 experiments

**Infarct Size**

20 studies

18 drugs

30 experiments
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Supplement IV: Study quality score report

1. Publication in a peer reviewed journal  
2. Random allocation to group  
3. Blinded induction of ischaemia  
4. Blinded assessment of outcome  
5. Use of comorbid animals  
6. Sample size calculation  
7. Compliance with animal welfare regulations  
8. Statement of a potential conflict of interest

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Study references


Han F, Shirasagi Y, Fukunaga K. 3-[2-[4-(3-Chloro-2-methylphenylmethyl)-1-piperazinyl]ethyl]-5,6-dimethoxy-1-(4-imidazolylmethyl)-1H-indazole dihydro-chloride 3.5 hydrate (DY-9760e) is neuroprotective in rat microsphere embolism: role of the cross-talk between calpain and caspase-3 through calpastatin. J Pharmacol Exp Ther. 2006;317:529-536.


Lapchak PA. The phenylpropanoid micronutrient chlorogenic acid improves clinical rating scores in rabbits following multiple infarct ischemic strokes: synergism with tissue plasminogen activator. Exp Neurol. 2007;205:407-413.

Lapchak PA. Efficacy and safety profile of the carotenoid trans sodium crocetinate administered to rabbits following multiple infarct ischemic strokes: a combination therapy study with tissue plasminogen activator. Brain Res. 2010;1309:136-145.


Lapchak PA, Han MK. Simvastatin improves clinical scores in a rabbit multiple infarct ischemic stroke model: synergism with a ROCK inhibitor but not the thrombolytic tissue plasminogen activator. Brain Res. 2010;1344:217-225.


Lapchak PA, Zivin JA. The lipophilic multifunctional antioxidant edaravone (radicut) improves behavior following embolic strokes in rabbits: a combination therapy study with tissue plasminogen activator. Exp Neurol. 2009;215:95-100.


Basic Sciences

对腔隙性卒中动物模型干预措施的系统回顾及荟萃分析
Systematic review and meta-analysis of interventions tested in animal models of lacunar stroke
Peddor H, Vesterinen HM, Macleod MR, Wardlaw JM

背景和目的：25% 的卒中为腔隙性卒中，它在病理生理学上与大动脉性卒中完全不同。尽管不断出现的证据显示其对躯体残疾和痴呆有相当的影响，但对其特异性治疗的关注却很少。如何最优化使用腔隙性卒中动物模型以检测某些干预措施，尚不清楚。

方法：我们对在腔隙性卒中动物模型中检测干预措施的研究进行了系统回顾和荟萃分析，使用随机 - 基于证据荟萃分析方法评估研究特征的影响并寻找发表偏倚的证据。

结果：57 篇文献描述了 43 项不同干预措施的疗效。在总分 8 分的研究质量评分列表中，中位数是 3 分（四分位范围，2-4）。许多模型表现的发病机制与腔隙性卒中相关性很小。对 27 项研究结果的荟萃分析显示，梗死体积和神经行为结局分别平均提高 34.2%(24.1 ~ 44.2)，平均标准差 0.82(0.51 ~ 1.14)。4 项干预措施能同时改善了梗死体积和神经行为结局，但缺乏充足的证据证明这些发现具有足够的说服力。在梗死面积方面，盲法研究中的疗效较低，随机研究中的疗效较高。在神经行为方面，随机研究中的疗效偏低。在梗死面积上，存在发表偏倚。

结论：尚无任何干预措施在足够广度和深度上可以转化到临床试验。仅有限的报道中治疗措施减少了偏倚风险，证据存在大量发表偏倚。

关键词：荟萃分析，系统性综述，腔隙性卒中
药时，我们认定治疗为首剂时间，剂量为头 24 小时的剂量总和。当报道的数据来自多个脑切片时，我们仅入选对照组梗死最大对应的切片。当报道的神经行为结局 >1 个时间点时，我们仅入选最晚时间评估作为临床最相关的终点。当数据以图表表示时，我们与作者联系寻找进一步信息，如果需要从图表上测量数值。

**质量评估**

使用实验性研究中动物数据荟萃分析和系统回顾的协作方法（CAMARADES）列表 10 项中的 8 项来评估偏倚风险。未包括温度控制和避免使用具有明显内在特性的麻醉剂的陈述，因为我们认为这些与腔隙性卒中模型的相关性很小。我们记录所选研究：①是否发表于同行评审杂志，及在哪发表；②试验是否随机分组；③实验期间是否保持盲法分组；④是否盲法评估结局；⑤实验动物是否有相关合并症；⑥是否有样本量计算的陈述；⑦是否有遵循管理要求的陈述；⑧是否有可能利益冲突的陈述。

数据统计

**结果**

**研究特征**

我们电子搜索找到 4379 篇文献，其中 4322 篇排除，剩余 57 篇入选系统回顾。

关于腔隙性卒中的诱发方法，57 项研究中，16 项（28%）为经颈内动脉微血栓注射，15 项（25%）为经颈内动脉微球注射，12 项（21%）为深部灰质注射内皮素。有 5 项研究（9%）应用了盐负荷加速 SHRSP 的自发卒中和无盐负荷 SHRSP 自发性卒中。所有研究中，41 项（72%）使用大鼠，其余为兔或小鼠。仅 47 项研究报道在诱导卒中过程中使用麻醉剂。

所有研究，按照总分 8 分的研究质量列表项评分的中位数为 3（四分位间距，2-4）。所有研究均发表在同行评审的杂志上。57 项研究中，26 项（46%）报道随机分配，10 项（18%）报道研究期盲法分组，26 项（46%）报道引法评估结局，12 项（21%）报道研究统计学相关共病（高血压）动物，7 项（12%）声明可能的利益冲突，5 项（9%）（来自同实验室的研究）报告了样本量计算，44 项（77%）陈述了遵循动物管理要求。

![图 1. 使用标准化平均差 (SMDs; A) 计算的 18 项干预措施的神经行为评分和使用归一化平均差计算的 18 种干预措施梗死体积改善 (B) 的森林图。水平误差短线代表 95% 置信区间 (CI)。垂直灰色条代表总体疗效的 95% CI。图形大小代表每个干预措施实验的动物相对数目。NDP-a-MSH 表示 [Nle(4), D-Phe(7)]<α>- 促黑激素；PPP 表示 (3-(3-羟苯基)-N-n-丙基哌啶)。](http://example.com/fig1.png)

**疗效**

总体上，在检验 18 种干预措施的实验中，神经行为评分改善了 0.82 个 SMD（95% CI 为 0.51 ~ 1.14，37 项比较，736 只动物），研究间的异质性巨大（$\chi^2 = 170.3, I^2 = 79\%$，df=36，$P<0.0057$）。干预措施分层分析显示，18 种措施中 8 种显著改善结局（$\chi^2 = 128.4, df=17, P<0.0057$，见图 1A）。

梗死体积在检验 18 种干预措施的实验中改善了 34.3（95% CI 为 24.2% ~ 44.4%，30 项比较，422 只动物），研究间的异质性同样巨大（$\chi^2 = 91.0, I^2 = 68\%$，df=29，$P<0.0057$）。干预措施分层分析显示，18 种措施中 8 种显著改善结局（$\chi^2 = 83.1, df=17, P<0.0057$，见图 1B）。

4 项干预措施同时改善了神经行为和梗死体积：丙克拉默（preclamol）（1 篇文献，68 只动物，研究质量评分 4 分）；法舒地尔（1 篇文献，44 只动物，研究质量评分 2 分）；烟剂（nicotiflorin）（1 篇文献，48 只动物，研究质量评分 4 分）；肝细胞生长因子（1 篇文献，21 只动物，研究质量评分 2 分）。其他化合物改善了神经行为（DY9760e、阿托伐他汀、氨丁三醇、奥扎格雷）或梗死体积（双肼酞嗪、西洛他唑、莫达非尼、二甲胺四环素），但非同时改善，虽然这其中有 4 种报道了神经行为（阿托伐他汀、奥扎格雷）或梗死体积（双肼酞嗪、西洛他唑）。

**研究质量**

关于神经行为评分，随机状态而非盲法状态的分层研究，解释了相当部分的观察到的异质性（图 2A）。非随机化相对增加了报道的功效 282%（95% CI 为 17% ~ 546%，见图 2C）。盲法相对减少功效 8%（95% CI 为 -110% ~ 94%）或使用相关共病动物（见图 2C）没有
对功效产生明显的影响。

关于梗死体积，随机或盲法状态的分层研究均解释了观察到的相当比例的异质性(见图2B)，非随机化使疗效相对减少25.5%(95%CI为-84.3%~33.1%)，非盲法使疗效相对增加28.4%(95%CI为-60%~116%，见图2C)。动物是否有合并症没有明显的差异。

总体而言，研究质量列表评分的得分解释了相当部分观察到的异质性，不论是神经行为(χ2=21.0，df=4，P<0.0057，见图3A)或是梗死体积(χ2=44.3，df=5，P<0.0057，见图3B)，最高质量的研究给出了疗效的最低估计值。

发表偏倚

漏斗图显示神经行为结局(见图4A)的明显不对称，而梗死体积则不然(见图4B)，但是 Egger 回归提示均有发表偏倚(见图4C和4D)。运用整理和填充分析，估计20项未发表的神经行为结局(见图4A)给出校正的总体功效为0.13SMD(95%CI为-0.13~0.55)，相较于1.04SMD(95%CI为0.60~2.52)，使用SHRSP的自发性卒中的研究报道的功效最高(1.56SMD，95% CI为1.05~2.07)。卒中后1周内评估结局的研究报道的功效最高(1.12SMD，95% CI为0.58~1.66)。评估时间越短，则功效越低，那些卒中后>1月进行结局评估的报告未见显著改善(-0.06SMD，95% CI为-0.60~0.48; χ2=18.7，df=4，P<0.0057，见图5C)。未见明显的治疗时间的影响。

关于梗死体积，在卒中发病3小时后给药功效差(20.8%，95% CI为6.8~34.7)，不及3小时(20.8%，95% CI为6.8~34.7)或发病前给药(30.6%，95% CI为17.1~70.1, X2=22.1, df=3, P<0.0057，见图6A)。卒中后1~3周进行结局评估者，功效显著低于其他时间(14.1%，95% CI为-4.7~32.8)，在首周内评估结局的研究的的功效最高(43.3%，95% CI为20.9~65.8, X2=42.4, df=2, P<0.0057，见图6B)。未见卒中诱导方法及途径有显著影响。
图 3. 随研究质量评分的上升，对下列方面的影响：A：对神经行为结局的疗效下降，使用标准平均差（SMDs；A）计算；B：对梗死体积的疗效下降，使用归一化平均差计算。垂直误差短线表示 95% 置信区间（CI）。水平灰色条表示总体疗效的 95%CI。条图宽度表示每个比较中动物的相对数目。

图 4. 使用 Trim and Fill 分析对发表结局（黑圈）和未发表结局研究（灰圈）估算的神经行为（A）和梗死体积（B）疗效的漏斗图。垂直灰线表示总体功效。Egger 回归图为神经行为（C）和梗死体积（D）的回归线及 95% 置信区间。
图 5. 常规给药（A）、卒中诱导方法（B）和结局评估时间（C）对神经行为评分的显著影响，使用标准平均差（SMDs）计算。垂直误差短线表示95％置信区间（CI），水平灰色条表示总体疗效的95％CI。条图宽度表示每个比较中动物的相对数目。

图 6. 给药时间（A）和评估时间（B）对梗死体积的显著影响，使用归一化平均差计算。垂直误差短线表示95％置信区间（CI），水平灰色条表示总体疗效的95％CI。条图宽度表示每个比较中动物的相对数目。
讨论

我们报道了在7种独立的腔隙性卒中动物模型中检验的22种干预措施的荟萃分析结果。14种措施改善梗死体积和（或）神经行为结局，其中10种已经获得美国食品药品监督管理局批准用于其它适应症治疗。然而，减少偏倚措施的使用水平很低妨碍了数据的内在可靠性，发表偏倚的可能性又妨碍了外延的可靠性。这些不足提示，即使是看似有前景的干预措施，在开始临床试验前仍然需要进一步高质量的相关模型的有效数据，特别是新的干预措施。

可能的不足

本研究为观察性，分析了既往收集的数据，研究发现仅是用于产生假设。可能观察到的差异是由一些与感兴趣变量共分离的因素所致，数据集太小以致不能做多因素分析。我们无法从11篇发表文献中获取数据。因为低质量的研究夸大了功效，而我们入选了所有研究，这将夸大治疗效应。许多实验模型反映的机制与多数无关。最与人类腔隙性卒中相关的模型是SHRSP，由于缺乏卒中发病时间而未能入选。

疗效

4项干预同时改善神经行为结局和梗死体积，其中仅法舒地尔有过临床试验，初步结果不错。其他一些措施经检验可以改善1项结局。对于局灶性缺血，虽然合并了约1000只动物的数据显示出稳定的效果，我们认为还需要更多更好的动物模型的疗效证据来帮助设计临床试验。

偏倚风险

虽然避免偏倚治疗措施的报告较少，但与卒中及其他神经疾病的系统性回顾仍有可能性。报道干预性治疗避免偏倚的研究却显示了治疗效果最好。非随机化研究对神经行为结局的疗效夸大，与以前的发现一致。虽然根据随机化和盲化对梗死体积的分析解释了观察到的很大比例的异质性，但在其他方面的分层还是不同的，例如，随机研究中开始治疗的中位时间非常短。除了随机治疗和神经行为评估，在有偏倚风险的研究中，疗效差异的95%置信区间包含零。在报道了神经行为结局和梗死体积的实验间，非随机化影响的差异，既可能是因其他变量的混淆效应，也可能确实存在差异，来源于非随机分组时神经行为分析能力的差异。我们认为有偏倚研究的影响的证据，特别是神经行为结局方面，反映了发表偏倚，支持对此应建立解决该问题的系统。

腔隙性卒中模型使用不同的损伤，还不清楚是否存在一个最好的模型，或不同模型是否适合不同的研究问题。血栓栓塞是人类腔隙性卒中的少见原因，但超过半数的研究使用栓塞模型。这明显限制了所报数据的临床相关性，也强调了使用相关性更好的已有模型和建立更好的腔隙性卒中模型的重要性。SHRSP的自发性卒中可能最类似于人类腔隙性卒中，所以关注该模型可能更有意义，未来试验设计应关注自发性腔隙性卒中模型中卒中发生时间的评估。腔隙性卒中患者的脑损伤通常不局限于卒中结构的脑组织，更多的是它的多样性，因为SVD具有弥散性。因此，像SHRSP这样的广泛性缺血动脉模型，似乎比药物试验更适合，因为它能模拟从卒中事件的恢复以及对SVD等其他脑血管病事件的效应。

在模型选择，时间恰当和偏倚风险低的实验中，即使有对结局小的改善，都能对开展临床试验提供有效的证据。然而，要使研究具有充分效力，需要进行更大样本量，而且需要通过多中心的动物实验完成。

基于循证的临床试验设计

腔隙性卒中是SVD领域的一部分，表现为急性腔隙性卒中综合征的患者可能应立即开始治疗以预防腔隙性卒中复发。然而，SVD的其他表现则是典型的静息性累积，患者除非发生认知功能或躯体功能衰减，否则不会治疗，然而在此阶段治疗的成功可能性已经下降。治疗防止腔隙性卒中进展是理想的，但仅有4项研究验证了卒中前治疗的疗效。

结论

我们的发现为进行进一步实验室和临床研究提供了参考。首先，最适合用于验证干预措施的腔隙性卒中动物模型还不明确，而目前最相关的模型还存在实验设计问题，诸如如何识别新发卒中及确定发病时间。其次，提高研究的内在（研究质量）和外在（发表偏倚）有效性可为向临床试验转化提供坚实基础，更进一步探索这些研究有效性的局限方面能够为入选和排除这类试验的标准提供启示。

参考文献