THE EFFECT OF ANGIOPOIETIN-1 UPREGULATION ON THE OUTCOME OF ACUTE ISCHAEMIC STROKE IN RODENT MODELS: A META-ANALYSIS

Cover title: *Angiopoietin-1 and experimental stroke outcomes*

Joseph V. Moxon PhD,[1,2] Alexandra F. Trollope PhD,[1,2,3] Brittany Dewdney MSc,[1,4] Catherine de Hollander BSc,[3] Domenico R. Nastasi,[1] Jane M. Maguire PhD,[4] Jonathan Golledge MChir FRCS FRACS,[1,2,5] *

1. Queensland Research Centre for Peripheral Vascular Disease, College of Medicine and Dentistry, James Cook University, Townsville, QLD 4811, Australia;
2. Centre for Molecular Therapeutics, The Australian Institute of Tropical Health and Medicine, James Cook University, Townsville, QLD, 4811, Australia;
3. Department of Anatomy, College of Medicine and Dentistry, James Cook University, Townsville, QLD 4811, Australia;
4. Faculty of Health, University of Technology Sydney, Sydney, NSW, 2007, Australia;
5. Department of Vascular and Endovascular Surgery, the Townsville Hospital, Townsville, QLD 4811, Australia

*Correspondence: Jonathan Golledge,
Queensland Research Centre for Peripheral Vascular Disease, College of Medicine and Dentistry, James Cook University, Townsville, QLD, Australia
Phone: +61 7 4781 4370; Fax: +61 7 4433 1767; email: jonathan.golledge@jcu.edu.au

ABSTRACT

Clinical studies report that low circulating angiopoietin-1 concentration at presentation predicts worse outcomes after ischemic stroke. Upregulating angiopoietin-1 may therefore have therapeutic benefit for ischemic stroke. This systematic review assessed whether upregulating angiopoietin-1 improved outcomes in rodent models of ischemic stroke. Random-effects models quantified the effect of angiopoietin-1 upregulation on stroke severity in terms of the size of cerebral infarction and the extent of blood-brain-barrier permeability. Eleven studies utilizing rat and mouse models of ischaemic stroke fulfilled the inclusion criteria. Meta-analyses demonstrated that angiopoietin-1 upregulation significantly reduced cerebral infarction size (standardized mean difference -3.02; 95% confidence intervals -4.41, -1.63, $p<0.001$; n=171 animals), and improved blood-brain barrier integrity (standardized mean difference -2.02; 95% confidence intervals -3.27, -0.77, $p=0.002$; n=129 animals). Subgroup analyses demonstrated that angiopoietin-1 upregulation improved outcomes in models of transient, not permanent cerebral ischaemia. Six studies assessed the effect of angiopoietin-1 upregulation on neurological function, however, inter-study heterogeneity prevented meta-analysis. In conclusion, published rodent data suggest that angiopoietin-1 upregulation improves outcome following temporary cerebral ischaemia by reducing cerebral infarction size and improving blood-brain barrier integrity. Additional research is required to examine the effect of angiopoietin-1 upregulation on neurological function during stroke recovery, and investigate the benefit and risks in patients.

Key words: Ischaemic stroke; angiopoietin-1; rodent model; cerebral infarction; blood-brain barrier permeability.

INTRODUCTION

Stroke is the world's third leading cause of death, and primary cause of adult disability [1]. Approximately 80% of all strokes result from acute cerebral ischaemia.[2] An important treatment for ischemic stroke is rapid restoration of the cerebral blood supply by mechanical or chemical thrombolysis [2]. Disadvantages of this therapy include a risk of substantial complications, need for specialist expertise and resources not available in many regions of the world, limited windows of effectiveness, and lack of suitability for many patients [3, 4]. Identifying novel therapies for ischemic stroke is a key research priority [5].

The angiopoietins (angpts) are growth factors which play an important role in maintaining the vasculature vascular function [6-9]. Angpts bind to the Tie-2 receptor which is predominantly expressed on the vascular endothelium [6-9]. An observational study previously reported that patients who suffered a recent ischemic stroke had significantly lower serum angpt-1 concentrations than healthy controls [10]. Patients with serum angpt-1 concentration in the lowest tertile were more likely to have died or have severe disability 3 months after the stroke than those in the highest tertile [10]. Experimental studies suggest that angpt-1 may improve the outcome of ischaemic stroke in several ways (please see [7, 8, 11] for detailed reviews). Firstly, Tie-2 activation has been reported to activate PI3/Akt, and inhibit NFκB to induce pro-survivor and anti-inflammatory pathways in endothelial cells [7, 8, 11]. Angpt-1 activation of the Tie-2 receptor is also important in regulating vascular integrity by maintaining the contacts between neighbouring endothelial cells, and the extracellular matrix [7, 8, 11]. Angpt-1 has also been demonstrated to induce the migration of endothelial cells [7, 8, 11]. Overall these studies suggest that angpt-1 inhibits inflammation, promotes angiogenesis and improves endothelial integrity which may act to limit the severity of cerebral infarction following stroke. Previous studies have examined the effect of upregulating or administering angpt-1 on the outcome of ischemic stroke in rodent models but findings have been inconsistent [12-22]. The current study systematically reviewed the efficacy of upregulating or

administering angpt-1 in improving recovery from experimentally induced ischemic stroke in rodent models.

METHODS

This systematic review was performed in line with the guidelines of PRISMA and the Systematic Review Center for Laboratory animal Experimentation (SYRCLE) statement. A protocol was developed following these guidelines [23] and was registered in the CAMARADES database (date of registration: 21-08-2017).

Search criteria

A systematic literature search was conducted to identify studies investigating the effects of upregulating angpt-1 in rodent ischemic stroke models (Supplement 1). Eligible studies reported original experiments involving angpt-1 administration or upregulation in a rodent model of acute ischemic stroke (regardless of dose, method, timing or frequency of administration). Studies also had to provide data on at least one of the following outcome measures: i) cerebral infarction size; ii) blood-brain-barrier (BBB) permeability; iii) neurobehavioural outcomes as assessed by a neurological deficit score. Review articles, editorials and publications in languages other than English were excluded. Studies which investigated the effect of angpt-1 in combination with other interventions were excluded unless data on the effects of angpt-1 upregulation alone could be extracted. In cases of ambiguity, authors were contacted to provide additional information. The final literature search was completed in January 2019.

Data extraction and quality assessment

Data extraction and quality assessment was independently conducted by three authors (JVM, AFT and DN). Information extracted from each study included the species, strain, age and/or body weight of rodents used, duration of cerebral ischaemia, the mechanisms by which angpt-1 was up-regulated, the interventions administered to control animals, and the

timing of administration relative to stroke induction. Information extracted for each outcome measure included the methods used to assess the outcome, timing of the assessment relative to stroke induction, and where possible detailed numerical data to quantify the results, in addition to reported p-values from comparison between groups. Behavioural assessments and molecular analyses were not reported in a standard way in the identified studies, and data relating to these outcomes could therefore not be meta-analysed.

The quality and potential bias of the included studies was assessed using a modified CAMARADES score (Supplement 2 and [24]). Individual scores were calculated based on the number of 'yes' answers. Studies were allocated a maximum score of 11, a score $\leq$3 was considered to denote a poor-quality study, those with a score of 4-7 were considered to be of moderate quality, and high-quality studies achieved scores $\geq$8. No articles were excluded based on their quality assessment score. Discrepancies in the extracted data were resolved at a consensus meeting.

Data analysis

The primary outcome measure for the meta-analysis was the difference in cerebral infarction size between rodents in which angpt-1 was upregulated compared to controls. This was chosen since it is a widely employed outcome measure used in rodent models and human studies. Secondary outcome measures were the differences in blood-brain barrier (BBB) permeability and neurological function (as assessed using a defined scoring system) between experimental groups. Data were extracted from publications, and where necessary were converted to provide common units for comparison between studies. If studies reported findings as mean and standard error, standard deviations were calculated by multiplying the standard error by the square root of the sample size. Where necessary, data were extrapolated from graphs using the analysis function of Photoshop CC 2018 (Adobe Inc, San Jose, California, USA).

Meta-analysis was conducted when comparable outcome data were available from at least 3 studies. As high inter-study variation was anticipated, random-effects models were constructed (RevMan V5, Cochrane Collaboration). Effect sizes were calculated as SMD and 95% confidence intervals (CI). Sub-analyses assessing the impact of angpt-1 upregulation on the outcome of stroke were performed in a) permanent versus temporary cerebral ischaemia models, and b) studies which up-regulated angpt-1 before, or after stroke induction. Inter-study variation was assessed using the I^2 statistic. Leave-one-out sensitivity analyses excluding data from individual studies were conducted to assess the robustness of findings. Significance for all analyses was accepted if p-values were <0.05. Funnel plots were generated to assess publication bias.

Results

Characteristics of included studies

The initial database search identified 642 potentially eligible studies. After duplicate removal, and title and abstract screening, the full-text of 18 studies was reviewed. After excluding seven studies, eleven were included in the current systematic review (Figure 1, Table 1) [12-22]. Eight studies used rats and three employed mice. Four studies induced stroke by occluding the middle cerebral artery for two hours, [12, 14, 17, 21], seven induced permanent cerebral ischaemia by inserting a filament into the middle cerebral artery [15, 16, 18, 19], or intravenous thrombus injection [13, 25]. Venkat and colleagues induced embolic cerebral ischaemia but did not specify their methodologies [20].

Nine studies tested a single angpt-1 intervention, two [19, 25] tested two angpt-1-upregulation approaches (Table 1, total of 13 interventions). Three of the interventions were virus-based, two were stem cell-based and eight utilized proteins or peptides. Variations in the dosage, methods of delivery and timing of the angpt-1 interventions were observed. Five interventions were administered prior to stroke induction, seven were administered following

cerebral ischaemia induction, and the timing of one was unclear (Table 1). Recombinant angpt-1 was co-administered with tissue plasminogen activator in one study [13], animals receiving tissue plasminogen activator plus a control protein were used as controls in the current meta-analysis.

Quality assessment

Three studies were of low quality, six were of moderate quality and two were of high quality (Supplement 2). Only two studies reported randomizing rodents to experimental groups [20, 21], only one provided a sample-size calculation [13], and only two reported blinding observers [20]. No studies provided data regarding the inter/intra-rater reproducibility of the reported outcome measures.

Primary outcome assessment: The effect of angpt-1 upregulation on cerebral infarction size

The effect of angpt-1 upregulation on cerebral infarction size was assessed in 10 studies [12-21, 25] (Table 2 and Supplement 3). Only 9 of the 11 studies meeting inclusion criteria for the systematic review provided data which could be included in the meta-analysis for the primary outcome. These studies (which included 171 rodents) measured cerebral infarction size by histological assessment of excised brains [12-15, 18-21, 25]. Of these, three studies expressed the infarction size as a percentage of the cerebral hemisphere, six provided absolute data estimating infarct volume. In the study authored by Shin *et al.,* cerebral infarction size was assessed by MRI and data could not be accurately extracted from the presented graphs; and therefore this study was excluded from the meta-analysis [17].

Meta-analysis suggested that upregulating angpt-1 significantly reduced cerebral infarction size (SMD for overall effect: -3.02, 95% CI: -4.41, -1.63; $p<0.001$, $I^2=89\%$; Figures 2A and B, number of rodents included in the meta-analysis: 171). Sub-analyses suggested that angpt-1 upregulation reduced infarct size in models of temporary, but not permanent ischaemia

(SMD: -8.46, 95% CI: -12.55, -4.37; p<0.001, I^2=89%; and SMD: -0.39 (95% CI: -0.84, 0.05; p=0.08; I^2=0%; respectively Figure 2A). Significant reductions in cerebral infarction sizes were found for studies that upregulated angpt-1 before or after ischaemia induction (SMD: -1.04, 95% CI:-1.86, -0.21; p=0.01, and -5.87, SMD:-8.91, -2.82; p<0.001, respectively; Figure 2B). Funnel plots revealed an asymmetrical distribution of reported SMD, suggesting potential publication bias (Supplement 4). Exclusion of any single study did not alter the overall finding that angpt-1 upregulation significantly reduced cerebral infarction size (Supplement 5). Removal of data reported by Yu *et al.* did, however, lead to a marked drop in the overall effect size. When the study authored by Meng *et al.* was excluded, the meta-analysis suggested that upregulating angpt-1 prior to stroke induction did not significantly reduce cerebral infarction size [14]. When the study authored by Yu *et al.* was excluded, the meta-analysis suggested that upregulating angpt-1 after stroke induction did not significantly reduce cerebral infarction size (Supplement 5) [21].

~~Subgroup analyses demonstrated that exclusion of data provided by Meng *et al.* and Yu *et al.* meant that upregulating angpt-1 before, or after stroke induction respectively, no longer significantly reduced infarct size [14, 21] (Supplement 4).~~

Secondary outcome assessment: The effect of angpt-1 upregulation on BBB permeability

Seven of the 11 eligible studies assessed the impact of angpt-1 administration on BBB permeability (Supplement 6) [12, 14, 16, 19-21, 25]. Four studies used experimental rats, and three utilised mice. Temporary cerebral ischaemia was induced in three studies [12, 14, 21] , while permanent cerebral ischaemia was induced in four studies [16, 19, 20, 25]. All studies assessed BBB permeability using Evan's blue staining, although the timing between stain administration and tissue collection varied. Data reported by Valable *et al.* could not be extracted from the original paper; no response to correspondence was received and data from this study was excluded from the meta-analysis [19]. All other studies reported data as

the amount of Evan's blue dye recovered per unit weight of cerebral tissue, however, results differed between studies by several orders of magnitude (Supplement 6).

Meta-analysis of findings from a total of 129 rodents from six studies demonstrated that BBB permeability was significantly lower in animals over-expressing angpt-1 compared to controls (overall SMD: -2.02, 95% CI: -3.27, -0.77; p=0.002; I^2: 85%; Figures 3A and B). Sub-analysis suggested that angpt-1 upregulation was effective in reducing BBB permeability in models of temporary, but not permanent, cerebral ischaemia (SMD: -3.74, 95% CI: -6.02, -1.45; p=0.001; and -0.65, -1.52, 0.21; p=0.14, respectively). The significant reduction in BBB permeability was not influenced by the timing of the angpt-1 upregulation (SMD: -0.95, 95% CI: -1.57, -0.34; p=0.002 and -3.71, -6.37, -1.04; p=0.006; for studies that up-regulated angpt-1 before, or after stroke respectively; Figure 3B). Funnel plots generated for this outcome revealed a symmetrical spread of reported SMD suggestive of low publication bias, however, one clear outlier was noted (Supplement 7). Removal of data reported by Yu *et al.* rendered the overall effect of angpt-1 on BBB permeability non-significant (Supplement 8). Exclusion of other studies did not influence the outcome of the overall analysis, however, sub-group analyses became non-significant following removal of the studies by Yu *et al.,* Zhang *et al.,* Venkat *et al.* and Gao *et al.* (Supplement 8).

Secondary outcome assessment: The effect of angpt-1 upregulation on neurological function

Six studies (96 animals) assessed the effect of angpt-1 upregulation on neurological function in the stroke models (Table 2, Supplement 9), using standardized scoring systems to measure neurological deficit [12-14, 17, 20, 21]. All studies were performed in rats. Cerebral ischemia was induced temporarily in four studies, [12, 14, 17, 21] and permanently in two studies [13, 20]. The timing of outcome assessment relative to cerebral ischaemia varied between studies. Four studies assessed neurological function repeatedly during follow-up [14, 17, 20, 21]. All studies utilized different methods to assess the severity of the neurological deficit, thereby prohibiting meta-analysis. Four studies reported that the severity of neurological

deficit was significantly less in rats receiving angpt-1 upregulation during at least one of the assessed time points [12, 14, 17, 20]. The remaining two studies reported no statistically significant differences in neurological function between groups [13, 21] (Table 2, Supplement 9).

DISCUSSION

This meta-analyses suggests that angpt-1 upregulation improves outcomes following temporary cerebral ischaemia, evidenced by significant reductions in cerebral infarction size and BBB permeability. Four studies also reported that angpt-1 up-regulation improved post-stroke neurological function, however, this outcome could not be meta-analysed. Care must be taken when interpreting these findings as inter-study heterogeneity in many aspects of study design including the choice of rodent model, methods used to induce cerebral ischaemia and mechanisms to upregulate angpt-1 was observed. Only two of the 11 included studies were considered to be of high quality [13, 21]. Funnel plots suggested potential publication bias for the primary outcome (cerebral infarction size), however, this was difficult to objectively assess as relatively few studies were included. Finally, only male rodents were included in the identified studies. It is therefore unclear whether findings of the current meta-analysis can be generalized to females.

Cerebral infarction size was the primary outcome measure for this meta-analysis as it was the most widely reported outcome measure, and has translational potential since cerebral infarction volume has been linked with stroke severity in clinical investigations [26]. Preserving hypo-perfused brain tissue following ischaemic stroke relies on rapid restoration of the cerebral blood supply before irreversible damage occurs [27]; clinically, this can be achieved through chemical and/or mechanical thrombolysis [2, 28]. Data from the current meta-analysis suggest that angpt-1 upregulation may augment endogenous physiological responses. Neovascularisation, through angiogenesis or arteriogenesis, occurs after stroke in order to

restore blood supply to the ischaemic tissue [29]. Angpt-1 is a key regulator of neovascularization and stabilizes blood vessels during formation and remodeling [7, 30]. Genetic data suggest a role for angpt-1 in the response to cerebral ischaemia as single nucleotide polymorphisms in the angpt-1 gene have been associated with the severity of cerebral infarction in both mice and humans [31]. Secondary analyses presented by Meng *et al.* and Shin *et al.* suggest that angpt-1 increases the survival and proliferation of endothelial and neuronal cells, and promotes neuronal cell differentiation [14, 17]. Toyama *et al.* also reported that cerebral capillary density within the infarction boundary zone was significantly higher in rodents receiving angpt-1, compared to controls [18]. Studies utilizing a range of rodent models (including skin grafting, hind limb ischaemia and muscle injury) have also reported enhanced revascularization in animals receiving angpt-1 when compared to controls [32-35]. Collectively, this suggests that the benefit of angpt-1 upregulation on cerebral infarction size may be due to enhanced revascularization and neuronal protection.

Ischaemic stroke also increases BBB permeability, and this has been suggested to promote inflammation and oedema formation [36, 37]. Clinical studies suggest that patients with the most severe BBB disruption after ischaemic stroke experience poorer functional outcomes, than those with greater BBB integrity [36, 38]. Importantly, cerebral reperfusion by mechanical or chemical thrombolysis promotes cerebral oedema [4, 13]. The ability of angpt-1 to reduce BBB leakiness after temporary cerebral ischemia may therefore have clinical importance.

The BBB comprises vascular endothelial cells, neurons and pericytes supported by an extracellular matrix [39]. Following ischaemia, BBB integrity is lost, although the mechanisms causing this are incompletely understood. Traditionally, stroke-related increases in vascular permeability are thought to result from disassembly of tight- and adherens-junctions between neighbouring endothelial cells and angpt-1 has been demonstrated to play an important role

in maintaining these junctions [7, 8, 37, 39-41]. Two studies included in the current review [16, 21], reported that cerebral expression of tight junction proteins such as ZO-1 and occludin was significantly higher in animals receiving angpt-1 than controls. Thus, the observed reduction in BBB permeability may be due to preservation of tight- and adherens-junctions between endothelial cells.

The severity of neurological dysfunction immediately following ischaemic stroke is strongly predictive of outcome at one year [42]. It has been recommended that animal stroke model research should assess neurological function [43, 44]. Thus, it is surprising that the effect of angpt-1 upegulation on the severity of neurological deficits was the least widely studied outcome. Moreover, there was marked heterogeneity in the methods used to measure neurological dysfunction, complicating overall interpretation of the findings; and similar heterogeneity has been noted in clinical studies [45, 46]. Assessing other aspects of physical function such as behaviour or exercise capacity may also further contextualise neurological function outcomes, however only four of the included studies presented such data [14, 15, 17, 18]. As with the neurological data, inter-study heterogeneity in the manner by which behavioural assessments were conducted and reported prevented detailed analysis of these outcomes. Further studies which directly assess the impact of angpt-1 upregulation on neurological and behavioural capacity are needed to provide greater insight into the translational potential of this intervention.

In conclusion, this meta-analysis suggests that angpt-1 upregulation significantly reduces cerebral infarction size and BBB leakiness following temporary cerebral ischemia. This systematic review highlights the need for high quality rodent model research that includes standardised assessment of neurological function and incorporates key clinical aspects, such as upregulating angpt-1 after (not before) stroke induction and including patient co-

morbidities, within the experimental design [47]. Adoption of the ARRIVE, and STAIR (Stroke Therapy Academic Industry Roundtable) guidelines will also improve the quality of evidence produced from future rodent studies [44, 48].

ACKNOWLEDGEMENTS

We thank Jieli Chen and Poornima Venkat for providing additional information, and Georgina Anderson, Stephen Anderson and Sam Rannard for assistance with the literature search strategy. JVM holds an Advance Queensland Fellowship from the Queensland Government. This research was supported by grants from the National Health and Medical Research Council (1098717, 1079369 and 1022752), Townsville Hospital and Health Service, and Queensland Government. JG holds a Practitioner Fellowship from the National Health and Medical Research Council (1117601) and a Senior Clinical Research Fellowship from the Queensland Government.

Disclosures/conflict of interests: The authors declare that there is no conflict of interests.

Supplementary material: Supplementary material supporting this paper have been made available through the JCBFM website.

REFERENCES

1. Howard R. The management of ischaemic stroke. *Anaesthesia Intensive Care Med* 2016; 17: 591-595.

2. Moretti A, Ferrari F and Villa RF. Neuroprotection for ischaemic stroke: current status and challenges. *Pharmacol Thera* 2015; 146: 23-34.

3. Dewdney B, Trollope A, Moxon J, *et al*. Circulating MicroRNAs as Biomarkers for Acute Ischemic Stroke: A Systematic Review. *J Stroke Cerebrovasc Dis* 2018; 27: 522-530.

4. Thiebaut AM, Gauberti M, Ali C, *et al*. The role of plasminogen activators in stroke treatment: fibrinolysis and beyond. *Lancet Neurol* 2018; 17: 1121-1132.

5. Prabhakaran S, Ruff I and Bernstein RA. Acute stroke intervention: a systematic review. *JAMA* 2015; 313: 1451-1462.

6. Matkar PN, Ariyagunarajah R, Leong-Poi H, *et al*. Friends Turned Foes: Angiogenic Growth Factors beyond Angiogenesis. *Biomolecules* 2017; 7. Pii E74.

7. Eklund L, Kangas J and Saharinen P. Angiopoietin-Tie signalling in the cardiovascular and lymphatic systems. *Clinical Sci* 2017; 131: 87-103.

8. Moss A. The angiopoietin:Tie 2 interaction: a potential target for future therapies in human vascular disease. *Cytokine Growth Factor Rev* 2013; 24: 579-592.

9. Brindle NP, Saharinen P and Alitalo K. Signaling and functions of angiopoietin-1 in vascular protection. *Circ Res* 2006; 98: 1014-1023.

10. Golledge J, Clancy P, Maguire J, *et al*. Plasma angiopoietin-1 is lower after ischemic stroke and associated with major disability but not stroke incidence. *Stroke* 2014; 45: 1064-1068.

11. van Meurs M, Kumpers P, Ligtenberg JJ, *et al*. Bench-to-bedside review: Angiopoietin signalling in critical illness - a future target? *Crit Care* 2009; 13: 207.

12. Gao X, Li HL, Li YQ, *et al*. Protective effect of angiopoietin-1 on the blood brain barrier after focal cerebral ischemia-reperfusion injury in rats. *J Int Transl Med* 2015; 3: 1-5.

13. Kawamura K, Takahashi T, Kanazawa M, *et al*. Effects of angiopoietin-1 on hemorrhagic transformation and cerebral edema after tissue plasminogen activator treatment for ischemic stroke in rats. *PLoS ONE* 2014; 9: e98639.

14. Meng Z, Li M, He Q, *et al*. Ectopic expression of human angiopoietin-1 promotes functional recovery and neurogenesis after focal cerebral ischemia. *Neuroscience* 2014; 267: 135-146.

15. Onda T, Honmou O, Harada K, *et al*. Therapeutic benefits by human mesenchymal stem cells (hMSCs) and Ang-1 gene-modified hMSCs after cerebral ischemia. *J Cereb Blood Flow Metab* 2008; 28: 329-340.

16. Shen F, Walker EJ, Jiang L, *et al*. Coexpression of angiopoietin-1 with VEGF increases the structural integrity of the blood-brain barrier and reduces atrophy volume. *J Cereb Blood Flow Metab* 2011; 31: 2343-2351.

17. Shin HY, Lee YJ, Kim HJ, *et al*. Protective role of COMP-Ang1 in ischemic rat brain. *J Neurosci Res* 2010; 88: 1052-1063.

18. Toyama K, Honmou O, Harada K, *et al*. Therapeutic benefits of angiogenetic gene-modified human mesenchymal stem cells after cerebral ischemia. *Exp Neurol* 2009; 216: 47-55.

19. Valable S, Montaner J, Bellail A, *et al*. VEGF-induced BBB permeability is associated with an MMP-9 activity increase in cerebral ischemia: both effects decreased by Ang-1. *J Cereb Blood Flow Metab* 2005; 25: 1491-1504.

20. Venkat P, Yan T, Chopp M, *et al*. Angiopoietin-1 Mimetic Peptide Promotes Neuroprotection after Stroke in Type 1 Diabetic Rats. *Cell Transplant* 2018; 27: 1744-1752.

21. Yu H, Wang P, An P, *et al*. Recombinant human angiopoietin-1 ameliorates the

expressions of ZO-1, occludin, VE-cadherin, and PKCalpha signaling after focal cerebral ischemia/reperfusion in rats. *J Mol Neurosci* 2012; 46: 236-247.

22. Zhang H, Lin S, Chen X, *et al*. The effect of age, sex and strains on the performance and outcome in animal models of stroke. *Neurochem Int* 2019; 127: 2-11.

23. de Vries RBM, Hoojimans CR, Langendam MW, *et al*. A protocol format for the preparation, registration and publication of systematic reviews of animal intervention studies. *Evidence-based Preclin Med* 2015; 1: 1-9.

24. Macleod MR, O'Collins T, Howells DW, *et al*. Pooling of animal experimental data reveals influence of study design and publication bias. *Stroke* 2004; 35: 1203-1208.

25. Zhang ZG, Zhang L, Croll SD, *et al*. Angiopoietin-1 reduces cerebral blood vessel leakage and ischemic lesion volume after focal cerebral embolic ischemia in mice. *Neuroscience* 2002; 113: 683-687.

26. Laredo C, Zhao Y, Rudilosso S, *et al*. Prognostic Significance of Infarct Size and Location: The Case of Insular Stroke. *Sci Rep* 2018; 8: 9498.

27. Baron JC. Protecting the ischaemic penumbra as an adjunct to thrombectomy for acute stroke. *Nat Rev Neurol* 2018; 14: 325-337.

28. Hankey GJ. Stroke. *Lancet* 2017; 389: 641-654.

29. Prakash R and Carmichael ST. Blood-brain barrier breakdown and neovascularization processes after stroke and traumatic brain injury. *Curr Opin Neurol* 2015; 28: 556-564. 2015/09/25.

30. Fagiani E and Christofori G. Angiopoietins in angiogenesis. *Cancer Lett* 2013; 328: 18-26.

31. Du R, Zhou J, Lorenzano S, *et al*. Integrative Mouse and Human Studies Implicate ANGPT1 and ZBTB7C as Susceptibility Genes to Ischemic Injury. *Stroke* 2015; 46: 3514-3522.

32. Shyu KG, Manor O, Magner M, *et al*. Direct intramuscular injection of plasmid DNA encoding angiopoietin-1 but not angiopoietin-2 augments revascularization in the rabbit ischemic hindlimb. *Circulation* 1998; 98: 2081-2087.

33. Jiang J, Jiangl N, Gao W, *et al*. Augmentation of revascularization and prevention of plasma leakage by angiopoietin-1 and vascular endothelial growth factor co-transfection in rats with experimental limb ischaemia. *Acta Cardiologica* 2006; 61: 145-153.

34. Byun SJ, Choi KS, Park SH, *et al*. Cartilage oligometric matrix protein-angiopoietin-1 promotes revascularization through increased survivin expression in dermal endothelial cells of skin grafts in mice. *Am J Pathol* 2007; 171: 1682-1690.

35. Mofarrahi M, McClung JM, Kontos CD, *et al*. Angiopoietin-1 enhances skeletal muscle regeneration in mice. *Am J Physiol Regul Integr Comp Physiol* 2015; 308: R576-589.

36. Lorberboym M, Lampl Y and Sadeh M. Correlation of 99mTc-DTPA SPECT of the blood-brain barrier with neurologic outcome after acute stroke. *J Nucl Med* 2003; 44: 1898-1904.

37. Krueger M, Mages B, Hobusch C, *et al*. Endothelial edema precedes blood-brain barrier breakdown in early time points after experimental focal cerebral ischemia. *Acta Neuropathol Comm* 2019; 7: 17.

38. Brouns R, Wauters A, De Surgeloose D, *et al*. Biochemical markers for blood-brain barrier dysfunction in acute ischemic stroke correlate with evolution and outcome. *Eur Neurol* 2011; 65: 23-31.

39. Haley MJ and Lawrence CB. The blood-brain barrier after stroke: Structural studies and the role of transcytotic vesicles. *J Cereb Blood Flow Metab* 2017; 37: 456-470.

40. Krueger M, Hartig W, Reichenbach A, *et al*. Blood-brain barrier breakdown after embolic stroke in rats occurs without ultrastructural evidence for disrupting tight junctions. *PLoS ONE* 2013; 8: e56419.

41. Krueger M, Bechmann I, Immig K, *et al*. Blood-brain barrier breakdown involves four distinct stages of vascular damage in various models of experimental focal cerebral ischemia. *J Cereb Blood Flow Metab* 2015; 35: 292-303.

42. Rost NS, Bottle A, Lee JM, *et al*. Stroke Severity Is a Crucial Predictor of Outcome: An International Prospective Validation Study. *J Am Heart Assoc* 2016; 5 piiL e002433.

43. Balkaya M, Krober JM, Rex A, *et al*. Assessing post-stroke behavior in mouse models of focal ischemia. *J Cereb Blood Flow Metab* 2013; 33: 330-338.

44. Recommendations for standards regarding preclinical neuroprotective and restorative drug development. *Stroke* 1999; 30: 2752-2758.

45. Borschmann K, Hayward KS, Raffelt A, *et al*. Rationale for Intervention and Dose Is Lacking in Stroke Recovery Trials: A Systematic Review. *Stroke Res Treat* 2018; 2018: 8087372.

46. Boyd LA, Hayward KS, Ward NS, *et al*. Biomarkers of stroke recovery: Consensus-based core recommendations from the Stroke Recovery and Rehabilitation Roundtable. *Int J Stroke* 2017; 12: 480-493.

47. Herson PS and Traystman RJ. Animal models of stroke: translational potential at present and in 2050. *Future Neurol* 2014; 9: 541-551.

48. Kilkenny C, Browne WJ, Cuthill IC, *et al*. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol* 2010; 8: e1000412.

49. Jeong SW, Chu K, Jung KH, *et al*. Human neural stem cell transplantation promotes functional recovery in rats with experimental intracerebral hemorrhage. *Stroke* 2003; 34: 2258-2263.

50. Zea M and Zuluaga R. [Mercury levels in auxiliary dental personnel in the CES Specialist Center (Sabaneta, Antioquia)]. *CES Odontol* 1989; 2: 47-50. 1989/01/01.

51. Garcia JH, Wagner S, Liu KF, *et al*. Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats. Statistical validation. *Stroke* 1995; 26: 627-635.

52. Chen J, Li Y, Wang L, *et al*. Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke* 2001; 32: 1005-1011.

53. Zausinger S, Hungerhuber E, Baethmann A, *et al*. Neurological impairment in rats after transient middle cerebral artery occlusion: a comparative study under various treatment paradigms. *Brain Res* 2000; 863: 94-105.

Figure Legends

Figure 1: PRISMA diagram outlining the literature search.

Figure 2: Meta-analysis comparing cerebral infarction size in rodents receiving angpt-1 interventions compared to controls split by A) Duration of ischaemia, and B) timing of intervention relative to stroke induction.

Figure 3: Meta-analysis comparing BBB permeability in rodents receiving angpt-1 interventions compared to controls split by A) Duration of ischaemia, and B) timing of intervention relative to stroke induction.

Table 1 – Characteristics of included studies

Lead author	Rodents Used	Ischaemia duration	Method of ANGPT1 upregulation	Control intervention used for this review	Timing of intervention relative to stroke induction
Venkat [20]	Male Wistar rats (type 1 diabetes induced, body weight 225-250 grams, age 8-12 weeks)	Permanent	Intra-peritoneal injection of mimetic peptide (3 µg/kg).	Saline	Thirty minutes pre-, and 8 and 24 hours post-stroke induction.
Gao [12]	Male Wistar rats (body weight 250-300 grams)	Two hours	Injection of recombinant protein (1µg/kg) into carotid artery.	Saline	Upon cerebral reperfusion, and every 12 hours thereafter.
Shin [17]	Male Sprague-Dawley rats (body weight 260-300 grams)	Two hours	Transvenous injection of andenoviral vector delivering a modified angpt-1 ($1x10^9$ plaque forming units/animal).	Viruses containing LacZ gene	Three days post stroke induction
Yu [21]	Male Wistar rats (body weight 250-300 grams)	Two hours	Injection of recombinant protein into right intra-cerebral artery (1µg/kg body weight).	Saline	Post (immediately upon reperfusion)
Meng [14]	Male Sprague-Dawley rats (body weight 180-230 grams)	Two hours	Intracerebral injection of a lentiviral vector encoding human Angpt1 ($2x10^9$ viral particles/animal).	Viruses containing empty vector	Two weeks prior to stroke induction
Onda [15]	Male Sprague-Dawley rats (body weight 250-300 grams)	Permanent	Intravenous infusion of angpt1 over-expressing mesenchymal stem cells	Human mesenchymal stem cells	Six hours post stroke induction
Shen [16]	Male CD-1 mice (age and body weight not specified)	Permanent	Intracerebral adenovirus expression	Virus containing LacZ gene	One hour post stroke induction
Toyama [18]	Male Sprague-Dawley rats (body weight 250-300 grams)	Permanent	Intravenous infusion of Angpt1 over-expressing human mesenchymal stem cells ($1x10^6$ cells/animal).	Human mesenchymal stem cells	Six hours post stroke induction
Valable [19]	OF1 mice (age/body weight and sex not specified)	Permanent	Intracerebroventricular injection of recombinant human Angpt1 (27 ng/animal). OR Intra-striatal infusion of recombinant human Angpt-1 via osmotic minipump (delivering 0.15 ng/hour for 7 days).	Saline None	Immediately prior to stroke induction. Unclear
Zhang [25]	Male C57 black 6 mice (body weight 25-30 grams)	Permanent	Intravenous adenoviral delivery ($1x10^9$ plaque forming units/animal) OR intraperitoneal injection of recombinant protein [Bow-Ang] groups (Dose not specified)	Adenovirus containing green fluorescent protein. Unspecified control protein	Three days prior to stroke induction; Twelve hours prior to stroke induction
Kawamura [13]	Male Sprague Dawley rats (body weight 250-300 grams)	Permanent	Intravenous injection of COMP-Angpt1 protein (30 µg/animal)	COMP (30µg/animal)	Four hours post stroke induction (immediately prior to tPA administration).

COMP: Cartilage Oligomeric Matrix Protein. tPA: Tissue plasminogen activator.

Table 2 – Summary of outcome assessments reported by each study.

	Lead author	Species	Angpt-1 timing	Infarction size			BBB permeability			Neurological function		
				Method	Timing	Reported outcome	Incubation time (hours)	Timing	Reported outcome	Method	Timing	Reported outcome
Temporary ischaemia	Gao [12]	R	Post	TTC stain	48h	↓	2		↓	Five point scale (no reference cited)	?	↑
	Shin [17]	R	Post	MRI	2, 10, 17 and 31 days	? *	--	--	--	Ten point scale based on [49]	Days -1 (baseline), 3, 6, 10, 17, 24 and 31	↑
	Yu [21]	R	Post	TTC stain	12h, 48h and 7 days	12h: ↓ 48h: ↓ 7 days: ↓	2	12h, 48h and 7 days	12h: ↓ 48h: ↓ 7 days: ↓	Five point scale based on [50] †	12h, 48h and 7 days	12h: ↔ 48h: ↔ 7 days: ↔
	Meng [14]	R	Pre	TTC stain	24h and 14 days	24h: ↓ 14 days: ↓	22		↓	Modified score based on [51]	24h and 14 days	24h: ↔ 14 days: ↑
Permanent ischaemia	Venkat [20]	R	Pre	H&E stain	48h	↓	4		↓	Modified score based on [52]	2h, 24h, 48h	2h: ↑ 24h: ↑ 48h: ↑
	Onda [15]	R	Post	TTC stain	7 days	↔	--	--	--	--	--	--
	Toyama [18]	R	Post	TTC stain	7 days	↔	--	--	--	--	--	--
	Zhang [25]	M	Pre	H&E stain/ TTC stain	24h	↔ ‡	6		Adenovirus: ↓ Protein: ↓	--	--	--
	Kawamura [13]	R	Post	TTC stain	24h	↔	--	--	--	Six point scale based on [53]	24h	↔
	Valable [19]	M	Pre	Thionin stain	24h	↔ §	3		? §	--	--	--
	Shen [16]	M	Post	--	--	--	1		↔	--	--	--

Species: R: rats; M: mice. ***Angpt-1 timing:*** Pre: angpt-1 first up-regulated prior to stroke induction; Post: angpt-1 first up-regulated after stroke induction. ***Timing:*** Timing of assessment relative to stroke induction; ***Reported outcome:*** ↑: Significant improvement in mice receiving angpt-1; ↔: No statistically significant difference between groups; ↓: Significantly decreased in animals receiving angpt-1; ?: Unclear based on presented information. TTC: 2,3,5-triphenyltetrazolium chloride; H&E: Haematoxylin and Eosin; * Presented analysis compares data from rodents receiving angpt-1 encoding viruses with a group receiving saline, rather than empty viral vector. † Unclear whether given reference is appropriate; ‡ Only data for adenovirus group presented. § Only assessed in animals receiving recombinant protein.