

## Central Lancashire Online Knowledge (CLOK)

|          |  |
|----------|--|
| Title    | Lithium and Stroke Recovery: A Systematic Review and Meta-Analysis of Stroke Models in Rodents and Human Data  |
| Type     | Article  |
| URL      | <a href="https://clock.uclan.ac.uk/id/eprint/43785/">https://clock.uclan.ac.uk/id/eprint/43785/</a>  |
| DOI      | <a href="https://doi.org/10.1161/strokeaha.122.039203">https://doi.org/10.1161/strokeaha.122.039203</a>  |
| Date     | 2022   |
| Citation | Almeida, Osvaldo P., Singulani, Monique P., Ford, Andrew H., Hackett, Maree, Etherton-Beer, Christopher, Flicker, Leon, Hankey, Graeme J., De Paula, Vanessa J.R., Penteado, Camila T. et al (2022) Lithium and Stroke Recovery: A Systematic Review and Meta-Analysis of Stroke Models in Rodents and Human Data. Stroke, 53 (9). pp. 2935-2944. ISSN 1524-4628 |
| Creators | Almeida, Osvaldo P., Singulani, Monique P., Ford, Andrew H., Hackett, Maree, Etherton-Beer, Christopher, Flicker, Leon, Hankey, Graeme J., De Paula, Vanessa J.R., Penteado, Camila T. and Forlenza, Orestes V.  |

It is advisable to refer to the publisher's version if you intend to cite from the work.  
<https://doi.org/10.1161/strokeaha.122.039203>

For information about Research at UCLan please go to <http://www.uclan.ac.uk/research/>

All outputs in CLOK are protected by Intellectual Property Rights law, including Copyright law. Copyright, IPR and Moral Rights for the works on this site are retained by the individual authors and/or other copyright owners. Terms and conditions for use of this material are defined in the <http://clock.uclan.ac.uk/policies/>

# **Lithium and stroke recovery: a systematic review and meta-analysis of stroke models in rodents and human data**

Osvaldo P. Almeida, MD, PhD<sup>1+</sup>, Monique P. Singulani, PhD<sup>2+</sup>, Andrew H. Ford, MD<sup>1</sup>, Maree Hackett, PhD<sup>3</sup>, Christopher Etherton-Beer, MBBS, PhD<sup>1</sup>, Leon Flicker, MBBS, PhD<sup>1</sup>, Graeme J. Hankey, MBBS, MD<sup>1</sup>, Vanessa J. R. De Paula, PhD<sup>2</sup>, Camila T. Penteado, MD<sup>2</sup>, Orestes V. Forlenza, MD, PhD<sup>2</sup>.

<sup>1</sup>Medical School, University of Western Australia, Perth, Australia.

<sup>2</sup>Laboratory of Neuroscience LIM27, Department and Institute of Psychiatry HCFMUSP, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.

<sup>3</sup>The George Institute for Global Health, the University of New South Wales, Sydney, Australia.

<sup>+</sup>These authors contributed equally to this work

Correspondence to: Orestes V. Forlenza, Instituto de Psiquiatria, Hospital das Clínicas da Faculdade de Medicina da USP, Rua Dr. Ovídio Pires de Campos 785, São Paulo, SP, 05403-903, Brazil.

E-mail: forlenza@usp.br.

Short title: Lithium and stroke recovery: a systematic review

## ABSTRACT

**Background:** Lithium has neuroprotective effects in animal models of stroke, but benefits in humans remain uncertain. **Aim:** To systematically review the available evidence of the neuroprotective and regenerative effects of lithium in animal models of stroke, as well as in observational and trial stroke studies in humans. **Methods:** This systematic review and meta-analysis was conducted according to PRISMA guidelines. We searched Medline, Embase, and PsycINFO for preclinical and clinical studies published between January 2000 and September 2021. A random-effects meta-analysis was conducted from observational studies. **Results:** From 1,625 retrieved studies, 42 were included in the systematic review. Of those, we identified 36 rodent models of stroke using pre- or post-insult treatment with lithium, and 6 studies conducted in human samples, of which 4 could be meta-analysed. The review of animal models was stratified according to the type of stroke and outcomes. Human data were subdivided into observational and intervention studies. Treatment of rodents with lithium was associated with smaller stroke volumes, decreased apoptosis, and improved post-stroke function. In humans, exposure to lithium was associated with a lower risk of stroke among adults with bipolar disorder in 2 of 4 studies. Two small trials showed equivocal clinical benefits of lithium post-stroke. **Conclusions:** Animal models of stroke show consistent biological and functional evidence of benefits associated with lithium treatment, whereas human evidence remains sparse and inconclusive. The potential role of lithium in post-stroke recovery is yet to be adequately tested in humans.

**Keywords:** stroke, lithium, ischaemic stroke, haemorrhagic stroke, neuroprotection.

## NON-STANDARD ABBREVIATIONS AND ACRONYMS

BD, Bipolar disorder; BCCAO, bilateral common carotid artery occlusion; MCAO, middle cerebral artery occlusion; tMCAO, transient middle cerebral artery occlusion; pMCAO, permanent middle cerebral artery occlusions; ICH, intracerebral haemorrhage; CD, could not be determined; i.p., intraperitoneal; Li, lithium; LiCl, lithium chloride; NA, not applicable or not available; ns, not significant; P, postnatal day; s.c., subcutaneous; SD, Sprague-Dawley.

For Stroke Peer Review. Do not distribute. Destroy after use.

## INTRODUCTION

John Cade, an Australian psychiatrist, observed that lithium salts had a tranquilizing effect on rodents<sup>1</sup>. This led him to test the clinical effects of lithium in people with ‘psychotic excitement’ and report in 1949 its antimanic properties<sup>1</sup>. Lithium salts have since become the ‘gold standard’ treatment for bipolar disorder (BD) worldwide<sup>2</sup>. More recent evidence suggests that the clinical effects of lithium may not be limited to its antimanic and mood-stabilizing properties in mood disorders. Lithium salts may have pleiotropic effects in preventing or delaying cognitive decline in the general population<sup>3</sup>, in older adults with BD<sup>4,5</sup>, and mild cognitive impairment<sup>6</sup>, in addition to enhancing motor recovery<sup>7</sup> and verbal memory<sup>8</sup> after stroke.

There is evidence from preclinical studies that treatment with lithium may reduce post-ischaemic neuronal apoptosis and mitigate the extent of brain injury after stroke<sup>9</sup>. Such effects have been associated with better functional recovery in some animal models<sup>10-15</sup>. However, the translation of these findings into clinical practice is uncertain<sup>7,8,16-20</sup>. Lithium exerts its effect through modification of multiple intracellular signalling systems, with emphasis on the inhibition of glycogen synthase kinase-3 $\beta$  and subsequent downstream effects reducing inflammation, promoting maintenance of glial and neuronal structures, and downregulating apoptosis<sup>21,22</sup>.

In this systematic review, we first sought to examine animal models of ischaemic and haemorrhagic stroke to determine if treatment with lithium decreased stroke volume and the apoptosis of neural cells, and increased post-stroke neurogenesis and function. Second, we reviewed observational data in humans and reported the association between exposure to lithium and the risk of stroke, and evaluated the results of clinical trials investigating the effects of treatment with lithium among adults after stroke.

## METHODS

The conduct and reporting of the current systematic review and meta-analysis conform to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines and recommendations<sup>23</sup>. This systematic review was not registered because of its concurrent inclusion of animal and human data. The results generated during the current study are available from the corresponding author upon reasonable request. This study was exempted from IRB approval.

#### Information sources and search strategy

We used Ovid to search the databases of Embase, MEDLINE, and PsycINFO. Database search details, report selection, synthesis of research findings, and statistical analysis are available in Expanded Materials & Methods S1.

#### Reporting bias assessment

The methodological quality of animal studies was assessed through the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE's RoB) tool<sup>24</sup>. The quality of studies in humans followed the NIH guidelines (<https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools>). The detail of the study quality assessment tools is presented in Expanded Materials & Methods S2.

## RESULTS

The initial search yielded 1625 documents. After removing the duplicates, we reviewed the titles/abstracts of 1443 articles, of which 1365 were excluded. The full text of the remaining 78 articles was retrieved and reviewed. Of these, 40 articles met the inclusion criteria for the systematic review. Additionally, 2 records were identified through other sources and included in the study. Therefore 42 records were selected for this systematic review, of which 36 were preclinical<sup>9-15,25-53</sup> and 6 included humans<sup>7,8,16-19</sup>. Of these, 4 studies were included for quantitative analysis (meta-analysis)<sup>16-19</sup>. The study selection process is described in Figure 1.

## Preclinical studies

A detailing of the 36 preclinical studies is presented in Table 1 and Table 2. Table 1 summarises the methodological characteristics of the studies, and Table 2 lists the key outcomes of each study. In addition, a description of the assessment methods, as well as the neurobiological and neurobehavioural effects of lithium treatment in animal models of stroke can be accessed in Table S1.

Briefly, as described in Table 1, most animal studies addressing the potential effects of lithium in experimental models of stroke were based on the induction of ischaemic insults (n=28, 77.7%), whereas haemorrhagic stroke was reported in eight studies (22.2%). Ischaemic stroke was induced by transient middle cerebral artery occlusion (tMCAO) (n=11, 39.2%) or bilateral common carotid artery occlusion (BCCAO) (n=8, 28.5%). Four studies used unilateral occlusion (14.2%), and three used permanent middle cerebral artery occlusions (pMCAO) (10.7%). Microsphere-induced cerebral embolism<sup>26</sup> or left middle cerebral artery electrocoagulation<sup>41</sup> were each used in one study (3.5% each). Of the eight studies that used models of intracerebral haemorrhage (ICH), five were based on the whole blood-induced ICH model (62.5%), and two used the collagenase-induced ICH model<sup>46,47</sup> (25%), and one used whole blood-induced intra-ventricular haemorrhagic model<sup>48</sup> (12.5%).

Concerning the treatment protocol, there was a large variation across studies in the frequency and duration of the intervention with lithium. Of all animal studies reviewed, six (16.6%) were based on pretreatment with lithium, twenty-four (66.6%) used post-insult interventions, and six studies (16.6%) performed both pre-and post-insult treatment. Most studies administered doses of lithium of 1.0mmol/kg (n=8, 22.2%), 1.5mmol/kg (n=6, 16.6%), or 2.0mmol/kg (n=9, 25%) at varying intervals. The lowest and the highest doses of lithium were respectively 0.03<sup>10</sup> and 12mmol/kg<sup>35</sup>, each used in one study (2.7%). Seven studies used more than one therapeutic dose of lithium (19.4%), and one study (2.7%) used lithium-treated extracellular vesicles<sup>45</sup> in a concentration of

2.5mM. Finally, treatment duration ranged from 14 days before the vascular insult to 56 days post-insult. Serum lithium concentrations were determined in only five studies<sup>10,11,25,27,46</sup> (13.8%) (for more information, see Table 1).

Overall, in animal models of ischaemic stroke, sixteen studies (57.1%) investigated the effects of lithium on stroke volume and all showed reduced infarct volume. Of the 22 studies (78.5%) that addressed the effect of lithium on apoptotic markers, nineteen reported findings indicative of inhibition of apoptosis, and three showed possible effects related to this outcome<sup>35,37,40</sup>. The effect of lithium on neurogenesis was investigated in thirteen studies, twelve of which indicated positive (42.8%) and another possible positive result (3.5%). Finally, eighteen (64.2%) of the nineteen studies that addressed the effect of lithium in the neurobehavioural response reported treatment-related improvements in various measures of function. Of the 8 animal studies investigating the effect of lithium on haemorrhagic stroke, three (37.5%) reported a reduction of hemispheric atrophy or haematoma volume<sup>46,49,53</sup>, seven (87.5%) described decreased apoptosis (5 positives and 2 possibly positive effects)<sup>46-52</sup>, one study (12.5%) reported increased neurogenesis<sup>53</sup>, one (12.5%) found no evidence of increased neurogenesis<sup>47</sup>, and seven (87.5%) showed improved neurobehavioural function<sup>46,47,49-53</sup> (Table 2).

The SYRCLE tool revealed that 65.8% of preclinical studies were associated with low risk of bias, 25% with a high risk of bias, and in 9.1% the risk of bias was unclear. Only two items related to blinding procedures ('performance' and 'detection') were associated with a high risk of bias in more than 50% of studies, and one item related to outcome data ('incomplete outcome data') was considered unclear in approximately in 70% of studies (Table S2 and Figure S1).

### Observational studies in humans

Table 3 describes the key findings of the 4 observational studies that reported data on the effect of lithium treatment on the risk of stroke in humans. These studies were largely based on samples of



patients with BD, representing the target population of lithium-treated adults, and accounted for the odds ratio of the occurrence of stroke among participants allocated in two groups (lithium/non-lithium). Two of these studies were retrospective, including a cohort<sup>16</sup> and a chart review study<sup>17</sup>, and one was a prospective study with a cross-over design<sup>18</sup>. The remaining study was based on the assessment of a population-wide electronic database of health records with over 700,000 people with BD, addressing the incidence of neurodegenerative and cerebrovascular outcomes<sup>19</sup>. The forest plot (Figure 2) indicates that lithium decreased the risk of stroke (odds ratio [OR]=0.71, 95%CI=0.54-0.93)<sup>16-19</sup>, although study heterogeneity was high ( $I^2=60.0\%$ ). Study quality was rated as good for one study<sup>16</sup>, fair for two of them<sup>18,19</sup>, and poor for another study<sup>17</sup> (Table S3).

#### Clinical trials in humans

We identified two interventional studies investigating the clinical, cognitive, and neuroimaging effects of treatment with lithium following a stroke (Table 4). The first reported the results of a single-site randomized, double-blind placebo-controlled treatment trial with oral lithium carbonate 300mg twice per day compared with placebo started within two days of the first-ever stroke and continued for 30 days (none of the participants underwent thrombolysis)<sup>7</sup>. Eighty adults aged 50-80 years were randomized (40 per group), but outcome data were reported for only 32 participants treated with lithium and 34 with placebo. After one month, modified National Institute of Health Stroke Scale scores of both treatment groups did not differ but hand motor function improved more among participants treated with lithium than with placebo.

The second intervention study was a small, uncontrolled open-label pre-post investigation of the effects of lithium carbonate (target serum concentration of 0.4-0.8mmol/L) for the treatment of 12 stroke survivors for 60 days, of whom 8 completed the trial<sup>8</sup>. The higher cumulative dose of lithium was correlated with improved scores on a task of delayed recall, and those who had received daily oral dosages of lithium  $\geq 300\text{mg}$  showed greater global grey matter volume than their counterparts

treated with <300mg. The respective quality of these two studies was rated as fair<sup>7</sup> and poor<sup>8</sup> (Table S4).

## DISCUSSION

Among multiple experimental approaches that have been proposed for the animal modelling of stroke<sup>54</sup>, we found evidence that treatment with lithium reduces stroke volume and apoptosis, and improves function in rodent models of ischaemic and haemorrhagic stroke. Results from published observational studies of people with BD suggest that stroke is less frequent among those treated with lithium, but the quantity and quality of available data are limited. The effects of short-term treatment with lithium on stroke recovery in one trial and a pre-post observational study were consistent with benefits, but their results were not compelling.

The plausibility of the hypothesis that lithium may contribute to improving the clinical outcomes of stroke survivors is supported by various observations<sup>22</sup>. Neuroimaging studies have shown that the chronic use of lithium is associated with increased grey matter volume in the brain of people with BD<sup>55</sup>, and there is cumulative evidence from experimental studies that lithium may also activate neuroprotective and trophic responses, with therapeutic implications for degenerative, traumatic, and vascular brain diseases<sup>4,6</sup>. The disruption of homeostasis caused by stroke activates signalling pathways that upregulate inflammatory, oxidative, and apoptotic responses, further increasing the magnitude of brain injury. At a molecular level, many signalling pathways modified by lithium may be relevant to the pathophysiology of stroke. These mechanisms include the inhibition of glycogen synthase kinase-3 $\beta$ , an important hub of signalling control in multiple cell types, with a central role in the regulation of apoptotic and neuro-regenerative processes<sup>21,22,56</sup>.

Animal models of stroke have produced data consistent with a neuroprotective effect of lithium, as inferred by the reduced volume of infarcts<sup>10,53</sup>, attenuation of post-ischaemic excitotoxicity<sup>25,51</sup>, and the enabling of post-stroke recovery<sup>10</sup>. Other studies have shown that lithium treatment is associated

with anti-apoptotic effects<sup>15,34,43,49,51,52</sup>, decreased expression of inflammatory markers<sup>31,42,43,45,50</sup>, reduced oxidative stress<sup>35,43,45</sup>, and activation of immuno-mediated responses involved in the restoration of blood-brain barrier integrity<sup>44,50</sup>. Finally, evidence of trophic responses is supported by the ability of lithium to up-regulate the brain-derived neurotrophic factor production<sup>39,52,53</sup>, to promote neuronal differentiation, axonal plasticity, and endogenous neurogenesis<sup>14,28,42,45</sup>, as well as to stimulate post-stroke angiogenesis<sup>53</sup>. According to the SYRCLE's tool, the overall assessment of the quality of animal studies included in the review was ranked as good, i.e., low risk of bias in most domains; except for 'performance-' and 'detection-blinding', where the risk of bias was observed in >50% of studies. Poor blinding may compromise the assessment of qualitative and quantitative outcomes, and lead to inaccuracies in the assessment of treatment effects<sup>24</sup>.

The recognition of lithium as a therapeutic compound that may promote neurotrophic and restorative responses is the rationale for its repurposing as a candidate drug for the adjunctive treatment of stroke. In fact, the potential neuroprotective effects of lithium in humans may not be limited to stroke<sup>5,22</sup>. A nested case-control study of the Danish population found that the incidence rate ratio of dementia was lowest for older adults exposed to lithium concentration in drinking water >15µg/L compared with concentrations ≤5µg/L and that this benefit was extended to dementia caused by cerebrovascular disease<sup>3</sup>. Moreover, a randomized, placebo-controlled trial of 61 older adults with mild cognitive impairment (serum concentration of lithium between 0.25-0.5mmol/L) found that treatment with lithium was associated with a lower rate of cognitive decline, and a statistical trend for better function and improved cerebrospinal fluid concentration of amyloid beta-peptide compared with placebo after 2 years<sup>6</sup>. These results suggest that treatment with lithium may contribute to preserving the integrity of neurons, a conclusion that is reinforced by the compelling evidence from basic science about the neuroprotective properties of lithium<sup>21</sup>. However, treatment with lithium cannot be seen as a panacea for neurodegenerative disorders. For example, a randomized, double-blind, placebo-controlled trial of lithium carbonate (target serum concentration of 0.4-0.8mmol/L) for the treatment of 214 adults with amyotrophic lateral sclerosis found no

evidence of increased survival among those treated with lithium for 18 months, although the medication was well tolerated by participants<sup>57</sup>.

## CONCLUSION

The rationale for the testing of lithium in the management of stroke survivors is strongly supported by data derived from *in vitro* and animal models of stroke. However, the translation from basic to clinical science is yet to be adequately tested. Published information in humans remains limited in quantity and quality. High-quality data from well-designed randomized trials are now required to establish whether lithium can improve the health outcomes of people recovering from a haemorrhagic or ischaemic stroke.

## SOURCES OF FUNDING

The study was supported by the National Institute for Biomarker Research in Neuropsychiatry (INBION) and INCT-CAPES (Institutos Nacionais de Ciência e Tecnologia; Grant no.465412/2014-9; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; Grant nos.88887.463672/2019-00 and 88887.514714/2020-00).

## DISCLOSURES

None.

## SUPPLEMENTAL MATERIAL

Expanded Materials & Methods S1-S2.

Tables S1-S4.

Figure S1.

Prisma Checklist

## REFERENCES

1. CADE JF. Lithium salts in the treatment of psychotic excitement. *Med J Aust.* 1949;2:349-352. doi:10.1080/j.1440-1614.1999.06241.x
2. Malhi GS, Gessler D, Outhred T. The use of lithium for the treatment of bipolar disorder: Recommendations from clinical practice guidelines. *J Affect Disord.* 2017;217:266-280. doi:10.1016/j.jad.2017.03.052
3. Kessing LV, Gerds TA, Knudsen NN, Jørgensen LF, Kristiansen SM, Vouthkova D, Ernstsens V, Schullehner J, Hansen B, Andersen PK, et al. Association of Lithium in Drinking Water With the Incidence of Dementia. *JAMA Psychiatry.* 2017;74:1005-1010. doi:10.1001/jamapsychiatry.2017.2362
4. Nunes PV, Forlenza OV, Gattaz WF. Lithium and risk for Alzheimer's disease in elderly patients with bipolar disorder. *Br J Psychiatry.* 2007;190:359-360. doi:10.1192/bjp.bp.106.029868
5. Kessing LV, Søndergård L, Forman JL, Andersen PK. Lithium treatment and risk of dementia. *Arch Gen Psychiatry.* 2008;65:1331-1335. doi:10.1001/archpsyc.65.11.1331
6. Forlenza OV, Radanovic M, Talib LL, Gattaz WF. Clinical and biological effects of long-term lithium treatment in older adults with amnesic mild cognitive impairment: randomised clinical trial. *Br J Psychiatry.* 2019;1-7. doi:10.1192/bjp.2019.76
7. Mohammadianinejad SE, Majdinasab N, Sajedi SA, Abdollahi F, Moqaddam MM, Sadr F. The effect of lithium in post-stroke motor recovery: a double-blind, placebo-controlled, randomized clinical trial. *Clin Neuropharmacol.* 2014;37:73-78. doi:10.1097/WNF.0000000000000028
8. Sun YR, Herrmann N, Scott CJM, Black SE, Swartz RH, Hopyan J, Lanctôt KL. Lithium Carbonate in a Poststroke Population: Exploratory Analyses of Neuroanatomical and Cognitive Outcomes. *J Clin Psychopharmacol.* 2019;39:67-71. doi:10.1097/JCP.0000000000000981
9. Li Q, Li H, Roughton K, Wang X, Kroemer G, Blomgren K, Zhu C. Lithium reduces apoptosis and autophagy after neonatal hypoxia-ischemia. *Cell Death Dis.* 2010;1:e56. doi:10.1038/cddis.2010.33
10. Ren M, Senatorov VV, Chen RW, Chuang DM. Postinsult treatment with lithium reduces brain damage and facilitates neurological recovery in a rat ischemia/reperfusion model. *Proc Natl Acad Sci U S A.* 2003;100:6210-6215. doi:10.1073/pnas.0937423100
11. Xu J, Culman J, Blume A, Brecht S, Gohlke P. Chronic treatment with a low dose of lithium protects the brain against ischemic injury by reducing apoptotic death. *Stroke.* 2003;34:1287-1292. doi:10.1161/01.STR.0000066308.25088.64
12. Xu XH, Zhang HL, Han R, Gu ZL, Qin ZH. Enhancement of neuroprotection and heat shock protein induction by combined prostaglandin A1 and lithium in rodent models of focal ischemia. *Brain Res.* 2006;1102:154-162. doi:10.1016/j.brainres.2006.04.111
13. Sheng R, Zhang LS, Han R, Gao B, Liu XQ, Qin ZH. Combined prostaglandin E1 and lithium exert potent neuroprotection in a rat model of cerebral ischemia. *Acta Pharmacol Sin.* 2011;32:303-310. doi:10.1038/aps.2010.211

14. Xiao Y, Fan M, Jin W, Li WA, Jia Y, Dong Y, Jiang X, Xu J, Meng N, Lv P. Lithium chloride ameliorated spatial cognitive impairment through activating mTOR phosphorylation and inhibiting excessive autophagy in the repeated cerebral ischemia-reperfusion mouse model. *Exp Ther Med*. 2020;20:109. doi:10.3892/etm.2020.9237
15. Ji YB, Gao Q, Tan XX, Huang XW, Ma YZ, Fang C, Wang SN, Qiu LH, Cheng YX, Guo FY, et al. Lithium alleviates blood-brain barrier breakdown after cerebral ischemia and reperfusion by upregulating endothelial Wnt/ $\beta$ -catenin signaling in mice. *Neuropharmacology*. 2021;186:108474. doi:10.1016/j.neuropharm.2021.108474
16. Lan CC, Liu CC, Lin CH, Lan TY, McInnis MG, Chan CH, Lan TH. A reduced risk of stroke with lithium exposure in bipolar disorder: a population-based retrospective cohort study. *Bipolar Disord*. 2015;17:705-714. doi:10.1111/bdi.12336
17. Prosser JM, Fieve RR. Patients receiving lithium therapy have a reduced prevalence of neurological and cardiovascular disorders. *Prog Neuropsychopharmacol Biol Psychiatry*. 2016;71:39-44. doi:10.1016/j.pnpbp.2016.06.006
18. Chen PH, Tsai SY, Pan CH, Chang CK, Su SS, Chen CC, Kuo CJ. Mood stabilisers and risk of stroke in bipolar disorder. *Br J Psychiatry*. 2019;215:409-414. doi:10.1192/bjp.2018.203
19. Harrison PJ, Luciano S. Incidence of Parkinson's disease, dementia, cerebrovascular disease and stroke in bipolar disorder compared to other psychiatric disorders: An electronic health records network study of 66 million people. *Bipolar Disord*. 2021;23:454-462. doi:10.1111/bdi.13022
20. Crilly S, Withers SE, Allan SM, Parry-Jones AR, Kasher PR. Revisiting promising preclinical intracerebral hemorrhage studies to highlight repurposable drugs for translation. *Int J Stroke*. 2021;16:123-136. doi:10.1177/1747493020972240
21. Singulani MP, De Paula VJR, Forlenza OV. Mitochondrial dysfunction in Alzheimer's disease: Therapeutic implications of lithium. *Neurosci Lett*. 2021;760:136078. doi:10.1016/j.neulet.2021.136078
22. Rybakowski JK, Suwalska A, Hajek T. Clinical Perspectives of Lithium's Neuroprotective Effect. *Pharmacopsychiatry*. 2018;51:194-199. doi:10.1055/s-0043-124436
23. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71. doi:10.1136/bmj.n71
24. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol*. 2014;14:43. doi:10.1186/1471-2288-14-43
25. Ma J, Zhang GY. Lithium reduced N-methyl-D-aspartate receptor subunit 2A tyrosine phosphorylation and its interactions with Src and Fyn mediated by PSD-95 in rat hippocampus following cerebral ischemia. *Neurosci Lett*. 2003;348:185-189. doi:10.1016/s0304-3940(03)00784-5
26. Sasaki T, Han F, Shioda N, Moriguchi S, Kasahara J, Ishiguro K, Fukunaga K. Lithium-induced activation of Akt and CaM kinase II contributes to its neuroprotective action in a rat

- microsphere embolism model. *Brain Res.* 2006;1108:98-106. doi:10.1016/j.brainres.2006.06.009
27. Bian Q, Shi T, Chuang DM, Qian Y. Lithium reduces ischemia-induced hippocampal CA1 damage and behavioral deficits in gerbils. *Brain Res.* 2007;1184:270-276. doi:10.1016/j.brainres.2007.09.054
  28. Yan XB, Hou HL, Wu LM, Liu J, Zhou JN. Lithium regulates hippocampal neurogenesis by ERK pathway and facilitates recovery of spatial learning and memory in rats after transient global cerebral ischemia. *Neuropharmacology.* 2007;53:487-495. doi:10.1016/j.neuropharm.2007.06.020
  29. Han R, Gao B, Sheng R, Zhang LS, Zhang HL, Gu ZL, Qin ZH. Synergistic effects of prostaglandin E1 and lithium in a rat model of cerebral ischemia. *Acta Pharmacol Sin.* 2008;29:1141-1149. doi:10.1111/j.1745-7254.2008.00873.x
  30. Kim YR, van Meer MP, Tejima E, Murata Y, Mandeville JB, Dai G, Chuang DM, Rosen BR, Lo EH. Functional MRI of delayed chronic lithium treatment in rat focal cerebral ischemia. *Stroke.* 2008;39:439-447. doi:10.1161/STROKEAHA.107.492215
  31. Li H, Li Q, Du X, Sun Y, Wang X, Kroemer G, Blomgren K, Zhu C. Lithium-mediated long-term neuroprotection in neonatal rat hypoxia-ischemia is associated with antiinflammatory effects and enhanced proliferation and survival of neural stem/progenitor cells. *J Cereb Blood Flow Metab.* 2011;31:2106-2115. doi:10.1038/jcbfm.2011.75
  32. Tsai LK, Wang Z, Munasinghe J, Leng Y, Leeds P, Chuang DM. Mesenchymal stem cells primed with valproate and lithium robustly migrate to infarcted regions and facilitate recovery in a stroke model. *Stroke.* 2011;42:2932-2939. doi:10.1161/STROKEAHA.110.612788
  33. Shin WJ, Gwak M, Baek CH, Kim KS, Park PH. Neuroprotective effects of lithium treatment following hypoxic-ischemic brain injury in neonatal rats. *Childs Nerv Syst.* 2012;28:191-198. doi:10.1007/s00381-011-1627-2
  34. Takahashi T, Steinberg GK, Zhao H. Lithium treatment reduces brain injury induced by focal ischemia with partial reperfusion and the protective mechanisms dispute the importance of akt activity. *Aging Dis.* 2012;3:226-233.
  35. Ozkul A, Sair A, Akyol A, Yenisey C, Dost T, Tataroglu C. Effects of lithium and lamotrigine on oxidative-nitrosative stress and spatial learning deficit after global cerebral ischemia. *Neurochem Res.* 2014;39:853-861. doi:10.1007/s11064-014-1281-7
  36. Xie C, Zhou K, Wang X, Blomgren K, Zhu C. Therapeutic benefits of delayed lithium administration in the neonatal rat after cerebral hypoxia-ischemia. *PLoS One.* 2014;9:e107192. doi:10.1371/journal.pone.0107192
  37. Boyko M, Nassar A, Kaplanski J, Zlotnik A, Sharon-Granit Y, Azab AN. Effects of Acute Lithium Treatment on Brain Levels of Inflammatory Mediators in Poststroke Rats. *Biomed Res Int.* 2015;2015:916234. doi:10.1155/2015/916234
  38. Fan M, Jin W, Zhao H, Xiao Y, Jia Y, Yin Y, Jiang X, Xu J, Meng N, Lv P. Lithium chloride administration prevents spatial learning and memory impairment in repeated cerebral ischemia-reperfusion mice by depressing apoptosis and increasing BDNF



- expression in hippocampus. *Behav Brain Res.* 2015;291:399-406. doi:10.1016/j.bbr.2015.05.047
39. Fan M, Song C, Wang T, Li L, Dong Y, Jin W, Lu P. Protective effects of lithium chloride treatment on repeated cerebral ischemia-reperfusion injury in mice. *Neurol Sci.* 2015;36:315-321. doi:10.1007/s10072-014-1943-x
  40. Silachev DN, Gulyaev MV, Zorova LD, Khailova LS, Gubsky LV, Pirogov YA, Plotnikov EY, Sukhikh GT, Zorov DB. Magnetic resonance spectroscopy of the ischemic brain under lithium treatment. Link to mitochondrial disorders under stroke. *Chem Biol Interact.* 2015;237:175-182. doi:10.1016/j.cbi.2015.06.012
  41. Silachev DN, Plotnikov EY, Babenko VA, Savchenko ES, Zorova LD, Pevzner IB, Gulyaev MV, Pirogov YA, Sukhikh GT, Zorov DB. Protection of Neurovascular Unit Cells with Lithium Chloride and Sodium Valproate Prevents Brain Damage in Neonatal Ischemia/Hypoxia. *Bull Exp Biol Med.* 2016;160:313-318. doi:10.1007/s10517-016-3159-y
  42. Taliyan R, Ramagiri S. Delayed neuroprotection against cerebral ischemia reperfusion injury: putative role of BDNF and GSK-3 $\beta$ . *J Recept Signal Transduct Res.* 2016;36:402-410. doi:10.3109/10799893.2015.1108338
  43. Doeppner TR, Kaltwasser B, Sanchez-Mendoza EH, Caglayan AB, Bähr M, Hermann DM. Lithium-induced neuroprotection in stroke involves increased miR-124 expression, reduced RE1-silencing transcription factor abundance and decreased protein deubiquitination by GSK3 $\beta$  inhibition-independent pathways. *J Cereb Blood Flow Metab.* 2017;37:914-926. doi:10.1177/0271678X16647738
  44. Haupt M, Zechmeister B, Bosche B, Lieschke S, Zheng X, Zhang L, Venkataramani V, Jin F, Hein K, Weber MS, et al. Lithium enhances post-stroke blood-brain barrier integrity, activates the MAPK/ERK1/2 pathway and alters immune cell migration in mice. *Neuropharmacology.* 2020;181:108357. doi:10.1016/j.neuropharm.2020.108357
  45. Haupt M, Zheng X, Kuang Y, Lieschke S, Janssen L, Bosche B, Jin F, Hein K, Kilic E, Venkataramani V, et al. Lithium modulates miR-1906 levels of mesenchymal stem cell-derived extracellular vesicles contributing to poststroke neuroprotection by toll-like receptor 4 regulation. *Stem Cells Transl Med.* 2021;10:357-373. doi:10.1002/sctm.20-0086
  46. Kang K, Kim YJ, Kim YH, Roh JN, Nam JM, Kim PY, Ryu WS, Lee SH, Yoon BW. Lithium pretreatment reduces brain injury after intracerebral hemorrhage in rats. *Neurol Res.* 2012;34:447-454. doi:10.1179/1743132812Y.0000000015
  47. Kang K, Kim YJ, Lee SH, Yoon BW. Lithium fails to enhance neurogenesis in subventricular zone and dentate subgranular zone after intracerebral hemorrhage in rats. *Neurol Res.* 2014;36:79-85. doi:10.1179/1743132813Y.0000000265
  48. Yuan Q, Bu XY, Yan ZY, Liu XZ, Wei ZY, Ma CX, Qu MQ. Combination of endogenous neural stem cell mobilization and lithium chloride treatment for hydrocephalus following intraventricular hemorrhage. *Exp Ther Med.* 2016;12:3275-3281. doi:10.3892/etm.2016.3778
  49. Zheng J, Liu Z, Li W, Tang J, Zhang D, Tang X. Lithium posttreatment confers neuroprotection through glycogen synthase kinase-3 $\beta$  inhibition in intracerebral hemorrhage rats. *J Neurosurg.* 2017;127:716-724. doi:10.3171/2016.7.JNS152995



50. Li W, Li R, Zhao S, Jiang C, Liu Z, Tang X. Lithium Posttreatment Alleviates Blood-Brain Barrier Injury After Intracerebral Hemorrhage in Rats. *Neuroscience*. 2018;383:129-137. doi:10.1016/j.neuroscience.2018.05.001
51. Liu Z, Li R, Jiang C, Zhao S, Li W, Tang X. The neuroprotective effect of lithium chloride on cognitive impairment through glycogen synthase kinase-3 $\beta$  inhibition in intracerebral hemorrhage rats. *Eur J Pharmacol*. 2018;840:50-59. doi:10.1016/j.ejphar.2018.10.019
52. Li M, Xia M, Chen W, Wang J, Yin Y, Guo C, Li C, Tang X, Zhao H, Tan Q, et al. Lithium treatment mitigates white matter injury after intracerebral hemorrhage through brain-derived neurotrophic factor signaling in mice. *Transl Res*. 2020;217:61-74. doi:10.1016/j.trsl.2019.12.006
53. Li R, Liu Z, Wu X, Yu Z, Zhao S, Tang X. Lithium chloride promoted hematoma resolution after intracerebral hemorrhage through GSK-3 $\beta$ -mediated pathways-dependent microglia phagocytosis and M2-phenotype differentiation, angiogenesis and neurogenesis in a rat model. *Brain Res Bull*. 2019;152:117-127. doi:10.1016/j.brainresbull.2019.07.019
54. Kumar A, Aakriti, Gupta V. A review on animal models of stroke: An update. *Brain Res Bull*. 2016;122:35-44. doi:10.1016/j.brainresbull.2016.02.016
55. Hajek T, Bauer M, Simhandl C, Rybakowski J, O'Donovan C, Pfennig A, König B, Suwalska A, Yucel K, Uher R, et al. Neuroprotective effect of lithium on hippocampal volumes in bipolar disorder independent of long-term treatment response. *Psychol Med*. 2014;44:507-517. doi:10.1017/S0033291713001165
56. Pap M, Cooper GM. Role of glycogen synthase kinase-3 in the phosphatidylinositol 3-Kinase/Akt cell survival pathway. *J Biol Chem*. 1998;273:19929-19932. doi:10.1074/jbc.273.32.19929
57. Morrison KE, Dhariwal S, Hornabrook R, Savage L, Burn DJ, Khoo TK, Kelly J, Murphy CL, Al-Chalabi A, Dougherty A, et al. Lithium in patients with amyotrophic lateral sclerosis (LiCALS): a phase 3 multicentre, randomised, double-blind, placebo-controlled trial. *Lancet Neurol*. 2013;12:339-345. doi:10.1016/S1474-4422(13)70037-1

## FIGURE LEGENDS

**Graphical Abstract.** Neuroprotective effects of lithium treatment in ischaemic and haemorrhagic stroke. Servier Medical Art images were used in the making of the image.

**Figure 1.** PRISMA flow diagram of search.

**Figure 2.** Forest plot depicting the results of studies investigating the effect of treatment with lithium on the risk of stroke in humans. Odds ratio values  $<1$  indicate decreased risk of stroke in association with lithium treatment.

**Table 1.** Methodological characteristics of the preclinical studies.

| Study                             | Animal model   |                               |  | Intervention   |  |   |
|-----------------------------------|----------------|-------------------------------|--|--|--|---|
|                                   | Type of Stroke | Animal strain (weight/age)    | Stroke model/Duration of occlusion-reperfusion | Lithium doses/Administration route                             | Treatment protocol (frequency; duration)/Time to the evaluation of results           | Serum lithium level                         |
| Ma et al.(2003) <sup>25</sup>     | Ischaemic      | Male SD rats/250-300g         | BCCAO/15min occlusion-6h reperfusion           | 2mg/kg(LiCl) (0.05mmol/kg)/i.p.                                | Pretreatment: 7 days, daily/6h post-insult   | 0.5-1.0 and 0.2-0.8mmol/L                   |
| Ren et al.(2003) <sup>10</sup>    | Ischaemic      | Male SD rats/250-300g         | tMCAO/60min occlusion-reperfusion              | 0.5, 1, 2, 3mEq/kg(LiCl) (0.03, 0.06, 0.1 and 0.2mmol/kg)/s.c. | Post-treatment: immediate, 3h and 7 days, daily/up to 14 days                        | 5-1.0mmol/L at 12h and 0.2-0.8mmol/L at 24h |
| Xu et al.(2003) <sup>11</sup>     | Ischaemic      | Male Wistar rats/200-230g     | tMCAO/90min occlusion-reperfusion              | 1mmol/kg(LiCl)/s.c.  | Pretreatment: 14 days, daily; post-treatment: 2 days, daily/up to 2 days post-insult | 0.40±0.06mmol/L                             |
| Sasaki et al.(2006) <sup>26</sup> | Ischaemic      | Male Wistar rats/220-270g     | Microsphere-embolism/permanent occlusion       | 3mEq/kg(LiCl) (0.2mmol/kg)/i.p.                                | Post-treatment: 30min/up to 72h  | NA  |
| Xu et al.(2006) <sup>12</sup>     | Ischaemic      | Male SD rats/280-300g         | Permanent MCAO/permanent                       | 1mEq/kg(Li) (1mmol/kg)/s.c.                                    | Pretreatment: 2 days, daily/24h post-insult  | NA  |
| Bian et al.(2007) <sup>27</sup>   | Ischaemic      | Male Mongolian Gerbils/55-70g | BCCAO/5min occlusion-reperfusion               | 3mEq/kg(LiCl) (0.2mmol/kg)/i.p.                                | Pretreatment: 7 days, daily; post-treatment: 24h/up to 7 days                        | 0.49±0.09mM                                 |
| Yan et al.(2007) <sup>28</sup>    | Ischaemic      | Male SD rats/200-220g         | BCCAO/15min occlusion-2h reperfusion           | 1mmol/kg(LiCl)/i.p.  | Pretreatment: 14 days, daily/up to 21 days post-insult                               | NA  |
| Han et al.(2008) <sup>29</sup>    | Ischaemic      | Male SD rats/280-300g         | Permanent MCAO/permanent                       | 0.5mEq/kg(Li) (0.5mmol/kg)/s.c.                                | Pretreatment: 24h before and immediate/up to 24h post-insult                         | NA  |

|                                      |           |   |                                     |                                      |  |    |
|--------------------------------------|-----------|---|-------------------------------------|--------------------------------------|--|----|
| Kim et al.(2008) <sup>30</sup>       | Ischaemic | Male SD rats/250-350g                   | tMCAO/NA                            | 1mmol/L(LiCl)/s.c.                   | Post-treatment: 12h and 14 days, daily/up to 14 days   | NA |
| Li et al.(2010) <sup>9</sup>         | Ischaemic | Male Wistar rats/9-day postnatal (P)    | Unilateral occlusion/50min hypoxia  | 2mmol/kg(LiCl)/i.p.                  | Post-treatment: immediate and 24h/up to 72h  | NA |
| Li et al.(2011) <sup>31</sup>        | Ischaemic | Male Wistar rats/P9                     | Unilateral occlusion/50min hypoxia  | 2mmol/kg(LiCl)/i.p.                  | Post-treatment: immediate and 6 days, daily/up to 7 weeks  | NA |
| Sheng et al.(2011) <sup>13</sup>     | Ischaemic | Male SD rats/260-300g                   | Permanent MCAO/permanent            | 0.5mmol/kg(Li)/i.v.                  | Post-treatment: immediate/up to 24h  | NA |
| Tsai et al.(2011) <sup>32</sup>      | Ischaemic | Male SD rats/190-230g                   | tMCAO/60min occlusion-reperfusion   | 2.5mmol/kg(LiCl)/i.p.                | Pretreatment: mesenchymal stem cells incubated for 24h; post-treatment: 14 days, daily/up to 15 days | NA |
| Shin et al.(2012) <sup>33</sup>      | Ischaemic | Male SD rats/12-16g/P7                  | Unilateral occlusion/150min hypoxia | 1mmol/kg(LiCl)/s.c.                  | Post-treatment: immediate and 14 days, daily/up to 14 days   | NA |
| Takahashi et al.(2012) <sup>34</sup> | Ischaemic | Male SD rats/270-350g                   | BCCAO/30min occlusion-reperfusion   | 3mg/kg(LiCl) (0.1mmol/kg)/i.p.       | Post-treatment: immediate/up to 48h  | NA |
| Ozkul et al.(2014) <sup>35</sup>     | Ischaemic | Female Wistar rats/188-260g/6-month-old | BCCAO/permanent occlusion           | 40-80mg/kg(Li) (6 or 12mmol/kg)/i.p. | Post-treatment: immediate/up to 28 days  | NA |
| Xie et al.(2014) <sup>36</sup>       | Ischaemic | Male Wistar rats/P8                     | Unilateral occlusion/45min hypoxia  | 2mmol/kg(LiCl)/i.p.                  | Post-treatment: 5 and 14 days, daily/up to 12 weeks  | NA |
| Boyko et al.(2015) <sup>37</sup>     | Ischaemic | Male Wistar rats/NA                     | tMCAO/NA                            | 100mg/kg(LiCl) (2mmol/kg)/i.p.       | Pretreatment: 2h before/ up to 24h post-insult   | NA |

|                                     |           |   |   |  |  |    |
|-------------------------------------|-----------|---|---|--|--|----|
| Fan et al.(2015) <sup>39</sup>      | Ischaemic | Male C57Bl/6 mice/22-25g/8-week-old     | BCCAO/repeated ischemia-reperfusion                       | 2-5mmol/kg(LiCl)/i.p.  | Pretreatment: 7 days; post-treatment: 28 days, daily/up to 34 days                                     | NA |
| Fan et al.(2015) <sup>38</sup>      | Ischaemic | Male C57Bl/6 mice/22-25g                | BCCAO/repeated ischemia-reperfusion                       | 2mmol/kg(LiCl)/i.p.  | Post-treatment: 2, 4, and 6 weeks, daily/up to 6 weeks   | NA |
| Silachev et al.(2015) <sup>40</sup> | Ischaemic | Male Outbred White rats/320-350g        | tMCAO/60min occlusion-reperfusion                         | 3mmol/kg(LiCl or Li succinate)/i.p.                          | Post-treatment: immediate and 3h/up to 24h   | NA |
| Silachev et al.(2016) <sup>41</sup> | Ischaemic | Male and Female rats/9-14g/P7           | Middle cerebral artery electrocoagulation/120m in hypoxia | 2mmol/kg(LiCl)/i.p.  | Post-treatment: immediate, 24 and 48h/up to 4 days   | NA |
| Taliyan et al.(2016) <sup>42</sup>  | Ischaemic | Male Wistar Rats/250g                   | tMCAO/60min occlusion-reperfusion                         | 40-60mg/kg(LiCl) (1 or 1.5mmol/kg)/i.p.                      | Post-treatment: 24h and 7 days, daily/up to 9 days   | NA |
| Doeppner et al.(2017) <sup>43</sup> | Ischaemic | Male C57Bl/6 mice/NA                    | tMCAO/45min occlusion-reperfusion                         | 1mmol/kg(LiCl)/i.p.  | Post-treatment: immediate, 3, 6, or 9h after and daily by 56 days with doses of 2mmol/kg/up to 56 days | NA |
| Haupt et al.(2020) <sup>44</sup>    | Ischaemic | Male C57Bl/6 mice/7-week-old            | tMCAO/45min occlusion-reperfusion                         | 1mmol/kg(LiCl)/i.p.  | Post-treatment: immediate and 2mmol/kg doses for 6, 24 and/or 48h/up to 72h                            | NA |
| Xiao et al.(2020) <sup>14</sup>     | Ischaemic | Male C57Bl/6 mice/22-26g/10-12-week-old | BCCAO/repeated ischemia-reperfusion                       | 2mmol/kg(LiCl)/i.p.  | Pretreatment: 7 days (84mg/kg); post-treatment: 2mmol/kg for 14 days, daily/up to 28 days              | NA |
| Haupt et al.(2021) <sup>45</sup>    | Ischaemic | Male C57Bl/6 mice/26-29g                | tMCAO/60min occlusion-reperfusion                         | 100µL of extracellular vesicles (2.5mM LiCl) (25mmol/L)/i.v. | Post-treatment: 1, 3 and 5 days/up to 3 months   | NA |
| Ji et al.(2021) <sup>15</sup>       | Ischaemic | Male C57Bl/6 mice/20-23g/8-10-week-old  | tMCAO/60min occlusion-reperfusion                         | 1.5, 3.0 or 6.0mmol/kg(LiCl)/i.p.                            | Post-treatment: immediate and 24h/up to 48h  | NA |

|                                  |              |                          |  |  |  |              |
|----------------------------------|--------------|--------------------------|--|--|--|--------------|
| Kang et al.(2012) <sup>46</sup>  | Haemorrhagic | Male SD rats/250-280g    | Collagenase-induced ICH model/CD                   | 2mEq/kg(LiCl) (0.1mmol/kg)/i.p                         | Pretreatment: daily 3 days and 24h before/up to 42 days post-insult          | 0.3-0.4mEq/l |
| Kang et al.(2014) <sup>47</sup>  | Haemorrhagic | Male SD rats/250-280g    | Collagenase-induced ICH model/CD                   | 1, 2 and 4mEq/kg(LiCl) (0.05, 0.1 and 0.2mmol/kg)/i.p. | Pretreatment: 14 days before; post-treatment: 14 days after/up to 28 days    | NA           |
| Yuan et al.(2016) <sup>48</sup>  | Haemorrhagic | Male SD rats/5-10g/P1-4  | Whole blood-induced intraventricular hemorrhage/CD | 3mmol/kg(LiCl)/i.p.                                    | Post-treatment: 2 days after/up to 32 days                                   | NA           |
| Zheng et al.(2017) <sup>49</sup> | Haemorrhagic | Male SD rats/250-300g    | Whole blood-induced ICH/CD                         | 30, 60, or 90mg/Kg(LiCl) (0.5, 1.5, 2mmol/kg)/i.p.     | Post-treatment: immediately, 2h, and twice a day for 21 days/up to 42 days   | NA           |
| Li et al.(2018) <sup>50</sup>    | Haemorrhagic | Male SD rats/230-280g    | Whole blood-induced ICH/CD                         | 60mg/kg(LiCl) (1.5mmol/kg)/i.p.                        | Post-treatment: 2h after/up to 3 days  | NA           |
| Liu et al.(2018) <sup>51</sup>   | Haemorrhagic | Male SD rats/250-300g    | Whole blood-induced ICH/CD                         | 60mg/kg(LiCl) (1.5mmol/kg)/i.p.                        | Post-treatment: 2h after and twice a day for 21 days/up to 21 days           | NA           |
| Li et al.(2019) <sup>53</sup>    | Haemorrhagic | Male SD rats/250-300g    | Whole blood-induced ICH/CD                         | 60mg/kg(LiCl) (1.5mmol/kg)/i.p.                        | Post-treatment: 2h and daily for 14 days/up to 14 days                       | NA           |
| Li et al.(2020) <sup>52</sup>    | Haemorrhagic | Male C57Bl/6 mice/23-30g | Whole blood-induced ICH/CD                         | 0.25-0.5mmol/L(LiCl)/i.p.                              | Post-treatment: every 24h after 1h of hemorrhage up to 21 days/up to 21 days | NA           |

SD, Sprague-Dawley; P, postnatal day; Li, lithium; LiCl, lithium chloride; i.p., intraperitoneal; i.v., intravenous; s.c., subcutaneous; NA, not applicable or not available; CD, could not be determined.

**Table 2.** Summary of the results from preclinical studies.

| Study                                | Main results                   |                            |                               |                          |
|--------------------------------------|--------------------------------|----------------------------|-------------------------------|--------------------------|
|                                      | <i>Decreased stroke volume</i> | <i>Decreased apoptosis</i> | <i>Increased neurogenesis</i> | <i>Improved function</i> |
| Ma et al.(2003) <sup>25</sup>        | NR                             | Yes                        | NR                            | NR                       |
| Ren et al.(2003) <sup>10</sup>       | Yes                            | Yes                        | Yes                           | Yes                      |
| Xu et al.(2003) <sup>11</sup>        | Yes                            | Yes                        | NR                            | Yes                      |
| Sasaki et al.(2006) <sup>26</sup>    | NR                             | Yes                        | Yes                           | NR                       |
| Xu et al.(2006) <sup>12</sup>        | Yes                            | NR                         | Yes                           | Yes                      |
| Bian et al.(2007) <sup>27</sup>      | NR                             | Yes                        | Yes                           | Yes                      |
| Yan et al.(2007) <sup>28</sup>       | NR                             | NR                         | Yes                           | Yes                      |
| Han et al.(2008) <sup>29</sup>       | Yes                            | NR                         | Yes                           | No                       |
| Kim et al.(2008) <sup>30</sup>       | NR                             | NR                         | Yes                           | NR                       |
| Li et al.(2010) <sup>9</sup>         | Yes                            | Yes                        | NR                            | NR                       |
| Li et al.(2011) <sup>31</sup>        | Yes                            | Yes                        | Yes                           | NR                       |
| Sheng et al.(2011) <sup>13</sup>     | Yes                            | Yes                        | NR                            | Yes                      |
| Tsai et al.(2011) <sup>32</sup>      | Yes                            | Yes                        | Yes                           | Yes                      |
| Shin et al.(2012) <sup>33</sup>      | Yes                            | Yes                        | NR                            | NR                       |
| Takahashi et al.(2012) <sup>34</sup> | Yes                            | Yes                        | NR                            | NR                       |
| Ozkul et al.(2014) <sup>35</sup>     | NR                             | Possible                   | NR                            | Yes                      |
| Xie et al.(2014) <sup>36</sup>       | Yes                            | NR                         | Yes                           | Yes                      |
| Boyko et al.(2015) <sup>37</sup>     | NR                             | Possible                   | NR                            | Yes                      |
| Fan et al.(2015) <sup>39</sup>       | NR                             | Yes                        | NR                            | Yes                      |
| Fan et al.(2015) <sup>38</sup>       | NR                             | Yes                        | NR                            | Yes                      |
| Silachev et al.(2015) <sup>40</sup>  | Yes                            | Possible                   | NR                            | Yes                      |
| Silachev et al.(2016) <sup>41</sup>  | Yes                            | NR                         | NR                            | NR                       |
| Taliyan et al.(2016) <sup>42</sup>   | NR                             | Yes                        | Possible                      | Yes                      |
| Doepfner et al.(2017) <sup>43</sup>  | Yes                            | Yes                        | Yes                           | Yes                      |
| Haupt et al.(2020) <sup>44</sup>     | NR                             | Yes                        | NR                            | NR                       |
| Xiao et al.(2020) <sup>14</sup>      | NR                             | Yes                        | NR                            | Yes                      |
| Haupt et al.(2021) <sup>45</sup>     | Yes                            | Yes                        | Yes                           | Yes                      |
| Ji et al.(2021) <sup>15</sup>        | Yes                            | Yes                        | NR                            | Yes                      |
| Kang et al.(2012) <sup>46</sup>      | Yes (Atrophy)                  | Yes                        | NR                            | Yes                      |
| Kang et al.(2014) <sup>47</sup>      | NR                             | Possible                   | No                            | Yes                      |
| Yuan et al.(2016) <sup>48</sup>      | NR                             | Yes                        | NR                            | NR                       |
| Zheng et al.(2017) <sup>49</sup>     | Yes (Atrophy)                  | Yes                        | NR                            | Yes                      |
| Li et al.(2018) <sup>50</sup>        | NR                             | Possible                   | NR                            | Yes                      |
| Liu et al.(2018) <sup>51</sup>       | NR                             | Yes                        | NR                            | Yes                      |
| Li et al.(2019) <sup>53</sup>        | Yes                            | NR                         | Yes                           | Yes                      |
| Li et al.(2020) <sup>52</sup>        | NR                             | Yes                        | NR                            | Yes                      |

NR, not reported.

**Table 3.** Characteristics of the observational studies in stroke humans.

| Study                                    | Setting and Population   | Design   | Measures  | N                                    | N<br>Lithium | Findings  |
|--|--|--|---|--------------------------------------|--------------|---|
| Lan et al.(2015) <sup>16</sup>           | <ul style="list-style-type: none"> <li>NHIRD Taiwan;</li> <li>Random sample of adults diagnosed with BD between 2001-2006;</li> <li>Propensity score-matched sub-sample of controls for age, gender, and comorbidities.</li> </ul> | Retrospective cohort study (participants followed until first stroke/death or December 2011) | <ul style="list-style-type: none"> <li>Recorded diagnosis of stroke according to ICD-9 (transient ischaemic attack excluded);</li> <li>Exposure to lithium.</li> </ul>  | 3681                                 | 635          | <ul style="list-style-type: none"> <li>18/635 on lithium and 307/3046 controls had a recorded diagnosis of stroke (OR=0.26, 95%CI=0.15, 0.42);</li> <li>18/635 on lithium and 68/1250 matched controls had a recorded diagnosis of stroke (OR=0.51, 95%CI=0.28, 0.87);</li> <li>Lithium use reduced the risk of stroke among participants with BD.</li> </ul> |
| Prosser et al.(2016) <sup>17</sup>       | <ul style="list-style-type: none"> <li>4 lithium outpatient clinics;</li> <li>Men and women aged&gt;18 years.</li> </ul>   | Chart review (1970-2015)   | <ul style="list-style-type: none"> <li>Recorded clinical diagnosis of stroke;</li> <li>Regular use of lithium (0.1-30 years).</li> </ul>  | 1028                                 | 577          | <ul style="list-style-type: none"> <li>6/577 on lithium and 7/451 clinic controls had a recorded diagnosis of stroke (OR=0.67; 95%CI=0.18, 2.34);</li> <li>A lower proportion of participants treated with lithium had a myocardial infarction.</li> </ul>  |
| Chen et al.(2019) <sup>18</sup>          | <ul style="list-style-type: none"> <li>NHIRD Taiwan;</li> <li>Bipolar people aged 15-65 years free of stroke before diagnosis.</li> </ul>  | Case cross-over  | <ul style="list-style-type: none"> <li>Outcome: stroke;</li> <li>Lithium exposure during four 14-day periods compared with 14-period preceding stroke;</li> <li>Analyses controlled for comorbidities and other medications.</li> </ul> | 609 BD participants who had a stroke | 609          | <ul style="list-style-type: none"> <li>62/609 were on lithium during the case-period. The adjusted risk ratio of stroke compared with control periods was 0.96 (95%CI=0.72, 1.28).</li> </ul>   |
| Harrison and Luciano(2021) <sup>19</sup> | <ul style="list-style-type: none"> <li>TriNetX health network records, USA;</li> <li>BD compared with mixed disorders.</li> </ul>  | Longitudinal data (1-18 years)   | <ul style="list-style-type: none"> <li>Outcome: stroke;</li> <li>Ever exposure to lithium.</li> </ul>   | 1,209,856 (stroke=11,221)            | 156,507      | <ul style="list-style-type: none"> <li>996/156,407 adults on lithium had a stroke compared with 10,225/1,053,449 not on lithium (OR=0.65, 95%CI=0.61,0.70).</li> </ul>  |

NHIRD, National Health Insurance Research Database.



**Table 4.** Characteristics of the trials with stroke survivors.

| Study                                       | Setting and Population  | Design   | Outcome   | Duration | N<br>lithium | N<br>placebo | Findings  |
|---|---|--|---|----------|--------------|--------------|---|
| Mohammadianinejad et al.(2014) <sup>7</sup> | <ul style="list-style-type: none"> <li>▪ Tertiary hospital in Iran;</li> <li>▪ First-ever acute ischaemic stroke (&lt;48h since onset) not eligible to receive antithrombotic treatment;</li> <li>▪ Age 50-80 years.</li> </ul> | Double-blind, placebo-controlled trial of lithium 300mg bd.                      | <ul style="list-style-type: none"> <li>▪ Modified National Institute of Health Stroke Scale score (mNIHSS);</li> <li>▪ 25% improvement of full-arm motor function on the hand score of the Fugl-Meyer Assessment (hFMA).</li> </ul> | 30 days  | 40           | 40           | <ul style="list-style-type: none"> <li>▪ 32-34 participants treated with lithium and placebo respectively completed the study;</li> <li>▪ No difference between the groups on the mNIHSS (<math>p=0.402</math>);</li> <li>▪ 14/32 and 5/34 participants treated with lithium and placebo showed <math>\geq 25\%</math> improvement in the hFMA (post-hoc OR=4.51; 5%CI=1.24, 18.41).</li> </ul> |
| Sun et al.(2019) <sup>8</sup>               | <ul style="list-style-type: none"> <li>▪ Ischaemic stroke during the preceding year;</li> <li>▪ Age&gt;40years</li> </ul>   | Pre-post open-label use of lithium with a target concentration of 0.4-0.8mmol/L. | <ul style="list-style-type: none"> <li>▪ Cognitive function;</li> <li>▪ Grey matter volume.</li> </ul>  | 60 days  | 12           | NA           | <ul style="list-style-type: none"> <li>▪ No cognitive benefits;</li> <li>▪ No change in grey matter volume;</li> <li>▪ Post-hoc: percentage increase in grey matter volume was higher for patients treated with 300mg/d compared with those on lower daily dosages.</li> </ul>  |

mNIHSS, Modified National Institute of Health Stroke Scale score.

Figure 1.

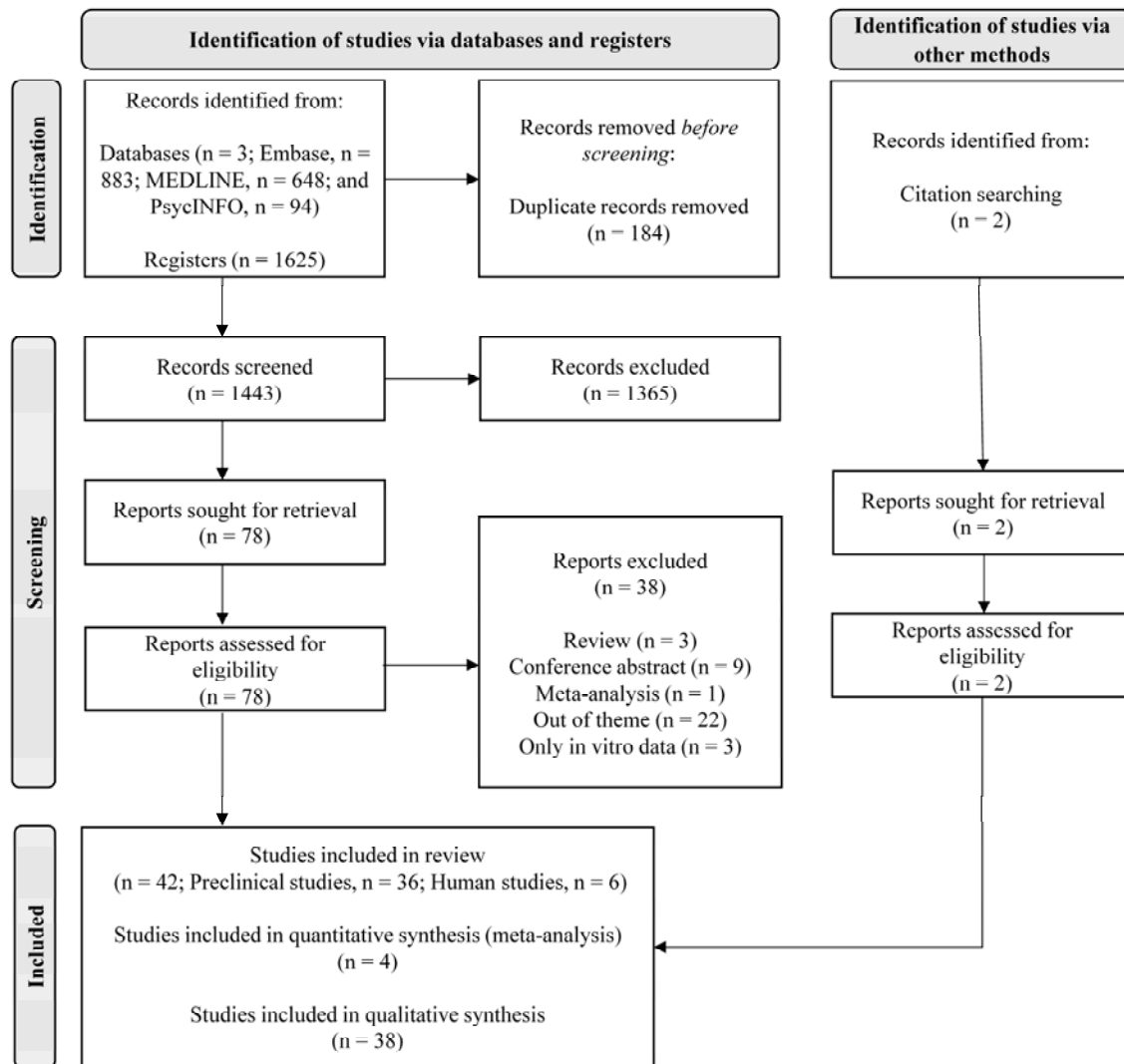
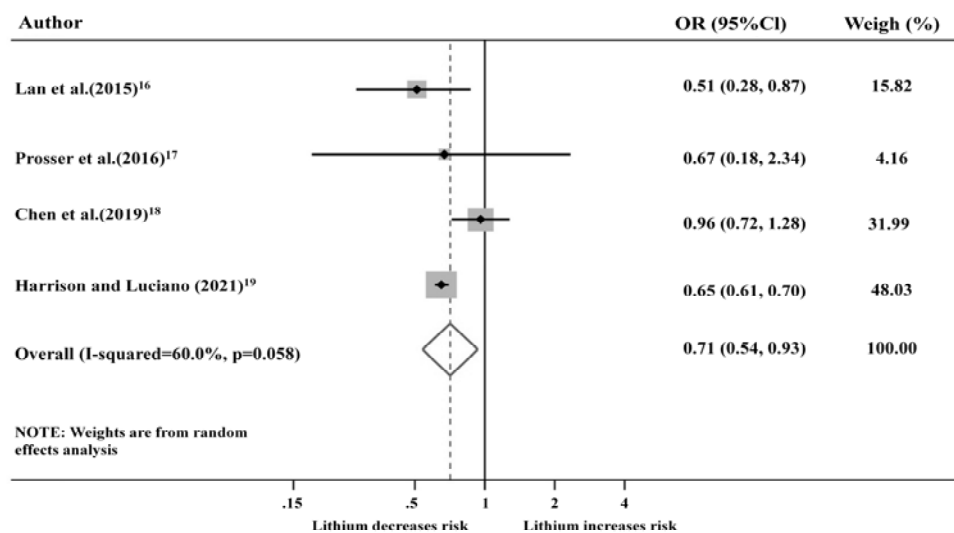


Figure 2.



For Stroke Peer Review. Do not  
after use.

