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Cerebrolysin for acute ischaemic stroke (Review)

Ziganshina LE, Abakumova T, Vernay L

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[Intervention Review]

Cerebrolysin for acute ischaemic stroke

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ABSTRACT

Background

Cerebrolysin is a mixture of low-molecular-weight peptides and amino acids derived from pigs' brain tissue, which has potential neuroprotective and neurotrophic properties. It is widely used in the treatment of acute ischaemic stroke in Russia, Eastern Europe, China, and other Asian and post-Soviet countries.

Objectives

To assess the benefits and risks of cerebrolysin for treating acute ischaemic stroke.

Search methods

In May 2016 we searched the Cochrane Stroke Group Trials Register, CENTRAL, MEDLINE, Embase, Web of Science Core Collection, with Science Citation Index, LILACS, OpenGrey, and a number of Russian Databases. We also searched reference lists, ongoing trials registers and conference proceedings, and contacted the manufacturer of cerebrolysin, EVER Neuro Pharma GmbH (formerly Ebewe Pharma).

Selection criteria

Randomised controlled trials (RCTs) comparing cerebrolysin, started within 48 hours of stroke onset and continued for any time, with placebo or no treatment in people with acute ischaemic stroke.

Data collection and analysis

Two review authors independently applied inclusion criteria, assessed trial quality and risk of bias, and extracted data.

Main results

We identified six RCTs (1501 participants) that met the inclusion criteria.

We evaluated risk of bias and judged it to be unclear for generation of allocation sequence in four studies and low in two studies; unclear for allocation concealment in five studies and low in one study; high for incomplete outcome data (attrition bias) in five studies and unclear in one study; unclear for blinding; high for selective reporting in four studies and unclear in two; and high for other sources of bias in three studies and unclear in the rest. The manufacturer of cerebrolysin, pharmaceutical company EVER Neuro Pharma,

supported three multi-centre studies, either totally, or providing cerebrolysin and placebo, randomisation codes, research grants, or statisticians.

None of the included trials reported on poor functional outcome defined as death or dependence at the end of the follow-up period or early death (within two weeks of stroke onset).

All-cause death: we extracted data from five trials (1417 participants). There was no difference in the number of deaths: 46/714 in cerebrolysin group versus 47/703 in placebo group; risk ratio (RR) 0.91 95% confidence interval (CI) 0.61 to 1.35 (5 trials, 1417 participants, moderate-quality evidence).

Serious adverse events (SAEs): there was no significant difference in the total number of SAEs with cerebrolysin (RR 1.16, 95% CI 0.81 to 1.67). This comprised no difference in fatal SAEs (RR 0.90, 95% CI 0.59 to 1.38) and an increase in the number of people with non-fatal SAEs (20/667 with cerebrolysin and 8/668 with placebo: RR 2.47, 95% CI 1.09 to 5.58, P = 0.03) (3 trials, 1335 participants, moderate-quality evidence).

Total number of people with adverse events: three trials reported on this. There was no difference in the total number of people with adverse events: 308/667 in cerebrolysin group versus 307/668 in placebo group; RR 0.97 95% CI 0.86 to 1.09, random-effects model (3 trials, 1335 participants, moderate-quality evidence).

Authors' conclusions

The findings of this Cochrane Review do not demonstrate clinical benefits of cerebrolysin for treating acute ischaemic stroke. We found moderate-quality evidence of an increase in non-fatal SAEs with cerebrolysin use but not in total SAEs.

PLAIN LANGUAGE SUMMARY

Cerebrolysin for acute ischaemic stroke

Review question

Are there any benefits of using cerebrolysin to treat people with acute ischaemic stroke, and are there any risks?

Background

Cerebrolysin, a mixture derived from pig brain tissue, is widely used in Russia, Eastern Europe, China, and other Asian and post-Soviet countries. We assessed evidence from randomised controlled trials (RCTs) investigating cerebrolysin in people with acute ischaemic stroke.

Study characteristics

This review included six RCTs with a total of 1501 participants that compared cerebrolysin with placebo (inactive medication) added to standard treatment of acute stroke, including thrombolysis. Three of them were large multicentre studies, two were small in size and were judged to be of unclear quality, and one did not include numerical results.

Key results

The evidence is current up to June 2016. This review of six trials involving 1501 participants showed no beneficial effect of cerebrolysin in terms of death in people with acute ischaemic stroke. There was no difference in the total number of people with adverse events but a concern that cerebrolysin may increase the risk of people having non-fatal serious adverse events compared with placebo.

Quality of the evidence

The medication and methodology of the majority of included trials were provided by the manufacturer of cerebrolysin creating a likely conflict of interest. There is moderate-quality evidence currently available that suggests cerebrolysin performs no better than placebo in terms of all-cause death when given to people with acute ischaemic stroke within 48 hours of stroke onset. There is moderate-quality evidence that raises concerns about the increase of serious adverse events with cerebrolysin use in people with acute ischaemic stroke. Further research is likely to have an important impact on our confidence in the estimate of cerebrolysin risks in contributing to serious adverse events in people with acute stroke.

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON [Explanation]

Cerebrolysin compared to placebo for acute ischaemic stroke

Patient or population: people with acute ischaemic stroke
Settings: inpatient health facilities in seven European countries: Austria, Croatia, the Czech Republic, Hungary, Russia, Slovakia, and Slovenia; and five Asian countries: China, Hong Kong, Iran, Myanmar, and South Korea
Intervention: cerebrolysin added to standard therapy (in most studies aspirin; in one study thrombolysis)
Comparison: placebo added to standard therapy (in most studies aspirin; in one study thrombolysis)

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Placebo	Cerebrolysin				
All-cause death - cerebrolysin dose 30 mL: 10 days; 10 mL: 10 days, 50 mL: 10 days, and cerebrolysin dose 50 mL: 21 days	**67 per 1000**	**61 per 1000** (41 to 90)	**RR 0.91** (0.61 to 1.35)	1417 (5)	⊕⊕⊕◯ Moderate[1,2,3,4]	
Total number of people with serious adverse events (SAEs) - cerebrolysin dose 30 mL: 10 days and cerebrolysin dose 50 mL: 21 days	**75 per 1000**	**87 per 1000** (61 to 125)	**RR 1.16** (0.81 to 1.67)	1335 (3)	⊕⊕⊕◯ Moderate[5,6,7,10]	
Total number of people with fatal SAEs - cerebrolysin dose 30 mL: 10 days and cerebrolysin dose 50 mL: 21 days	**63 per 1000**	**57 per 1000** (37 to 87)	**RR 0.90** (0.59 to 1.38)	1335 (3)	⊕⊕⊕◯ Moderate[5,6,8,10]	

Total number of people with non-fatal SAEs - cerebrolysin dose 30 mL: 10 days and cerebrolysin dose 50 mL: 21 days	**12 per 1000**	**30 per 1000** (13 to 67)	**RR 2.47** (1.09 to 5.58)	1335 (3)	⊕⊕⊕◯ Moderate[5,6,9,10]
Total number of people with adverse events - cerebrolysin dose 30 mL: 10 days and cerebrolysin dose 50 mL: 21 days	**460 per 1000**	**446 per 1000** (404 to 478)	**RR 0.97** (0.86 to 1.09)	1335 (3)	⊕⊕⊕◯ Moderate[11,12,13,14]

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **RR:** Risk ratio

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1]Downgraded by one for risk of bias. Five trials were considered at high risk of bias due to high levels of exclusions from the final analyses and one small trial was at unclear risk of bias. Trial authors did not report on time of death. Only two trials reported on causes of death. The manufacturer of cerebrolysin supported three studies, for other two trials we were unclear on manufacturer involvement. No conflict of interest statement provided in these three trials.
[2]No serious inconsistency. Five eligible trials contributed to the outcome All-cause death, we did not detect any heterogeneity.
[3]No serious imprecision. The five trials synthesised together with 1417 participants had enough power in total to detect difference, there was no difference: 46 deaths in cerebrolysin group (out of 714 randomised participants) and 47 deaths in placebo group (out of 703 randomised participants). Though the CIs were wide.
[4]No serious indirectness. The studies were conducted in seven European countries: Austria, Croatia, the Czech Republic, Hungary, Russia, Slovakia, and Slovenia; and five Asian countries: China, Hong Kong, Iran, Myanmar, and South Korea . The results may be generalisable to other populations and situations between 2003 and 2014.

[5]Downgraded by one for risk of bias. Three multicentre studies, which contributed to the outcomes 'Total number of people with serious adverse events (SAEs)', 'Total number of people with fatal SAEs', and 'Total number of people with non-fatal SAEs', were considered at high risk of bias due to high levels of exclusions from the final analyses. The manufacturer of cerebrolysin supported CASTA 2012, provided a statistician for CERE-LYSE-1 2012, and provided the study medication (cerebrolysin) and the placebo, as well as the randomisation codes (procedure) for Ladurner 2005.

[6]No serious inconsistency. Three eligible multicentre studies contributed to the outcomes 'Total number of people with serious adverse events (SAEs)', 'Total number of people with fatal SAEs', and 'Total number of people with non-fatal SAEs'. We detected no statistical heterogeneity for the outcomes 'Total number of people with serious adverse events (SAEs)' and 'Total number of people with fatal SAEs'; we detected a moderate level of heterogeneity for the outcome 'Total number of people with non-fatal SAEs'.

[7]No serious imprecision. The three multicentre studies synthesised together, with 1335 participants, had enough power to detect difference: 58 serious adverse events (SAEs) in the cerebrolysin group (667 randomised participants) and 50 SAEs in the placebo group (668 randomised participants); although the CIs for Ladurner 2005 were wide and the direction of the effect was opposite, but this did not result in statistical heterogeneity.

[8]No serious imprecision. The three multicentre studies synthesised together, with 1335 participants, had enough power to detect difference: 38 fatal SAEs in the cerebrolysin group (667 randomised participants) and 42 fatal SAEs in the placebo group (668 randomised participants); although the CIs of Ladurner 2005 were wide, there was no heterogeneity.

[9]No serious imprecision. The three multicentre studies synthesised together, with 1335 participants, had enough power to detect difference: 20 non-fatal SAEs in the cerebrolysin group (667 randomised participants) and 8 non-fatal SAEs in the placebo group (668 randomised participants); although the CIs of Ladurner 2005 were wide and the direction of the effect was opposite, contributing to moderate level of heterogeneity.

[10]No serious indirectness. These three multicentre studies were conducted in six European countries: Austria, Croatia, the Czech Republic, Hungary, Slovakia, and Slovenia; and four Asian countries: China, Hong Kong, Myanmar, and South Korea. The results may be generalisable to other populations and situations.

[11]Downgraded by one for risk of bias. Three trials were considered at high risk of bias due to high levels of exclusions from the final analyses. The manufacturer of cerebrolysin supported CASTA 2012, provided a statistician for CERE-LYSE-1 2012, and provided the drug and randomisation codes for Ladurner 2005 .

[12]No serious inconsistency. Three eligible trials contributed to the outcome 'Total number of people with adverse events', we detected a moderate level of heterogeneity.

[13]No serious imprecision. The three trials synthesised together with 1335 participants had enough power in total to detect difference. There was no difference: 308 people with adverse events in the cerebrolysin group (667 randomised participants) and 307 people with adverse events in the placebo group (668 randomised participants); although the CIs of Ladurner 2005 were wide and the direction of the effect was opposite, contributing to moderate level of heterogeneity.

[14]No serious indirectness. The studies were conducted in six European countries: Austria, Croatia, the Czech Republic, Hungary, Slovakia, and Slovenia; and four Asian countries: China, Hong Kong, Myanmar, and South Korea. The results may be generalisable to other populations and situations.

BACKGROUND

Effective, simple, and reliable treatment methods are urgently needed to reduce stroke mortality and disability. Many clinical trials and Cochrane Reviews have addressed the question of benefits and risks of potential pharmacological treatment options for acute ischaemic stroke. However, strategies with proven therapeutic effects and an acceptable benefit-to-risk ratio are still lacking. Potential strategies can be grouped according to the existing evidence of their benefits and harms determining their role in clinical practice.

Evidence of benefit

Aspirin at a dose of 160 mg to 300 mg daily (orally or per rectum), started within 48 hours of onset of presumed ischaemic stroke appears to be the only effective treatment for early secondary prevention, reducing the risk of early recurrent ischaemic stroke without a major risk of early haemorrhagic complications, and improving long-term outcomes (Sandercock 2014). Despite the positive overall conclusions of a Cochrane Review (Wardlaw 2014) and individual patient data meta-analysis (Emberson 2014) of thrombolysis in acute ischaemic stroke, there is still some debate regarding the optimal use of intravenous recombinant tissue plasminogen activators (rtPA) (Alper 2015). It is estimated that for each person with a good stroke outcome at six months, another person would have symptomatic intracranial bleeding, and for every three to four people without neurological deficits at six months, there is an excess of one death after thrombolysis (Appelros 2015; Brunström 2015). The evidence is inadequate to conclude whether lower doses of thrombolytic agents are more effective than higher doses, whether one agent is better than another, or which route of administration is the best for treatment of people who have had an acute ischaemic stroke (Wardlaw 2013).

Evidence of harm

Glycoprotein IIb-IIIa inhibitors (abciximab and tirofiban) increase the risk of intracranial haemorrhage without evidence of any reduction in death or disability in stroke survivors (Ciccone 2014). These data do not support their routine use in clinical practice. Abciximab contributed 89% of the total number of participants of the Cochrane Review (Ciccone 2014). Anticoagulants (standard unfractionated heparin, low-molecular-weight heparins, heparinoids, oral anticoagulants, and thrombin inhibitors) as immediate therapy for acute ischaemic stroke are not associated with net short- or long-term benefit. Reduced rate of recurrent stroke, deep vein thrombosis, and pulmonary embolism with anticoagulant therapy was offset by the increased risk of intracranial haemorrhage and extracranial bleeding. The data do not support the routine use of any of the currently available anticoagulants in acute ischaemic stroke (Berge 2002; Sandercock 2008a; Sandercock 2008b). Long-term anticoagulant therapy in people with presumed non-cardioembolic ischaemic stroke or transient ischaemic attack was not associated with any benefit, but there was a significant bleeding risk (Sandercock 2009).

Tirilazad, an amino steroid inhibitor of lipid peroxidation, increased the combined end-point of 'death or disability' in people with acute ischaemic stroke (TISC 2001). Lubeluzole, an ion channel modulator of glutamate release that has a benzothiazole structure with potential neuroprotective properties, did not reduce death or dependency in acute ischaemic stroke patients. In contrast, it increased heart-conduction disorders (Q-T prolongation) (Gandolfo 2002).

Evidence of lack of benefit

The evidence of the lack of benefit has accumulated for the following treatment options, which were tested in clinical trials and the results of which were systematically reviewed: corticosteroids (Sandercock 2011); calcium antagonists (Horn 2000); haemodilution (Chang 2014); excitatory amino acid antagonists, including ion channel modulators and N-methyl-D-aspartic acid (NMDA) antagonists (Muir 2003); piracetam (Ricci 2012a); and a free radical trapping agent NXY-059 (Shuaib 2007). There is no evidence that colloids lead to lower odds of death or dependence after stroke compared with crystalloids (Visvanathan 2015).

Role in clinical practice

There is still inadequate evidence from RCTs for the following antithrombotic agents: oral antiplatelet drugs other than aspirin (clopidogrel, ticlopidine, cilostazol, satigrel, sarpolgrelate, KBT 3022, iisbogrel) (Sandercock 2014); and fibrinogen-depleting agents (ancrod and defibrase) (Hao 2012).

The list of interventions of agents tested in clinical trials with subsequent Cochrane Reviews of results that documented inadequate evidence to establish a role in clinical practice includes: ginkgo biloba (Zeng 2005); gamma aminobutyric acid (GABA) receptor agonists (Liu 2016); percutaneous vascular interventions, including intra-arterial thrombolysis with urokinase and pro-urokinase (O'Rourke 2010); sonothrombolysis (Ricci 2012b); glycerol (Righetti 2004); mannitol (Bereczki 2007); naftidrofuryl, a 5-HT2 serotonergic antagonist (Leonardi-Bee 2007); theophylline or methylxanthine derivatives (Bath 2004a; Bath 2004b); nitric oxide donors (Bath 2002); blood pressure-altering interventions (BASC 2000; BASC 2001; Bath 2014); prostacyclin and its analogues (Bath 2004c); vinpocetine (Bereczki 2008); gangliosides (Candelise 2001); colony stimulating factors (Bath 2013); or stem cells (Boncoraglio 2010); Chinese herbal medicine Sanchi (Chen 2008), puerarin (Tan 2008), mailuoning (Yang 2009), tongxinluo capsules (Zhuo 2008); and the neuroprotective agent edaravone

(Feng 2011), which are widely used for ischaemic stroke in China. Cerebrolysin belongs to this category (Ziganshina 2015).

Description of the condition

Ischaemic stroke occurs when the brain loses its blood and energy supply, resulting in damage to brain tissue; it is a brain equivalent of a heart attack. Most strokes (87%) are ischaemic (AHA 2014). Worldwide every year 15 million people suffer a stroke: five and a half million people die and another five million are left permanently disabled, placing a burden on family and community (WHO 2014). Stroke is one of the major causes of disability and mortality (AHA 2014; Bonita 1992; WHO 2014). It is the third most common cause of death in the developed world after coronary disease and cancer. The World Health Organization (WHO) stroke statistics registered the number of deaths from stroke to be more than 200,000 in the Russian Federation, as well as in China and in India, with the highest number of 1,652,885 in China and 517,424 in Russia in 2002 (WHO 2014). According to the Russian data there are between 400,000 to 450,000 cases of acute stroke registered in the Russian Federation annually (Gusev 2003) with the incidence of 3.36 per 1000 population and standardised incidence of 2.39 (3.24 in men and 2.24 in women) per 1000 population (Gusev 2013). The case fatality rate of stroke is 40.37% (61.4% for haemorrhagic stroke and 21.8% for ischaemic stroke). The north-west regions had the highest stroke incidence of 7.43 per 1000, followed by some cities in middle areas of the country (5.37 per 1000) and the far east (4.41 per 1000) (Gusev 2003; Vilenskii 2006b). The stroke recurrence rate is 30% (Suslina 2009). Stroke survivors experience serious neurological disorders (loss of vision, speech or both; paralysis; and confusion) and these are not restored in 30% to 66% of cases six months after a stroke (French 2007). In Russia, stroke is the number one cause of disability in adults: 32 cases per 100,000 population. By the end of one year 25% to 30% of stroke survivors develop dementia. Stroke presents a huge financial burden for the health system (Martynchik 2013).

Description of the intervention

Cerebrolysin is a mixture of low-molecular-weight peptides and amino acids derived from pigs' brain tissue, which has potential neuroprotective and neurotrophic properties. Its manufacturer promotes it for multiple neurological conditions, and it is widely used in the treatment of acute ischaemic stroke in Russia, China, and other Asian and post-Soviet countries.

How the intervention might work

The term 'neuroprotection' is used to describe the putative effect of interventions protecting the brain from pathological damage. In ischaemic stroke the concept of neuroprotection includes inhibition of pathological molecular events leading to calcium influx, activation of free radical reactions and cell death. Knowledge of pathophysiology in acute ischaemic stroke stimulated development of a number of potential neuroprotective agents. Many neuroprotective agents have proven to be efficacious in animal studies. Cerebrolysin is a mixture of low-molecular-weight peptides (80%) and free amino acids (20%) derived from pig brain tissue, with proposed neuroprotective and neurotrophic properties similar to naturally occurring growth factors (nerve growth factor, brain-derived neurotrophic factor) (Alvarez 2000; Fragoso 2002).

Results of in vitro and animal studies of cerebrolysin have been traditionally used to suggest its potential for treating acute ischaemic neuronal damage (Masliah 2012). For example, cerebrolysin was shown to be effective in tissue culture models of neuronal ischaemia dose-dependently increasing neuronal survival (Schauer 2006). In brain slices it counteracted necrotic and apoptotic cell death induced by glutamate (Riley 2006). Cerebrolysin also demonstrated neuroprotective activity in a rat model of haemorrhagic stroke (Makarenko 2005) and ischaemic stroke (Zhang 2010), as well as spinal cord trauma (Sapronov 2005). One randomised double blind placebo-controlled trial showed no effect of cerebrolysin in acute haemorrhagic stroke on chosen efficacy measures (Barthel Index (BI), Unified Neurological Stroke Scale, and Syndrome Short Test (SST)) (Bajenaru 2010).

Why it is important to do this review

Despite the effectiveness of neuroprotective agents in animal models of stroke, clinical trials of neuroprotective agents in humans have provided disappointing results (European Ad Hoc Consensus 1998). More recent Cochrane Reviews of the effects of individual neuroprotective agents and pharmacological groups confirmed this (Gandolfo 2002; Muir 2003; Ricci 2012a; TISC 2001). Other means of neuroprotection are being sought. Cerebrolysin is well accepted by Russian and Asian physicians. It is widely used in the treatment of acute ischaemic stroke and other neurological disorders (Chukanova 2005; Gromova 2006; Onishchenko 2006). Research data from observational studies and clinical trials of cerebrolysin in acute stroke or head injury, with most performed in Russia and China, have accumulated (Chukanova 2005; Gafurov 2004; Gromova 2006; Ladurner 2005; Skvortsova 2004; Wong 2005). We carried out a Cochrane Systematic Review, which did not find sufficient evidence to support cerebrolysin use in practice (Ziganshina 2010a).

Cerebrolysin, as assessed in a Cochrane Systematic Review for vascular dementia, may have positive effects on cognitive function and global function in elderly people with mild to moderate dementia, but the review authors do not recommend it for routine use in vascular dementia due to the limitations of the studies and the resulting review: small number of included trials, wide variety of treatment durations, and short-term follow-up (Chen 2013b).

Cerebrolysin has also been proposed for treatment of people with Alzheimer's disease (Fragoso 2002). Trials of cerebrolysin in acute haemorrhagic stroke have been assessed in a meta-analysis (Shu 2012), concluding on its safety and supporting implementation of new trials for definitive efficacy assessment.

The previous versions of this Cochrane Review, based on one eligible trial only, did not find evidence of cerebrolysin benefit in acute ischaemic stroke (Ziganshina 2010a; Ziganshina 2015). More research data from clinical trials of cerebrolysin in acute ischaemic stroke have accumulated with cerebrolysin used for varying periods of time, majority of them for less than 14 days, as specified by the protocol of the review, and at various doses (10 mL, 30 mL, and 50 mL). We decided to use a more inclusive approach and to refine our inclusion criteria to allow inclusion of the accumulated research data.

The aim of this Cochrane Review update is to establish whether the available evidence from controlled trials indicates if cerebrolysin is beneficial and safe for the treatment of acute ischaemic stroke.

OBJECTIVES

To assess the benefits and risks of cerebrolysin for treating acute ischaemic stroke.

METHODS

Criteria for considering studies for this review

Types of studies

We included all published randomised controlled trials (RCTs), comparing cerebrolysin with placebo or no treatment in people with acute ischaemic stroke. We excluded uncontrolled studies, as well as quasi-RCTs where allocation to treatment or control was not concealed (e.g. allocation by alteration, open random number list, date of birth, day of the week, or hospital number).

Types of participants

People with acute ischaemic stroke, irrespective of age, gender, or social status, whose symptom onset was less than 48 hours previously. Stroke symptoms include: sudden weakness or numbness of the face, arm, or leg, often unilateral; confusion; difficulties in speaking or seeing with one or both eyes; difficulties walking; loss of balance or co-ordination; severe no-cause headache; fainting or loss of consciousness. Stroke diagnosis confirmation with neuroimaging was not a required eligibility criterion.

Types of interventions

We compared cerebrolysin with placebo or no treatment added to standard treatment versus standard treatment alone. Standard treatment is not defined precisely and differs between studies. Study medication must have been started within 48 hours of stroke onset and continued for any period of time. We added a separate analysis for the comparison: cerebrolysin versus other neuroprotective agents. We planned to combine data for cerebrolysin with data for newer peptide-mixtures, which we have named 'cerebrolysin-like agents'.

Types of outcome measures

Primary outcomes

1. All-cause death

Secondary outcomes

1. Poor functional outcome defined as death or dependence at the end of the follow-up period
2. Early death (within two weeks of stroke onset)
3. Quality of life, if assessed in the included studies
4. Time to restoration of capacity for work

Adverse events and effects

1. A serious adverse event (SAEs), as defined according to the International Council for Harmonisation (ICH) guideline, is "any untoward medical occurrence that, at any dose, results in death, is life-threatening, requires inpatient hospitalisation or results in prolongation of existing hospitalisation, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect, or is a medically important event or reaction." (ICH 2003). We confirmed the definition of SAEs used by researchers and the numbers of people with SAEs in the CASTA 2012 trial though correspondence with the manufacturer of cerebrolysin and the lead author of this trial (Professor WD Heiss); and extracted data from the CERE-LYSE-1 2012 trial report that used MedDRAcoded SOC (System Organ Class) and Preferred Term (PT) (MedDRA 2011), developed under the auspices of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH 2003). We used the following outcomes for SAEs.
    i) Total number of people with SAEs
    ii) Total number of people with fatal SAEs
    iii) Total number of people with non-fatal SAEs
2. Adverse effects specifically associated with cerebrolysin, such as hypersensitivity reactions
3. Total number of people with adverse events

Search methods for identification of studies

See the 'Specialized register' section in the Cochrane Stroke Group module. We attempted to identify all relevant trials regardless of language or publication status, and arranged translation of relevant papers where necessary.

Electronic searches

We searched the Cochrane Stroke Group Trials Register (May 2016); the Cochrane Central Register of Controlled Trials (CENTRAL) (the Cochrane Library 2016, Issue 5: searched May 2016; Appendix 1); MEDLINE (1966 to May 2016; Appendix 2); Embase (1974 to May 2016; Appendix 3); Web of Science Core Collection, which includes Science Citation Index (1940 to May 2016; Appendix 4); LILACS (Latin American and Caribbean Health Sciences Literature) (1982 to May 2016; Appendix 5); OpenGrey (System for Information on Grey Literature in Europe; http://www.opengrey.eu; 1980 to May 2016; Appendix 6); and the following Russian Databases: e-library (http://elibrary.ru; 1998 to May 2016); and EastView (http://online.ebiblioteka.ru/index.jsp; 2006 to May 2016; Appendix 7).

We also searched the following ongoing trials and research registers (May 2016): the Stroke Trials Registry (www.strokecenter.org/trials/), ClinicalTrials.gov (clinicaltrials.gov/), World Health Organization (WHO) International Clinical Trials Registry Platform Search Portal (apps.who.int/trialsearch/), and ISRCTN Registry (isrctn.com).

The Cochrane Stroke Group Information Specialist developed the search strategies for CENTRAL, MEDLINE, Embase, and Web of Science and we adapted the MEDLINE strategy for the other databases.

Searching other resources

In an effort to identify further published, unpublished, and ongoing trials and obtain additional trial information we:

1. checked the reference lists of all trials identified by the above methods;
2. searched the following neurology conference proceedings held in Russia: Chelovek i Lekarstvo (2006 to 2016), National'niy congress cardiologov (2006 to 2016), Rossiyskiy Mezhdunarodniy Congress Cerebrovascularnaya patologiya i insult (2008 to 2016);
3. contacted the manufacturer of cerebrolysin, pharmaceutical company EVER Neuro Pharma GmbH (July 2016).

Data collection and analysis

Selection of studies

Two review authors (LEZ and LV) independently examined titles and abstracts of records from the electronic searches and excluded obviously irrelevant studies. We used Covidence software (www.covidence.org/) allowing us to quickly detect and solve conflicts between the reviewers. We obtained the full text of the remaining papers and the same two review authors independently selected studies for inclusion based on the pre-determined inclusion criteria refined for this update. We resolved disagreements through discussion. We excluded studies that did not meet the inclusion criteria and gave the reasons for exclusion in the Characteristics of excluded studies table, generated by Covidence.

Data extraction and management

Two review authors (LEZ and LV) independently extracted data using Covidence. We extracted data on the methods of the studies, participants, interventions, and outcomes. We resolved any differences in the extracted data by referring to the original articles and through discussion. We extracted data to allow an intention-to-treat (ITT) analysis (including all the participants in the groups to which they were originally randomly allocated) and we presented the data in the Characteristics of included studies table, generated by Covidence. We calculated the percentage loss to follow-up and presented it in the 'Risk of bias' tables.

For binary outcomes, we extracted the number of participants with the event in each group. For continuous outcomes, we planned to use arithmetic means and standard deviations for each group.

Assessment of risk of bias in included studies

We (LEZ and LV) independently evaluated methodological quality in terms of generation of allocation sequence, allocation concealment, blinding, loss to follow-up of participants, and other risks of bias using the Cochrane 'Risk of bias' assessment tool (Higgins 2011) in Covidence.

We followed the guidance to assess whether adequate steps had been taken to reduce the risk of bias across seven domains: generation of allocation sequence; allocation concealment; blinding of participants and personnel; blinding of outcome assessors; incomplete outcome data (attrition bias); selective outcome reporting; and other sources of bias. We have categorised these judgments as 'low', 'high', or 'unclear' risk of bias. We considered loss to follow-up to be acceptable (low risk of bias) if it was less than 10%.

For the assessment of other sources of bias we looked at the way the study authors described funding sources for their trials and, if described at all, how they presented their conflict of interest statements. We judged the risk of bias to be high in cases of clear cerebrolysin manufacturer sponsorship, involvement of the manufacturer with trial planning and design, sequence generation, medication provision, statistical procedures, blinding of personnel and outcome assessors, and involvement in reporting, as well as in cases of declared relationship of study authors with the manufacturer of cerebrolysin. Where there was no mention of the funding sources

and no conflict of interest statements we judged the risk of bias to be unclear.
We resolved any disagreements arising at any stage by discussion.

Measures of treatment effect

For binary outcomes we extracted the number of participants with the event in each group; for continuous outcomes we planned to use arithmetic means and standard deviations for each group. We presented dichotomous data and we combined them using risk ratios (RRs). We showed RRs accompanied by 95% confidence intervals (CIs).

Unit of analysis issues

We only included studies that randomised individual participants.

Dealing with missing data

We undertook analysis according to the ITT principle: we used the number of initially randomised participants as a denominator. We extracted the total numbers of people who died or had serious adverse events and we used them as numerators. We used the data on the number of deaths in both comparison groups to generate the primary outcome of all-cause death and we used the number of people initially randomised into each comparison group as the denominator. This approach assumes that all missing participants had a positive outcome (did not die, did not experience an adverse event) and in this sense represents the best-case scenario.

Assessment of heterogeneity

We tested for homogeneity or heterogeneity of effect sizes between studies by inspecting the forest plots, and using the I^2 statistic (Higgins 2003), with a value of 30% to 60% used to denote moderate levels of heterogeneity (Deeks 2011).

Assessment of reporting biases

If there were a sufficient number of studies, we planned to use funnel plots to examine asymmetry that may have been caused by publication bias or heterogeneity.

Data synthesis

We undertook analysis according to the ITT principle. We used Review Manager to analyse the data (RevMan 2014). We used RR as a measure of effect for binary outcomes and used a fixed-effect model for pooling the data in cases of no heterogeneity, or a low level of heterogeneity.
Where we detected heterogeneity (forest plot inspection and I^2 statistic > 30%) and it was still appropriate to pool the data, we used the random-effects model. We used it for the outcome 'total number of people with non-fatal serious adverse events'. For other outcomes we used a fixed-effect model of pooling data.

We used and presented 95% confidence intervals (CIs) for risk ratios (RRs) of all studied outcomes.

Subgroup analysis and investigation of heterogeneity

We investigated potential sources of heterogeneity using the following subgroups.

1. Cerebrolysin dose
2. Length of treatment

Sensitivity analysis

We performed a sensitivity analysis to test the robustness of our results by investigating the impact of methodological study quality on the results: high risk of bias versus unclear risk. We examined the resulting forest plot for direction of cerebrolysin effect, for the effects sizes in studies with high risk of bias versus unclear risk of bias.

Summarising and interpreting results

We used the GRADE approach to interpret findings (Schünemann 2011). We used GRADE Profiler Software (GRADEPro GDT 2015), and imported data from RevMan 2014, to create 'Summary of findings for the main comparison' for the primary outcome: all-cause death; and for adverse events: total number of people with serious adverse events (SAEs), total number of people with fatal SAEs, total number of people with non-fatal SAEs, and the total number of people with adverse events.

Summary of findings for the main comparison includes information on overall quality of the evidence from the trials and information of importance for healthcare decision making. The GRADE approach determines the quality of evidence on the basis of an evaluation of eight criteria (risk of bias, inconsistency, indirectness, imprecision, publication bias, effect size, presence of plausible confounding that will change effect, and dose-response gradient). We used the criteria of risk of bias, inconsistency, indirectness, and imprecision to guide our conclusions and recommendations.

RESULTS

Description of studies

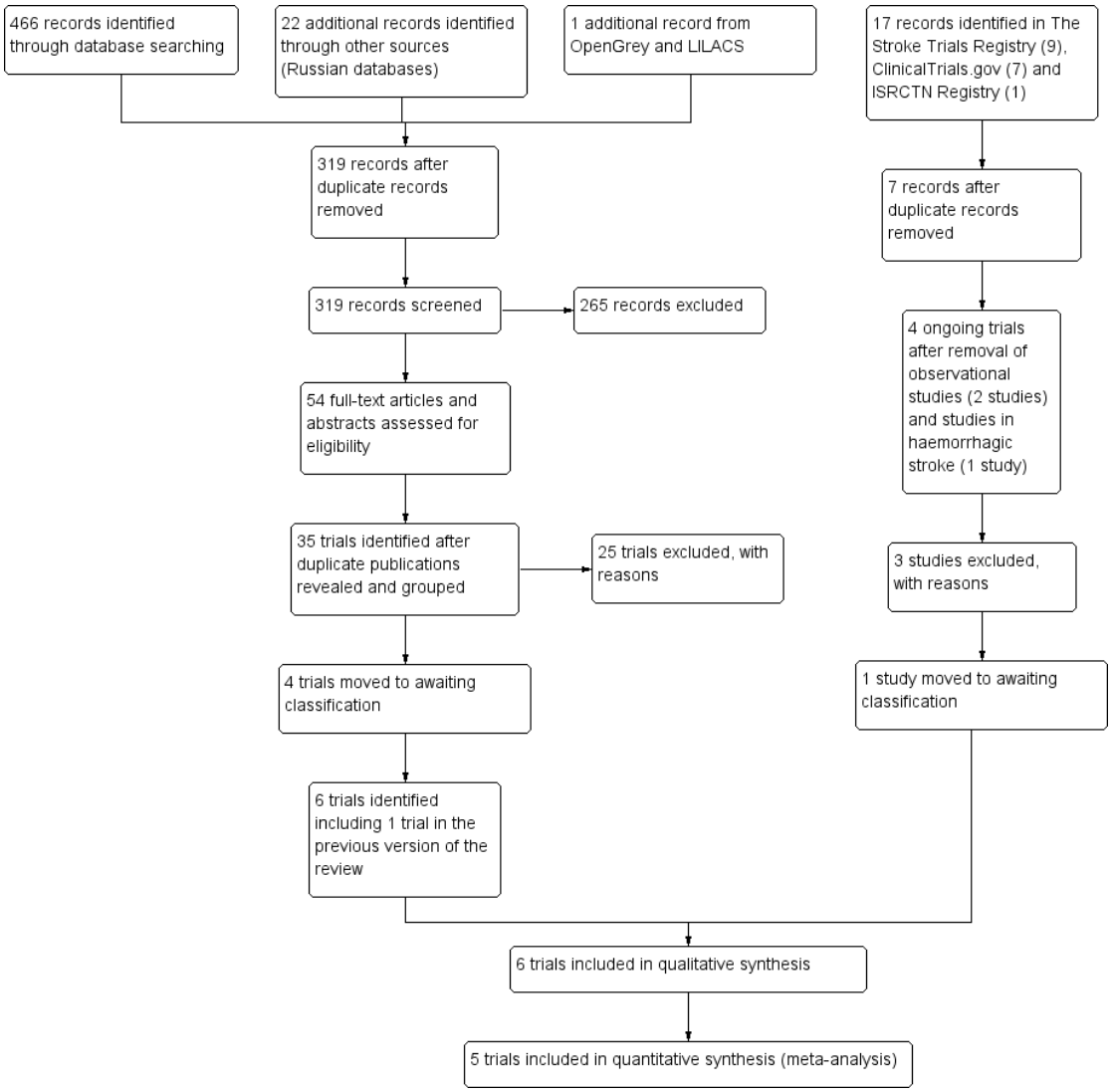
Results of the search

We identified 466 records through database searches and 22 additional records from other sources (Russian databases, Open Grey and LILACS). After we removed duplicates, 319 records remained, which we screened and excluded 265 records. We retrieved 54

full-text articles and abstracts. After controlling for multiple publications of the same trial we identified 35 trials and assessed them for eligibility as per protocol with refined inclusion criteria. We excluded 25 studies with reasons (see 'Characteristics of excluded studies'), moved four trials to awaiting classification (see 'Characteristics of studies awaiting classification') and identified six eligible trials. One was the same RCT included in the previous versions of this review (Ziganshina 2010a; Ziganshina 2015) and five new trials. We present details of these six trials in 'Characteristics of included studies'.

We identified 17 records in clinical trials registries, controlled for multiple records and identified seven studies. We removed three studies as irrelevant, excluded three trials with reasons (see 'Characteristics of excluded studies') and one trials is awaiting classification (see 'Characteristics of studies awaiting classification'). We illustrated these results in the study flow diagram (Figure 1).

Figure 1. Study flow diagram



Included studies

Six trials met the inclusion criteria.

Amiri Nikpour 2014 was performed in Iran. The trial compared cerebrolysin with placebo (normal saline) in 46 people (23 participants in each group) with acute ischaemic stroke confirmed by computed tomography (CT) scan or magnetic resonance imaging (MRI), or both. Cerebrolysin was started within 24 hours of stroke onset and continued for 10 days as a once-daily intravenous infusion of 30 mL in addition to standard treatment of 100 mg of aspirin daily. The average age of the trial participants was 60 years. There were no significant differences between the two groups in terms of baseline characteristics. The duration of follow-up was 90 days; one participant in the cerebrolysin group and two participants in the placebo group died within 30 days of trial initiation.

CASTA 2012 was a multicentre placebo-controlled trial performed in four countries: China, Hong Kong, South Korea, and Myanmar. The trial compared cerebrolysin with placebo added to standard base-line therapy in 1070 people with acute ischaemic stroke with CT or MRI results compatible with a clinical diagnosis of acute hemispheric stroke (529 participants in the cerebrolysin group and 541 participants in the control group). Cerebrolysin was started within 12 hours of stroke onset and continued for 10 days as a once-daily intravenous infusion of 30 mL diluted in saline (total of 100 mL) in addition to standard treatment of 100 mg of aspirin daily. Placebo was 100 mL saline as daily intravenous infusion for 10 days starting within 12 hours of stroke onset. The average age of the trial participants was 65 years. The duration of follow-up was 90 days; 180 participants were lost to follow-up (16.8%). There were differences between the two groups in terms of baseline prognostic variables: more people with chronic diseases were in the placebo group: 293 versus 251 (55% versus 46% of randomised participants): more people with diabetes (117 (21.7%) versus 108 (20.5%)), arrhythmia (90 (16.7%) versus 71 (13.5%)) and coronary heart disease (86 (16.0%) versus 72 (13.7%)) were in the placebo group compared with the cerebrolysin group. The trial was supported by the manufacturer of cerebrolysin, EVER Neuro Pharma GmbH.

CERE-LYSE-1 2012 was also a multicentre placebo-controlled trial performed in five countries: Austria, Croatia, Czech Republic, Slovakia, and Slovenia. The trial compared cerebrolysin with placebo in 119 people (60 in the cerebrolysin group and 59 participants in the control group) with acute hemispheric ischaemic stroke after exclusion of brain haemorrhage by CT. Cerebrolysin was started within two hours of stroke onset and continued for 10 consecutive days as a once-daily intravenous infusion of 30 mL mixed with 70 mL of normal saline (total volume 100 mL over a time period of 30 minutes), starting immediately one hour after thrombolytic treatment (alteplase). The placebo consisted of 100 mL normal saline. The average age of the trial participants was 66 years; there were no significant differences between treatment groups in terms of baseline prognostic variables. The duration of follow-up was 90 days; 19 participants of 119 (16%) were lost to follow-up. The authors did not report any information on funding sources of the trial, including provision of cerebrolysin. The statistician of the study was contracted by EVER Neuro Pharma GmbH - the manufacturer of cerebrolysin.

Ladurner 2005 was a multicentre placebo-controlled trial conducted in Austria, the Czech Republic and Hungary. The trial compared cerebrolysin with placebo (100 mL normal saline) added to standard baseline therapy in 146 people with acute ischaemic stroke with clinical symptoms of the middle cerebral artery area. Cerebrolysin (50 mL mixed with 50 mL of normal saline) and placebo were started within 24 hours of stroke onset and continued for 21 days as a once-daily intravenous infusion over a period of 20 minutes. The same basic therapy was used in the treatment group and the control group (pentoxifylline and acetylsalicylic acid): cerebrolysin plus basic therapy; 78 participants; and placebo plus basic therapy; 68 participants. The average age of the trial participants was 65 years. The duration of follow-up was 90 days; 25 participants (17%) were lost to follow-up, nine of whom were in the treatment group and the remaining 16 were in the control group. There were no significant differences between the two groups in terms of baseline characteristics. The trial was supported by the manufacturer of cerebrolysin, EVER Neuro Pharma GmbH, who also provided the study centres with cerebrolysin.

Skvortsova 2004 was performed in Russia. The trial compared cerebrolysin with placebo added to standard baseline therapy in 36 people with acute ischaemic stroke in the basin of the internal carotid artery, confirmed by CT or MRI. Cerebrolysin was started within 12 hours of stroke onset and continued for 10 days as a once-daily intravenous infusion of either 10 mL or 50 mL. There were three groups, 12 participants in each, treated with: 10 mL cerebrolysin, 50 mL cerebrolysin, or placebo. Standard baseline therapy consisted of aspirin 100 mg a day, haemodilution, pentoxifylline, and heparin (when needed). There were no significant differences between the groups in terms of baseline characteristics. The average age of the trial participants was 69 years; the duration of follow-up was 30 days; there were no losses to follow-up. No information on funding sources for the trial and no conflict of interest statement was provided.

Xue 2016 was performed in China. The trial compared cerebrolysin with placebo and another neuroprotective agent (DL-3-n-butylphthalide; NBP) in 60 people with acute ischaemic stroke, confirmed by CT or MRI (20 participants each). There were no significant differences between the two groups in terms of baseline characteristics. Cerebrolysin was administrated for 10 days as a once-daily intravenous infusion of 30 mL mixed with 70 mL of normal saline, the infusions lasted over 50 to 70 minutes. Participants of the control group received intravenous infusions of 100 mL of normal saline, the other study group received an intravenous infusion of 100 mL of 25 mg NBP in normal saline, twice daily for 10 days starting within 12 hours after stroke onset. Standard baseline therapy consisted of antithrombotics, hypoglycemics, an-

tilipaemic agents, antihypertensives, and dehydration, according to local current guidelines for the management of ischaemic stroke in neurological intensive care units; and 100 mg aspirin orally. The duration of follow-up was 90 days.

We have presented details of the included trials in the 'Characteristics of included studies' table.

There are no trials awaiting assessment.

Excluded studies

We excluded 25 studies because of:

1. wrong study design, including lack of randomisation or control arm;
2. wrong patient population, including participants with treatment initiation exceeding the protocol-specified 48 hours after stroke onset;
3. wrong research question: research questions not relevant, such as effects of cerebrolysin on stroke volume.

We have presented the reasons for excluding these studies in the 'Characteristics of excluded studies' table.

Risk of bias in included studies

Six RCTs met the inclusion criteria.

Allocation

For sequence generation we judged four trials to be at unclear risk of bias because the authors did not provide any information on sequence generation (Amiri Nikpour 2014; CASTA 2012; CERE-LYSE-1 2012; Skvortsova 2004). We carefully reviewed the published protocol of the CASTA 2012 study, which was published as Hong 2009 and we remain unclear whether the described procedure for sequence generation was indeed used when performing the trial.

We judged Xue 2016 at low risk of bias since the sequence generation was performed with computer-generated numbers by a third party. Though it was unclear who the third party was, we decided that we would consider this lack of clarity in the domain of 'other sources of bias'.

In Ladurner 2005 the manufacturer of cerebrolysin, EVER Neuro Pharma GmbH, provided the randomisation method: a computer-generated randomisation code. We acknowledge that a computer-generated randomisation code is normally considered to have a low risk of bias.

For allocation concealment we judged that all the included trials were at unclear risk of bias since the authors did not provide any relevant information, except for Ladurner 2005, where the trial authors used sealed envelopes with information on the actual treatment dispensed, and provided these envelopes to the investigator for emergency cases. The published report described that all envelopes remained sealed throughout the study. Although the trial authors did not describe the envelopes as opaque, we judged the allocation concealment to be at low risk of bias.

Blinding

None of the included trials provided clear information on blinding either of outcome assessors, or participants and personnel, or blinding by outcome. The trials authors consistently reported that investigators and all study personnel were blinded across all six included trials. However, it was impossible to assess blinding by outcome in any of the trials.

Incomplete outcome data

We judged that all included trials, except for Skvortsova 2004 (no loss to follow-up), were at high risk of attrition bias because in CASTA 2012 180/1070 randomised participants were lost to follow-up (16.8%); in Ladurner 2005 25/146 (17%) randomised participants were lost to follow-up; in CERE-LYSE-1 2012 19/119 randomised participants (16%) were lost to follow up; in Xue 2016 24/84 randomised participants (29%) were lost in the trial report. Amiri Nikpour 2014 did not report on adverse events at all, although the study authors informed the readers that one participant in the cerebrolysin group and two participants in the placebo group died before day 30 and were excluded from the final analysis. We judged this to be the source for a high risk of attrition bias.

Selective reporting

We judged the risk of bias for selective outcome reporting to be high for four studies and unclear for two studies (CASTA 2012; Skvortsova 2004).

The study protocol for CASTA 2012 was available and all of the pre-specified (primary and secondary) outcomes, which are of interest to the review, were reported accordingly. However the study authors did not describe the causes of deaths, and the Kaplan-Meier mortality curve presented only the subgroup of trial participants with an NIH score greater than 12.

Skvortsova 2004 described the causes of deaths (pulmonary embolism, pneumonia, and pyelonephritis and brain stem syndrome secondary to the brain oedema), but without a precise indication of the time when the deaths occurred and without a clear indication to which study group the participants belonged, nor the confirmed cause of death. The study authors did not report on adverse events. The timing of the outcomes presented in a table and a graph in the publication was also unclear.

Amiri Nikpour 2014 did not describe the causes of death, and provided no information on clinically relevant outcomes.

Ladurner 2005 did not report on the time when the deaths occurred, and did not assess potential causality with administered medicines. We compared, using the ITT principle, the number

of deaths extracted from the safety section of the trial report and presented data as all-cause death.

CERE-LYSE-1 2012 was stopped because no significant result for the main study outcome criteria was reached. According to the study authors, there was no causal relationship to the study drug for any of the deaths observed. Neither the reasons for the deaths, nor the timing of the deaths were presented; the timing of adverse events and serious adverse events were also not presented.

Xue 2016, which was the only study that compared cerebrolysin and another neuroprotective agent (NBP), had a questionable design with 84 participants at the trial initiation. However, the authors presented data for 60 participants (20, 20 and 20 people in each comparison group) only without any explanation for the loss of 24 participants. We could not include any data in the quantitative synthesis.

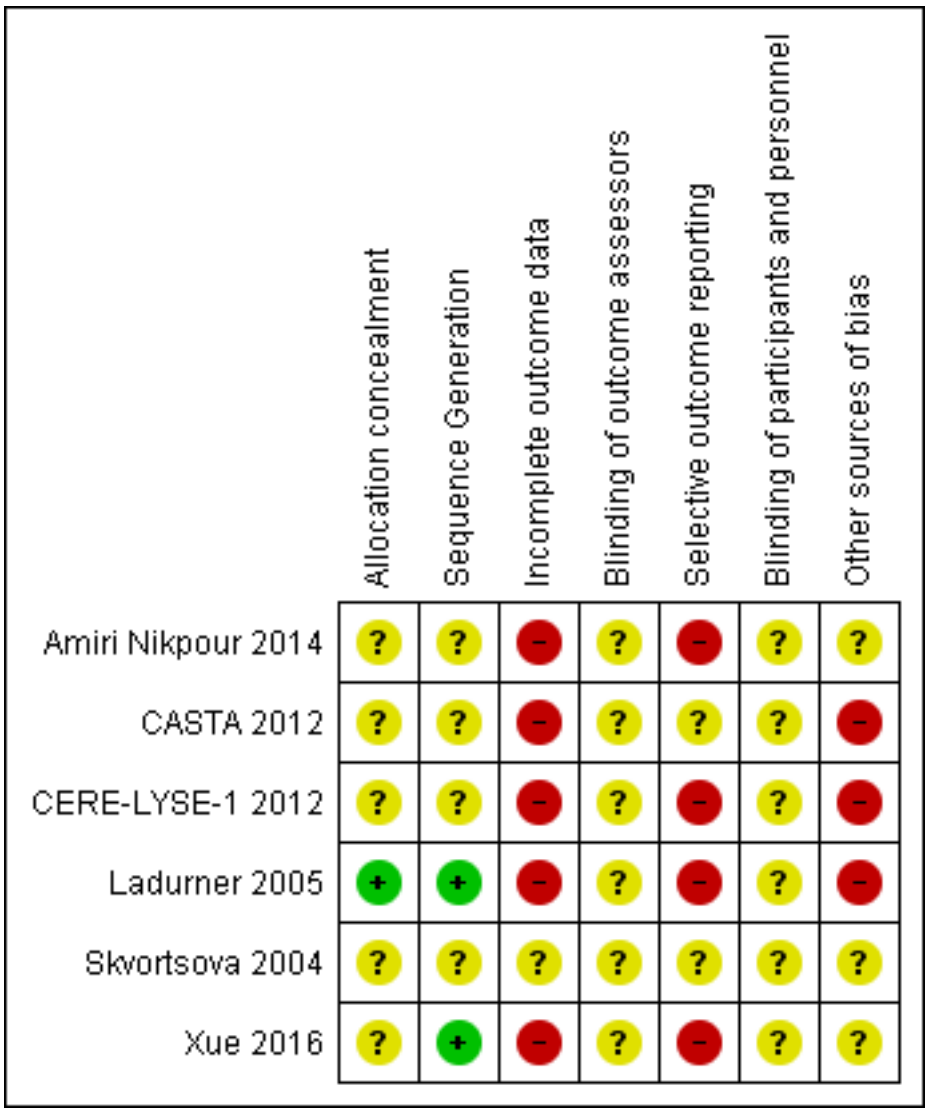
We judged all these to indicate a high risk of selective reporting bias.

Other potential sources of bias

CASTA 2012 and CERE-LYSE-1 2012 were totally dependent on pharmaceutical company EVER Neuro Pharma, which provided not only cerebrolysin, but for CERE-LYSE-1 2012 also the study statistician and research grants. For Ladurner 2005 the manufacturer of cerebrolysin, EVER Neuro Pharma, provided the study medication cerebrolysin and the placebo, as well as the randomisation codes (procedure). We judged this to be a source of high risk of bias. Further involvement of the pharmaceutical company in the trial design, the execution of the trial, or in the analyses was not described in the published trial report. The trial authors did not provide any information on funding sources for the trial itself, report drafting, nor a conflict of interest statement. We judged these facts to be sources of high risk of bias in these three studies. The authors of Amiri Nikpour 2014, Skvortsova 2004 and Xue 2016 did not present a conflict of interest statement nor clear information on funding sources of their trials in their published reports, which we judged as unclear risk of bias.

We illustrated these judgements in the 'Risk of bias' summary plot (Figure 2).

Figure 2. Risk of bias summary: review authors' judgements about each risk of bias item for each included study



Effects of interventions

See: **Summary of findings for the main comparison** Cerebrolysin compared to placebo for acute ischaemic stroke

None of the included trials reported on the secondary outcomes: poor functional outcome (defined as death or dependence at the end of the follow-up period), early death (within two weeks of stroke onset), quality of life, or the time to restoration of capacity for work.

The studies did report on the numbers of deaths in various sections of their trial reports, including description of adverse events. We used these data on the number of deaths in the comparison groups to generate the primary outcome of all-cause death.

All-cause death

We found no difference in all-cause death between the cerebrolysin and placebo groups: 46 deaths in the 714 cerebrolysin-treated participants and 47 deaths in the 703 placebo-treated participants; RR 0.91, 95% CI 0.61 to 1.35 (5 trials 1417 participants). The test for heterogeneity revealed no heterogeneity: I^2 = 0% (Analysis

1.1).

Causes of death in the included trials

In Amiri Nikpour 2014, the study authors did not describe causes of death, except for mentioning that one participant in the cerebrolysin group and two participants in the placebo group died before day 30; the authors excluded these three participants from their final analysis: we used these data for the all-cause death assessment.

In CASTA 2012, 28/529 randomised participants died in the cerebrolysin group, and 32/541 randomised to placebo. The study authors did not describe either the causes of deaths or the time when the deaths occurred.

In Ladurner 2005 6/78 participants died in the cerebrolysin group and 6/68 participants died in the placebo group. The study authors reported on the following causes of death: cerebral infarct (four in the cerebrolysin group and two in the placebo group), heart failure (two in the cerebrolysin group and one in the placebo group), pulmonary embolism (two in the placebo group), and pneumonia (one in the placebo group). The trial authors did not report on the time when those deaths occurred.

In CERE-LYSE-1 2012 four participants died in each group: 4/60 in the cerebrolysin group and 4/59 in the placebo group. The study authors did not describe either the cause, or the time when the deaths occurred, and did not find any relationship in any of the cases to the study medication.

The authors of Skvortsova 2004 described the causes of deaths. These were the causes which the authors did not attribute to the stroke: pulmonary embolism, pneumonia, and pyelonephritis in three participants in the cerebrolysin group and one in the placebo group (not clear which of these), and the cause of death associated with the strokes: brain oedema with secondary brain-stem syndrome, which occurred in two participants in both the cerebrolysin and placebo groups. The deaths occurred within 30 days after the stroke onset; the study authors did not report precisely on the time of each death. It was not possible to know to which cerebrolysin subgroup by dose these participants belonged, 10 mL or 50 mL.

In Xue 2016 one death occurred in the DL-3-n-butylphthalide (NBP) group.

Adverse events and effects

Serious adverse events

There was no difference in the total number of people with SAEs: RR1.16, 95% CI 0.81 to 1.67 (Analysis 1.2), as there was no difference in the total number of people with fatal SAEs (people who died): RR 0.90, 95% CI 0.59 to 1.38 (Analysis 1.3). However we found a greater than two-fold increase in the number of people with non-fatal SAEs receiving cerebrolysin treatment: 20/667 participants randomised to cerebrolysin and 8/668 participants randomised to placebo: RR 2.47, 95% CI 1.09 to 5.58, P = 0.03, no heterogeneity, I^2 = 0%; with test for subgroup differences between two cerebrolysin dosing regimens (30 mL for 10 days versus 50 mL for 21 days): Chi^2 = 1.85, df = 1 (P = 0.17), I^2 = 46.0% (Analysis 1.4).

The study authors did not describe the nature of adverse events in CASTA 2012. In CERE-LYSE-1 2012 the study authors described the serious adverse events. For the cerebrolysin-treated participants these included: acute coronary syndrome, atrial fibrillation, cardiac failure, gastric ulcer, pneumonia (three cases), rectal cancer, coma, pleural effusion, aspiration pneumonia (two cases), cerebral haematoma, and pulmonary embolism. For the placebo-treated participants adverse events included: cardiac arrest, cardiac failure, hepatic cirrhosis, infective arthritis, pneumonia, sepsis, renal failure, respiratory failure, cerebral haemorrhage and haemorrhagic stroke (one case each).

The Ladurner 2005 study authors reported only one serious non-fatal adverse event in the placebo group: haematemesis.

Total number of people with adverse events

We found information on this outcome in three included studies (CASTA 2012; CERE-LYSE-1 2012; Ladurner 2005). The synthesis of the data from these studies revealed no difference between cerebrolysin and placebo: RR 0.97, 95% CI 0.86 to 1.09 (Analysis 1.5).

In CASTA 2012 the study authors reported that 242/529 participants in the cerebrolysin group and 243/541 participants in the placebo group experienced adverse events: RR 1.02, 95% CI 0.89 to 1.16.

CERE-LYSE-1 2012 described the overall evaluation of safety, stating that 88% of cerebrolysin-treated participants and 97% of the placebo participants reported at least one adverse event. We recalculated from this for the outcome total number of people with adverse events: 53/60 participants in the cerebrolysin group and 57/59 participants in the placebo group: RR 0.91, 95% CI 0.82 to 1.01.

In Ladurner 2005 the study authors reported the overall incidence of adverse events: 16.4% in the cerebrolysin group and 10.3% in the placebo group. We recalculated from this for the outcome total number of people with adverse events: 13/78 participants in the cerebrolysin group and 7/68 participants in the placebo group: RR 1.62, 95% CI 0.69 to 3.82. The trial authors did not report on any adverse effects specifically associated with cerebrolysin, for example, hypersensitivity reactions.

Subgroup analysis and investigation of heterogeneity

We investigated potential sources of heterogeneity using the following subgroups of studied treatment regimes, which differ in

cerebrolysin dose and the length of treatment for the outcomes 'all-cause death' and 'total number of people with adverse events':

- cerebrolysin dose 30 mL for 10 days (Amiri Nikpour 2014; CASTA 2012; CERE-LYSE-1 2012; Xue 2016); Xue 2016 did not contribute data to the quantitative analyses;
- cerebrolysin dose 50 mL for 21 days (Ladurner 2005);
- cerebrolysin does 10 mL and 50 mL for 10 days (Skvortsova 2004).

For the outcome 'all-cause death' we found no heterogeneity between the subgroups: $I^2 = 0$ (Analysis 1.1).

We found a moderate level of heterogeneity ($I^2 = 34\%$) for the outcome 'total number of people with adverse events' between the two subgroups of cerebrolysin dose (30 mL for 10 days and 50 mL for 21 days). There was a suggestion that the higher dose and the longer duration may be associated with higher risk of adverse event (Analysis 1.5) but this did not achieve conventional levels of statistical significance.

Interestingly, we found moderate levels of heterogeneity ($I^2 = 37\%$) of the effect sizes for this outcome also within a subgroup of the lower cerebrolysin dose and shorter duration of treatment (30 mL for 10 days), which was mainly due to the opposite directions of the effect in the two multicentre studies but not to the magnitude of the effect (CASTA 2012; CERE-LYSE-1 2012; Analysis 1.5).

For the outcome 'serious adverse events' we could not perform a subgroup analysis, but would like to mention here that there was no heterogeneity ($I^2 = 0\%$) between the two studies contributing to this outcome (CASTA 2012; CERE-LYSE-1 2012), both showing an increase in the number of serious adverse events with cerebrolysin treatment.

Sensitivity analyses

We performed a single sensitivity analysis to investigate the effect of methodological study quality: high risk of bias (Amiri Nikpour 2014; CASTA 2012; CERE-LYSE-1 2012; Ladurner 2005) versus unclear risk of bias (Skvortsova 2004) for the outcome 'all-cause death'. We examined the resulting forest plot to see the opposite direction of cerebrolysin effect in studies with high risk of bias compared with the single small trial with unclear risk of bias (Analysis 1.6). However the effect sizes were small and the resulting statistical index of potential heterogeneity remained the same, equalling zero ($I^2 = 0$).

We could not use funnel plots to examine asymmetry and small study effects because we had only six eligible studies and only five studies contributed data to the quantitative analyses, namely for the outcome 'all-cause death'. Excluding data from two small studies did not change the direction, magnitude, or CIs of the estimate of effect (Amiri Nikpour 2014; Skvortsova 2004).

DISCUSSION

The WHO collection of National Essential Medicines Lists (EML) includes the latest acting country editions, which recommend cerebrolysin for treating various neurological conditions, including acute ischaemic stroke. These include the National EMLs of the Russian Federation (GovRu 2016), Slovakia, Ukraine, Vietnam, and Syrian Arab Republic (WHO 2016).

However, the potential benefits of cerebrolysin for improving clinical outcomes in people with acute ischaemic stroke and risks of its use have not been systematically evaluated on the basis of research synthesis of RCTs of acceptable quality.

In this Cochrane Review we have assessed the benefits and harms of cerebrolysin when added to standard treatment for acute ischaemic stroke, focusing on clinically relevant and widely accepted outcomes, and specifically excluding assessment methods with numerous varying scales.

Summary of main results

We identified six RCTs, involving 1501 participants, that met the inclusion criteria, five of which contributed to quantitative analyses.

None of the six included trials (three of which were directly supported by the manufacturer of cerebrolysin, EVER Neuro Pharma GmbH (formerly Ebewe Pharma), and three for which we were unclear about the manufacturer's involvement) provided sufficient evidence of the effects of cerebrolysin on clinically relevant outcome measures for acute ischaemic stroke, such as poor functional outcome (death or dependence by the end of the follow-up period) and early death (within two weeks of stroke onset).

However, in this update of the review, we found moderate quality evidence that cerebrolysin did not influence risk of death (Analysis 1.1). We also found an increase in the number of people with non-fatal serious adverse events (Analysis 1.4, moderate quality evidence).

For the total number of people with adverse events, we did not find a statistically significant difference between cerebrolysin groups and placebo groups, but found moderate levels of heterogeneity between the three trials contributing to this outcome (Analysis 1.5).

When we performed a sensitivity analysis of high risk of bias versus unclear risk of bias we noticed an opposite direction of effect estimates for the outcome 'all-cause death': the single trial with unclear risk of bias (Skvortsova 2004) favoured placebo with a lower death rate in the placebo group compared with the cerebrolysin group (Analysis 1.6).

Despite the lack of evidence of benefits in acute ischaemic stroke, cerebrolysin is still widely used in Russia, Eastern European countries, China, and other Asian countries.

Therefore, the routine use of cerebrolysin in people with acute ischaemic stroke is not supported by any evidence from the existing clinical trials.

Any further studies, if conducted in this area, must be well-designed RCTs assessing clinical outcome measures rather than stroke scale parameters or other surrogate outcomes, such as infarct volume. The studies should be reported in full to allow the wider scientific community to gain a better understanding of potential risks of cerebrolysin in acute ischaemic stroke.

In view of the lack of evidence of benefit of cerebrolysin for acute ischaemic stroke, current thinking on the potential benefit of neuroprotection in acute ischaemic stroke needs to be re-assessed.

Overall completeness and applicability of evidence

In this update, we loosened the protocol inclusion criteria to allow various durations of cerebrolysin use, and included all trials that recruited people with confirmed acute ischaemic stroke, for whom trialists initiated treatment within 48 hours of stroke onset. We followed the protocol in all other aspects, but did not restrict the review to trials in which cerebrolysin or placebo was used for at least 14 days (two weeks).

The six eligible studies, three of which were international multicentre studies, were carried out in multiple clinical centres in Europe (seven countries): Austria, Croatia, the Czech Republic, Hungary, , Russia, Slovakia, and Slovenia; and in Asia (five countries): China, Hong Kong, Iran, Myanmar, and South Korea. The participant populations were geographically diverse. The included studies were conducted in high-income, middle-income, and low-income countries, which means the results of this Cochrane Review are likely to be applicable to settings where the burden of stroke and stroke deaths is high. Of particular importance is the fact that the results of this update are likely to be applicable to settings of low-income countries, where the burden of stroke deaths and disability is even higher (WHO 2014) and poses huge financial demand on health systems and society (Martynchik 2013), and where cerebrolysin is in widespread use. The included studies tested three various doses of cerebrolysin (10 mL, 30 mL, and 50 mL) and treatment duration with cerebrolysin varied from 10 days to 21 days. We did not find any clear evidence that cerebrolysin can improve clinical outcomes in acute ischaemic stroke with any of the tested treatment regimens. Treatment strategies for acute ischaemic stroke should be reviewed in the light of this evidence.

Given the poor prognosis of people with stroke, further evidence relating to the use of cerebrolysin in conjunction with aspirin would be welcome to better clarify the risks associated with its use in acute ischaemic stroke. Within the five eligible trials that contributed to quantitative analyses, reporting of the outcomes was selective in three studies (Amiri Nikpour 2014; CERE-LYSE-1 2012; Ladurner 2005) and incomplete in four studies (Amiri Nikpour 2014; CASTA 2012; CERE-LYSE-1 2012l; Ladurner 2005).

Reporting of data on death and safety parameters without clarification on the time of death and development of adverse events with data of many enrolled participants missing (attrition bias) brings confusion to meaningful interpretation of these data. Harmonised reporting standards for these and other outcomes in stroke trials would be welcome. However, the power of analysis for at least two outcomes (all-cause death and serious adverse events) raises concerns about cerebrolysin safety in acute ischaemic stroke. There was no significant reduction in death and an increase in non-fatal serious adverse events.

None of the included studies reported on cerebrolysin-specific adverse effects, such as hypersensitivity or emotional disturbances such as arousal and aggression or fatigue, tiredness and apathy or sleeplessness, convulsive preparedness, rise or fall in blood pressure, shortness of breath, flu-like syndrome, or reactions on immediate intravenous administration like feelings of chills or heat, cold sweat, dizziness and tachycardia, or redness and itching at the site of administration, gastrointestinal disturbances, and others (Registry of Medicines 2015).

Quality of the evidence

We assessed the quality of the evidence using the GRADE approach (Guyatt 2008) and we presented the results in Summary of findings for the main comparison.

For this table we asked the following question: should cerebrolysin be used in acute ischaemic stroke to improve clinical outcomes?

From the five studies that contributed to quantitative analysis, there is no evidence that cerebrolysin added to standard therapy reduces death in people with confirmed acute ischaemic stroke (Amiri Nikpour 2014; CASTA 2012; CERE-LYSE-1 2012; Ladurner 2005; Skvortsova 2004). There is moderate-quality evidence that cerebrolysin performs no better or no worse than placebo in preventing 'all-cause death' in people with acute ischaemic stroke if started within 48 hours of stroke onset and continued for 10 to 21 days as once-daily intravenous infusions of 10 mL, 30 mL, or 50 mL (Summary of findings for the main comparison).

However, further research is likely to have an important impact on our confidence in the estimate of cerebrolysin being no better than placebo in preventing all-cause death in acute stroke.

On the basis of the two multicentre studies that studied cerebrolysin added to standard therapy of acute ischaemic stroke, including thrombolysis, the review raises concerns about an increased risk of serious adverse events (CASTA 2012; CERE-LYSE-1 2012). There is moderate quality evidence that cerebrolysin presents harms, increasing the risks of non-fatal serious adverse events by more than twice compared with placebo (Summary of findings for the main comparison).

However, further research is likely to have an important impact on our confidence in the estimate of cerebrolysin risks in contributing to serious adverse events in people with acute stroke.

From three studies we know that cerebrolysin added to standard therapy of acute ischaemic stroke is no different from placebo

in the number of people with any adverse events (CASTA 2012; CERE-LYSE-1 2012; Ladurner 2005). There is moderate-quality evidence that cerebrolysin performs no better or no worse than placebo in terms of the number of people with any adverse events (Summary of findings for the main comparison).

However, further research is likely to have an important impact on our confidence in the estimate of cerebrolysin being no better or no worse than placebo for the number of people with any adverse events in acute stroke.

Potential biases in the review process

We performed the data extraction unblinded. The included trials are published and we obtained unpublished data on serious adverse events through feedback received from the manufacturer of cerebrolysin - EVER Neuro Pharma GmbH (formerly Ebewe Pharma).

Agreements and disagreements with other studies or reviews

We asked whether cerebrolysin has a role in improving the treatment outcomes for people diagnosed with acute ischaemic stroke. The original version of this review did not provide evidence that cerebrolysin was effective (Ziganshina 2010a).

These unfavourable results caution against its widespread use and its inclusion on national EMLs in Russia (GovRu 2016), Ukraine, Slovakia, Vietnam, and the Syrian Arab Republic (WHO 2016).

As new research data has accumulated, we have updated the review, having performed new literature searches. The conclusions have been changed by the results of this updated Cochrane Review.

In this review update, we did not find any evidence to support cerebrolysin use as a treatment option for acute ischaemic stroke. Estimates from six eligible studies suggest that all-cause death is not improved with cerebrolysin use compared with placebo, and reported numbers of people with any adverse events were not statistically different.

However, the review raises concerns about the increased risk of serious non-fatal adverse events with cerebrolysin use in people with acute ischaemic stroke. There was consistency between the two recent large multicentre trials performed in European (CERE-LYSE-1 2012) and Asian (CASTA 2012) countries that reported this outcome.

The methodological quality of most clinical trials of cerebrolysin is insufficient for inclusion in this Cochrane Review.

One study excluded from this review, a multi-centre prospective controlled study of cerebrolysin versus placebo in 277 people with acute ischaemic stroke, performed in Russia, showed a trend towards higher death rates in the cerebrolysin group compared with the placebo group (seven versus one). The cerebrolysin treatment regimen was 10 mL intravenously for 10 days (Skvortsova 2006). The study authors reported on the safety of cerebrolysin use and its benefit for scales' indices.

We included in this update two multicentre studies (CASTA 2012; CERE-LYSE-1 2012). The first one was a large study with participants from China, Hong Kong, South Korea, and Myanmar (CASTA 2012). There was no difference in death, described by the trial report authors in the safety section as fatal adverse events not related to the study drug. The number of serious non-fatal adverse events was 50 in the cerebrolysin group and 39 in the placebo group. The manufacturer of cerebrolysin provided funding support for the trial and 23% of participants were lost to follow-up.

The other multicentre study tested cerebrolysin in addition to alteplase in people with acute ischaemic hemispheric stroke in Austria and four Eastern European countries (CERE-LYSE-1 2012). The manufacturer of cerebrolysin provided research grants for the study and the study statistician. The study was terminated early and did not find any benefits of adding cerebrolysin to alteplase. Four participants died in each of the cerebrolysin and placebo groups. There was a pattern of more participants having serious adverse events in the cerebrolysin group than in placebo (12 versus 7).

With these multiple risks of bias these two studies confirmed our previous findings on the lack of benefits of cerebrolysin for acute ischaemic stroke, and in pooled analysis of serious adverse events contributed to our new finding of cerebrolysin harms - the increase in the number of people with non-fatal serious adverse events. Yet, the authors of both multicentre studies in their conclusions advocate for the safe use of cerebrolysin in acute ischaemic stroke (CASTA 2012) and in combination with alteplase (CERE-LYSE-1 2012).

Thus, the published reports on cerebrolysin use in people with acute ischaemic stroke advocate in their conclusions and abstracts that cerebrolysin is safe and well tolerated. Most studies reported on beneficial changes in surrogate efficacy measurements and various stroke scales, which were reported inconsistently by investigators and are not universally accepted. These have been reported despite the lack of evidence of benefit from cerebrolysin on the clinically relevant outcomes of death, serious adverse events, and total number of people with adverse effects.

Noteworthy is the fact that all later studies administered cerebrolysin in smaller doses (30 mL) and for shorter periods of time (10 days) whereas the first multicentre study administered cerebrolysin in larger doses (50 mL) for a longer period of time (21 days) with the result that the total number of people with adverse events in the cerebrolysin group was nearly twice that in the placebo group (Ladurner 2005).

We could not identify any clear evidence that cerebrolysin can improve clinical outcomes in acute ischaemic stroke.

AUTHORS' CONCLUSIONS

Implications for practice

The findings of this Cochrane Review do not demonstrate clinical benefits of cerebrolysin for treating acute ischaemic stroke. The review found moderate-quality evidence of an increase in non-fatal serious adverse events (SAEs) with cerebrolysin use but not in total SAEs.

Implications for research

Future research, if any at all, should focus on well-designed randomised controlled trials (RCTs) to assess the risks of cerebrolysin in acute ischaemic stroke.

The trial investigators must ensure that they use pragmatic clinical outcome measures including all-cause death, early death, dependency, and adverse events. They must provide a detailed description of any basic or routine therapy used concurrently with cerebrolysin (these should be the same in both the intervention and control groups). The trials should be reported in full and conform to the Consolidated Standards of Reporting Trials (CONSORT) statement (Moher 2001).

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REFERENCES

References to studies included in this review

Amiri Nikpour 2014 *{published data only}*

Amiri-Nikpour MR, Nazarbaghi S, Ahmadi-Salmasi B, Mokari T, Tahamtan U, Rezaei Y. Cerebrolysin effects on neurological outcomes and cerebral blood flow in acute ischemic stroke. *Neuropsychiatric Disease and Treatment* 2014;**10**:2299–306.

CASTA 2012 *{published data only}*

Bornstein NM. CASTA - the background for the study and protocol design. *International Journal of Stroke* 2010;**5** (Suppl 2):50.

Brainin M, Heiss WD, Bornstein N, Tuomilehto J, Hong Z. CASTA - results of a double-blind, placebo-controlled, randomized trial with cerebrolysin on patients with acute ischaemic stroke in Asia. *Cerebrovascular Diseases* 2012;**33** (Suppl 2):459–60.

Heiss WD, Brainin M, Bornstein N, Zhen H. Cerebrolysin in patients with acute ischemic stroke - the CASTA study. *International Journal of Stroke* 2010;**5**(Suppl 2):71.

* Heiss WD, Brainin M, Bornstein NM, Tuomilehto J, Hong Z, Cerebrolysin Acute Stroke Treatment in Asia (CASTA) Investigators. Cerebrolysin in patients with acute ischemic stroke in Asia: results of a double-blind, placebo-controlled randomized trial. *Stroke* 2012;**43**(3):630–6.

Hong Z, Moessler H, Bornstein N, Brainin M, Heiss W-D, CASTA Investigators. A double-blind, placebo-controlled, randomized trial to evaluate the safety and efficacy of cerebrolysin in patients with acute ischaemic stroke in Asia - CASTA. *International Journal of Stroke* 2009;**4**(5):406–12.

Zhen H. The safety and efficacy of cerebrolysin in patients with acute ischemic stroke (CASTA). www.ClinicalTrials.gov 2014.

CERE-LYSE-1 2012 *{published data only}*

* Lang W, Stadler CH, Poljakovic Z, Fleet D, Lyse Study Group. A prospective, randomized, placebo-controlled, double-blind trial about safety and efficacy combined treatment with alteplase (rt-PA) and cerebrolysin in acute ischaemic hemispheric stroke. *International Journal of Stroke* 2012;**8**(2):95–104.

Windisch M, Lang W. Combined treatment with alteplase (rt-PA) and cerebrolysin in acute ischemic hemispheric stroke (CERE-LYSE-1). www.ClinicalTrials.gov 2014.

Ladurner 2005 *{published data only}*

Ladurner G. Neuroprotection in acute ischaemic stroke. *Stroke 2001* 2001;**32**:323.

Ladurner G, Gmeinbauer R, Moessler H. Cerebrolysin in acute ischaemic stroke: a randomized, placebo-controlled trial with a neuroprotective agent. *Cerebrovascular Diseases* 2001;**11**(4):75.

* Ladurner G, Kalvach P, Moessler H, Cerebrolysin Study Group. Neuroprotective treatment with cerebrolysin in patients with acute stroke: a randomised controlled trial. *Journal of Neural Transmission* 2005;**112**(3):415–28.

Skvortsova 2004 *{published data only}*

Skvortsova VI, Shamalov NA, Stakhovskaya LV, Gubsky LV, Tikhonova IV, Smichkov AS. Cerebrolysin in acute ischaemic stroke: results of randomised, double blind, placebo-controlled study. *Cerebrovascular Diseases* 2005;**19** (Suppl 2):76.

* Skvortsova VI, Stakhovskaia LV, Gubskii LV, Shamalov NA, Tikhonova IV, Smychkov AS. A randomized, double-blind, placebo-controlled study of Cerebrolysin safety and efficacy in the treatment of acute ischemic stroke. *Zhurnal nevrologii i psikhiatrii imeni S.S. Korsakova / Ministerstvo zdravookhraneniia i meditsinskoi promyshlennosti Rossiiskoi Federatsii, Vserossiiskoe obshchestvo nevrologov [i] Vserossiiskoe obshchestvo psikhiatrov* 2004;**Suppl 11**:51–5.

Skvortsova VI, Stakhovskaya LV, Gubsky LV, Shamalov NA, Tikhonova IV, Smychkov AS. Evaluation of safety and efficacy of cerebrolysin for treating acute ischaemic stroke [ИССЛЕДОВАНИЕ БЕЗОПАСНОСТИ И ЭФФЕКТИВНОСТИ ЦЕРЕБРОЛИЗИНА ДЛЯ ЛЕЧЕНИЯ ОСТРОГО ИШЕМИЧЕСКОГО ИНСУЛЬТА]. Рецепт *[Recept]* 2007;**5**(55):94–8.

Xue 2016 *{published only}*

Xue LX, Zhang T, Zhao YW, Geng Z, Chen JJ, Chen H. Efficacy and safety comparison of DL-3-n-butylphthalide and cerebrolysin: effects on neurological and behavioral outcomes in acute ischemic stroke. *Experimental and Therapeutic Medicine* 2016;**11**(5):2015–20.

References to studies excluded from this review

Barolin 1996 *{published only}*

Barolin GS, Koppi S, Kapeller E. Old and new aspects of stroke treatment with emphasis on metabolically active medication and rehabilitative outcome. *Eurorehab* 1996;**3**: 135–43.

Bavarsad Shahripour 2011 *{published only}*

Bavarsad Shahripour R, Shalil Ahmadi A. The efficacy of cerebrolysin in patients with acute ischemic stroke. Proceedings of the 20th European Stroke Conference. 2011 May 24-27; Hamburg, Germany 2011.

Bayer 1980 *{published only}*

Bayer E. Therapeutic use of a brain hydrolysate. *Medizinische Welt* 1980;**31**(17):636–7.

Belkin 2011 *{published and unpublished data}*

Belkin AA. Navigation brain stimulation for evaluation of the neuroprotective drug efficiency in patients after ischemic stroke. ClinicalTrials.gov (accessed 19 July 2016).

Belkin 2015 *{published and unpublished data}*

Belkin AA, Alasheev AM. Comprehensive reparative therapy in ischemic stroke complex repair in ischemic stroke - arm. ClinicalTrials.gov Accessed 19 July 2016.

Chang 2016 *{published data only}*

Chang WH, Park CH, Kim DY, Shin YI, Ko MH, Lee A, et al. Cerebrolysin combined with rehabilitation promotes motor recovery in patients with severe motor impairment after stroke. Effects of cerebrolysin on motor recovery in patients with subacute stroke (E-COMPASS). *BMC Neurology* 2016;**2**(16):31. [DOI: 10.1186/s12883-016-0553-z

Cuparnecu 2001 *{published data only}*

Cuparnecu B. Efficacy of cerebrolysin in patients with ischaemic stroke of the middle cerebral artery. *Pharmacology and Toxicology* 2001;**89 Suppl 1**:136.

Domzal 1995 *{published data only}*

Domzal T, Zaleska B. Cerebrolysin in treatment of acute ischemic stroke. *Neurologia i Neurochirurgia Polska* 1995;**29** (3):325–31.

E-COMPASSII 2016 *{published and unpublished data}*

Kim YH. Effects of cerebrolysin combined with rehabilitation on motor recovery in stroke. www.strokecenter.org (accessed 18 July 2016).

Ershov 2011 *{published data only}*

* Ershov VI. Comparative aspects of using neuroprotectors in the management of patients with ischemic stroke. *Zhurnal Nevrologii I Psikhiatrii Imeni S.S. Korsakova* 2011; **111**(8 Pt 2):41–4.

Ershov VI. Comparative characteristics of efficacy of neuroprotectors for treating ischaemic stroke. *Nevrologichesky Vestnik. Zhurnal im. VM Bekhtereva* 2009; **41**(3):65–9.

Guekht 2015 *{published data only}*

Guekht A, Heiss WD, Bajenaru OA, Popescu C, Gusev E, Vester JC, et al. Cerebrolysin and recovery after stroke (CARS): a meta-analysis of two randomized, controlled, double-blind trials assessing the combination of Cerebrolysin and standardized rehabilitation on motor recovery. *European Journal of Neurology* 2015;**22**:598.

Guekht 2015a *{published data only}*

Guekht A, Heiss D, Gusev E, Vester J, Doppler E, Muresanu D. Cerebrolysin and recovery after stroke (CARS 2): a randomized, placebo-controlled, double-blind, multicenter clinical study. *Journal of the Neurological Sciences* 2015;**357**: e103.

Haffner 2001 *{published data only}*

Haffner Z, Gmeinbauer R, Moessler H. A randomized, double-blind, placebo-controlled trial with cerebrolysin in acute ischaemic stroke. *Cerebrovascular Diseases* 2001;**11 Suppl 4**:76.

Jianu 2010 *{published data only}*

Jianu DC, Muresanu DF, Bajenaru O, Popescu BO, Deme SM, Moessler H, et al. Cerebrolysin adjuvant treatment in Broca's aphasics following first acute ischemic stroke of

the left middle cerebral artery. *Journal of Medicine and Life* 2010;**3**(3):297–307.

Jianu 2015 ***{published data only}***
Jianu DC, Jianu SN, Petrica L, Deme SM, Dan F, Falup Pecurariu C, et al. Cerebrolysin adjuvant treatment in Broca's aphasics following first acute ischemic stroke of the left middle cerebral artery. *International Journal of Stroke* 2015;**10**:187.

Kim 2014 ***{published data only}***
Kim YH, Kim DY, Shin YI, Ko MH, Chang WH, Lee AH. Effects of cerebrolysin on motor recovery in patients with subacute stroke. *International Journal of Stroke* 2014;**9**: 107–8.
* Kim YH, Park CH, Kim DY, Shin YI, Ko MH, Chang WH, et al. Effects of cerebrolysin on motor recovery of stroke patients: a diffusion tensor imaging study. *International Journal of Stroke* 2014;**9**:49–9.

Kim 2015 ***{published data only}***
Kim YH, Kim DY, Shin YI, Ko MH, Chang WH, Lee AH. Effects of cerebrolysin on motor recovery in patients with severe stroke motor impairment after stroke. *International Journal of Stroke* 2015;**10**(Suppl 2):90.

Martinez Sanchez 2015 ***{published data only}***
Martinez Sanchez BR, Hernandez Hernandez JJ, Franco Del Aguila DI, Gryzbowski Gainza E, Paz Ballesteros WC, Herrera Rojas J, et al. An open label, one arm study to evaluate the efficacy and safety of cerebrolysin in patients with acute severe ischemic stroke in Mexico. *Value in Health* 2015;**18**(7):A827–8.

Muresanu 2016 ***{published data only}***
Muresanu D, Heiss WD, Bajenaru O, Popescu CD, Vester J, Guekht A. Cerebrolysin and recovery after stroke (CARS): a randomized, placebo-controlled, double-blind, multicenter, phase II clinical study. *International Journal of Stroke* 2015; **10**(Suppl 2):92.
* Muresanu DF, Heiss WD, Hoemberg V, Bajenaru O, Popescu CD, Vester JC, et al. Cerebrolysin and Recovery After Stroke (CARS): a randomized, placebo-controlled, double-blind, multicenter trial. *Stroke* 2016;**47**(1):151–9.

Pushkarev 2015 ***{published data only}***
Pushkarev K, Tsoy R. Neuroprotective therapy for lacunar stroke - which is better Ceraxon or Cerebrolysin?. *European Journal of Neurology* 2015;**22**:775.

Shamalov 2005 ***{published data only}***
Shamalov NA, Stakhovskaya LV, Gubsky LV, Tikhonova IV, Smichkov AS, Skvortsova VI, et al. Effects of the neuroprotective drug cerebrolysin on the infarct volume after acute ischemic stroke. *Cerebrovascular Diseases* 2005; **19 Suppl 2**:107.

Shamalov 2010 ***{published data only}***
Shamalov NA, Stakhovskaia LV, Burenchev DV, Kichuk IV, Tvorogova TV, Botsina AI, et al. The effect of cerebrolysin in dosage 50 ml on the volume of lesion in ischemic stroke. *Zhurnal nevrologii i psikhiatrii imeni S.S. Korsakova / Ministerstvo zdravookhraneniia i meditsinskoi promyshlennosti Rossiiskoi Federatsii, Vserossiiskoe obshchestvo nevrologov [i] Vserossiiskoe obshchestvo psikhiatrov* 2010;**110**(12 Pt 2):34–7.

Shishkova 2015 ***{published data only}***
Shishkova V, Zotova LI, Remennik AYU. Association between BDNF-196 G>A and BDNF-270 C>T polymorphisms, BDNF concentration, and cerebrolysin treatment outcome after ischemic stroke. *International Journal of Stroke* 2015;**10 Suppl 2**:170.

Skvortsova 2006 ***{published data only}***
Skvortsova VI, Stakhovskaia LV, Shamolov NA, Kerbikov OB. Results of the multicenter prospective study of cerebrolysin safety and efficacy in acute stroke. *Zhurnal Nevrologii I Psikhiatrii Imeni SS Korsakova* 2006;**Suppl 16**: 41–5.

Stan 2013 ***{published data only}***
Stan AD, Badisor A, Birle C, Blesneag AV, Opincariu I, Iancu M, et al. The influence of neurotrophic factors treatment on stroke volume. *Romanian Journal of Neurology* 2013;**XII**(3):124–9.

Vilenskii 1999 ***{published data only}***
Vilenskii BS, Semenova GM, Shirokov EA. The application of cerebrolysine in ischemic stroke. *Zhurnal nevrologii i psikhiatrii imeni S.S. Korsakova / Ministerstvo zdravookhraneniia i meditsinskoi promyshlennosti Rossiiskoi Federatsii, Vserossiiskoe obshchestvo nevrologov [i] Vserossiiskoe obshchestvo psikhiatrov* 1999;**99**(4):65–9.

Yavorskaya 2008 ***{published data only}***
Yavorskaya VA, Bondar OB. Clinical features of cerebrolysin application in patients in acute period of ischemic stroke. *International Journal of Stroke* 2008;**3 Suppl 1**:141.

Zhu 2003 ***{published data only}***
Zhu GX, Hong Z, Yao JL, Yu LY. Double-blind and randomized placebo-controlled trial of cerebrolysin in improvement of nerve function and living ability in patients with ischemic stroke. *Chinese Journal of Clinical Rehabilitation* 2003;**7**(22):3084–5.

References to studies awaiting assessment

Dobi 2010 ***{published data only}***
Dobi D, Xhaxho S, Kapisyzi M, Papadhopulli Z. The effect of cerebrolysin in acute ischemic stroke. *Revue Neurologique* 2010;**166**(3):355.

Hong 2005 ***{published data only}***
Hong Z, Zhu G, Chen H. The clinical efficacy of cerebrolysin in the treatment of acute ischemic stroke. *Chinese Journal of Geriatric Heart Brain and Vessel Diseases* 2005;**7**(5):331–3.

IRCT138803272042N1 2014 ***{published and unpublished data}***
IRCT138803272042N1. The efficacy of cerebrolysin in the treatment of acute ischemic stroke. Iranian Registry of Clinical Trials (en.search.irct.ir/view/1090) (accessed 15 December 2014).

Nazarbaghi 2014 *{published data only}*
Nazarbaghi S, Nikpoor MA. Efficiency of cerebrolysin in treatment of patients with acute ischemic stroke. *International Journal of Stroke* 2014;**9 Suppl 3**:91–2.

Skvortsova 2008 *{published data only}*
Jech M, Shamalov NA, Moessler H, Skvortsova VI. Safety and efficacy of cerebrolysin as adjuvant therapy in patients suffering from acute ischemic stroke. *Cerebrovascular Diseases* 2009;**27**(Suppl 6):84.
* Skvortsova VI, Shamalov NA, Moessler H, Doppler E. Safety and efficacy of cerebrolysin as adjuvant therapy in patients suffering from acute ischemic stroke. *European Journal of Neurology* 2008;**15 Suppl 3**:253.
Skvortsova VI, Shamalov NA, Moessler H, Novak PH. Beneficial effects of the neurotrophic drug cerebrolysin on the infarct volume after acute stroke. *Cerebrovascular Diseases* 2008;**25 Suppl 2**:145.
Skvortsova VI, Shamalov NA, Moessler H, Novak PH. Positive impacts of the neurotrophic drug cerebrolysin on the infarct volume after acute stroke. *International Journal of Stroke* 2008;**3 Suppl 1**:137.

Additional references

AHA 2014
Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Blaha MJ, et al. Heart disease and stroke disease statistics - 2014 update: a report from the American Heart Association. *Circulation* 2014;**129**(3):e28–e292.

Alper 2015
Alper BS, Malone-Moses M, McLellan JS, Prasad K, Manheimer E. Thrombolysis in acute ischaemic stroke: time for a rethink?. *BMJ* 2015;**350**:h1075. [DOI: http://dx.doi.org/10.1136/bmj.h1075

Alvarez 2000
Alvarez XA, Lombardi VR, Corzo L, Pérez P, Pichel V, Laredo M, et al. Oral cerebrolysin enhances brain alpha activity and improves cognitive performance in elderly control subjects. *Journal of Neural Transmission* 2000;**59 Suppl**:315–28.

Appelros 2015
Appelros P, Terént A. Thrombolysis in acute stroke. *Lancet* 2015;**385**(9976):1394. [DOI: http://dx.doi.org/10.1016/S0140-6736(15)60714-0

Bajenaru 2010
Bajenaru O, Tiu C, Moessler H, Antochi F, Muresanu D, Popescu BO, et al. Efficacy and safety of cerebrolysin in patients with hemorrhagic stroke. *Journal of Medicine and Life* 2010;**3**(2):137–43.

BASC 2000
Blood pressure in Acute Stroke Collaboration (BASC). Vasoactive drugs for acute stroke. *Cochrane Database of Systematic Reviews* 2000, Issue 4. [DOI: 10.1002/14651858.CD002839

BASC 2001
Blood pressure in Acute Stroke Collaboration (BASC). Interventions for deliberately altering blood pressure in acute stroke. *Cochrane Database of Systematic Reviews* 2001, Issue 3. [DOI: 10.1002/14651858.CD000039

Bath 2002
Bath PMW, Willmot M, Leonardi-Bee J, Bath-Hextall FJ. Nitric oxide donors (nitrates), L-arginine, or nitric oxide synthase inhibitors for acute stroke. *Cochrane Database of Systematic Reviews* 2002, Issue 4. [DOI: 10.1002/14651858.CD000398

Bath 2004a
Bath PMW. Theophylline, aminophylline, caffeine and analogues for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2004, Issue 3. [DOI: 10.1002/14651858.CD000211.pub2

Bath 2004b
Bath PMW, Bath-Hextall FJ. Pentoxifylline, propentofylline and pentifylline for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2004, Issue 3. [DOI: 10.1002/14651858.CD000162.pub2

Bath 2004c
Bath PMW. Prostacyclin and analogues for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2004, Issue 3. [DOI: 10.1002/14651858.CD000177.pub2

Bath 2013
Bath PMW, Sprigg N, England T. Colony stimulating factors (including erythropoietin, granulocyte colony stimulating factor and analogues) for stroke. *Cochrane Database of Systematic Reviews* 2013, Issue 6. [DOI: 10.1002/14651858.CD005207.pub4

Bath 2014
Bath PMW, Krishnan K. Interventions for deliberately altering blood pressure in acute stroke. *Cochrane Database of Systematic Reviews* 2014, Issue 10. [DOI: 10.1002/14651858.CD000039.pub3

Bereczki 2007
Bereczki D, Fekete I, Prado GF, Liu M. Mannitol for acute stroke. *Cochrane Database of Systematic Reviews* 2007, Issue 3. [DOI: 10.1002/14651858.CD001153.pub2

Bereczki 2008
Bereczki D, Fekete I. Vinpocetine for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2008, Issue 1. [DOI: 10.1002/14651858.CD000480.pub2

Berge 2002
Berge E, Sandercock P. Anticoagulants versus antiplatelet agents for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2002, Issue 4. [DOI: 10.1002/14651858.CD003242

Boncoraglio 2010
Boncoraglio GB, Bersano A, Candelise L, Reynolds BA, Paratti EA. Stem cell transplantation for ischemic stroke. *Cochrane Database of Systematic Reviews* 2010, Issue 9. [DOI: 10.1002/14651858.CD007231.pub2

Bonita 1992
Bonita R. Epidemiology of stroke. *Lancet* 1992;**339**(8789):342–4.

Brunström 2015
Brunström M, Carlberg B. Thrombolysis in acute stroke. *Lancet* 2015;**385**(9976):1394-5. [DOI: http://dx.doi.org/10.1016/S0140-6736(15)60715-2

Candelise 2001
Candelise L, Ciccone A. Gangliosides for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2001, Issue 4. [DOI: 10.1002/14651858.CD000094

Chang 2014
Chang TS, Jensen MB. Haemodilution for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2014, Issue 8. [DOI: 10.1002/14651858.CD000103.pub2

Chen 2008
Chen X, Zhou M, Li Q, Yang J, Zhang Y, Zhang D, et al. Sanchi for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2008, Issue 4. [DOI: 10.1002/14651858.CD006305.pub2

Chen 2013b
Chen N, Yang M, Guo J, Zhou M, Zhu C, He L. Cerebrolysin for vascular dementia. *Cochrane Database of Systematic Reviews* 2013, Issue 1. [DOI: 10.1002/14651858.CD008900.pub2

Chukanova 2005
Chukanova EI. The effect of cerebrolysin on the clinical symptoms and the course of ischemic encephalopathy. *Zhurnal Nevrologii i Psikhiatrii Imeni S.S. Korsakova* 2005;**105**(1):42–5.

Ciccone 2014
Ciccone A, Motto C, Abraha I, Cozzolino F, Santilli I. Glycoprotein IIb-IIIa inhibitors for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2014, Issue 3. [DOI: 10.1002/14651858.CD005208.pub3

Deeks 2011
Deeks JJ, Higgins JPT, Altman DG (editors). Chapter 9: Analysing data and undertaking meta-analyses. In: Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from handbook.cochrane.org.

Emberson 2014
Emberson J, Lees KR, Lyden P, Blackwell L, Albers G, Bluhmki E, et al. Effect of treatment delay, age, and stroke severity on the effects of intravenous thrombolysis with alteplase for acute ischaemic stroke: a meta-analysis of individual patient data from randomised trials. *Lancet* 2014;**384**(9958):1929–35.

European Ad Hoc Consensus 1998
The European Ad Hoc Consensus Group. Neuroprotection as initial therapy in acute stroke. Third report of an Ad Hoc Consensus Group Meeting. *Cerebrovascular Diseases* 1998;**8**(1):59–72.

Feng 2011
Feng S, Yang Q, Liu M, Li W, Yuan W, Zhang S, et al. Edaravone for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2011, Issue 12. [DOI: 10.1002/14651858.CD007230.pub2

Fragoso 2002
Fragoso Y, Dantas DC. Cerebrolysin for Alzheimer's disease. *Cochrane Database of Systematic Reviews* 2002, Issue 3. [DOI: 10.1002/14651858.CD003801

French 2007
French B, Thomas LH, Leathley MJ, Sutton CJ, McAdam J, Forster A, et al. Repetitive task training for improving functional ability after stroke. *Cochrane Database of Systematic Reviews* 2007, Issue 4. [DOI: 10.1002/14651858.CD006073

Gafurov 2004
Gafurov BG, Alikulova NA. Clinical and pathogenetical peculiarities and treatment policy in ischemic stroke of elderly and old age [Russian]. *Zhurnal Nevrologii i Psikhiatrii Imeni S.S. Korsakova* 2004;**104 Suppl 11**:44–6.

Gandolfo 2002
Gandolfo C, Sandercock P, Conti M. Lubeluzole for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2002, Issue 1. [DOI: 10.1002/14651858.CD001924

GovRu 2016
Essential Medicines List for 2016. Government of the Russian Federation RASPORIESE 26 December 2015. government.ru/media/files/MzyV0Mmm2FoDAP7AWAAVLLpjoLoiShib.pdf (accessed 12 August 2016).

GRADEPro GDT 2015 [Computer program]
McMaster University (developed by Evidence Prime, Inc.). Available from www.gradepro.org. GRADEPro Guideline Development Tool. McMaster University (developed by Evidence Prime, Inc.). Available from www.gradepro.org, 2015.

Gromova 2006
Gromova OA, Tret'iakov VE, Moshkovskiĭ SA, Gusev EI, Nikonov AA, Val'kova LA, et al. An oligopeptide membrane fraction of cerebrolysin. *Zhurnal Nevrologii I Psikhiatrii Imeni S.S. Korsakova* 2006;**106**(7):68–70.

Gusev 2003
Gusev EI, Skvortsova VI, Stakhovskaia LV. Epidemiology of stroke in Russia. *Zhurnal Nevrologii I Psikhiatrii Imeni S.S. Korsakova* 2003;**Suppl 8**:4–9.

Gusev 2013
Gusev EI, Martynov MYu, Kamtchatnov PR. Ischemic stroke: current status [Ishemichesky insult. Sovremennoye sostoyaniye problemy]. *Nevrologiya* 2013;**83**(5):2–7.

Guyatt 2008
Guyatt GH, Oxman AD, Vist G, Kunz R, Falck-Ytter Y, Alonso-Coello P, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* 2008;**336**(7650):924–6.

Hao 2012
Hao Z, Liu M, Counsell C, Wardlaw JM, Lin S, Zhao X. Fibrinogen depleting agents for acute ischaemic stroke.

Cochrane Database of Systematic Reviews 2012, Issue 3. [DOI: 10.1002/14651858.CD000091.pub2

Higgins 2003
Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003;**327**: 557–60.

Higgins 2011
Higgins JPT, Altman DG, Sterne JAC (editors). Chapter 8: Assessing risk of bias in included studies. In: Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from handbook.cochrane.org.

Hong 2009
Hong Z, Moessler H, Bornstein N, Brainin M, Heiss W-D, CASTA Investigators. A double-blind, placebo-controlled, randomized trial to evaluate the safety and efficacy of cerebrolysin in patients with acute ischaemic stroke in Asia - CASTA. *International Journal of Stroke* 2009;**4**(5):406–12.

Horn 2000
Horn J, Limburg M. Calcium antagonists for acute ischemic stroke. *Cochrane Database of Systematic Reviews* 2000, Issue 1. [DOI: 10.1002/14651858.CD001928

ICH 2003
ICH Expert Working Group. International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use. Post-approval safety data management: definitions and standards for expedited reporting E2D. ICH harmonised tripartite guideline, Step 4 version 2003.

Leonardi-Bee 2007
Leonardi-Bee J, Steiner T, Bath-Hextall F. Naftidrofuryl for acute stroke. *Cochrane Database of Systematic Reviews* 2007, Issue 2. [DOI: 10.1002/14651858.CD005478.pub2

Liu 2016
Liu J, Wang LN, Ma X, Ji X. Gamma aminobutyric acid (GABA) receptor agonists for acute stroke. *Cochrane Database of Systematic Reviews* 2016, Issue 10. [DOI: 10.1002/14651858.CD009622.pub4

Makarenko 2005
Makarenko AN, Kositsin NS, Nazimov IV, Svinov MM, Goloborod'ko EV, Pasikova NV. A comparative study of antistroke activity of the new drug "cerebral" and its fractions in rats. *Eksperimental'naia i Klinicheskaia Farmakologiia* 2005;**68**(2):15–20.

Martynchik 2013
Martynchik SA, Sokolova OV. Medical and economic assessment and rationale for improving organization of inpatient care for cerebral stroke [Medico–economicheskaya otsenka i obosnovaniye sovershenstvovaniya organizatsionnykh form okazaniya stacionarnoy pomoschi pri mozgovom insulte]. Social Aspects of Population Health (vestnik.mednet.ru/content/view/473/30/lang.ru/) 2013; Vol. 30, issue 2.

Masliah 2012
Masliah E, Díez-Tejedor E. The pharmacology of neurotrophic treatment with cerebrolysin: brain protection and repair to counteract pathologies of acute and chronic neurological disorders. *Drugs of Today* 2012;**48**(Suppl A): 3–24. [DOI: 10.1358/dot.2012.48(Suppl.A).1739716

MedDRA 2011
MedDRA Maintenance and Support Services Organization. Introductory Guide to MedDRA Version 14.0. Chantilly, Virginia, March, 2011.

Moher 2001
Moher D, Schulz KF, Altman DG. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. *Lancet* 2001;**357**(9263):1191–4.

Muir 2003
Muir KW, Lees KR. Excitatory amino acid antagonists for acute stroke. *Cochrane Database of Systematic Reviews* 2003, Issue 3. [DOI: 10.1002/14651858.CD001244

O'Rourke 2010
O'Rourke K, Berge E, Walsh CD, Kelly PJ. Percutaneous vascular interventions for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2010, Issue 10. [DOI: 10.1002/14651858.CD007574.pub2

Onishchenko 2006
Onishchenko LS, Gaĭ kova ON, Ianishevski ĭ SN. Changes in the focus of experimental ischemic stroke under the influence of neuroprotective drugs. *Morfologiia* 2006;**130**(6):40–6.

Registry of Medicines 2015
Registry of Medicines. Cerebrolysin. www.rlsnet.ru/tn_index_id_3528.htm#pobochnye-dejstviya (accessed 31 January 2015).

RevMan 2014 [Computer program]
The Nordic Cochrane Centre, The Cochrane Collaboration. Review Manager (RevMan). Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.

Ricci 2012a
Ricci S, Celani MG, Cantisani TA, Righetti E. Piracetam for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2012, Issue 9. [DOI: 10.1002/14651858.CD000419.pub3

Ricci 2012b
Ricci S, Dinia L, Del Sette M, Anzola P, Mazzoli T, Cenciarelli S, et al. Sonothrombolysis for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2012, Issue 10. [DOI: 10.1002/14651858.CD008348.pub3

Righetti 2004
Righetti E, Celani MG, Cantisani T, Sterzi R, Boysen G, Ricci S. Glycerol for acute stroke. *Cochrane Database of Systematic Reviews* 2004, Issue 2. [DOI: 10.1002/14651858.CD000096.pub2

Riley 2006
Riley C, Hutter-Paier B, Windisch M, Doppler E, Moessler H, Wronski R. A peptide preparation protects cells in organotypic brain slices against cell death after glutamate intoxication. *Journal of Neural Transmission* 2006;**113**(1): 103–10.

Sandercock 2008a
Sandercock PAG, Counsell C, Kamal AK. Anticoagulants for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2008, Issue 4. [DOI: 10.1002/14651858.CD000024.pub3

Sandercock 2008b
Sandercock P, Counsell C, Tseng MC. Low-molecular-weight heparins or heparinoids versus standard unfractionated heparin for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2008, Issue 3. [DOI: 10.1002/14651858.CD000119.pub3

Sandercock 2009
Sandercock PAG, Gibson LM, Liu M. Anticoagulants for preventing recurrence following presumed non-cardioembolic ischaemic stroke or transient ischaemic attack. *Cochrane Database of Systematic Reviews* 2009, Issue 2. [DOI: 10.1002/14651858.CD000248.pub2

Sandercock 2011
Sandercock PAG, Soane T. Corticosteroids for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2011, Issue 9. [DOI: 10.1002/14651858.CD000064.pub2

Sandercock 2014
Sandercock PAG, Counsell C, Tseng MC, Cecconi E. Oral antiplatelet therapy for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2014, Issue 3. [DOI: 10.1002/14651858.CD000029.pub3

Sapronov 2005
Sapronov NS, Bul'on VV, Kuznetsova NN, Selina EN. The neuroprotector effect of a new taurine derivative on a model of compression spinal cord trauma in rats [Russian]. *Eksperimental'naia i Klinicheskaia Farmakologiia* 2005;**68** (6):45–8.

Schauer 2006
Schauer E, Wronski R, Patockova J, Moessler H, Doppler E, Hutter-Paier B, et al. Neuroprotection of Cerebrolysin in tissue culture models of brain ischemia: post lesion application indicates a wide therapeutic window. *Journal of Neural Transmission* 2006;**113**(7):855–68.

Schünemann 2011
Schunemann HJ, Oxman AD, Vist GE, Higgins JPT, Deeks JJ, Glasziou P, et al. Chapter 12: Interpreting results and drawing conclusions. In: Higgins JPT, Green S (editors), Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011). The Cochrane Collaboration, 2011. Available from www.handbook.cochrane.org. Available from www.cochrane–handbook.org. The Cochrane Collaboration.

Shu 2012
Shu LLS, San Jose CZ, Pasco PP. The efficacy and safety of cerebrolysin in acute hemorrhagic stroke: a meta analysis. *Cerebrovascular Diseases* 2012;**34**(Suppl 1):36.

Shuaib 2007
Shuaib A, Lees KR, Lyden P, Grotta J, Davalos A, Davis SM, et al. NXY-059 for the treatment of acute ischemic stroke. *New England Journal of Medicine* 2007;**357**(6):562–71.

Suslina 2009
Suslina ZA, Varakin YY, Vereshchagin NV. Clinical and epidemiological research as a perspective direction of cerebral pathology study (the first report) [Clinico–epidemiologicheskiye issledovaniya – perspectivnoye napravleniye izucheniya cerebralnoi patologii (soobscheniye pervoiye)]. *Annals of Neurology* 2009;**3**:4–11.

Tan 2008
Tan Y, Liu M, Wu B. Puerarin for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2008, Issue 1. [DOI: 10.1002/14651858.CD004955.pub2

TISC 2001
Bath PM, Iddenden R, Bath FJ, Orgogozo JM, Tirilazad International Steering Committee. Tirilazad for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2001, Issue 4. [DOI: 10.1002/14651858.CD002087

Vilenskiĭ 2006b
Vilenskiĭ BS, Iakhno NN. The problem of cerebral stroke: its contemporary state. *Vestnik Rossiĭskoĭ Akademii Meditsinskikh Nauk* 2006;**9-10**:18–24.

Visvanathan 2015
Visvanathan A, Dennis M, Whiteley W. Parenteral fluid regimens for improving functional outcome in people with acute stroke. *Cochrane Database of Systematic Reviews* 2015, Issue 9. [DOI: 10.1002/14651858.CD011138.pub2

Wardlaw 2013
Wardlaw JM, Koumellis P, Liu M. Thrombolysis (different doses, routes of administration and agents) for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2013, Issue 5. [DOI: 10.1002/14651858.CD000514.pub3

Wardlaw 2014
Wardlaw JM, Murray V, Berge E, del Zoppo GJ. Thrombolysis for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2014, Issue 7. [DOI: 10.1002/14651858.CD000213.pub3

WHO 2014
World Health Organization. The Atlas of Heart Disease and Stroke. www.who.int/cardiovascular_diseases/resources/atlas/en/ (accessed November 2014).

WHO 2016
National Essential Medicines Lists. www.who.int/selection_medicines/country_lists (accessed 12 August 2016).

Wong 2005
Wong GK, Zhu XL, Poon WS. Beneficial effect of cerebrolysin on moderate and severe head injury patients:

result of a cohort study. *Acta Neurochirurgica Supplement* 2005;**95**:59–60.

Yang 2009
Yang W, Hao Z, Zhang S, Dong W, Wu T, Liu GJ, et al. Mailuoning for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2009, Issue 2. [DOI: 10.1002/14651858.CD007028.pub2

Zeng 2005
Zeng X, Liu M, Yang Y, Li Y, Asplund K. Ginkgo biloba for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2005, Issue 4. [DOI: 10.1002/14651858.CD003691.pub2

Zhang 2010
Zhang CL, Chopp M, Cui YS, Wang L, Zhang RL, Zhang L, et al. Cerebrolysin enhances neurogenesis in the ischemic brain and improves functional outcome after stroke. *Journal of Neuroscience Research* 2010;**88**(15):3275–81.

Zhuo 2008
Zhuo Q, Yang X, Wu T, Liu G, Zhou L. Tongxinluo capsule for acute stroke. *Cochrane Database of Systematic Reviews* 2008, Issue 4. [DOI: 10.1002/14651858.CD004584.pub2

References to other published versions of this review

Ziganshina 2010a
Ziganshina LE, Abakumova T, Kuchaeva A. Cerebrolysin for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2010, Issue 4. [DOI: 10.1002/14651858.CD007026.pub2

Ziganshina 2010b
Ziganshina LE, Abakumova T, Kuchaeva A. Cerebrolysin for acute ischaemic stroke. *International Journal of Risk and Safety in Medicine* 2010;**22**(4):179–93.

Ziganshina 2013
Ziganshina LE, Abakumova TR. Cerebrolysin for acute ischemic stroke. *Vestnik Rossiĭskoĭ Akademii Meditsinskikh Nauk* 2013;**1**:21–9.

Ziganshina 2015
Ziganshina LE, Abakumova T. Cerebrolysin for acute ischaemic stroke. *Cochrane Database of Systematic Reviews* 2015, Issue 6. [DOI: 10.1002/14651858.CD007026.pub3

* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Amiri Nikpour 2014

Methods	Study design: RCT Study grouping: parallel group
Participants	Total number of participants: 46. However, 3 participants died before day 30: 1 participant in the cerebrolysin group and 2 participants in the placebo group: 43 participants included in the final analysis Baseline characteristics Cerebrolysin • Participants: 22 • Mean age: 60 year (SD ± 9.6) • Men: 12 (54.5%) • Women: 10 (45.5%) • Risk factor: ischaemic heart disease: 4 (18.2%); diabetes mellitus: 8 (36.4%); hypertension: 13 (59.1%); dyslipidaemia: 11 (50%); smoking: 3 (13.6%) • Drug histories: beta-blockers: 4 (18.2); ACE-1: 3 (13.6%); angiotensin receptor blocker: 8 (36.4%); calcium channel blocker: 0 (0%); diuretic: 3 (13.6%); statin: 12 (54.5%); antidiabetic: 8 (36.4%); antidiabetic plus statin: 3 (13.6%); antidiabetic plus antihypertensive: 4 (18.2%); antihypertensive plus statin: 4 (18.2%) • Stroke location: anterior circulation: 14 (63.6%); posterior circulation: 8 (36.4%) Placebo • Participants: 21 • Mean age: 60.1 years (SD ± 10) • Men: 10 (47.6%) • Women: 11 (52.4%) • Risk factor: ischaemic heart disease: 3 (14.3%); diabetes mellitus: 10 (47.6%); hypertension: 13 (61.9%); dyslipidaemia: 12 (57.1%); smoking: 3 (14.3%) • Drug histories: beta-blockers: 5 (23.8%); ACE-1: 2 (9.5%); angiotensin receptor blocker: 5 (23.8%); calcium channel blocker: 1 (4.8%); diuretic: 6 (28.6%); statin: 12 (57.1%); antidiabetic: 10 (47.6%); antidiabetic plus statin: 7 (33.3%); antidiabetic plus antihypertensive: 2 (9.5%); antihypertensive plus statin: 5 (23.8%) • Stroke location: anterior circulation: 16 (76.2%); posterior circulation: 5 (23.8%) Inclusion criteria: both sexes, 18-85 years; focal neurological injury; ischaemic stroke within 6-24 h before admission; acute focal ischaemic stroke detected by CT or MRI or both; NIHSS score of 6-22 at presentation Exclusion criteria: rapid improvement of signs and symptoms, or complete resolution, or both, within 24 h; seizure upon the development of stroke; any conditions interfering with neurological examination, such as severe dementia or psychological diseases; severe heart failure; acute myocardial infarction; pregnancy or breast-feeding; significant systemic diseases associated with disability and decreased well-being; systolic and diastolic blood pressure above 220 mm/Hg and 120 mm/Hg respectively; CT or MRI suggesting acute or chronic hemorrhagic stroke or neoplasm, or both; hernia in the brain or increased intracranial pressure; contraindication or sensitivity to aspirin or cerebrolysin, or both; taking other neuroprotective agents such as piracetam; and taking vasodilators

Amiri Nikpour 2014 (*Continued*)

	such as nimodipine Pretreatment: no difference
Interventions	Cerebrolysin • Frequency of dosage: intravenous injection of 30 mL of cerebrolysin diluted in normal saline once a day for 10 days • Standard treatment: 100 mg of aspirin daily Placebo • Frequency of dosage: normal saline, as placebo, with a prescription order similar to the main drug • Standard treatment: 100 mg of aspirin daily
Outcomes	All-cause death (dichotomous outcome)
Identification	Sponsorship source: Urmia University of Medical Sciences grant Country: Iran Setting: hospital (inpatient setting) Author: Mohammad Reza Amiri- Nikpour Institution: Seyyed-al-Shohada Heart Centre Email: yousefrezaei1986@gmail.com
Notes	

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Allocation concealment	Unclear risk	Quote: "All patients who met inclusion criteria were randomly assigned into two groups to receive intravenously either 30 mL of cerebrolysin diluted in normal saline once a day for 10 days (n = 23) or normal saline, as placebo, with a prescription order similar to the main drug (n = 23)." Comment: insufficient information to permit judgement of 'low risk' or 'high risk'. The method of concealment was not described
Sequence Generation	Unclear risk	Quote: "In a randomised, double-blinded, placebo-controlled clinical trial, patients who had signs and symptoms of acute brain stroke were assessed from March 2013 to March 2014." Comment: there was no information about the sequence generation

Amiri Nikpour 2014 (*Continued*)

Incomplete outcome data All outcomes	High risk	Quote: "After receiving treatments, one patient in the cerebrolysin-received group and two patients in the placebo-received group died before day 30 (4.3% versus 8.7%); they were excluded from the final analysis due to lack of measuring their outcomes at 90-day follow-up" Comment: adverse events not reported
Blinding of outcome assessors All outcomes	Unclear risk	Comment: there was no information showing if outcome assessors were aware of the allocated interventions
Selective outcome reporting	High risk	Comment: causes of death were not described; no information on clinically relevant outcomes
Blinding of participants and personnel All outcomes	Unclear risk	Quote: "In a randomised, double-blinded, placebo-controlled clinical trial, ..." Comment: there was insufficient information to permit judgement of 'low risk' or 'high risk'
Other sources of bias	Unclear risk	Quote: "We thank the vice-chancellor of research in Urmia University of Medical Sciences for providing the grant of this study. Moreover, we would like to greatly thank all members of emergency department of Imam Khomeini Hospital, Urmia, West Azerbaijan Province, Iran, for helping us in collecting the study data." Comment: there was no clear information on funding sources

CASTA 2012

Methods	Study design: phase IV clinical trial designed as a multicentre, randomised, double-blind placebo-controlled parallel-group study Study grouping: parallel group Losses to follow-up: 180 participants
Participants	Total number of participants: 1070 Baseline characteristics Cerebrolysin • Men: 314 (59.6%) • Mean age: 65.0 years (SD 12.22) • Mean body mass index: 23.7 kg/m^2 (SD 3.04) • Mean time until hospital admission: 5.6 h (SD 3.00)

	• Mean time until start of treatment, calculated from stroke onset: 7.7 h (SD 5.97)
	• Thrombolysis treatment: 50 (9.49%)
	• Prevalence of risk factors: 582
	  ○ Hypertension: 331 (62,8%)
	  ○ Diabetes: 108 (20.5%)
	  ○ Arrhythmia: 71 (13.5%)
	  ○ Coronary heart disease: 72 (13.7%)
	• Baseline efficacy criteria, median (range)
	  ○ NIHSS maximum (range, 0-42 points): 9 (6-33)
	  ○ Barthel Index maximum (range, 0-100 points): 30 (0-100)
	  ○ Modified Rankin Scale maximum (range, 0-6 points): 4 (0-5)
	Placebo
	• Men: 326 (60.4%)
	• Mean age: 65.5 years (SD 11.71)
	• Mean body mass index: 24.0 kg/m^2 (SD 3.20)
	• Mean time until hospital admission: 5.6 h (SD 3.75)
	• Mean time until start of treatment, calculated from stroke onset: 7.6 h (SD 3.69)
	• Thrombolysis treatment: 44 (8.1%)
	• Prevalence of risk factors: 625
	  ○ Hypertension: 332 (61.6%)
	  ○ Diabetes: 117 (21.7%)
	  ○ Arrhythmia: 90 (16.7%)
	  ○ Coronary heart disease: 86 (16%)
	• Baseline efficacy criteria, median (range)
	  ○ NIHSS maximum (range, 0-42 points): 9 (6-26)
	  ○ Barthel Index maximum (range, 0-100 points): 30 (0-100)
	  ○ Modified Rankin Scale maximum (range, 0-6 points): 4 (0-5)
	Inclusion criteria: men and women, aged 18-85 years with focal neurological deficit and a clinical diagnosis of acute hemispheric ischaemic stroke with CT or MRI results compatible with a clinical diagnosis of acute hemispheric stroke, NIHSS score between 6 and 22 (both inclusive), and functionally independent before stroke with a pre-stroke Rankin Scale score of 0 or 1. Randomisation and treatment with the trial medication initiated within 12 h after stroke onset. Signed informed consent was obtained from the participant or the participant's legally accepted representative
	Exclusion criteria: evidence on CT/MRI of intracranial haemorrhage, decreased consciousness (defined as score of $\geq$ 2 on NIHSS Question 1a), neurological signs and symptoms that were likely to resolve completely within 24 h, systolic blood pressure $\geq$ 220 mm Hg or diastolic blood pressure $\geq$ 120 mm Hg on repeated measurement, severe congestive heart failure or presentation with acute myocardial infarction, pre-existing systemic disease significantly limiting life expectancy, concomitant treatment with other neuroprotective or nootropic drugs, and intolerance or contraindication to aspirin or cerebrolysin
	Pretreatment: more participants with diabetes (117 (21.7%) versus 108 (20.5%)); arrhythmia (90 (16.7%) versus 71 (13.5%)); and coronary heart disease (86 (16.0%) versus 72 (13.7%)) in the placebo group

Interventions	Cerebrolysin • Frequency of dosage: daily intravenous infusion of 30 mL cerebrolysin diluted in saline (total of 100 mL) for 10 days starting within 12 h of stroke onset

	• Standard treatment: 100 mg aspirin orally as standard treatment every day Placebo • Frequency of dosage: daily intravenous infusion of placebo (100 mL saline) for 10 days starting within 12 h of stroke onset • Standard treatment: 100 mg aspirin orally as standard treatment every day
Outcomes	• Poor functional outcome defined as death or dependence at the end of the follow-up period (dichotomous outcome) • Early death (dichotomous outcome) • All-cause death (dichotomous outcome) • Adverse effects specially associated with cerebrolysin (dichotomous outcome) • Total number of participants with adverse events (dichotomous outcome) • Serious adverse events (dichotomous outcome)
Identification	Sponsorship source: EVER Neuro Pharma GmbH (Oberburgau 3, Austria) Country: China, Hong Kong, South Korea, Myanmar Setting: inpatient (hospital) Comments: all study authors were closely bound with EVER Neuro Pharma. Dr Heiss is an advisor for the company, Dr Brainin has received financial support from EVER Neuro Pharma, Dr Bornstein is a consultant for EVER Neuro Pharma, Dr Tuomilehto is active in the Speakers Bureau of EVER Neuro Pharma, and Dr Hong received a research grant from EVER Neuro Pharma Authors: Wolf-Dieter Heiss and Zhen Hong Institution: Max-Planck Institut fur Neurologie and Hua Shan Hospital, Department of Neurology Email: wdh@nf.mpg.de; profzhong@sina.com
Notes	

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Allocation concealment	Unclear risk	Quote: "From September 2005 to September 2009, 1070 patients were randomised. Of 1069 patients who received at least 1 infusion of study medication, 529 patients (49.5%) received cerebrolysin and 540 patients (50.5%) placebo" Comment: no description of allocation concealment, we used the published protocol Hong 2009
Sequence Generation	Unclear risk	Quote: "From September 2005 to September 2009, 1070 patients were randomised. Of 1069 patients who received at least 1 infusion of study medication, 529 patients (49.5%) received cerebrolysin and 540 patients (50.5%) placebo"

		Comment: no description of sequence generation, we used the published protocol Hong 2009
Incomplete outcome data All outcomes	High risk	Quote: "to Visit 6 (Day 90). Eighty-nine serious adverse events occurred after start of the treatment (cerebrolysin 50 serious adverse events, placebo 39 serious adverse events). Sixty of 1069 patients sustained fatal adverse events (cerebrolysin 28 patients [5.3%] and placebo 32 patients [5.9%]). Of 1069 patients, 85 patients (8.0%) discontinued the study due to adverse events, 39 patients in the cerebrolysin group" Quote: "Sixty patients died and 890 (83.2% of all randomised patients) completed the 90-day follow-up." Comment: 16.8% of participants were lost to follow-up. However, the proportion of missing outcomes compared with observed event risk was not enough to have a clinically relevant impact on the intervention effect estimate
Blinding of outcome assessors All outcomes	Unclear risk	Quote: "Before unblinding the study, a blind review of the data was performed. The review was within the framework of the requirements of the ICH Guideline E9.17" Quote: "Patients and investigators remained strictly blinded to the treatment assignments, and the occurrence or nature of adverse events did not compromise the blinding either." Comment: impossible to assess blinding by outcome, described as randomised, double-blind placebo-controlled parallel-group study in the study. We used blinding specifics as described in the published protocol Hong 2009
Selective outcome reporting	Unclear risk	Comment: no causes of death were described in the trial report, Kaplan-Meier mortality curve presented only for the subgroup of patients NIH > 12. The study protocol is available and all of the study's pre-specified (primary and secondary) outcomes that are of interest in the review have been reported in the pre-specified way

Blinding of participants and personnel All outcomes	Unclear risk	Quote: "Patients and investigators remained strictly blinded to the treatment assignments, and the occurrence or nature of adverse events did not compromise the blinding either. Missing data were handled according to international standards or guidelines." Comment: we judged this unclear because this statement about strict blinding appeared only in the discussion section of the trial report. Other details were available in the protocol published as Hong 2009, but there was nothing whatsoever on blinding in the methods and/or results sections of the trial report
Other sources of bias	High risk	Quote: "This study was funded by EVER Neuro Pharma GmbH, Oberburgau 3, Austria. The steering committee, safety committee, and other study investigators were working independently. The sponsor assisted in the writing of the protocol, selection of study sites, data collection, and project management. The statistical data analysis was carried out by an independent statistical consultant from Idv Gauting, Germany. The interpretation of results and conclusions are those of the authors, and these and writing of the article were not influenced by the sponsor. The article was reviewed and approved by the independent steering committee and safety committee. The authors received an honorarium related to this work from the sponsor and support for travel." Quote: "Dr Heiss is an advisor for EVER Neuro Pharma and received honoraria for this activity. He is active in the speaker's bureau of EVER Neuro Pharma and CoAxia and he receives support from the Wolf-Dieter Heiss Foundation. Dr Brainin has received financial support for research grants from EVER Neuro Pharma and Boehringer Ingelheim and other research support from the European Research Foundation and Life Science Krems. He is in the speaker's bureau of Allergan, Boehringer Ingelheim, Ferrer, Pfizer, and EVER Neuro

CASTA 2012 (*Continued*)

		Pharma. He is active as a consultant and advisor for Allergan and EVER Neuro Pharma. Dr Bornstein is a consultant for EVER Neuro Pharma and received honoraria for this activity. He is also active in the speaker's bureau of EVER Neuro Pharma. Dr Tuomilehto is active in the speaker's bureau of EVER Neuro Pharma and received honoraria for this activity from EVER Neuro Pharma. Dr Hong received a research grant from EVER Neuro Pharma."

CERE-LYSE-1 2012

Methods	Study design: prospective, randomised, placebo-controlled, double-blind trial Study grouping: parallel group Losses to follow-up: 19

Participants	Baseline Characteristics
	Cerebrolysin
	• Participants: 60
	• Mean age: 65.5 years (SD 11.30)
	• Smokers: 15 (25%)
	• Men: 40 (66.7%)
	• Mean time from first symptoms to rt-PA infusion: 142.4 minutes (SD 27.39)
	• Mean NIHSS Score: 12.3 (SD 5.39)
	• Medical history:
	  ○ Hypertension: 46 (76.7%)
	  ○ Hyperlypoidemia: 20 (33.3%)
	  ○ Arrhythmia: 17 (28.3%)
	  ○ Coronary heart disease: 15 (25%)
	  ○ Obesity: 12 (20%)
	  ○ Diabetes of old age: 10 (16.7%)
	  ○ Earlier TIA: 6 (10.0%)
	• Mean time from first symptoms to hospital admission: 82.6 minutes (SD 38.91)
	• Mean time from first symptoms to rtPA infusion: 142.4 minutes (SD 27.39)
	• Mean time from hospital admission to rtPA infusion: 59.9 minutes (SD 36.59)
	Placebo
	• Participants: 59
	• Mean age: 67.0 years (SD 10.56)
	• Smokers: 12 (20.7%)
	• Men: 37 (62.7%)
	• Mean NIHSS Score: 11.0 (SD 5.44)
	• Medical history:
	  ○ Hypertension: 41 (69.5%)
	  ○ Hyperlypoidemia: 16 (27.1%)
	  ○ Arrhythmia: 17 (28.8%)

	○ Coronary heart disease: 12 (20.3%) ○ Obesity: 9 (15.3%) ○ Diabetes of old age: 7 (11.9%) ○ Earlier TIA: 6 (10.2%) • Mean time from first symptoms to hospital admission: 72.5 minutes (SD 30.86) • Mean time from first symptoms to rtPA infusion: 133.4 minutes (SD 34.37) • Mean time from hospital admission to rtPA infusion: 60.9 minutes (SD 29.04) Inclusion criteria: men and women, 18-80 years, who had a clinical diagnosis of acute ischaemic hemispheric stroke that had commenced within 3 h prior to initiation of administration of rt-PA, and had stroke symptoms being present for at least 30 minutes with no significant improvement before treatment, were eligible (further inclusion and exclusion criteria, see Table 1). All participants had to meet the admission standards of the European Medicines Agency (EMA) consensus criteria for the application of thrombolytic therapy with alteplase (rt-PA): (1) clinical diagnosis of ischaemic stroke causing a measurable neurological deficit defined as impairment of language, motor function, cognition and/or gaze, vision or neglect. Ischaemic stroke is defined as an event characterised by the sudden onset of an acute focal neurologic deficit presumed to be due to cerebral ischaemia after CT scan excluded haemorrhage, (2) informed consent Exclusion criteria: evidence of intracranial haemorrhage on the CT scan; participation in another therapeutic clinical trial 3 months before baseline; people with any history of prior stroke and concomitant diabetes; prior stroke within the last 3 months; platelet count of below 100-103/mm3; blood glucose < 50 or > 400 mg/dL (< 2·77 or > 22·15 mmol/L); known haemorrhagic diathesis; manifest or recent severe or dangerous bleeding; known bacterial endocarditis, pericarditis; acute pancreatitis; documented ulcerative gastrointestinal disease during the last 3 months, oesophageal varices, arterial-aneurysm, arterial/venous malformation; neoplasm with increased bleeding risk; severe liver disease, including hepatic failure, cirrhosis, portal hypertension, oesophageal varices, and active hepatitis; major surgery or significant trauma in past 3 months; multiple serious drug allergies; hypersensitivity or allergy to 1 of the components of the drug; severe renal impairment; systolic blood pressure > 185 mmHg or diastolic blood pressure > 110 mmHg, or aggressive management (intravenous medication repeatedly) necessary to reduce BP to these limits; recent (less than 10 days) traumatic external heart massage, obstetrical delivery, recent puncture of a non-compressible blood vessel (e.g. subclavian or jugular vein puncture); chronic intoxication or chronic substance use disorder with pharmaceuticals, drugs, alcohol, or industrial poisons; symptoms of ischaemic attack began more than 3 h prior to start of thrombolytic therapy or if time of symptom onset is unknown; minor neurological deficit or symptoms rapidly improving before start of infusion; severe stroke as assessed clinically (e.g. NIHSS > 25) and/or by appropriate imaging techniques; epilepsy; symptoms suggestive of subarachnoid haemorrhage, even if the CT scan is normal; known history of or suspected intracranial haemorrhage; suspected subarachnoid haemorrhage or condition after subarachnoid haemorrhage from aneurysm; any history of central nervous system damage (i.e. neoplasm, aneurysm, intracranial or spinal surgery); haemorrhagic retinopathy, e.g. in diabetes (vision disturbances may indicate haemorrhagic retinopathy); administration of heparin within the previous 48 h and a thromboplastin time exceeding the upper limit of normal for laboratory; people receiving oral anticoagulants, e.g. warfarin, sodium; people receiving nifedipine for acute treatment Pretreatment: the 2 groups were well balanced with respect to baseline prognostic variables and no significant differences between treatment groups were observed

CERE-LYSE-1 2012 (*Continued*)

Interventions	Cerebrolysin • Frequency of dosage: once daily for 10 consecutive days: intravenous infusion of 30 mL of cerebrolysin diluted with 70 mL of 0.9% physiological saline to a total volume of 100 mL. Cerebrolysin starting immediately 1 h after thrombolytic treatment • Standard treatment: the thrombolytic therapy with rt-PA was administered as intravenous infusion over 60 min. Immediately thereafter, the first intravenous infusion of the study medication (cerebrolysin/placebo) was administered over a time period of 30 min Placebo • Frequency of dosage: once daily for 10 consecutive days: an identical amount of physiological saline (100 mL) was used as placebo • Standard treatment: the thrombolytic therapy with rt-PA was administered as intravenous infusion over 60 min. Immediately thereafter, the first intravenous infusion of the study medication (cerebrolysin/placebo) was administered over a time period of 30 min
Outcomes	• Poor functional outcome defined as death or dependence at the end of the follow-up period (dichotomous outcome) • Early death (dichotomous outcome) • All-cause death (dichotomous outcome) • Serious adverse events (dichotomous outcome) • Adverse effects specially associated with cerebrolysin (dichotomous outcome) • Total number of participants with adverse events (dichotomous outcome)
Identification	Sponsorship source: not mentioned at all. Only the Conflict of Interest statement: Wilfried Lang has served as consultant for Bayer, Boehringer Ingelheim, EVER, MSD, Sanofi-Aventis and Pfizer and has received speaking honoraria from these companies. Christian Stadler has received speaker honoraria from EVER. Zdavka Poljakovic received Principal Investigator fee for the clinical study. David Fleet is a freelance consultant statistician undertaking statistical contracts on behalf of pharmaceutical/biotechnology organizations and as such was contracted by EVER. All authors have no other financial interest in the company or its products Country: 5 countries: Austria, Croatia, Czech Republic, Slovakia, Slovenia Setting: inpatient (hospital) Author: Wilfried Lang Institution: Department of Neurology, Hospital St. John, Austria Email: wilfried.lang@bbwien.at
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment	Unclear risk	Quote: "The vials containing the study drug and the placebo were visually identical." Comment: no mention of allocation concealment

CERE-LYSE-1 2012 (*Continued*)

Sequence Generation	Unclear risk	Quote: "according to a pre-compiled 1:1 randomization schedule, stratified by centre."
Incomplete outcome data All outcomes	High risk	Quote: "Two patients received the incorrect study medication assignment." Quote: "Based on statistical information from the third interim analysis, it was decided to terminate the study, as no significant result for the main outcome criteria was expected to be reached." Quote: "All patients were included in the ITT population with 60 patients being assigned to cerebrolysin and 59 assigned to placebo. In the PP population, 100 patients were included with 49 receiving cerebrolysin and 51 receiving placebo (Fig. 1). " Comment: 19 participants of 119 (16%) were lost to follow-up. Attrition bias. Impossible to access by outcome
Blinding of outcome assessors All outcomes	Unclear risk	Quote: "All study personnel and participants were blinded to treatment assignment for the duration of the study." Comment: insufficient information to permit judgement about blinding by outcomes
Selective outcome reporting	High risk	Quote: "one adverse event. There were no obvious differences between either treatment arms. In each treatment group, four patients died, but in none of the cases was any relationship to the study medication seen. The number of patients with serious adverse events was slightly higher in the cerebrolysin group compared to the placebo group (12 vs. 7, respectively). In total, 19 (16%) patients experienced at least one serious adverse event (Table 5). " Comment: the study was stopped because of no significant result for the main outcome criteria. According to the study authors, there was no causal relationship to the study drug for any of the deaths observed. Neither reasons of death, nor timing of death is presented. Timing of adverse events, serious adverse events not presented

CERE-LYSE-1 2012 (*Continued*)

Blinding of participants and personnel All outcomes	Unclear risk	Quote: "All study personnel and participants were blinded to treatment assignment for the duration of the study." Comment: insufficient information to permit judgement about blinding by outcomes
Other sources of bias	High risk	Quote: "Ljubljana, Ljubljana/Slovenia) ClinicalTrials.gov identifier: NCT00840671 Con icts of interest: Wilfried Lang has served as consultant for Bayer, Boehringer Ingelheim, EVER, MSD, Sano -Aventis and P zer and has received speaking honoraria from these companies. Christian Stadler has received speaker honoraria from EVER. Zdavka Poljakovic received Principal Investigator fee for the clinical study. David Fleet is a freelance consultant statistician undertaking statistical contracts on behalf of pharmaceutical/ biotechnology organizations and as such was contracted by EVER. All authors have no other financial interest in the company or its products. DOI: 10.1111/j.1747-4949.2012.00901.x Research © 2012" Comment: no information on funding sources for the trial. Statistician was contracted by EVER - the manufacturer of cerebrolysin. There is no information about the provider of cerebrolysin

Ladurner 2005

Methods	Study design: multicentre, randomised, double-blind controlled trial Mean duration of follow-up: 90 days Study grouping: parallel group
Participants	Baseline characteristics Cerebrolysin • Age: 65 years ± 1.17 • Men: 47 (60.3%) • Women: 31 (39.7%) • Total number: 78 • Handedness: left: 1 (1.3%); right: 77 (98.7%) • Stroke location: left hemisphere: 41 (52.6%); right hemisphere: 37 (47.4%) • Duration of symptoms (values are means ± SEM): 12.3 h ± 0.73 • CNS (values are means ± SEM): 6.88 ± 0.09

	• GCS (values are means ± SEM): 14.1 ± 0.20 Placebo • Age: 65 years ± 1.32 • Male : 38 (55.9%) • Female : 30 (44.1%) • Total number: 68 • Handedness: left: 0 (0%); right: 68 (100%) • Stroke location: left hemisphere: 31 (45.6%); right hemisphere: 37 (54.4%) • Duration of symptoms (values are means ± SEM): 13.5 h ± 1.16 • CNS (values are means ± SEM): 6.68 ± 0.14 • GCS (values are means ± SEM): 14.4 ± 0.16 Inclusion criteria: men and women suffering from their first acute ischaemic stroke with clinical symptoms of middle cerebral artery area were enrolled in this study. Patients were eligible if they were admitted to the hospital and received the first dose of study medication within 24 h of the onset of the stroke and were between 45 and 85 years of age at study entry. Participants were also required to have a GCS score of greater than 10 and a CNS score between 4.5-8.0 at baseline Exclusion criteria: people with haemorrhagic strokes, transient ischaemic attacks, uncontrollable hypertension, acute myocardial infarction, congestive heart failure, moderate-severe dementia prior to the stroke, coma or stupor, other severe concomitant diseases, impaired renal function, and people with a history of prior stroke Pretreatment: no significant group differences of the demographic characteristics were observed at baseline and the severity of the stroke at study entry was comparable between the 2 groups
Interventions	Cerebrolysin • Frequency of dosage: cerebrolysin 50 mL was administered once daily for 21 days by intravenous infusion in a peripheral vein over a period of 20 min. Cerebrolysin mixed with 50 mL of normal saline • Standard treatment: pentoxifylline (300 mg/day, intravenous) and acetylsalicylic acid (250 mg/day, orally) for the first 21 days and pentoxifylline (2400 mg/day, orally) and acetylsalicylic acid (250 mg/day, orally) from day 22 to the end of the study at day 90 Placebo • Frequency of dosage: placebo was administered once daily for 21 days by intravenous infusion in a peripheral vein over a period of 20 min. Placebo contained 100 mL of normal saline • Standard treatment: pentoxifylline (300 mg per day, intravenous) and acetylsalicylic acid (250 mg/day, orally) for the first 21 days and pentoxifylline (2400 mg/day, orally) and acetylsalicylic acid (250 mg/day, orally) from day 22 to the end of the study at day 90
Outcomes	• Poor functional outcome defined as death or dependence at the end of the follow-up period (dichotomous outcome) • Early death (dichotomous outcome) • All-cause death (dichotomous outcome) • Serious adverse events (dichotomous outcome) • Adverse effects specially associated with cerebrolysin (dichotomous outcome) • Total number of participants with adverse events (dichotomous outcome)

Ladurner 2005 (*Continued*)

Identification	Sponsorship source: EBEWE Pharma Country: Austria, Czech Republic, and Hungary Setting: inpatient (hospital) Authors: Dr G Ladurner and H Moessler Institution: Department of Neurology, Christian-Doppler Hospital, Salzburg, Austria Email: g.ladurner@lks.at and herbert.moessler@ebewe.com
Notes	Population: concomitant use of nootropic drugs (e.g. piracetam), drugs with dilatating effects on peripheral blood vessels (naftidrofuryl, cinnarizine, flunarizine, nimodipine), as well as chronic intake of anti-depressants, tranquillizers, sedatives or CNS stimulants was prohibited throughout the study

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Allocation concealment	Low risk	Quote: "For each patient a sealed envelope with information on the actual treatment dispensed was provided to the investigator for emergency cases. All envelopes remained sealed throughout the study." Comment: sealed envelopes were used to conceal allocation
Sequence Generation	Low risk	Quote: "Patients who met all entry criteria were assigned to the treatment groups in a 1:1 ratio, according to a randomisation code generated by a computer software (EBEWE Pharma, Unterach, Austria). The randomisation was carried out in blocks of 12 patients, stratified by study centre." Comment: the computer software used to generate random numbers was provided by EBEWE Pharma which is also the provider of cerebrolysin, which contributes to the other sources of bias
Incomplete outcome data All outcomes	High risk	Quote: "146 patients were randomised to two treatment groups and constituted the ITT population: 78 patients to the cerebrolysin group and 68 patients to the placebo group. Of these patients, 67 of the cerebrolysin group and 52 of the placebo group completed the study. Reasons for the 25 cases of study discontinuation were death (6 cerebrolysin, 6 placebo), serious adverse event (1 placebo), and consent withdrawn (3 cerebrolysin; 9 placebo)."

		Comment: attrition bias: 25 participants out of 146 randomised were lost to follow-up (17%). Information on the outcomes of interest to this review was available only for serious adverse events including death
Blinding of outcome assessors All outcomes	Unclear risk	Comment: there was no information about blinding of outcome assessment concerning efficacy assessments. Study authors stated that safety measures were done under blinded conditions but there were no more details about the protocol used. Impossible to assess blinding by outcomes
Selective outcome reporting	High risk	Quote: "Twelve patients died during the study: 6 in the cerebrolysin group (7.69%) and 6 in placebo group (8.83%). None of the deaths was reportedly related to the study drug administration." Quote: "With the exception of one SAE (hematemesis) in the placebo group which was rated to be likely related to the study drug, there was no causal relationship to the study drug for any other of the SAEs, as per the investigator's assessment." Comment: the trial authors did not report on the time when deaths occurred, and did not assess potential causality with administered medicines
Blinding of participants and personnel All outcomes	Unclear risk	Quote: "The investigators and all other study personnel were blind as to the random code assignment until the completion of the statistical analysis." Comment: impossible to assess blinding by outcomes
Other sources of bias	High risk	Quote: "The participants of the cerebrolysin study group were as follows: G. Ladurner, Christian-Doppler Clinic, Salzburg, Austria; K. Niederkorn, University Hospital for Neurology, Graz, Austria; I. Szirmai, Semmelweis University of Medicine, Budapest, Hungaria; P. Kalvach, Charles University, FNKV, Department of Neurology, Prague; F. Stockenhuber, Landeskrankenhaus, Oberpullendorf, Austria; Z. Haffner, Petz Alada ´ Megyei Koorha'z, Gyoor, Hungaria; P. Ridzon, Thomayer's

Ladurner 2005 (*Continued*)

		Hospital, Praha, Czech Republic; E. Diabl, Linz General Hospital, Linz, Austria." Quote: "The study medication was provided to the study centres by EBEWE Pharma in the form of a ready-to-use infusion solution. The active medication contained 50 mL cerebrolysin mixed with 50 mL of normal saline." Comment: there was no information on funding sources for the trial and no conflict of interest statement was provided. EBEWE Pharma provided the medication and randomisation codes

Skvortsova 2004

Methods	Study design: RCT Study grouping: parallel group
Participants	Cerebrolysin • Participants: 12 • Men: 6 • Women: 6 • Mean age: 68.7 years ± 10.6 • Ratio of participants with lesions of the left and right hemispheres: 8/4 • Period since the stroke to admission in hospital: 9.2 h ± 2.9 • NIH score prior to intervention: 11.2 ± 4.7 • Rankin score prior to intervention: 3.5 ± 1.1 • Number of participants with a NIH score: more than 14 (severe stroke): 3 (25%); 14 and less: 9 (75%) • Average volume of brain lesions: 17.5 cm^3 ± 14.7 • Number of participants with a lesion volume between 7 $cm^3$and 64 cm^3: 8 Placebo • Participants: 12 • Men: 9 • Women: 3 • Mean age: 69.4 years ± 9.5 • Ratio of participants with lesions of the left and right hemispheres: 8/4 • Period since the stroke to admission in hospital: 8.6 h ± 2.9 • NIH score prior to intervention: 12.2 ± 2.8 • Rankin score prior to intervention: 3.8 ± 0.9 • Number of participants with a NIH score more than 14 (severe stroke): 3 (25%); 14 and less: 9 (75%) • Average volume of brain lesions: 21.7 cm^3± 23.1 • Number of participants with a lesion volume between 7 cm^3 and 64 cm^3: 7 Inclusion criteria: people with the first in life-time ischaemic stroke in the basin of internal carotid artery, aged 45-85 years, admitted to the ICU within 12 h of stroke symptoms onset

	Exclusion criteria: disappearance of symptoms within 4 h from the beginning of stroke; people with haemorrhagic stroke or stroke in the vertebrobasilar system; people with blood pressure levels higher than 200/100 mmHg; people with acute myocardial infarction, with a priori severe dementia; pregnant women and participants in other studies Pretreatment: no difference
Interventions	Cerebrolysin • Frequency of dosage: diluted with 40 mL of saline infused by slow drip over 1 h for 10 days after stroke onset (within 12 h) • Standard treatment: aspirin 100 mg/day, haemodilution, pentoxifylline, heparin (when needed) Placebo • Frequency of dosage: physiological saline • Standard treatment: aspirin 100 mg/day, haemodilution, pentoxifylline, heparin (when needed)
Outcomes	• Poor functional outcome defined as death or dependence at the end of the follow-up period (dichotomous outcome) • Early death (dichotomous outcome) • All-cause death (dichotomous outcome) • Serious adverse events (dichotomous outcome) • Adverse effects specially associated with cerebrolysin (dichotomous outcome) • Total number of participants with adverse events (dichotomous outcome)
Identification	Sponsorship source: not reported Country: Russia Setting: inpatient Authors name: Skvortsova Institution: Department of basic and clinical neurology, Russian State medical University Address: Moscow
Notes	

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Allocation concealment	Unclear risk	Quote: "Всем пациентам рандомизированно и вслепую было назначено плацебо или церебролизин в дозе 10 либо 50 мл (по 12 человек в каждой группе)." ["All patients were randomly and blindly assigned to placebo or cerebrolysin at 10 or 50 mL (12 in each group)."] The method of concealment was not described
Sequence Generation	Unclear risk	Comment: there was no information about the process of the generation of a ran-

		domised sequence. Randomisation mentioned: "all patients randomly and blindly were given either placebo, or cerebrolysin"
Incomplete outcome data All outcomes	Unclear risk	Comment: information on the outcomes that are of interest in the review was available only for deaths. No losses to follow-up, the causes of death described in detail, although precise timing of each death was not provided
Blinding of outcome assessors All outcomes	Unclear risk	Comment: No information about the blinding of outcome assessors was provided
Selective outcome reporting	Unclear risk	Quote: "причины смерти 3 из 5 больных, получавших церебролизин, а также одного пациента из группы плацебо не были связаны с инсультом (тромбоэмболия легочной артерии, пневмония, пиелонефрит)". ["The causes of death for 3 of 5 patients who received cerebrolysin and 1 patient in the placebo group were not associated with stroke (pulmonary embolism, pneumonia, pyelonephritis)".] The time when deaths occurred was not reported. Furthermore, study authors considered that deaths were not drug-related. Adverse events were not reported. For the outcomes presented in a table and a graph the timing was not clear, although these are not the outcomes of interest for the review
Blinding of participants and personnel All outcomes	Unclear risk	Quote: "Всем пациентам рандомизированно и вслепую было назначено плацебо или церебролизин в дозе 10 либо 50 мл (по 12 человск в каждой группе)". ["All patients were randomly and blindly assigned to placebo or cerebrolysin at 10 or 50 mL (12 in each group)."] There was no information about blinding of personnel. No description of blinding. The text states: "all patients randomly and blindly were given either placebo, or cerebrolysin"
Other sources of bias	Unclear risk	Comment: no information on funding sources for the trial and no conflict of interest statement was provided

Xue 2016

Methods	Study design: RCT Study grouping: parallel group
Participants	Cerebrolysin • Participants: 20 • Age: 66.5 years (SD ± 8.1) • Men: 9 • Women: 11 • Time until admission: 5 h (SD ± 3.3) • Time until treatment: 7.6 h (SD: ± 3.6) • Systolic blood pressure: 150.7 mmHg (SD ± 13.7) • Diastolic blood pressure: 85.1 mmHg (SD ± 13.6) • Thrombolysis treatment: 7 (35%) • Previous history: hypertension: 6 (30%); diabetes: 7 (35%); coronary heart disease: 8 (40%) • NIHSS Score: 10.6 (SD ± 4.75) • Barthel Index Score: 22.25 (SD ± 7.16) Placebo • Participants: 20 • Age: 68.4 years (SD ± 4.2) • Men: 10 • Women: 10 • Time until admission: 4.8 h (SD ± 3.7) • Time until treatment: 5.6 (SD ± 3.0) • Systolic blood pressure: 152.5 mmHg (SD ± 12.8) • Diastolic blood pressure: 87.2 mmHg (SD ± 12.5) • Thrombolysis treatment: 6 (30%) • Previous history: hypertension: 10 (50%); diabetes: 6 (30%); coronary heart disease: 9 (45%) • NIHSS Score: 10.20 (SD ± 3.72): • Barthel Index Score: 20.0 (SD ± 6.96) Other neuroprotective agent • Participants: 20 • Age: 67.1 years (SD ± 6.3) • Men: 9 • Women: 11 • Time until admission: 5.4 h (SD ± 3.0) • Time until treatment: 7.7 h (SD ± 5.9) • Systolic blood pressure: 148.6 mmHg (SD ± 14.6) • Diastolic blood pressure: 88.7 mmHg (SD ± 10.7) • Thrombolysis treatment: 5 (25%) • Previous history: hypertension: 7 (35%); diabetes: 8 (40%); coronary heart disease: 6 (30%) • NIHSS Score: 12.4 (SD ± 4.38) • Barthel Index Score: 19.75 (SD ± 6.38) Inclusion criteria: participants included in the study suffered from acute ischaemic stroke for the first time < 12 h prior to entry into the study, and had a score of 6-25 on the NIHSS. Prior to randomisation, all participants were evaluated using cranial CT or MRI scanning and were followed with serial neurological examinations to confirm acute

Xue 2016 (*Continued*)

	ischaemic stroke Exclusion criteria: people with lacunar infarction, cerebral haemorrhagic infarction, epilepsy or epileptic seizures, history of neurological diseases, myocardial infarction, renal and hepatic abnormalities, metabolic diseases, and contraindications to antiplatelet treatments Pretreatment: comparison of baseline characteristics among the treatment groups revealed no significant differences (P > 0.05)
Interventions	Cerebrolysin • Frequency of dosage: intravenous infusion of 30 mL cerebrolysin/day in 100 mL normal saline for 10 days, the infusion lasted 50-70 min • Standard treatment: routine treatments including antithrombotic drugs, hypoglycaemic agents, antilipaemic agents, antihypertensive(s) and dehydration, according to guidelines for the management of ischaemic stroke in the neurological ICU (14); 100 mg aspirin orally as standard treatment Placebo • Frequency of dosage: 100 mL saline intravenous infusion once daily for 10 days • Standard treatment: routine treatments including antithrombotic drugs, hypoglycaemic agents, antilipaemic agents, antihypertensive(s) and dehydration, according to guidelines for the management of ischaemic stroke in the neurological ICU (14); 100 mg aspirin orally as standard treatment Other neuroprotective agent • Frequency of dosage: intravenous infusion of 100 mL NBP and sodium chloride injection, which contained 25 mg NBP and 0.9 g sodium chloride, twice daily during 10 days starting within 12 h after stroke onset • Standard treatment: routine treatments including antithrombotic drugs, hypoglycaemic agents, antilipaemic agents, antihypertensive(s) and dehydration, according to guidelines for the management of ischaemic stroke in the neurological ICU (14); 100 mg aspirin orally as standard treatment
Outcomes	• Poor functional outcome defined as death or dependence at the end of the follow-up period (dichotomous outcome) • Early death (dichotomous outcome) • All-cause death (dichotomous outcome) • Serious adverse events (dichotomous outcome) • Adverse effects specially associated with cerebrolysin (dichotomous outcome) • Total number of participants with adverse events (dichotomous outcome)
Identification	Sponsorship source: this study was supported by the Shanghai Jiao Tong University Affiliated Sixth People's Hospital (grant nos. 1462 and 1583) and the Shanghai Science and Technology Council (grant no. 13411951401) Country: China Setting: quote: "from January 2010 to May 2010, a randomised, double- blind trial was conducted, which involved patients with acute ischaemic stroke in the neurology ward of Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Shanghai, China)" Comments: there were 3 treatment groups: NBP, cerebrolysin, or placebo. The review authors could not understand the number randomised and evaluated, and thus we think that numerical results are meaningless for the review purposes Authors name: Dr Hao Chen

Xue 2016 (*Continued*)

	Institution: Department of Neurosurgery, Shanghai Jiao Tong University, Affiliated Sixth People's Hospital Email: chenhao_316@aliyun.com
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Allocation concealment	Unclear risk	Quote: "The random numbers were placed in concealed envelopes." Comment: concealed envelopes: not clear concealed by whom and from whom and who might have a chance to get to the envelopes
Sequence Generation	Low risk	Quote: "Patients were randomly assigned to the NBP group, cerebrolysin group or placebo group." Quote: "Randomization was performed by means of computer- generated numbers through software by a third party who was not involved in patient management." Comment: The investigators describe a random component (computer random-number generator) in the sequence generation process. Unclear who the third party was
Incomplete outcome data All outcomes	High risk	Quote: "During the trial period, 84 patients with AIS underwent randomization. Among these, 60 patients who received study intervention were included in the efficacy analysis. The NBP group contained 9 male and 11 female patients, whose ages ranged from 53 to 79 years. The cerebrolysin group contained 9 males and 11 females, and their ages ranged from 54 to 85 years. The placebo group contained 10 males and 10 females, whose ages were from 52 to 87 years." Comment: 84 - 60 = 24, which is 29% of randomised participants were lost in the trial report, no description of why only rounded numbers 20, 20 and 20 were included in any data presentation

Xue 2016 (*Continued*)

Blinding of outcome assessors All outcomes	Unclear risk	Quote: "Patients and methods: patient selection. From January 2010 to May 2010, a randomised, double- blind trial was conducted, which involved patients with AIS in the Neurology Ward of Shanghai Jiao Tong University Affiliated Sixth People's Hospital (Shanghai, China)." Comment: impossible to assess blinding by outcomes, no description of blinding at all. Insufficient information to permit judgement of 'low risk' or 'high risk'
Selective outcome reporting	High risk	Quote: "Missing values were substituted by last observation carried forward. P < 0.05 was considered to indicate a statistically significant result." Comment: 84 - 60 = 24, which is 29% of randomised participants were lost in the trial report, no description of why only rounded numbers 20, 20 and 20 were included in any data presentation
Blinding of participants and personnel All outcomes	Unclear risk	Quote: "a randomised, double- blind trial was conducted, ..." Comment: no description of blinding, impossible to assess blinding by outcomes
Other sources of bias	Unclear risk	Comment: no conflict of interest statement was provided

ACE: angiotensin converting enzyme
AIS: acute ischaemic stroke
CNS: central nervous system
CT: computed tomography
GCS: Glasgow Coma Score
h: hour/s
ICU: intensive care unit
MRI: magnetic resonance imaging
NBP: DL-3-n-butylphthalide
NIHSS: National Institutes of Health Stroke Scale
RCT: randomised controlled trial
rt-PA: recombinant tissue plasminogen activator
SD: standard deviation
SEM: standard error of the mean

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Barolin 1996	Wrong study design, not an RCT
Bavarsad Shahripour 2011	Reported as an abstract only; wrong patient population: the time-window not specified (review protocol specifies that symptom onset should be less than 48 h from the onset of stroke)
Bayer 1980	Wrong study design, not an RCT
Belkin 2011	Wrong question and patient population: neuroprotective drug efficiency in people after ischaemic stroke; people from 3-6 months after ischaemic stroke
Belkin 2015	Wrong question and patient population: effect of cerebrolysin at the level of paresis; the time from the stroke onset to the introduction of the drug was 72 h
Chang 2016	Wrong question and patient population; cerebrolysin started within 7 days after stroke onset
Cuparnecu 2001	Reported as an abstract only, no further full-text publications; no follow-up data
Domzal 1995	Wrong study design, not an RCT
E-COMPASSII 2016	Wrong question and patient population: effects of cerebrolysin on motor recovery in people with severe motor involvement at subacute phase of stroke; subacute stage (less than 1 week after stroke)
Ershov 2011	Wrong study design: no randomisation
Guekht 2015	Reported as an abstract only; not an RCT, a meta-analysis of CARS1 and CARS2
Guekht 2015a	Reported as an abstract only; wrong question and population: early rehabilitation after stroke
Haffner 2001	Reported as an abstract only; efficacy assessment with stroke scales; no information on death
Jianu 2010	Wrong study design: randomisation not described; therapeutic time-window was 72 h (review protocol specifies 48 h)
Jianu 2015	Wrong study design: no randomisation, therapeutic time-window was 72 h
Kim 2014	Reported as an abstract only. Not a relevant condition - subacute stroke; treatment initiated after 8 days of stroke onset
Kim 2015	Reported as an abstract only. Cerebrolysin given 7 days after stroke onset
Martinez Sanchez 2015	Wrong study design; not an RCT: "Open label, one arm, and dose decreasing exploratory study"
Muresanu 2016	Wrong question and wrong timing of cerebrolysin initiation after stroke onset

(*Continued*)

Pushkarev 2015	Wrong study design; not an RCT: "An analysis of 42 case histories of patients from the period 2000 to 2014 with the diagnosis of lacunar stroke who were hospitalised in a stroke center."
Shamalov 2005	Wrong question: effects on infarct volume after acute ischaemic stroke
Shamalov 2010	Wrong question: change in stroke volume of lesion detected by MRI, "Effect of cerebrolysin at a dose of 50 mL on morphometric picture of brain damage in ischemic stroke"
Shishkova 2015	Wrong question; wrong population: "60 patients with hand paresis and 60 with aphasia were randomly assigned to treatment with cerebrolysin (25 mL/daily) or placebo group (which received saline infusions) "
Skvortsova 2006	Wrong study design: no randomisation
Stan 2013	Wrong question: change in stroke volume
Vilenskii 1999	Wrong study design, not an RCT
Yavorskaya 2008	Wrong patient population, wrong question: participants with cognitive disorders
Zhu 2003	Wrong question and population: cerebrolysin used in people with stroke episode duration of 28 ± 7 days; efficacy assessment with stroke scales only

MRI: magnetic resonance imaging
NIHSS: National Institutes of Health Stroke Scale
RCT: randomised controlled trial

Characteristics of studies awaiting assessment *[ordered by study ID]*

Dobi 2010

Methods	RCT
Participants	124 participants with acute stroke were randomised within 24 h of stroke onset
Interventions	Cerebrolysin 10 mL in 100 mL NaCl 0.9% for 5 days + aspirin protect 100 mg/day
Outcomes	Barthel Index scores to evaluate the participants pre- and post-treatment
Notes	

Hong 2005

Methods	RCT
Participants	287 participants with acute ischaemic stroke in the carotid artery territory
Interventions	0.9% sodium chloride injection 500 mL containing cerebrolysin 50 mL for 10 days
Outcomes	NIH stroke scores on day 11, 21, and 28
Notes	

IRCT138803272042N1 2014

Methods	Randomised double-blind trial
Participants	100 participants, both male and female, aged 45-85 years, the occurrence of acute cerebral ischaemic attack (embolic or thrombotic), hospitalisation during 12 h of first symptoms of stroke, systolic blood pressure < 200 and diastolic < 100 mmHg Exclusion criteria: recovery of neurologic symptoms after 4 h of attack, haemorrhagic stroke or the occurrence of stroke in vertebrobasilar system with blood pressure ≥ 200/100 mmHg, seizures, papilledema or rising intracranial pressure (RICP), neck stiffness or symptoms of brain stimulation, the condition of consciousness stupor and coma (GCS ≤ 6), acute myocardial infarction, NIHSS < 7 and > 24, hepatic or renal failure, heart failure, dementia, acute infectious disease, doubt the involvement in subsequent brain area (posterior circulation), pregnant women, symptoms of progressive neurological defects, people who are in other trials, people who received piracetam or calcium channel blockers, people who received rt-PA treatment during first 4 h of symptoms
Interventions	Cerebrolysin (30 mg for first 5 days during the first week and 10 mg for first 5 days in the second, third and fourth weeks) adding to routine therapy
Outcomes	Clinical evaluation of motor ability (speech and motor ability of participants) daily; modified Rankin Scale, NIHSS; improvement in participants' understanding during the treatment on days 3, 7, 15, and 30; assessment of Clinical Global Impression Scale, Patient Global Satisfaction Score and Mini Mental State Examination by neurologist
Notes	

Nazarbaghi 2014

Methods	RCT
Participants	Ischaemic stroke patients admitted to URMIA Emam Khomeini educational medical centre, based on inclusion and exclusion criteria
Interventions	Cerebrolysin (30 mL) injection for 10 days added to standard treatment (antiplatelet, anticoagulant), and control group receiving placebo
Outcomes	NIHSS score criteria at 3 months and at baseline (day 1) and days 30, 60, and 90
Notes	

Skvortsova 2008

Methods	Unclear
Participants	People with acute ischaemic stroke
Interventions	Cerebrolysin
Outcomes	MRI infarct volume as efficacy measure
Notes	

GCS: Glasgow Coma Score
MRI: magnetic resonance imaging
NIHSS: National Institutes of Health Stroke Scale
RCT: randomised controlled trial
rt-PA: recombinant tissue plasminogen activator

DATA AND ANALYSES

Comparison 1. Cerebrolysin versus placebo

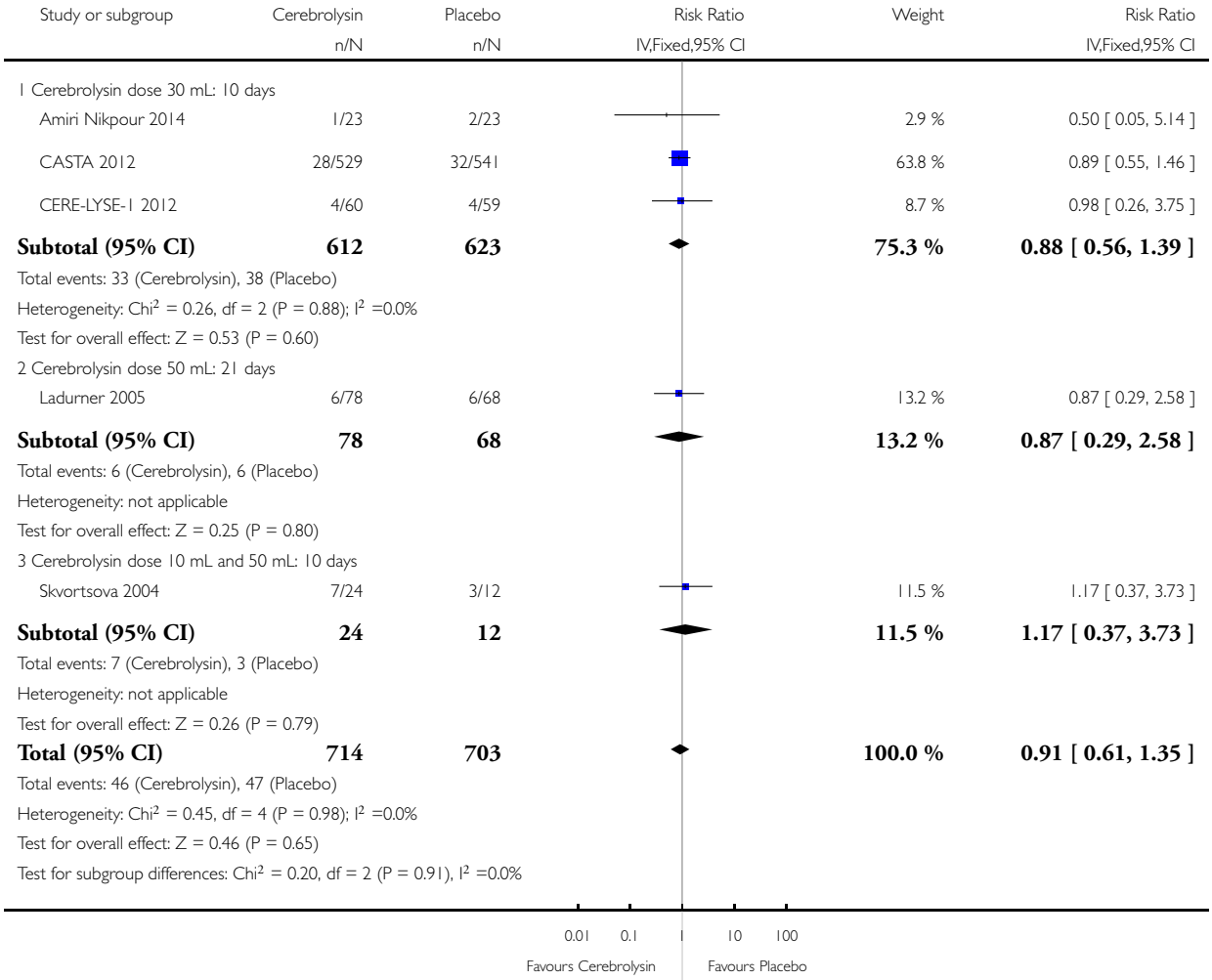
Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 All-cause death	5	1417	Risk Ratio (IV, Fixed, 95% CI)	0.91 [0.61, 1.35]
1.1 Cerebrolysin dose 30 mL: 10 days	3	1235	Risk Ratio (IV, Fixed, 95% CI)	0.88 [0.56, 1.39]
1.2 Cerebrolysin dose 50 mL: 21 days	1	146	Risk Ratio (IV, Fixed, 95% CI)	0.87 [0.29, 2.58]
1.3 Cerebrolysin dose 10 mL and 50 mL: 10 days	1	36	Risk Ratio (IV, Fixed, 95% CI)	1.17 [0.37, 3.73]
2 Total number of people with serious adverse events (SAEs)	3	1335	Risk Ratio (IV, Fixed, 95% CI)	1.16 [0.81, 1.67]
2.1 Cerebrolysin dose 30 mL: 10 days	2	1189	Risk Ratio (IV, Fixed, 95% CI)	1.23 [0.84, 1.81]
2.2 Cerebrolysin dose 50 mL: 21 days	1	146	Risk Ratio (IV, Fixed, 95% CI)	0.75 [0.26, 2.12]
3 Total number of people with fatal SAEs	3	1335	Risk Ratio (IV, Fixed, 95% CI)	0.90 [0.59, 1.38]
3.1 Cerebrolysin dose 30 mL: 10 days	2	1189	Risk Ratio (IV, Fixed, 95% CI)	0.90 [0.57, 1.44]
3.2 Cerebrolysin dose 50 mL: 21 days	1	146	Risk Ratio (IV, Fixed, 95% CI)	0.87 [0.29, 2.58]
4 Total number of people with non-fatal SAEs	3	1335	Risk Ratio (IV, Random, 95% CI)	2.47 [1.09, 5.58]
4.1 Cerebrolysin dose 30 mL: 10 days	2	1189	Risk Ratio (IV, Random, 95% CI)	2.86 [1.23, 6.66]
4.2 Cerebrolysin dose 50 mL: 21 days	1	146	Risk Ratio (IV, Random, 95% CI)	0.29 [0.01, 7.03]
5 Total number of people with adverse events	3	1335	Risk Ratio (IV, Random, 95% CI)	0.97 [0.86, 1.09]
5.1 Cerebrolysin dose 30 mL: 10 days	2	1189	Risk Ratio (IV, Random, 95% CI)	0.96 [0.86, 1.06]
5.2 Cerebrolysin dose 50 mL: 21 days	1	146	Risk Ratio (IV, Random, 95% CI)	1.62 [0.69, 3.82]
6 All-cause death sensitivity	5	1417	Risk Ratio (IV, Fixed, 95% CI)	0.91 [0.61, 1.35]
6.1 High risk of bias	4	1381	Risk Ratio (IV, Fixed, 95% CI)	0.88 [0.58, 1.34]
6.2 Unclear risk of bias	1	36	Risk Ratio (IV, Fixed, 95% CI)	1.17 [0.37, 3.73]

Analysis 1.1. Comparison 1 Cerebrolysin versus placebo, Outcome 1 All-cause death.

Review: Cerebrolysin for acute ischaemic stroke

Comparison: 1 Cerebrolysin versus placebo

Outcome: 1 All-cause death

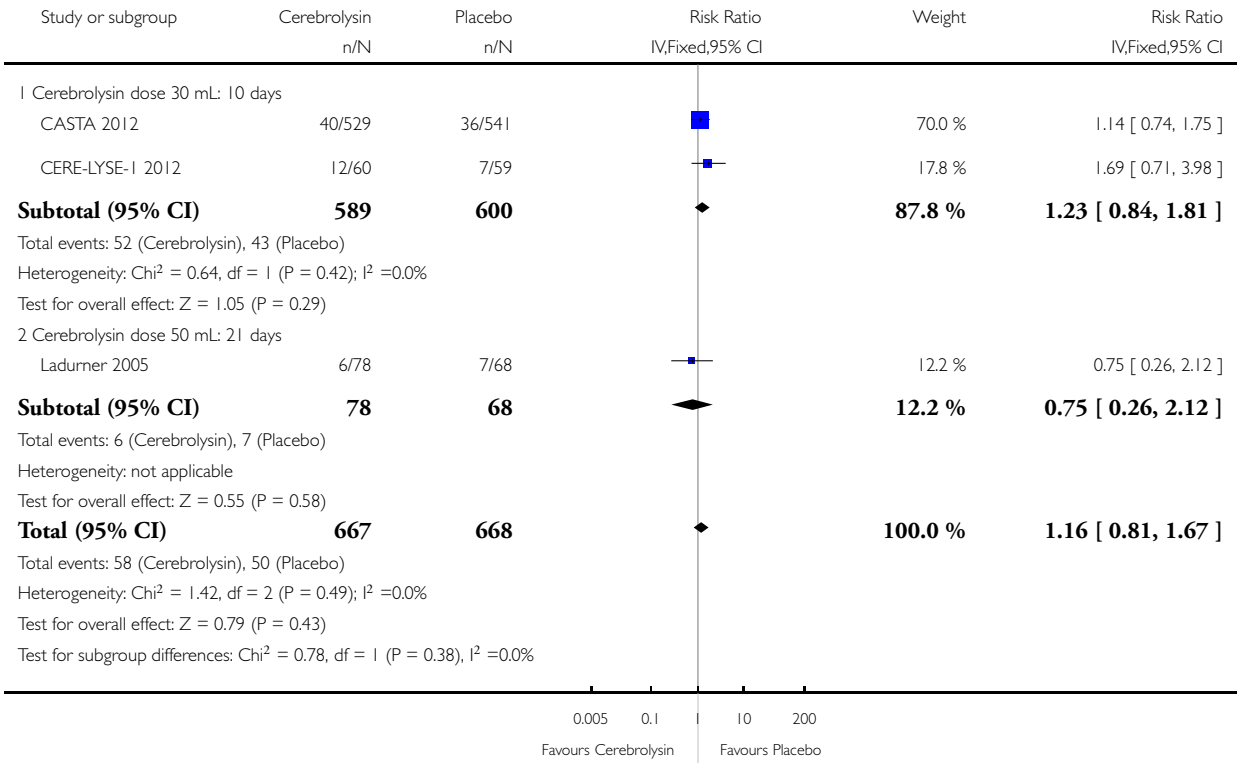


Analysis 1.2. Comparison 1 Cerebrolysin versus placebo, Outcome 2 Total number of people with serious adverse events (SAEs).

Review: Cerebrolysin for acute ischaemic stroke

Comparison: 1 Cerebrolysin versus placebo

Outcome: 2 Total number of people with serious adverse events (SAEs)

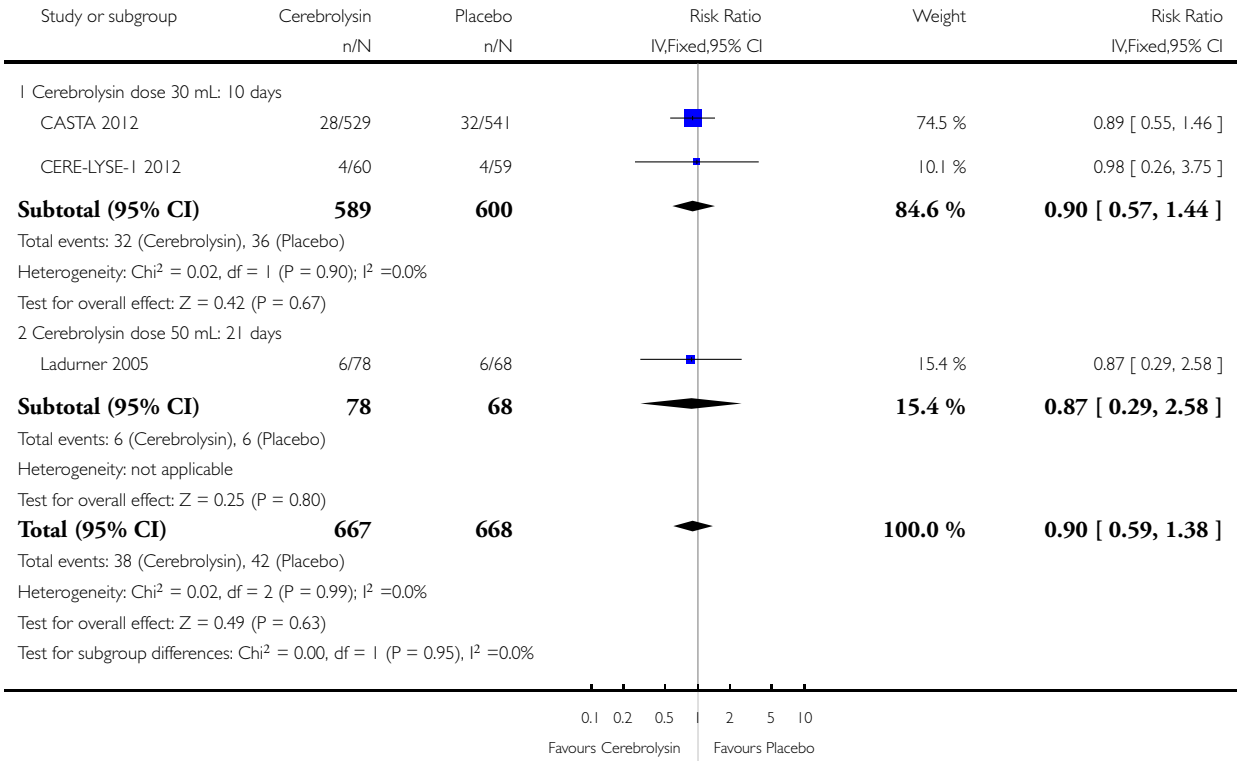


Analysis 1.3. Comparison 1 Cerebrolysin versus placebo, Outcome 3 Total number of people with fatal SAEs.

Review: Cerebrolysin for acute ischaemic stroke

Comparison: 1 Cerebrolysin versus placebo

Outcome: 3 Total number of people with fatal SAEs

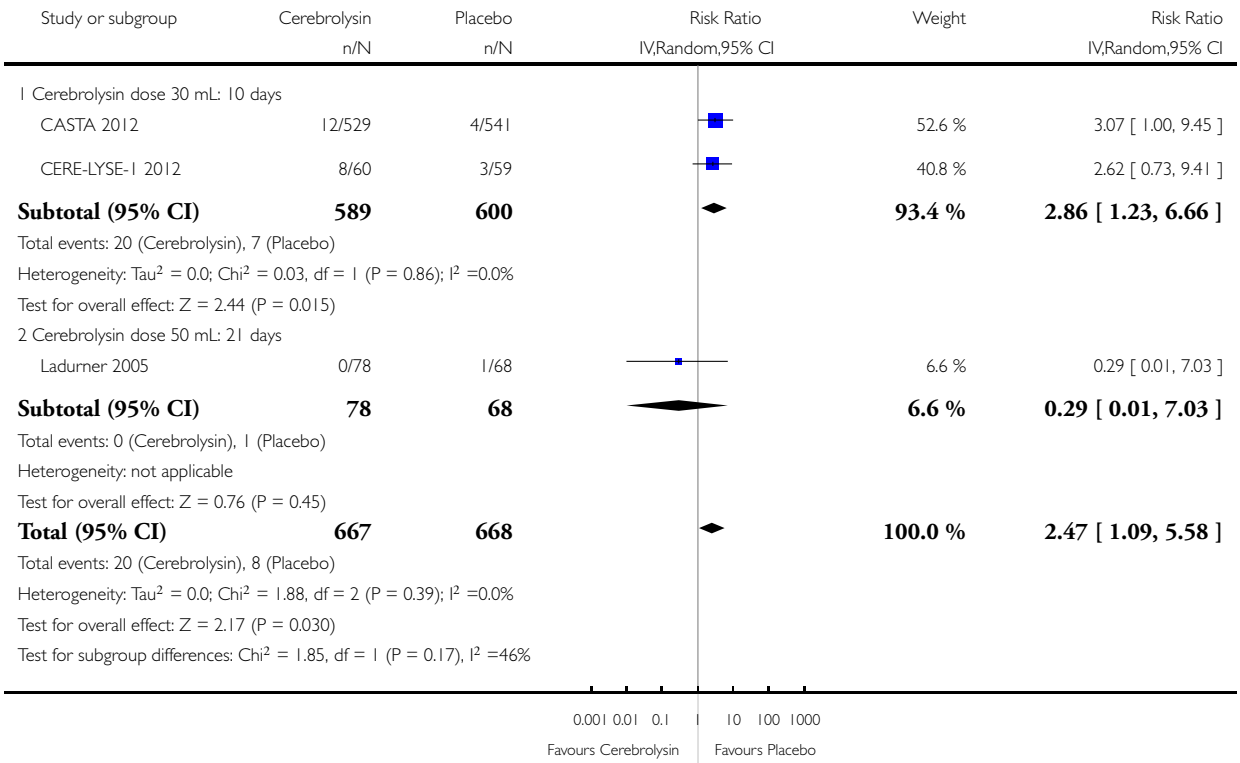


Analysis 1.4. Comparison 1 Cerebrolysin versus placebo, Outcome 4 Total number of people with non-fatal SAEs.

Review: Cerebrolysin for acute ischaemic stroke

Comparison: 1 Cerebrolysin versus placebo

Outcome: 4 Total number of people with non-fatal SAEs

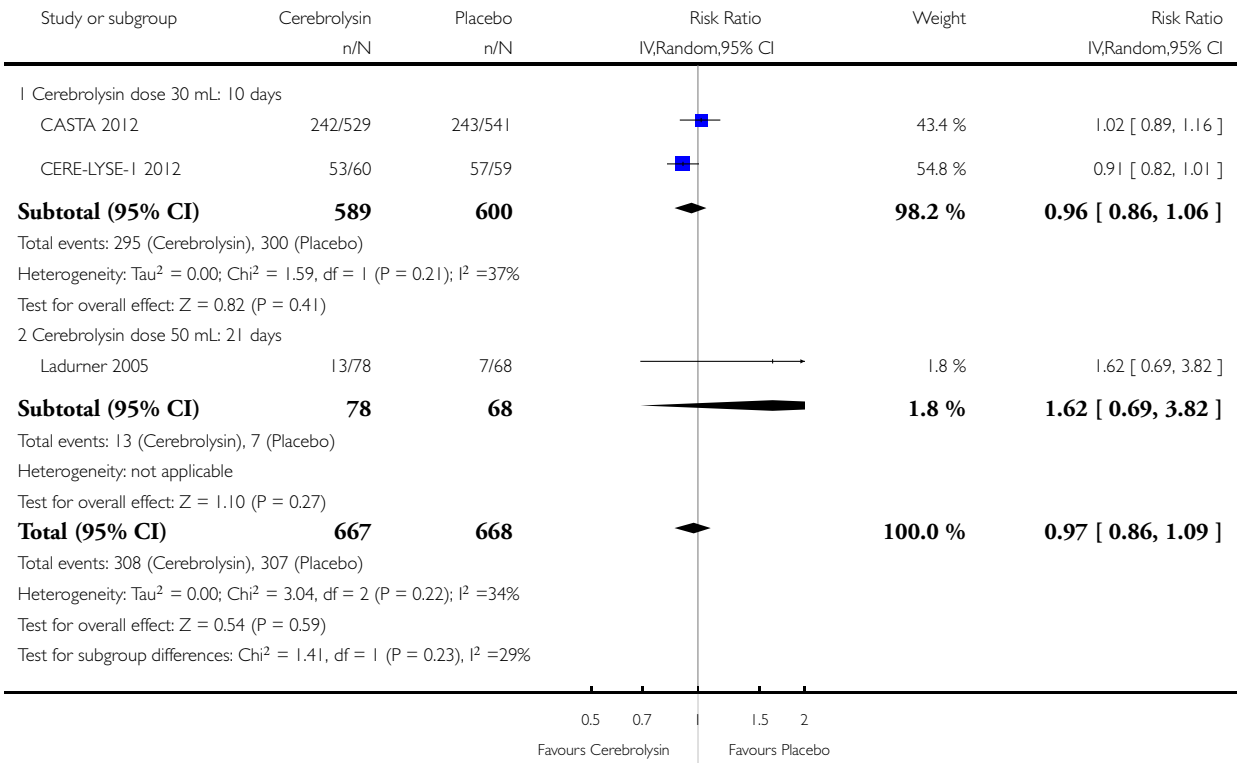


Analysis 1.5. Comparison 1 Cerebrolysin versus placebo, Outcome 5 Total number of people with adverse events.

Review: Cerebrolysin for acute ischaemic stroke

Comparison: 1 Cerebrolysin versus placebo

Outcome: 5 Total number of people with adverse events

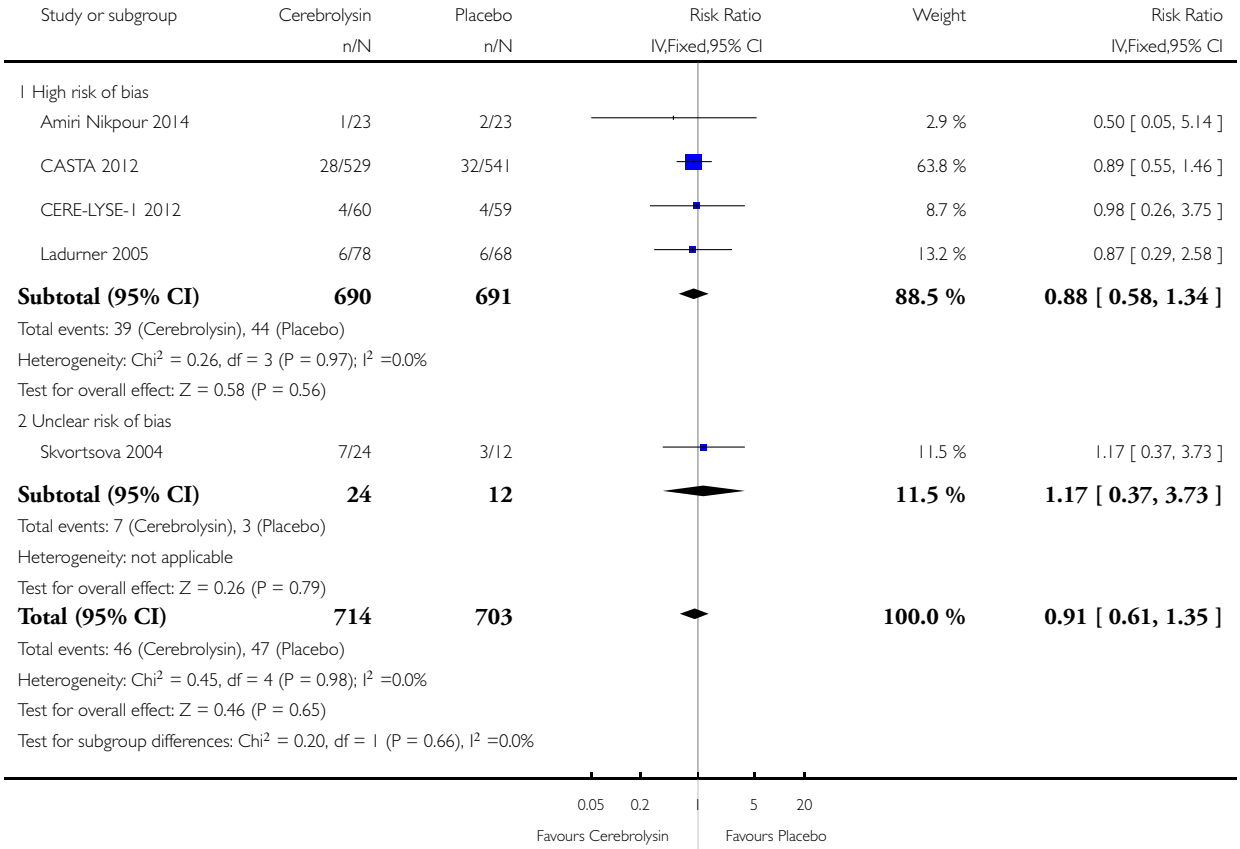


Analysis 1.6. Comparison 1 Cerebrolysin versus placebo, Outcome 6 All-cause death sensitivity.

Review: Cerebrolysin for acute ischaemic stroke

Comparison: 1 Cerebrolysin versus placebo

Outcome: 6 All-cause death sensitivity



APPENDICES

Appendix 1. CENTRAL (the Cochrane Library) search strategy

#1 [mh ^"cerebrovascular disorders"] or [mh ^"basal ganglia cerebrovascular disease"] or [mh "brain ischemia"] or [mh ^"carotid artery diseases"] or [mh ^"carotid artery thrombosis"] or [mh ^"carotid artery, internal, dissection"] or [mh ^"stroke, lacunar"] or [mh ^"intracranial arterial diseases"] or [mh ^"cerebral arterial diseases"] or [mh ^"infarction, anterior cerebral artery"] or [mh ^"infarction, middle cerebral artery"] or [mh ^"infarction, posterior cerebral artery"] or [mh "intracranial embolism and thrombosis"] or [mh ^stroke] or [mh "brain infarction"] or [mh ^"vertebral artery dissection"]
#2 ((brain or cerebr* or cerebell* or vertebrobasil* or hemispher* or intracran* or intracerebral or infratentorial or supratentorial or middle next cerebr* or mca* or anterior next circulation) near/5 (isch*emi* or infarct* or thrombo* or emboli* or occlus* or hypoxi*)):ti,ab
#3 (isch*emi* near/6 (stroke* or apoplex* or cerebral next vasc* or cerebrovasc* or cva or attack*)):ti,ab
#4 #1 or #2 or #3
#5 (cerebrolysin* or CERE or "FPF-1070" or FPF1070 or "FPF 1070" or "FPF 10-70"):ti,ab
#6 #4 and #5

Appendix 2. MEDLINE (Ovid) search strategy

1. cerebrovascular disorders/ or basal ganglia cerebrovascular disease/ or exp brain ischemia/ or carotid artery diseases/ or carotid artery thrombosis/ or carotid artery, internal, dissection/ or stroke, lacunar/ or intracranial arterial diseases/ or cerebral arterial diseases/ or infarction, anterior cerebral artery/ or infarction, middle cerebral artery/ or infarction, posterior cerebral artery/ or exp "intracranial embolism and thrombosis"/ or stroke/ or exp brain infarction/ or vertebral artery dissection/
2. ((brain or cerebr$ or cerebell$ or vertebrobasil$ or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or middle cerebr$ or mca$ or anterior circulation) adj5 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypoxi$)).tw.
3. (isch?emi$ adj6 (stroke$ or apoplex$ or cerebral vasc$ or cerebrovasc$ or cva or attack$)).tw.
4. 1 or 2 or 3
5. (cerebrolysin$ or CERE or FPF-1070 or FPF1070 or FPF 1070 or FPF 10-70).tw.
6. 4 and 5
7. exp animals/ not humans.sh.
8. 6 not 7

Appendix 3. Embase (Ovid) search strategy

1. cerebrovascular disease/ or brain infarction/ or brain stem infarction/ or cerebellum infarction/ or exp brain ischemia/ or carotid artery disease/ or exp carotid artery obstruction/ or cerebral artery disease/ or exp cerebrovascular accident/ or exp occlusive cerebrovascular disease/ or stroke patient/
2. ((brain or cerebr$ or cerebell$ or vertebrobasil$ or hemispher$ or intracran$ or intracerebral or infratentorial or supratentorial or middle cerebr$ or mca$ or anterior circulation) adj5 (isch?emi$ or infarct$ or thrombo$ or emboli$ or occlus$ or hypoxi$)).tw.
3. (isch?emi$ adj6 (stroke$ or apoplex$ or cerebral vasc$ or cerebrovasc$ or cva or attack$)).tw.
4. 1 or 2 or 3
5. cerebrolysin/
6. (cerebrolysin$ or CERE or FPF-1070 or FPF1070 or FPF 1070 or FPF 10-70).tw.
7. 5 or 6
8. 4 and 7
9. (exp animals/ or exp invertebrate/ or animal experiment/ or animal model/ or animal tissue/ or animal cell/ or nonhuman/) not (human/ or normal human/ or human cell/)
10. 8 not 9

Appendix 4. Web of Science Core Collection (including Science Citation Index) search strategy

#1. TOPIC: (stroke* or apoplex* or cerebral vasc* or cerebrovasc* or cva)
#2. TOPIC: (cerebrolysin*)
#3. #2 AND #1

Appendix 5. LILACS search strategy

cerebrolysin or CERE or FPF-1070 or FPF1070 or cortexin or CORT or N-PEP-12F

Appendix 6. OpenGrey search strategy

cerebrolysin or CERE or FPF-1070 or FPF1070 or cortexin or CORT or N-PEP-12F

Appendix 7. Russian databases search strategy

#1. инсульт or цереброваск * or церебральн * or цвб *
#2. церебролизин or ЦЕРЕ or кортексин or КОРТ
#3. #1 and #2

WHAT'S NEW

Date	Event	Description
11 April 2017	New citation required and conclusions have changed	Conclusions changed.
11 April 2017	Amended	In response to feedback, we refined the outcome serious adverse events (SAEs) and replaced it with: total number of people with SAEs; total number of people with fatal SAEs; and total number of people with non-fatal SAEs

HISTORY

Date	Event	Description
27 May 2016	New citation required and conclusions have changed	The conclusions of the review have changed.
27 May 2016	New search has been performed	We refined the inclusion criteria to allow inclusion of trials with the length of cerebrolysin use not restricted to 14 days (any length of use). We performed a new search and included five new trials. The review now has six included studies involving 1501 participants. Ludivine

(*Continued*)

		Vernay joined the author team. We used Covidence for managing records, papers and trials, to extract data and to assess risks of bias, and to resolve conflicting opinions of the authors. We refined the conclusions
27 January 2015	New citation required but conclusions have not changed	We performed a new search. The conclusions have not changed.
15 July 2008	Amended	Converted to new review format.

CONTRIBUTIONS OF AUTHORS

Liliya-Eugenevna Ziganshina (LEZ) prepared the protocol and was the author of the original review and was responsible for this update. All authors were involved in the conception of this review update. Tatyana R Abakumova (TRA) performed literature searches of the Russian language studies. LEZ, LV (Ludivine Vernay) and TRA assessed citations, abstracts, and full texts of trial reports for eligibility; LEZ and LV extracted data, assessed the risk of bias, managed the references using Covidence, and imported data from Covidence to RevMan. LEZ drafted the review text with input from the other authors.

DECLARATIONS OF INTEREST

LEZ: none known

LV: none known

TRA: none known

SOURCES OF SUPPORT

Internal sources

- Kazan Federal (Volga Region) University, Russian Federation.

Research and Educational Centre of Evidence-Based Medicine "Cochrane Russia", Department of Basic and Clinical Pharmacology. This work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.

- Cochrane Stroke Group, UK.
- Liverpool School of Tropical Medicine, UK.

External sources

- No sources of support supplied

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

2010, Issue 4 (first review version): we followed the Cochrane protocol precisely.

2015, Issue 6 (second review version): we did not incorporate changes to the structure of the previously published version of the review, we updated searches, followed the protocol precisely and confirmed the conclusions.

2016, Issue 11 (third review version): we changed the inclusion criteria to allow varying durations of cerebrolysin use and included six studies in total with one comparison: cerebrolysin versus placebo for acute ischaemic stroke. We restructured the outcomes: all-cause death became the primary outcome, the remainder are listed as secondary outcomes. We reworded "total number of adverse events" as "total number of people with adverse events". Ludivine Verney joined the team as a co-author.

2017, Issue 4 (fourth review version): we refined the outcome serious adverse events (SAEs) and replaced it with three outcomes: total number of people with SAEs; total number of people with fatal SAEs; and total number of people with non-fatal SAEs.

INDEX TERMS

Medical Subject Headings (MeSH)

Acute Disease; Amino Acids [adverse effects; *therapeutic use]; Cause of Death; Neuroprotective Agents [adverse effects; *therapeutic use]; Randomized Controlled Trials as Topic; Stroke [*drug therapy; mortality]

MeSH check words

Humans