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Review

Neuroprotective agents in Acute Ischemic Stroke—A Reality Check

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1. Introduction

Stroke remains to be one of the leading causes of adult disability and the third leading cause of mortality worldwide, and acute ischemic stroke (AIS) accounts for 80% of all strokes [1]. The main cause of ischemic stroke is the clot that obstructs the blood vessel within the brain leading to impaired blood flow and neuronal cell death. The increase in the ageing population has led to the requirement of a substantial therapy that can restrain the mortality and morbidity rate among AIS patients. Till date the only US FDA-approved drug therapy for ischemic stroke is the intravenous (iv.) thrombolysis with tissue plasminogen activator (t-PA); that must be administered within 4.5 hours of the symptom onset. The short window period of the therapeutic intervention limits its usage and only 2–5% of the population gets the benefit of the drug [2]. Moreover, the recently reported clinical trials have raised concerns regarding the utility of other acute treatment options such as the modern endovascular therapy with mechanical thrombectomy and intra-arterial fibrinolysis [3,4]. Therefore, there is a substantial need for the development of an effective and safe therapy that will benefit a large number of ischemic stroke patients.

1.1. Neuroprotection

The concept of neuroprotection has gained significant recognition over the past decades. “Neuroprotection is specifically defined as the “protection of neurons” and is a strategy used to potentially protect the brain in a number of different cerebral conditions including Parkinson’s disease, traumatic brain injury and ischemic stroke” [5]. Till date, more than 1000 neuroprotective agents have shown promising results in preclinical models. However, the translation of these neuroprotective agents has failed in the clinical setting. More than 200 neuroprotection clinical trials have been completed or are ongoing, but none of the neuroprotective agents have achieved success [6]. In this systematic review, we seek to summarize the current status and challenges of neuroprotection in ischemic stroke.

2. Methodology

2.1. Protocol and registration

The protocol of this systematic review was not registered in any registry.

2.2. Data Sources

All the registered Randomized Controlled Trials (RCTs) and published studies were identified inclusive of full text and abstract, using the search engines PubMed and Clinicaltrials.gov.

2.3. Eligibility criteria

We mainly reviewed the RCTs (Phase I/II/III), English language articles and interventional studies using pharmacological therapies. We included studies that assessed the effect of neuroprotective agents in AIS. The studies were included only if the development of the molecule was continuing. There were no limits on the type of controls used and outcomes analyzed in the studies.

2.4. Search and Selection of Studies

A systematic review was done using the following search terms: “Acute Ischemic Stroke AND Minocycline”, “Acute Ischemic Stroke AND Magnesium Sulfate”, “Acute Ischemic Stroke AND Cerebrolysin”, “Acute Ischemic Stroke AND Edaravone”, “Acute Ischemic Stroke AND Ebselen”, “Acute Ischemic Stroke AND Antioxidants”, “Acute Ischemic Stroke AND Haematopoietic growth factors”, “Acute Ischemic Stroke AND Erythropoietin”, “Acute Ischemic Stroke AND Atorvastatin”, “Acute Ischemic Stroke AND Neuroaid”. All the RCTs that were fitting the eligibility criteria were included for further discussion. The studies which were unrelated, non-English articles, surgical/dietary supplements/procedural, and animal studies were excluded from further analysis. The included studies had explored the administration of

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neuroprotective therapeutic agents on AIS patients. The studies did not have any restriction on the dosage, route of administration, timing of administration and the type of comparator used.

2.5. Data extraction

The information was extracted on study population, interventions, comparator, sample size, and outcome measures.

2.6. Risk of bias in individual studies

The risk of bias was assessed and the risk of bias table was plotted using Review Manager 5.3 [7]. Meta-analysis was performed for some of the studies due to the presence of remarkable heterogeneity in reporting the study outcomes. The random sequence generation and allocation concealment were assessed for the evaluation of risk of selection bias. The risk of performance bias was rated by assessing the blinding of participants and personnel. The reporting bias and attrition bias was measured by assessing the selective reporting and incomplete data respectively.

3. Results

3.1. Search results & study characteristics

A total of 2383 articles were identified in the initial search. After conducting a systematic review 309 articles were selected after an initial title and abstract screening which were relevant to the topic. 2074 articles were excluded, which comprised of studies which were unrelated to the topic, animal studies, and non-English articles. Among the 309 articles, 254 articles were excluded as they were studies done on surgical/procedural/dietary supplements/failed therapies/duplicates. Finally, a total of 55 articles were included in the final analysis, where we identified the newly emerging drug therapies for the treatment of AIS (Fig. 1). The molecules which were identified as most promising as in active clinical investigation for its neuroprotective benefit in stroke patients include minocycline, magnesium sulfate, edaravone, erythropoietin, cerebrolysin, and MCLC601 (NeuroAiD). We have briefly summarized the current status and the challenges of the novel drug therapies (Supplementary Table 1). The overall quality assessment of the RCTs was done (Table 1).

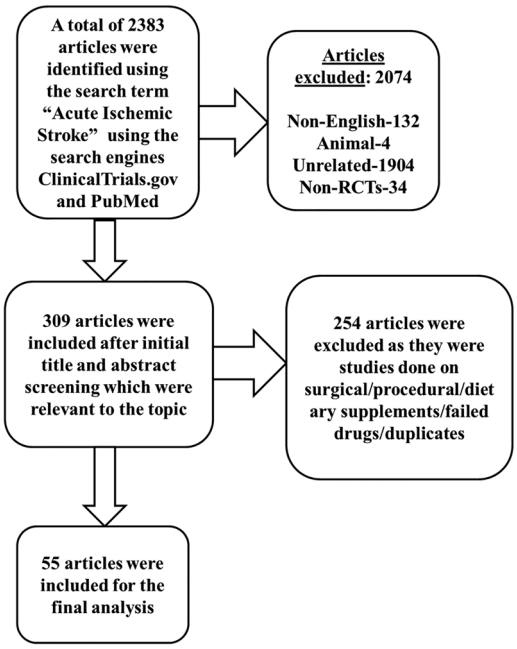

Fig. 1. PRISMA Flow diagram for Literature Search.

4. Discussion

4.1. Minocycline

Minocycline a second generation, tetracycline derivative is an antibiotic, which is widely used for the treatment of infections. Tetracycline derivatives are known for their antibiotic properties, but these derivatives have also exhibited biological actions that are independent of their anti-microbial activity which include the anti-inflammatory and antiapoptotic activities, and their inhibitory effects on proteolysis, angiogenesis, and tumor metastasis (Fig. 2). Minocycline has been explored for several indications such as dermatitis, periodontitis, atherosclerosis, autoimmune disorders such as rheumatoid arthritis and inflammatory bowel disease [8]. Minocycline has also shown promising neuroprotective benefit in animal models induced with focal cerebral ischemia, traumatic brain injury (TBI), spinal cord injury (SCI) and intracerebral hemorrhage (ICH) confirming the neuroprotective effect of minocycline [9].

Minocycline inhibits the enzymatic activity of enzymes such as iNOS, MMPs, COX-2, and PLA2 that contributes to inflammation. It also suppresses apoptosis through the inhibition of caspase-1 and caspase-3 activation and reduces the microglial activation. Treatment with minocycline has the ability to suppress the microglial activation and attenuates the infarct volume and neurological deficits, leading to a reduction in blood-brain barrier disruption and haemorrhage. Moreover, it has the ability to cross the blood-brain barrier, by inhibiting the activation of matrix metalloproteinases, has a good safety profile, reduces migration of T-cells and has a delayed therapeutic window period. Its anti-apoptotic like properties inhibit the apoptotic-like neuronal cell death that involves the anti-apoptotic Bcl-2/cytochrome-c- pathway [10].

Minocycline appears to be an ideal neuroprotective agent based on its established safety profile in pre-clinical studies, central nervous system penetration and its low cost. In an open-label, evaluator-blinded study, the administration of 200 mg of oral minocycline led to significant improvement in the National Institutes of Health Stroke Scale (NIHSS), modified Rankin Scale (mRS) and the Barthel Index (BI) scores in the treatment group compared to the placebo group [11]. Two similar studies replicated the same findings [12,13]. Another, multicenter prospective, a pilot study was performed where patients with ischemic or hemorrhagic stroke were randomized to receive 100 mg of intravenous minocycline (n = 47) or no treatment (n = 48). However, there were no significant differences in the neurological outcomes between the two groups [14].

Although clinical studies have shown promising results, the efficacy of minocycline for the treatment of AIS is far from established. The overall quality assessment was done for the studies (Table 1). Most of the studies had a high risk of bias with respect to random sequence generation, allocation concealment and blinding of participants/study personnel. Two of the four trials reported detection bias. Thus, the quality of future trials on minocycline has to be improved. In spite of studies reporting uniform and standard outcome measures, there is a limitation with respect to their duration of follow-up. In all the studies the longest follow-up period was 90 days. Meta-analysis was performed to assess the outcome measures NIHSS, mRS and BI scale on day 90 [11,12]. Minocycline showed improvement in the outcome measures NIHSS (MD: -4.04; 95% CI: -4.86 to -3.22; I2: 92%; $p < 0.00001$; FE model), mRS (MD: −0.99; 95% CI: −1.29 to −0.69; I2: 70%; $p < 0.00001$; FE model) and BI scale (MD: 13.79; 95% CI: 8.99 to 18.60; I2: 71%; $p < 0.00001$; FE model) (Fig. 3).

Investigators evaluated the effect of the drug which was administered to patients within 24 hours of symptom onset. However, early drug administration in the developing countries is a challenging process as many of these patients fail to seek immediate care. Therefore, it is

Table 1
Quality of selected studies based on the degree of bias.

Drug Class & Author Name	Random sequence generation (Selection Bias)	Allocation Concealment (Selection Bias)	Blinding of participants and personnel (Performance Bias)	Blinding of outcome assessment (Detection Bias)	Incomplete outcome Data (Attrition Bias)	Selective Reporting (Reporting Bias)	Follow-up (Days)
Antibiotics (Minocycline)							
Srivastava et al. [12]	No	Yes	Yes	Yes	No	No	7,30 & 90
Reza et al. [13]	Yes	Yes	Yes	Yes	No	No	30,60 & 90
Lamp et al. [11]	No	Yes	Yes	No	No	No	7, 30 & 90
Kohler et al. [14]	No	Yes	Yes	No	No	No	90
Anti-excitotoxicity							
Magnesium Sulfate ($MgSO_4$)							
Saver et al. [19]	No	No	No	No	No	No	90
Afshari et al. [20]	No	Yes	NC	Yes	No	No	90
Lampl et al. [17]	No	Yes	No	Yes	No	No	2,4,8&30
Muir et al. [18]	No	No	No	No	No	No	90
NA-1 (Tat-NR2B9c)							
Hill et al. [53]	No	No	No	No	No	No	30
Cerebrolysin							
Heiss et al. [54]	Yes	Yes	No	No	No	No	90
Jianu et al. [55]	Yes	Yes	Yes	Yes	No	No	1,3,7,14,21 & 90
Lang et al. [25]	No	Yes	No	No	No	No	1,5,10,30 & 90
Hong et al. [56]	No	No	No	No	No	No	90

Yes: Risk of bias is present; No: Risk of bias is absent; NA: Risk of bias is unclear

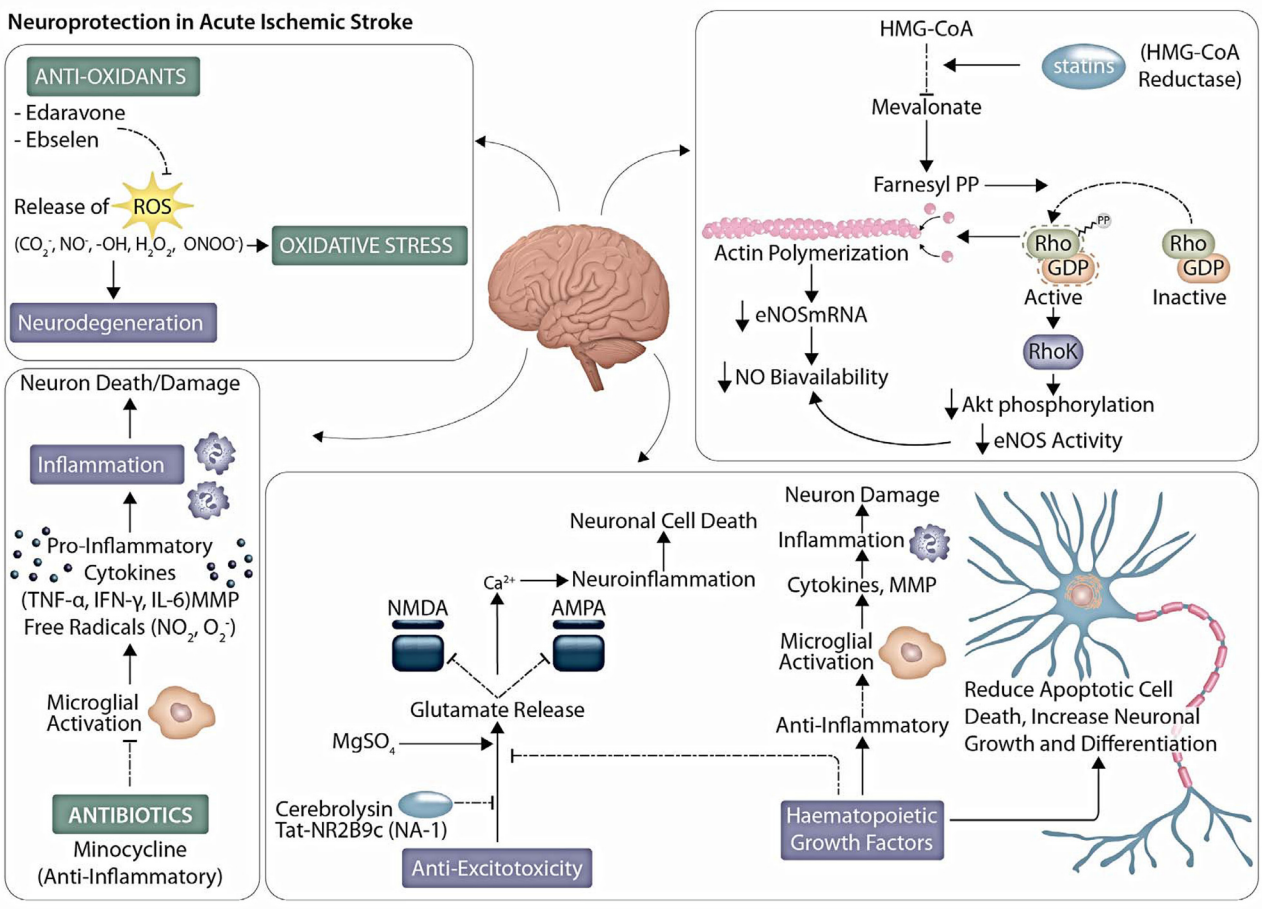

Fig. 2. Neuroprotection in Acute Ischemic Stroke.

necessary to design drug molecules that can be evaluated with a wider therapeutic window period. Another limitation is that the studies were conducted on a smaller population and only one of the studies calculated the sample size a priori [14]. Thus, the studies were not well-powered to measure the clinical efficacy of minocycline in AIS. Therefore, larger trials are required to determine the treatment effect of minocycline. The studies had varied inclusion criteria. Stroke subtyping was not done for the study population. Therefore, it is not clear which type of stroke will receive the maximum therapeutic benefit. Conversely, it can also be considered that minocycline will be effective in all the types of stoke. Hence, the inclusion criteria must be pre-defined, uniform and precise. The advantages and disadvantages of

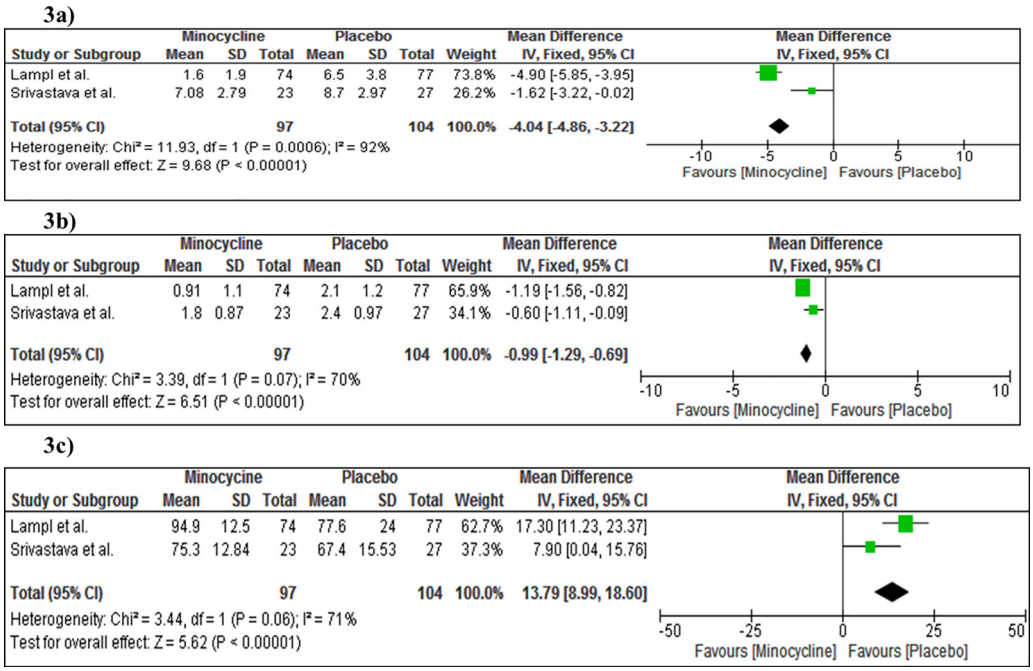

Fig. 3. (a) Effect of Minocycline on National Institutes of Health Stroke Scale at day 90. (b) Effect of Minocycline on modified Rankin Scale at day 90. (c) Effect of Minocycline on Barthel Index at day 90.

Table 2
Pros and Cons of novel neuroprotective agents in Acute Ischemic Stroke.

Name of Neuroprotective agents	Advantages	Disadvantages
Magnesium sulfate ($MgSO_4$) [52,57]	Magnesium is easily available, well-tolerated and is inexpensive	The oral intake of magnesium might result in diarrhoea, although there is no increase in the serum concentrations unless the patients suffer from renal insufficiency. In patients with renal failure, magnesium may get accumulated quickly resulting in hazardous side effects. Increase in the serum concentrations > 6 mmol/l can result in coma, respiratory insufficiency and cardiac arrest. As lower blood pressure could lead to a worsening of ischemic damage in states of cerebral ischemia, care should be taken that high doses of magnesium are avoided for neuroprotection. Efficacy diminishes with delay in administration.
Edaravone [31,58]	Compared to other cerebro-protective agents, edaravone can be started even as late as 72 hours of symptom onset. The drug has a shorter duration of treatment up to two weeks.	There is an increased risk of renal toxicity which is reversible with the stoppage of treatment. The drug molecule is available only as intravenous therapy. There will also be an increased frequency of hemorrhagic transformation when edaravone is administered to patients with cardiogenic embolism.
Minocycline [8]	Minocycline a safe and inexpensive drug. It is an oral therapy with (95-100% absorption) and reaches most of the compartments of the body, including the central nervous sytem.	The most common side-effects of minocycline include nausea, vertigo and mild dizziness that occur in the early stage after its administration. However, these side-effects were eliminated after the discontinuation of the treatment.
Granulocyte-Colony Stimulating Factor (G-CSF) [39,59]	G-CSF has a well-known pharmacological profile and displays excellent safety profile. The administration of G-CSF after longer time intervals can improve the functional recovery among AIS patients. This neuroprotective molecule could be used as a supportive treatment in the rehabilitation phases after the stroke event.	Elevation in the number of leukocytes which might produce a negative impact on brain inflammation or blood flow. Effect of G-CSF on the platelets might affect the coagulation.

treatment with minocycline have been illustrated in (Table 2). In conclusion, the efficacy and safety of minocycline need to be confirmed from well-powered trials with robust methodology before it could be approved as an agent for neuroprotection in stroke.

4.2. Anti-excitotoxicity

4.2.1. Magnesium Sulfate ($MgSO_4$)

$MgSO_4$ is currently approved for the clinical treatment of eclampsia, atrial paroxysmal tachycardia, cerebral edema, barium poisoning, glomerulonephritis or hypothyroidism. However, $MgSO_4$ has gained attention as a potential neuroprotective agent that preserves the neuronal function and mitigates the neuronal loss following the acute brain injury. The mechanisms that have postulated for the neuroprotective role of magnesium (Mg) include the blockade of N-methyl-D-aspartate (NMDA) receptor and calcium channels, decrease in the release of glutamate and hypothermia. Mg acts presynaptically and inhibits the release of excitatory amino acids (EAAs) and acts postsynaptically through the non-competitive voltage-dependent inhibition of NMDA receptor-mediated Ca^{2+} release. Based on its antagonistic effects, this therapy can reduce the infarct size and improve the cerebral blood flow. Mg produced significant protection in pre-clinical models of stroke

supporting the role of Mg in neuroprotection [15,16].

Successful pre-clinical studies, easy availability of Mg, adequate tolerance, and its low cost has led to considerable interest in evaluating the molecule clinically. A pilot clinical trial showed that intravenous $MgSO_4$ administered for five consecutive days had a significant positive effect on the neurological outcome in stroke patients [17]. But in one of the largest trials ever done with Mg till date (n = 2386), namely the Intravenous Magnesium Efficacy in Stroke (IMAGES) Study, the drug did not show improvement in outcomes. The authors surmised that the poor response to magnesium was due to delay in its timely administration [18]. The Field Administration of Stroke Therapy-Magnesium (FAST-MAG) phase 3 trial was designed to surmount the limitation of the IMAGE trial, where $MgSO_4$ was administered by paramedics in the field within 2 hours of stroke onset. Unfortunately, the results of the trial did not show the superiority of $MgSO_4$ over placebo [19]. In another placebo-controlled, randomized double-blind trial, administration of intravenous $MgSO_4$ significantly improved neurological outcome in AIS patients. In the sub-group analysis, magnesium significantly lowered the NIHSS score among patients receiving Mg within 2-5 hours of onset than that of patients who were treated after 6-12 hours of stroke onset [20].

The above-mentioned studies with magnesium seem to suggest a great degree of dissonance. We performed a meta-analysis for three studies [17, 19, and 20] to assess the difference in the mRS scores between the magnesium and placebo group. However, the administration of magnesium did not show a significant difference in the mean mRS score between both the groups (MD: -0.48; 95% CI: -1.15 to 0.19; I2: 91%; p = 0.16; FE model). Mortality was assessed among 2696 ischemic stroke patients who received either magnesium or placebo. There was no significant difference in the total number of mortality events (RD: 0.01; 95% CI: −0.04 to 0.07; I2: 31%; p = 0.64; FE model) between the magnesium and placebo group (Fig. 4) [18,20].

The larger trials demonstrated unfavorable effect whereas the smaller trials demonstrated the beneficiary effect of $MgSO_4$. The lack of early drug administration as suggested by the IMAGE investigators is a greater challenge in developing countries where delay in bringing patients to the hospital is more the norm than an exception. In this scenario, developing molecules that can act for longer than the expected short window period of three to four hours is the need of the hour.

The study population between the four trials was remarkably heterogeneous from each other. The study population was different in terms of the type of stroke, neurological assessment score, and demographic features. This diversity makes it challenging to come out with a meaningful conclusion. The studies which demonstrated positive outcomes of $MgSO_4$ were not well-powered. Hence, trials conducted in a smaller study population may include large variability in study population and safety cannot be measured. The quality assessment of studies was done based on the risk of bias (Table 1). It was observed that risk of bias was low in the IMAGE and FAST-MAG trial, whereas the risk of bias was higher in the smaller trials. Allocation concealment and blinding of outcome assessment were not done for the trials. Hence, there could have been selection bias hindering the benefits of randomization. Further, the outcome assessor being unblinded may have tempted the investigator to manipulate the outcome measures. Hence, the results of these studies should be viewed with caution. The outcomes evaluated in the studies were contrasting and were measured at different time points, making it harder to draw a meaningful conclusion. Designing studies with similar outcomes and follow-up period should be encouraged in further trials. In conclusion, magnesium's role as a neuroprotective agent from the meta-analysis performed with the existing literature has shown rather unsatisfactory results. It remains to be seen if further trials with magnesium in combination with other agents could change the bleak status of this molecule as a neuroprotective agent.

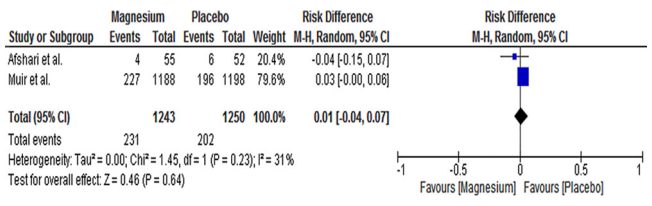

Fig. 4. Effect of Magnesium on Mortality.

4.3. Cerebrolysin

Cerebrolysin is currently approved for the treatment of ischemic and hemorrhagic stroke in 45 countries. The molecule is a porcine brain-derived preparation of low molecular weight neuropeptides and free amino acids. The neuroprotective properties of this drug molecule include anti-excitotoxicity, inhibition of free radical formation, microglia activation, and apoptosis. Additionally, it also exhibits neurotrophic action, promotes neuronal sprouting, improves cellular survival and stimulates neurogenesis [21]. Animal studies have shown that this molecule can improve the neurological function and reduce the infarct size, by preventing the free radical formation and counteracting excitotoxicity that can prevent cell death [22–24]. Clinical studies were conducted to study the safety and efficacy of cerebrolysin in AIS patients.

In spite of positive outcomes in pre-clinical studies, clinical trials with cerebrolysin have yielded rather mediocre results. For example, in the large phase IV, cerebrolysin Acute Stroke Treatment in Asia (CASTA) trial (n = 1070), cerebrolysin failed to provide any benefit in comparison to placebo with respect to composite outcome scores of the stroke scales. In another phase III trial, the combined treatment of rt-PA and cerebrolysin did not improve the neurological outcome score in comparison to placebo [25]. Further, in a recently published meta-analysis, cerebrolysin showed no significant effect on the neurological outcomes. There were no concerns regarding the safety of the drug molecule [26]. However, the combined treatment of cerebrolysin and other standard therapies seems promising for ischemic stroke therapy. In a recently published phase IV RCT, the combination of cerebrolysin and standard rehabilitation therapy has demonstrated an added benefit on motor recovery and plastic changes of the corticospinal tract among patients with severe motor impairment [27]. Thus, the available literature does not favor cerebrolysin as a potential neuroprotective agent. It appears that the lack of better alternatives could have made the regulators less stringent in evaluating the efficacy of the molecule. It remains to be seen if treatment with cerebrolysin before 6 hours could translate to better outcomes.

4.4. Antioxidants

The excessive production of free radicals stimulates cell injury that leads to cerebral ischemia. Antioxidants are a class of drugs which have the ability to inhibit the production of free radicals, scavenging of free radicals and increase the degradation of free radical scavengers [28]. Several antioxidants have failed to show success in the clinical studies (Table 3). Presently edaravone is being investigated as a neuroprotective agent for AIS.

4.4.1. Edaravone

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, MCI-186, Radicut), a strong free radical scavenger molecule is marketed by Mitsubishi Tanabe Pharma Corporation (Osaka, Japan). This neuroprotective agent was approved for the treatment of AIS in Japan in the year 2001. However, it is not yet approved for treatment in the USA and Europe. Edaravone displays anti-apoptotic, anti-necrotic, and anti-inflammatory effects. The antioxidant effect of edaravone inhibits the hydroxyl radical-dependent and independent lipid peroxidation. Thus, the antioxidant activity of the drug molecule suggested that it can offer

Table 3
List of drugs that failed to become a potential therapeutic agent for Acute Ischemic Stroke.

Name of drug	Comments
Tirilazad Mesylate	Tirilazad mesylate increased the mortality rate and disability by about one fifth when given to patients with acute ischemic stroke [60].
NXY-09	There was discrepancy between the animal and clinical studies with respect to the protocol differences i.e. (the therapeutic window period was longer in the clinical studies when compared to the animal studies), methodological quality and poor permeability across the blood brain barrier [51].
Human Albumin	The phase III Randomized Multicenter Clinical Trial of High-Dose Human Albumin Therapy for Neuroprotection in Acute Ischemic Stroke was terminated and Recruitment halted by DSMB following interim analysis [61].
Deferoxamine mesylate	A Double-blind, Randomized, Placebo Controlled, Dose-finding Phase 2 Clinical Trial of Intravenous Deferoxamine in Patients With Acute Ischemic Stroke Treated With Tissue Plasminogen Activator was completed. However, the results of the study seem to be unpublished and no further trials have been registered in clinicaltrilas.gov [62].
Dapsone	A phase III studying safety and efficacy of Dapsone among acute ischemic stroke patients has been registered. However, the recruitment status of this study is unknown and the completion date has passed and the status has not been verified in more than two years. No further trials have been registered in clinicaltrilas.gov [63].
Dextrorphan	The drug was safe and tolerated. However, no further improvements were observed [64].
Citicoline	The drug was not effective in the treatment of moderate to severe acute ischemic stroke [65].
Enlimomab	Poor outcomes were observed in the study drug population. The adverse events caused due to the drug included headache, vomiting, extra-systoles and anaphylactoid reaction [59].
Sipatrigine	The drug showed increased toxicity and had poor efficacy [66].
Nimodipine	The drug did not display any clinical benefits [67].
Piracetam	A study to evaluate the efficacy and safety of piracetam on aphasia after acute ischemic cerebral artery stroke was terminated after the interim analysis was performed. The interim analysis had demonstrated that the less than a 20% chance in showing a 15% difference in the frenchay aphasia screening test (FAST) score between the between placebo and piracetam groups [68].
Aptiganel hydrochloride	The drug failed to show a neuroprotective effect on stroke patients and the clinical development of this drug was halted. Moreover, the prolonged infusions of this drug lead to several side-effects such as hypertension, sedation and confusions or hallucinations [69–71].

protection against free radical-related injuries following AIS. Moreover, edaravone being a low molecular weight agent, having both water and lipid soluble properties, it can readily cross the blood-brain barrier. These properties of edaravone explain the neurovascular protective effects of edaravone among AIS patients, distinguishing the drug molecule from other free radical scavengers [29]. Therefore, edaravone is considered to be a suitable neuroprotective agent for AIS.

Two RCTs were conducted to study the safety and efficacy of edaravone in ischemic stroke patients. In an open-label, randomized, controlled pilot study, the continuous intravenous infusions of either edaravone 30 mg twice daily for 3 days (short-term group) or 10–14 days (long-term group) effectively improved the severity of disuse muscle atrophy and leg locomotor dysfunction [30]. Another comparative RCT study was conducted to compare two neuroprotective agents' edaravone and citicoline among AIS patients; edaravone showed a significant improvement in mRS and NIHSS when compared to citicoline and conventional treatment [31].

Clinical trial data have demonstrated that the administration of edaravone within 72 hours of stroke onset significantly improved the neurological outcomes. This should be a welcome advantage considering the fact that most other antioxidants require administration within 6-12 hours of stroke onset. In Japan it is recommended that edaravone should be administered for a duration of 14 days, however, the duration of administration could be shortened provided patients present with mild symptoms [32]. Studies have shown that neuronal cells will not survive after several hours of ischemia and the cycle of neuronal cell death will continue for a few days after the stroke event [33,34]. Based on these studies it was suggested that neuroprotective drugs will exert protective effects if it was administered within several hours after an ischemia and continued for a few days. Thus, the administration of edaravone for the duration of three days was found to be sufficient to provide neuroprotection. However, radical-related disuse muscle atrophy is known to begin among stroke patients due to a longer period of bed rest immobilization [35]. Therefore, it is highly recommended to provide edaravone therapy for a longer period to reduce the development of radical-related disuse muscle atrophy.

The administration of edaravone among AIS patients is associated with certain limitations (Table 2). The clinical data obtained so far show that the administration of edaravone has not been systematically evaluated. The edaravone trials are limited to heterogeneity, smaller sample size and the risk of bias summary of the trials was found to be moderate (Table 1). Therefore, the safety and efficacy of edaravone should be evaluated in well-controlled high-quality trials that have standardization of dosage, duration of treatment and the time to treatment.

4.5. Haematopoietic growth factors (HGFs)

HGFs such as granulocyte-colony-stimulating factor (G-CSF; AX200; Filgrastim), stem cell factor (SCF) and erythropoietin (EPO) have been extensively used in clinical practice for oncology and hematology. Recent studies have suggested that HGFs can provide neuroprotection through reduction of glutamate-induced excitotoxicity, stimulation of angiogenesis and neurogenesis, and through their anti-apoptotic and anti-inflammatory effects. G-CSF and EPO have shown promising results for pre-clinical experimental models of stroke [36,37]. Presently clinical studies are being conducted for the evaluation of G-CSF and EPO as suitable neuroprotective agents.

4.5.1. Granulocyte colony-stimulating factor (G-CSF)

G-CSF, a member of the cytokine family of growth factors, is a licensed drug molecule which is widely being used in clinical practice for the treatment of chemotherapy-associated neutropenia. With its anti-inflammatory, anti-apoptotic, and neurogenesis-promoting properties, it is being considered as a novel candidate for neuroprotection and neuroregeneration. G-CSF activates various intracellular pathways such as the Janus kinase (JAK)/signal transducer and activator of transcription (STAT), the Ras/MAPK and phosphatidylinositol 3-kinase (PI3K)/protein kinase B. The activation of these pathways restores the proliferation, differentiation, and survival of hematopoietic cells. The administration of G-CSF showed a significant reduction in the brain oedema through the suppression of IL-1, TNF-α, and endothelial nitric oxide synthase (eNOS) mRNA. The decreased production of pro-inflammatory cytokines caused the T-cell infiltration and the up-regulation of anti-inflammatory molecules, which resulted in the reduction of damage occurring due to the blood-brain barrier. Additionally, G-CSF mobilized the exogenous CD34+ stem cells into the peripheral bloodstream in subacute ischemic stroke. This homing of the cells to the site of brain damage improved the functional outcomes [38]. Thus, the multimodal properties of G-CSF make it a useful candidate for the treatment of AIS. Pre-clinical studies have shown that G-CSF can improve the stroke outcome by weakening the stroke damage and improving the post-stroke brain repair.

A total of one phase I and four phase IIa studies evaluating the safety

and tolerability of G-CSF among AIS patients have been completed. The studies did not show improvement in the neurological outcomes (NIHSS, mRS, and BI) scores. However, there was no increase in the number of thromboembolic events in the treatment group and there was no increase in drug-related SAEs [39,40]. Some of the advantages and disadvantages of the neuroprotective molecule G-CSF have been illustrated (Table 2). G-CSF appears to be a promising neuroprotective molecule for the treatment of AIS. However, data on efficacy is sparse at the moment, and larger phase III trials are required to establish the safety and efficacy of G-CSF in AIS therapy.

4.5.2. Erythropoietin (EPO)

EPO is currently used in clinical practice for treatment of anemic patients of various etiologies, especially among patients with uremia. The pleiotropic effects of EPO include the anti-ischemic and anti-apoptotic properties, promotion of neo-vascularization, mobilization of endothelial progenitor cells (EPCs), and enhancement of angiogenesis. The inhibition in the production of pro-inflammatory cytokines and reactive oxygen species exerted an anti-inflammatory and antioxidant effect respectively [41]. An animal model of AIS demonstrated that the administration of EPO enhanced the angiogenesis/vasculogenesis, neurogenesis, attenuated inflammation, oxidative stress, apoptosis and enhanced neurogenesis. Thus, there was a reduction in the brain infarct size and improvement in the neurological outcome [37].

Phase II studies were conducted to assess the safety and tolerability of EPO among AIS patients (Supplementary Table 1). In the German multicenter EPO stroke trial, EPO was administered to ischemic stroke patients with/without systemic thrombolysis. The study revealed no significant differences in the neurological outcomes (BI and NIHSS, mRS and MRI parameters) in both the groups [42]. In contrast in a Phase IIa trial evaluating the safety of Beta-hCG and EPO (B-E therapy), the domain-specific endpoints [NIHSS, BI, and arm and Fugl-Meyer Arm and Leg motor scores, Boston Naming Test, Line Cancellation Test, Action Research Arm Test, Trailmaking A and B] showed finer resolution of specific deficits. The clinical and laboratory data of the B-E therapy showed no safety concerns and no SAEs were reported [43]. In a recent study where evaluation of two consecutive doses of EPO was done; the long-term recurrent stroke or mortality did not vary between the two treatment groups. However, the long-term major adverse neurological event (MANE: death, recurrent stroke or long-term Barthel index) was lower in the EPO group when compared to the placebo group [44]. EPO therapy increased the number of circulating endothelial progenitor cells (EPCs) which in turn contributed to the repair of the cerebral vasculature. The circulating EPCs were responsible for the maintenance and repair of cerebral vasculature in cerebral ischemia [45]. At day 21, treatment with EPO significantly enhanced the number of circulating EPCs in the EPO treated group when compared to the placebo group [44]. Therefore, it can be proposed that an increase in the levels of circulating EPCs will strengthen local angiogenesis and vasculogenesis. This, in turn, will increase the cerebral blood flow and improve the neurological function in ischemic stroke patients. Studies have also shown that treatment of anemia can improve the neurological outcome of stroke, as most patients are likely to have a drop in hematocrit or red blood cells count after AIS. In conclusion, EPO has shown contrasting results in clinical studies. Therefore, more data is required to establish that EPO can be used for treating patients suffering from ischemic stroke. Thereby, more multicentre phase III trials with a larger population are necessary to establish the positive effect of HGFs in AIS.

4.6. MCLC601 (NeuroAiD)

MCLC601 (NeuroAiD), a Traditional Chinese Medicine (TCM) is a combination of 9 herbal (radix astragali, radix salvia mitorrhizae, radix paeoniae rubrae, rhizoma chuanxiong, radix angelicae sinensis, Carthamus tinctorius, Prunus persica, radix polygalae and rhizoma acori tatarinowii) and 5 animal components (Hirudo, Eupolyphaga seu Steleophaga, calculus bovis artifactus, Buthus martensii and Cornu saigae tataricae) in the capsule form which have demonstrated neuroprotective effects in both *in-vitro* and *in-vivo* studies. The drug was first launched at Singapore in the year 2006 and has gained tremendous acceptance as an innovative product for the post-stroke recovery. Later on, the drug has been marketed in several other countries such as South-East Asia, the Middle-East, Africa and Europe [46]. Animal studies have demonstrated that this neuroprotective agent attenuated the infarct size, improved the functional recovery and survival rate and protected the neurons against glutamate-induced injury. The above treatment exerts neuroprotective and neurodegenerative action through its multimodal action that comprises of the activation of Akt survival pathway and K_{ATP} channels, decrease of excitotoxicity and functional deficits, and reduction of necrosis, apoptosis, oxidative stress [47]. A multicenter, double-blind, placebo-controlled trial performed among 1100 patients demonstrated no difference among MCLC601 and placebo group in improving the 3 months outcomes when administered among patients with acute ischemic stroke of intermediate severity [48]. However, the Chinese Medicine NeuroAiD Efficacy on Stroke Recovery-Extension Study revealed the long term benefits of the drug over a period of two years. Nevertheless, more robust trials with subject selection based on poor prognosis with respect to severe NIHSS score and longer onset to treatment time with longer follow-up periods are required to study the effect of these neuroprotective or neurorestorative therapies [49].

5. The discrepancy between animal and human studies for neuroprotective agents

Several neuroprotective agents have shown promising results in pre-clinical studies; however, the success of the experimental findings has failed to translate into clinical evidence (Table 3). The prominent reasons for the failure could be due to the differences between the animal models and human studies, the difference in the quality of experimental and clinical studies, and the differences in the outcome measures between the studies. It has been observed that the outcome measures being evaluated in animal and clinical studies are heterogeneous from each other. The most commonly used outcome measures in clinical studies are mortality and neurological outcome scores (NIHSS, mRS, and BI) [31,39–40]. Whereas in animal studies the outcome measures are short-term that usually include infarct size, and the utility of neurological secondary outcomes are limited [50,59]. Hence, outcome measures being different in the experimental and clinical studies, the expected improvement may not be similar. Another discrepancy between the animal models and clinical studies is the differences in the study population; the experimental animal models are younger than the humans who suffer from AIS. Moreover, the animals are affected by fewer comorbidities such as such as hypertension, diabetes, and hyperlipidemia than the human population. The animal model will have homogenous aetiologies, whereas the human study population will consist of varying aetiologies. The appropriate drug dose for stroke is another issue in AIS treatment, leading to incorrect dose administration of the drug. Moreover, enough number of dose-response studies is not carried out in pre-clinical stroke models. Another intriguing factor is the origin and nature of the ischemic stroke, in the animal models, the ischemic territory usually begins from the middle cerebral artery whereas among the human population the ischemic territory is not restricted to the middle cerebral artery. Thus, these factors might affect the treatment of neuroprotective agents. In animal model studies, there is a control over the therapeutic window period giving room for better recovery among ischemic animal models. Conversely, in clinical studies, there is relatively lesser control over the therapeutic window period with delayed neuroprotective treatment. Therefore, the delayed treatment time could reduce the therapeutic efficacy of neuroprotective agents among ischemic stroke patients. Moreover, the humans would

have been exposed to other drugs whereas the animal models will not be treated with any other drugs previously. The drug-drug interactions may also constrain the efficacy of neuroprotective agents. The other factors that might be the reason for the failure of neuroprotective agents in clinical trials might be the route of administration, the availability of the drugs to the target areas and the scope for the dose optimization. All the above-mentioned factors may be the reason for the failure of neuroprotective agents [5,51–52]. A possible change in the current scenario could be an improvement in the animal models of stroke such that they are more reflective of the human counterpart.

6. Study Limitations

One of the major limitations of our study was that we were able to obtain data only from data sources such as PubMed and Clinicaltrial.gov. The exact status with regard to the history of development and the current status were missing for some of the drugs; this might be due to the fact that most of the pharmaceutical companies are reluctant towards publishing negative results. We could not perform meta-analysis for all the studies due to the presence of remarkable heterogeneity in reporting the study outcomes.

7. Conclusion

From the line of evidence it appears that neuroprotective agents have a rather bleak future in the treatment of AIS. Pre-clinical studies have shown that the administration of neuroprotective agents offers significant improvement in the reduction of brain infarct size and improvement in the functional outcomes. However, these findings failed to translate into clinical findings. Among the neuroprotective agents being studied, edaravone, EPO, G-CSF and MCLC601 (NeuroAiD) seems to be promising neuroprotective agents. However, larger well-designed, adequately powered, multi-centered RCTs and longer-term follow-up studies are required to establish neuroprotective agents for the treatment of AIS.

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Conflict ofinterest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopha.2018.11.041.

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