REVIEW

BIOLOGICAL MOLECULES IN CLINICAL STROKE TRIALS

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ABSTRACT

Stroke remains a leading cause of disability and mortality all over the world despite the efforts made towards improving treatment. Most of the clinical studies have not shown significant beneficial effects in the evaluation of various molecules for their neuroprotection and neurorecovery promoting properties. The new concept of multimodal, pleiotropic drugs has opened new perspectives in this field. This review focuses on clinical stroke studies with biologically active molecules such as erythropoietin, granulocyte-colony stimulating factor and Cerebrolysin.

Key words: neuroprotection, neurorecovery, multimodal, pleiotropic drugs

INTRODUCTION

Stroke remains a leading cause of disability and mortality all over the world (1) despite intense efforts made towards improving treatment. Why? The lack of an answer can be very frustrating for the doctors and the patients, too. What is the missing link?

In the last fifty years, tens of thousands of experimental and clinical studies have been performed to discover and understand the pathophysiologic mechanisms of stroke or to develop/enhance new drugs/techniques that may be able to cure or reduce the resulting neurologic disability.

Thorough knowledge of each step in the ischemic cascade and how they influence each other, together with deep understanding of the mechanistic action of various drugs, led to the conclusion that molecules with potential beneficial effects are able to simultaneously control multiple pathophysiological processes in the stroke cascade (2).

The new concept of a multimodal, pleiotropic drug refers to its ability to have both neuroprotective and neuroregenerative effects (2). Neurotrophic factors and growth factors are the best documented biological molecules with multimodal, pleiotropic effects, which are able to modulate but not suppress pathophysiological processes (2). This characteristic is very important because many processes, such as glutamate induced-excitotoxicity and neurotrophicity and neuroplasticity, rely on NMDA receptor activity as their common driver. Therefore, suppression of excitotoxicity will also affect the neurorecovery processes (2).

A pleiotropic mechanism refers to the control of multiple pathophysiological processes in a biological cascade (2). A multimodal drug is able to simultaneously promote neuroprotection and retain the capability for promoting neuroplasticity and neurorecovery (2).

The neuroprotective effects are due to the modulation of the ischemic cascade at different levels. Biological molecules have pleiotropic, neuroprotective effects. In other words, biological molecules can simultaneously modulate multiple pathological cascades (anti-excitotoxic, anti-inflammatory, anti-apoptotic, anti-oxidant, and more) (2).

The neurorecovery promoting properties of these drugs are mostly due to the stimulation of angiogenesis and neurogenesis. The known neurogenic zones are the subventricular and subgranular zones of the dentate gyrus (3). These zones have

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low oxygenation. Thus, neuronal stem cells may survive in the critical phase of ischemia, and hypoxia can regulate their cellular differentiation (3). After stroke, neural stem cells proliferate, migrate to and differentiate at the injury site, which interferes with structural and functional recovery. These pluripotent stem cells have the capacity to differentiate into neurons, astrocytes, oligodendrocytes, and endothelial cells. Experimental stroke studies demonstrated that stem cells can survive, integrate, and even operate as neurons (4-6). The common, crucial driver for all of these processes is the local presence of neurotrophic and vascular growth factors (3,7). Biologically active molecules and their receptors regulate differentiation, growth and development of different cell types, including nervous and vascular system cells.

ERYTHROPOIETIN (EPO)

The growth factor EPO, a hormone produced by fetal liver and adult kidney, is involved in the proliferation and differentiation of erythroid progenitor cells (8). Initially used in the treatment of anemia secondary to end-stage renal insufficiency, its efficacy spectrum was extended later to other forms of anemia, including cancer-related anemia (9). EPO crosses the brain blood barrier (10) and has important neuroprotective and neuroregenerative effects (11)[1]. These beneficial properties are mediated via the neuronal EPO-receptor (EPO-R) (10-12). EPO-R is different from the receptor involved in erythropoiesis, and it is expressed in neuro-epithelial tissue. EPO-R expression is upregulated following various types of brain injury, including hypoxia (10-13).

The multimodal, neuroprotective effects of EPO are the result of its anti-apoptotic, antioxidant, and anti-inflammatory effects(9,10,12,13. EPO inhibits apoptosis and lipid peroxidation. EPO also attenuates inflammation by reducing reactive astrocytosis and microglia activation (9,14. EPO's anti-apoptotic effects were demonstrated by a reduction in the number of apoptotic cells (12,13) and its interference with caspase-3 activity (15). In the ischemic brain, EPO has been reported to protect BBB integrity through the attenuation of inflammatory cells, thereby demonstrating its anti-inflammatory properties (16,17).

EPO also has important neuroregenerative properties which can enhance angiogenesis and neurogenesis (13,15). In a recent experimental study on neonatal stroke rats, Gonzales et al, demonstrated that EPO stimulates the proliferation and migration of neural progenitor cells from the subventricular zone (18). Neurogenesis and oligodendrogliosis were prominently stimulated at both early and late time points (18), confirming the results from previous studies (19,20).

In a meta-analysis of sixteen experimental studies, Minnerup et al. showed that EPO and its analogues significantly reduced infarct size by more than one-third and improved neurobehavioral deficits when administered after the onset of ischemia (11). EPO was more effective when administered during the first 6 hours after stroke compared to a later treatment initiation (11).

The first clinical trial, published in 2002, demonstrated that an intravenous high-dose of recombinant human EPO (rh-EPO) administered once daily for the first 3 days after stroke is well tolerated and is associated with improved clinical outcomes at 1 month. (21)

The double-blind, placebo-controlled, randomized German Multicenter EPO Stroke Trial (Phase II/III) enrolled 522 patients with acute ischemic stroke. The patients received EPO intravenously at 6, 24 and 48 hours after stroke. EPO treatment had no favorable effects and had an increased incidence of death (particularly in patients also receiving systemic thrombolysis) compared with the placebo-group (22).

Tseng et al. studied the effects of systemic rh-EPO on cerebral auto-regulation and the incidence of delayed ischemic deficits following aneurysmal subarachnoid hemorrhage (SAH) (23). Eighty patients with aneurysmal SAH received intravenous rh-EPO three times during the first week. The results showed that EPO reduced the delayed cerebral ischemia following aneurismal SAH and improved functional outcomes. The mechanism was through decreasing the severity of vasospasm and shortening the length of time for auto-regulation impairment (23).

Even though the clinical studies lack beneficial findings to date, the experimental studies using EPO are very promising.

GRANULOCYTE COLONY STIMULATING FACTOR

Granulocyte colony stimulating factor (G-CSF) is a growth factor that acts on hematopoietic $CD34^+$ stem cells to regulate neutrophil progenitor proliferation and differentiation (24). G-CSF is currently used in oncology to accelerate the recovery from neutropenia after chemotherapy. G-CSF is also used in hematology to increase the number of he-

matopoietic stem cells for subsequent autologous or allogenic infusion (24,25).

G-CSF is neuroprotective and promotes neurorecovery through different mechanisms including the mobilization of hematopoietic stem cells, reduction apoptosis and inflammation, anti-excitotoxic effects, and stimulation of neurogenesis and angiogenesis (24,25).

In a systematic review of 19 publications incorporating 666 animals, England TJ et al. showed that G-CSF is able to reduce lesion size significantly in transient but not permanent focal models of ischemia (26). Secondary, G-CSF reduced the motor deficit and death (26).

In human adults, CD34+ cells may differentiate into both hematopoietic stem cells and endothelial progenitor cells, which may contribute to angiogenesis after stroke (27). For this reason, G-CSF was studied using various dosages and administration routes to demonstrate its neuroregenerative effects.

In the AXIS trial (A Trial of Intravenous Granulocyte Colony-Stimulating Factor in Acute Ischemic Stroke) Schäbitz et al. evaluated high intravenous doses of G-CSF in patients with acute stroke (28). They concluded that G-CSF was well-tolerated even at high dosages in patients with acute ischemic stroke without significant adverse events compared with the placebo treatment. Additionally, the authors noted a G-CSF dose-dependent beneficial effect in patients with DWI lesions >14–17 cm (subgroup analysis) (28).

The AXIS II trial enrolled 328 patients with acute ischemic stroke that were within 9 hours from onset (29). The study failed to demonstrate any beneficial effects as measured using the mRS and NIHSS scores in patients treated with AX200 (29).

Boys et al. published the results of the first phase I/IIa open-labeled trial in which standard IV thrombolysis was compared with daily administration of subcutaneous G-CSF for 5 consecutive days (30). Twenty patients with acute ischemic stroke were enrolled and G-CSF treatment was initiated within the first 12 hours after stroke onset. G-CSF treatment increased the mobilization of CD34+ stem cells into the peripheral blood, but a dose-response relationship between subcutaneous G-CSF administration and CD34+ stem cell mobilization was not established. Additionally, G-CSF seems to improve the patients' neurocognitive functions. This study demonstrated that tPA seems to be safe when it is associated with G-CSF, which opens new directions in this field (30).

Overall, the clinical trials indicate that G-CSF is well tolerated, appears to be safe, and significantly increases white blood cell counts (28.29.30). We now need data from larger clinical trials aimed at confirming the safety and demonstrating the efficacy of G-CSF treatment.

CEREBROLYSIN

Cerebrolysin is a peptide produced by a biotechnological process: a standardized enzymatic breakdown of purified, lipid-free brain proteins. It consists of low molecular weight neuropeptides (<10 kDa) and free amino acids (31). Cerebrolysin is approved for the treatment of stroke, traumatic brain injuries and dementia in a number of European and Asian countries.

Cerebrolysin is another multimodal, pleiotropic drug. Due to its pleiotropic effects, Cerebrolysin modulates (without suppression) pathological processes such as excitotoxicity, inflammation, and apoptotic-like processes (2).

Cerebrolysin mimics the action of neurotrophic factors and exerts a neuroprotective (32) (59) and neurotrophic action (33,34). Cerebrolysin interacts with receptors of inhibitory neurotransmitters (anti-excitotoxic effects) and blocks the formation of free oxygen radicals by modulating the gene expression of antioxidant enzymes (antioxidant effects) (31). Cerebrolysin treatment stabilizes cytoskeletal proteins such as MAP2, which are degraded following ischemic events, which preserves the structural cell integrity. This effect is through the Cerebrolysin-mediated inhibition of calpain (31).

In addition, by mimicking the action of naturally occurring neurotrophic factors, Cerebrolysin exerts a neurotrophic effect and promotes neuroregeneration. The compound stimulates neuronal differentiation, growth and sprouting and supports neuronal survival and the formation of synaptic contacts, which enhances neurogenesis in the dentate gyrus of the hippocampus (31,35,36). Cerebrolysin reduces infarct volume and improves the neurological outcome measures in a rat model of acute focal ischemia (31). In 2013, Zhang et al. demonstrated that Cerebrolysin significantly increased neural progenitor cell proliferation and differentiation into neurons and myelinating oligodendrocytes (37). The authors demonstrated that the Shh pathway mediates Cerebrolysin-enhanced neurogenesis and white matter remodeling and improves functional recovery in rats after stroke.

In light of these promising findings, Cerebrolysin was studied in many clinical trials. All the clinical trials showed that the drug is well tolerated and has a good safety profile, even when administered with tPA (38,39).

In the RCT, double-blind, CASTA trial (Cerebrolysin Acute Stroke Treatment in Asia), a total of 1070 patients with ischemic hemispheric stroke were randomized (within 12 hours of symptom onset) to active treatment (30 mL Cerebrolysin daily) or placebo treatment (saline solution) given intravenously for 10 days in addition to aspirin (100 mg daily) (40). The results were neutral between the treatment groups. However, Cerebrolysin treatment trended favorably for the severely affected patients (NIHSS>12) (39).

In a study by Lang W et al., the combination of Cerebrolysin with tPA did not improve outcome measures at day 90 (mRS), but significantly more patients had favorable responses in neurological outcome measures (an improvement of 6 or more points on NIHSS) in the Cerebrolysin group (39). The regimen consisted of daily intravenous infusion of 30 ml Cerebrolysin or placebo administered one hour after thrombolysis and given for 10 consecutive days (39). The safety profile of Cerebrolysin was very good (39).

Cerebrolysin has demonstrated promising beneficial properties regarding neuroprotection and neurorecovery both in experimental and clinical studies. Cerebrolysin is still undergoing investigation in larger clinical trials.

CONCLUSIONS

Even though there are some discrepancies between the results of experimental clinical studies, biological molecules with multimodal, pleiotropic effects are very promising and show positive trends in clinical studies. Larger, well conducted clinical studies with improved designs taking into account possible comorbidities are needed to confirm the beneficial results from experimental studies.

Conflicts of interests

The authors declare no conflicts of interest.

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