REVIEW

Neurotherapeutic potential of erythropoietin after ischemic injury of the central nervous system

Florian Simon*, Nicolaos Floros, Wiebke Ibing, Hubert Schelzig, Artis Knapsis
Department of Vascular and Endovascular Surgery, Heinrich-Heine-University of Düsseldorf, Düsseldorf, Germany

Abstract

Erythropoietin (EPO) is one of the most successful biopharmaceuticals in history and is used for treating anemia of different origins. However, it became clear that EPO could also work in a neuroprotective, antiapoptotic, antioxidative, angiogenetic and neurotropic way. It causes stimulation of cells to delay cell apoptosis, especially in the central nervous system. In rodent models of focal cerebral ischemia, EPO showed an impressive reduction of infarct size by 30% and improvement of neurobehavioral outcome by nearly 40%. A large animal model dealing with ischemia and reperfusion of the spinal cord showed that EPO could reduce the risk of spinal cord injury significantly. In addition, some clinical studies tested whether EPO works in real live clinical settings. One of the most promising studies showed the innocuousness and improvements in follow-up, outcome scales and in infarct size, of EPO-use in humans suffering from ischemic stroke. Another study ended unfortunately in a negative outcome and an increased overall death rate in the EPO group. The most possible reason was the involvement of patients undergoing simultaneously systemic thrombolysis with recombinant tissue plasminogen activator. An experimental study on rats demonstrated that administration of EPO might exacerbate tissue plasminogen activator-induced brain hemorrhage without reducing the ischemic brain damage. This case shows clearly how useful animal models can be to check negative side effects of a treatment before going into clinical trials. Other groups looked in human trials at the effects of EPO on the outcome after ischemic stroke, relation to circulating endothelial progenitor cells, aneurysmal subarachnoid hemorrhage, traumatic brain injury, hemoglobin transfusion thresholds and elective first-time coronary artery bypass surgery. Most of the results were positive, but are based mostly on small group sizes. However, some of the most neglected facts when focusing on experimental setups of ischemia of the central nervous system are issues like age and comorbidities. It might be extremely worthy to consider these points for future projects, because EPO might influence all these factors.

Key Words: *erythropoietin; apoptosis; central nervous system; cerebral ischemia; animal model; stroke; human trials; age; comorbidity; spinal cord ischemia*

Correspondence to:
Florian Simon, PhD,
florian.simon@med.uni-duesseldorf.de.

orcid:
0000-0001-5743-8554
(Florian Simon)

doi: *10.4103/1673-5374.253507*

Received: *December 19, 2018*
Accepted: *January 21, 2019*

Erythropoietin (EPO) is one of the most successful biopharmaceuticals in history. Carnot and Deflandre (1906) assumed already at the beginning of the 20th century that there must be a humoral factor for red blood cell production. However, it took several decades until the substance itself was isolated and purified by Goldwasser (1996) in the 1970's out of the urine of patients suffering from aplastic anemia and finally cloned and transferred into mammalian cells at the end of the 1980's (Lin et al., 1985). EPO has since then been used for treating anemia of different origins. However, there is more about EPO than merely keeping up oxygen saturation in tissues by controlling production and apoptosis of red blood cells. The ability of EPO to protect tissue against harmful influence was unknown for a long time and treating anemia was the only focus linked to the application of EPO. This point of view changed as it became clear that EPO could also work in a neuroprotective, antiapoptotic, antioxidative, angiogenetic and neurotropic way (Maiese et al., 2004; Nakano et al., 2007). In fact, many different organs other than the kidneys produce EPO and it shows action in those tissues. It causes stimulation of cells in a paracrine and even autocrine manner to delay cell apoptosis (Maiese et al., 2005). Especially in the central nervous system (CNS) EPO producing cells as well as EPO receptor presenting cells can be found as one of the most important lines of defence against temporary tissue damage *e.g.*, ischemia that would otherwise result in cell apoptosis. Those cells are mostly astrocytes, neurons, oligodendrocytes and neural progenitor cells (Juul et al., 1999). This article presents a review of latest trials and research progress for EPO in treatment of CNS damages. We have performed a PubMed literature search of articles published in the period January 2000 – December 2018 on erythropoietin during ischemic injury of the central nervous system.

Studies emerged to clarify whether EPO administration can help to overcome damages of neuronal tissue. Indeed, preclinical studies revealed an enormous potential for EPO as a neuroprotective drug. Regarding ischemic strokes, high doses of EPO administration showed that cells lying in the ischemic penumbra of the brain profit from antiapoptotic EPO effects (Siren et al., 2001). In a systematic review and meta-analysis, assessing the efficacy of EPO in rodent models of focal cerebral ischemia, EPO showed an impressive reduction of infarct size by 30% and improvement of neurobehavioral outcome by nearly 40% (Jerndal et al., 2010). The authors own working group showed that EPO administration also works in a large animal model dealing with ischemia and reperfusion of the spinal cord caused by cross clamping of the thoracic aorta. This surgical maneuver mimics daily challenges in a vascular surgery department. A

second feature of this study is the use of a nonhematopoietic derivative of EPO, the so-called cEPO-FC. This molecule consists of two EPO molecules fused to the FC part of an immunoglobulin IgG1 with a consecutive carbamylation of the whole molecule to lose the ability to stimulate red cell count, but keeping up cell protective properties (Schriebl et al., 2006). Additionally, the half-life of this molecule extends so that EPO might interact much longer with its receptors. In order to evaluate the extension of neuronal damage and to assess the clinical outcome of the animals, histological staining and lower limbs moving ability were analyzed. The results of this experimental setup showed that EPO and cEPO-FC administrated shortly before and again directly after ischemia can reduce the risk of spinal cord injury significantly (Simon et al., 2011). This work is of special interest as large animal models are much more similar to daily clinical situations than models using small animals like *e.g.*, mice. Other effects of EPO were reduction of inflammation as response to injury. Therefore, it may help to overcome secondary damage to neuronal tissue during reorganization after a primary insult or during the appearance of a negative side effect *e.g.*, after surgical impact (Villa et al., 2003).

It does actually need much larger doses of EPO to bring positive non-hematopoietic effects to life than of those needed to treat anemia. However, there is convincing proof in experimental setups that justifies taking EPO serious for treating neuronal disorders. This is especially true in cases of acute hypoxia of neuronal tissue. Some clinical studies tested whether EPO works in real live clinical settings.

Certainly one of the most promising studies was the work of Ehrenreich from 2002. The group showed in a safety trial with 13 patients the innocuousness of EPO-use (3.3×10^4 IU/50 mL per 30 minutes once daily for the first 3 days after stroke) in humans, suffering from ischemic stroke. In a second step with 40 patients, the scientists used EPO or saline in a double-blinded and randomized proof-of-concept trial. Interestingly, Ehrenreich found improvements in follow-up and outcome scales and in infarct size as well proven by magnetic resonance imaging imaging (Ehrenreich et al., 2002). Based on these encouraging results the same working group of Ehrenreich et al. (2002) undertook a second study with 522 patients suffering from acute ischemic stroke in the middle cerebral artery territory. These patients received EPO within the first 6 hours of symptom onset as well as 24 and 48 hours (40,000 IU each time). In contrast to the good results of the first study, the effects of this trial ended in a negative outcome of the Barthel Index on day 90 and an increased overall death rate in the EPO group compared to the placebo group. The authors state that the involvement of patients undergoing simultaneously systemic thrombolysis with recombinant tissue plasminogen activator might have caused the negative outcome of the trial. Nevertheless, the trial raised safety concerns of EPO treatment affecting future clinical trials (Ehrenreich et al., 2009). One year later an experimental study on rats demonstrated exactly this crucial point when using EPO. Jia et al. (2010) were able to show that administration of EPO (5000 IU/kg) exacerbates tissue plasminogen activator-induced brain hemorrhage in rats without reducing the ischemic brain damage. This case shows clearly how useful animal models can be to check negative side effects of a treatment before going into clinical trials. However, these negative studies, clinical and experimental ones, should not lead to the conclusion that it might be better to avoid future studies on EPO. In fact, it is encouraging to initiate new studies as long as the scientists scrutinize precisely their study design in advance to prevent such fatal errors.

Another group looked at the effects of EPO (5000 IU at 48 and 72 hours after acute ischemic stroke) regarding the outcome after ischemic stroke and whether there is an effect on the count of the circulating endothelial progenitor cells as they might have a positive impact on neurological regeneration. Research staff was aware of the correlation of a too wide range of inclusion criterions as they might cause severe side effects not necessarily linked to EPO as described above. Some of the most important criteria might be the exclusion of intracranial hemorrhage, hematologic disorders and malignancies, renal insufficiency, recombinant tissue plasminogen activator therapy or hemoglobin levels > 15 mg/dL. In total 167 patients took part in this study and received either the EPO or the placebo drug preparation. This working group found that EPO significantly increased the circulating endothelial progenitor cell level and additionally improved the 90-day clinical outcome and lowered major adverse neurological events (Yip et al., 2011).

Human studies on EPO dealing with neurological outcome are still rare and even those published are not necessarily sounding or they suffer from the fact that the group of patients was too small. Springborg et al. (2007) published a study showing this is a crucial point of study design. They intended to examine the effects of EPO therapy (500 IU/kg per day for 3 days) in aneurysmal subarachnoid hemorrhage. The group ended recruitment prematurely, because the number of enrolled patients was increasing too slowly and therefore results were not reliable enough to conclude whether a clinical application of EPO in subarachnoid hemorrhage is advisable or not. The key findings were that administration of EPO increasing its concentration in the CNS by 600 times seems to be safe and that further studies with larger patient numbers in terms of a multiple center study should be performed (Springborg et al., 2007). Another setting dealing with subarachnoid hemorrhage looked at the delayed ischemic deficits connected with this disease (EPO 30,000 IU every 48 hours for a total of 90,000 IU). The authors described a decreased incidence of severe vasospasm, reduced delayed ischemic deficits, an improved autoregulation and a better outcome at discharge, but revealed no better overall outcome after 6 months (Tseng et al., 2009). Similar effects showed a study dealing with traumatic brain injury. The scientists compared the effects of EPO (500 IU/kg) and two hemoglobin transfusion thresholds (7 and 10 g/dL), because patients with severe traumatic brain injury often develop anemia, what might cause second injury worsening neurological outcome. No interventions, unfortunately, improved

neurological outcome at 6 months (Glasgow Outcome Scale score was used to measure neurological outcome) (Robertson et al., 2014). In a study focusing on patients with acute ischemic stroke two consecutive doses of EPO (5000 IU/dose, subcutaneously administered at 48 and 72 hours after acute stroke) were given. The results showed no difference in the long-term recurrent stroke and mortality rate. However, the long-term major adverse neurological events and the long-term severe neurological deficits were significantly lower in the treatment group. This is of special interest, because EPO was given not earlier than 48 hours after stroke, when most of the cells suffering from ischemia must be already dead. This shows the importance of ongoing apoptotic reactions in the penumbra of an ischemic region in the CNS (Tsai et al., 2015).

Neuronal tissue damage might also occur as side effect in surgery although the CNS itself is not the therapeutic target like *e.g.*, in heart surgery. Haljan et al. (2009) describe in their study the results of patients that underwent an elective first-time coronary artery bypass surgery what is often associated with neurocognitive dysfunction. The authors investigated whether EPO given in three different daily doses (375, 750 and 1500 IU/kg) can improve neurological function at discharge as well as after two months. They found no negative side effects of EPO administration, but also no benefits concerning the neurocognitive dysfunction at discharge. After two months, there was a trend of reduced neurocognitive dysfunction in association with EPO administration. Despite this positive result, the crucial part again was the small group size that makes it impossible to draw reliable conclusions from the study. The authors themselves demand a multicenter randomized controlled trial to clarify the specific questions of clinical reliability of the EPO administration (Haljan et al., 2009).

Some of the most neglected facts when focusing on experimental setups of ischemia of the CNS are issues like age and comorbidities. In humans, age is one of the most important risk factors for cerebral ischemia, but also plays a major role in recovery and neurological outcome of patients after stroke. Although there are no explicit studies on EPO in combination with age and comorbidities of the CNS yet, it might be extremely worthy to consider these points for future projects. Experiments with aged rats suffering from ischemic stroke showed a wide range of differences in comparison to young and healthy animals after an ischemic insult. For example, there are massive changes in neuroinflammation with increased brain-blood-barrier permeability, accelerated apoptosis and precipitous infarct development in the aged animals. There are also significant deviations in the gene expression especially down-regulation of anti-apoptotic related genes, upregulation of pro-inflammatory mediators and dysregulation of CNS remodeling. Another affected factor is angiogenesis with delayed sprouting and basal lamina build-up with decreased vascular density in the periinfarcted area. All these factors result in limited behavioral recovery of the aged animals compared to younger ones. The reason why age and comorbidities are very interesting for future EPO trials is that EPO influences all these factors mentioned above, because EPO is antinflammatic, antiapoptotic, antioxidative, angiogenetic and neurotropic as described above (Popa-Wagner et al., 2018).

In summary, it seems that EPO has many interesting effects justifying further projects (**Table 1**). On the one hand there are very promising experimental studies showing the range of the EPO area of use. On the other hand, it is apparent that EPO might have a deep clinical impact on neuronal tissue damage, too. Therefore, further studies both experimental and clinical are needed. When going into humans it is of special importance that study designs have to be planned very carefully, because medication, treatment, age and comorbidities of the patients might interact negatively with each other. Most EPO effects are of antiapoptotic and antiinflammatic origin so the most promising study areas seem to be that of acute occurring diseases like ischemia and reperfusion or traumatic injuries of the brain or the spinal cord to protect cells located in the penumbra of the damaged tissue. Finally, the list of possible drugs that might have positive effects is very short so that a promising drug like EPO is worth not to be left aside before its entire healing potential has been explored.

Author contributions: *Manuscript writing: FS; manuscript reviewing: AK, NF, WI and HS.*
Conflicts of interest: *All authors declare no conflicts of interest.*
Financial support: *None.*
Copyright license agreement: *The Copyright License Agreement has been signed by all authors before publication.*
Plagiarism check: *Checked twice by iThenticate.*
Peer review: *Externally peer reviewed.*

Open peer reviewers: *Aurel Popa-Wagner, University Medicine Rostock, Germany; Ramon Navarro, Cleveland Clinic Abu Dhabi, United Arab Emirates.*
Additional file: *Open peer review report 1.*

References

Carnot P, Deflandre C (1906) Sur l'activité hemopoïètique du sérum au cours de la régénération du sang. Compt Rend Acad Sci 143:348-386.

Ehrenreich H, Hasselblatt M, Dembowski C, Cepek L, Lewczuk P, Stiefel M, Rustenbeck H-H, Breiter N, Jacob S, Knerlich F, Bohn M, Poser W, Rüther E, Kochen M, Gefeller O, Gleiter C, Wessel TC, De Ryck M, Itri L, Prange H, et al. (2002) Erythropoietin therapy for acute stroke is both safe and beneficial. Mol Med 8:495-505.

Ehrenreich H, Weissenborn K, Prange H, Schneider D, Weimar C, Wartenberg K, Schellinger PD, Bohn M, Becker H, Wegrzyn M, Jahnig P, Herrmann M, Knauth M, Bahr M, Heide W, Wagner A, Schwab S, Reichmann H, Schwendemann G, Dengler R, et al. (2009) Recombinant human erythropoietin in the treatment of acute ischemic stroke. Stroke 40:e647-656.

Goldwasser E (1996) Erythropoietin: a somewhat personal history. Perspect Biol Med 40:18-32.

Haljan G, Maitland A, Buchan A, Arora RC, King M, Haigh J, Culleton B, Faris P, Zygun D (2009) The erythropoietin neuroprotective effect: assessment in CABG surgery (TENPEAKS): a randomized, double-blind, placebo controlled, proof-of-concept clinical trial. Stroke 40:2769-2775.

Table 1 Summary of studies, main findings and limitations

Study	Main findings	Limitations
Jerndal et al. (2010)	Reduction of infarct size by 30% and improvement of neurobehavioral outcome by nearly 40%	Rodent models
Simon et al. (2011)	Erythropoietin (EPO) and nonhematopoietic derivative of EPO (cEPO-FC) administrated shortly before and again directly after ischemia can reduce the ischemic damage of the spinal cord significantly	Large animal model
Ehrenreich et al. (2002)	Improvements in follow-up, outcome scales and in infarct size	Small group size
Ehrenreich et al. (2009)	Negative outcome of the Barthel Index on day 90 and an increased overall death rate	Involving patients undergoing systemic thrombolysis (recombinant tissue plasminogen activator)
Jia et al. (2010)	Administration of EPO exacerbates tissue plasminogen activator-induced brain hemorrhage	Rodent model
Yip et al. (2011)	EPO significantly increased the circulating endothelial progenitor cell level and additionally improved the 90-day clinical outcome and lowered major adverse neurological events	Wide range of exclusion criterions
Springborg et al. (2007)	EPO increased its concentration in the central nervous system by 600 times and seems to be safe in treating aneurysmal subarachnoid hemorrhage	Small group size
Tseng et al. (2009)	Decreased incidence of severe vasospasm, reduced delayed ischemic deficits, improved autoregulation and better outcome at discharge, but no better overall outcome after 6 months	Small group size
Robertson et al. (2014)	No improved neurological outcome at 6 months	Traumatic brain injury instead of ischemic stroke
Tsai et al. (2015)	No difference in long-term recurrent stroke and mortality rate, but long-term major adverse neurological events and long-term severe neurological deficits were significantly lower	EPO was given not earlier than 48 hours after stroke
Halian et al. (2009)	No benefits concerning the neurocognitive dysfunction at discharge, but after two months, there was a trend of reduced neurocognitive dysfunction	Small group size

Jerndal M, Forsberg K, Sena ES, Macleod MR, O'Collins VE, Linden T, Nilsson M, Howells DW (2010) A systematic review and meta-analysis of erythropoietin in experimental stroke. J Cereb Blood Flow Metab 30:961-968.

Jia L, Chopp M, Zhang L, Lu M, Zhang Z (2010) Erythropoietin in combination of tissue plasminogen activator exacerbates brain hemorrhage when treatment is initiated 6 hours after stroke. Stroke 41:2071-2076.

Juul SE, Yachnis AT, Rojiani AM, Christensen RD (1999) Immunohistochemical localization of erythropoietin and its receptor in the developing human brain. Pediatr Dev Pathol 2:148-158.

Lin FK, Suggs S, Lin CH, Browne JK, Smalling R, Egrie JC, Chen KK, Fox GM, Martin F, Stabinsky Z, et al. (1985) Cloning and expression of the human erythropoietin gene. Proc Natl Acad Sci U S A 82:7580-7584.

Maiese K, Li F, Chong ZZ (2004) Erythropoietin in the brain: can the promise to protect be fulfilled? Trends Pharmacol Sci 25:577-583.

Maiese K, Li F, Chong ZZ (2005) New avenues of exploration for erythropoietin. JAMA 293:90-95.

Nakano M, Satoh K, Fukumoto Y, Ito Y, Kagaya Y, Ishii N, Sugamura K, Shimokawa H (2007) Important role of erythropoietin receptor to promote VEGF expression and angiogenesis in peripheral ischemia in mice. Circ Res 100:662-669.

Popa-Wagner A, Glavan DG, Olaru A, Olaru DG, Margaritescu O, Tica O, Surugiu R, Sandu RE (2018) Present status and future challenges of new therapeutic targets in preclinical models of stroke in aged animals with/without Comorbidities. Int J Mol Sci 19:E356.

Robertson CS, Hannay HJ, Yamal JM, Gopinath S, Goodman JC, Tilley BC, Epo Severe TBI Trial Investigators, Baldwin A, Rivera Lara L, Saucedo-Crespo H, Ahmed O, Sadasivan S, Ponce L, Cruz-Navarro J, Shahin H, Aisiku IP, Doshi P, Valadka A, Neipert L, Waguspack JM, et al. (2014) Effect of erythropoietin and transfusion threshold on neurological recovery after traumatic brain injury: a randomized clinical trial. JAMA 312:36-47.

Schriebl K, Trummer E, Lattenmayer C, Weik R, Kunert R, Muller D, Katinger H, Vorauer-Uhl K (2006) Biochemical characterization of rhEpo-Fc fusion protein expressed in CHO cells. Protein Expr Purif 49:265-275.

Simon F, Scheuerle A, Gröger M, Vcelar B, McCook O, Moller P, Georgieff M, Calzia E, Radermacher P, Schelzig H (2011) Comparison of carbamylated erythropoietin-FC fusion protein and recombinant human erythropoietin during porcine aortic balloon occlusion-induced spinal cord ischemia/reperfusion injury. Intensive Care Med 37:1525-1533.

Siren AL, Fratelli M, Brines M, Goemans C, Casagrande S, Lewczuk P, Keenan S, Gleiter C, Pasquali C, Capobianco A, Mennini T, Heumann R, Cerami A, Ehrenreich H, Ghezzi P (2001) Erythropoietin prevents neuronal apoptosis after cerebral ischemia and metabolic stress. Proc Natl Acad Sci U S A 98:4044-4049.

Springborg JB, Moller C, Gideon P, Jorgensen OS, Juhler M, Olsen NV (2007) Erythropoietin in patients with aneurysmal subarachnoid haemorrhage: a double blind randomised clinical trial. Acta Neurochir (Wien) 149:1089-1101.

Tsai TH, Lu CH, Wallace CG, Chang WN, Chen SF, Huang CR, Tsai NW, Lan MY, Sung PH, Liu CF, Yip HK (2015) Erythropoietin improves long-term neurological outcome in acute ischemic stroke patients: a randomized, prospective, placebo-controlled clinical trial. Crit Care 19:49.

Tseng MY, Hutchinson PJ, Richards HK, Czosnyka M, Pickard JD, Erber WN, Brown S, Kirkpatrick PJ (2009) Acute systemic erythropoietin therapy to reduce delayed ischemic deficits following aneurysmal subarachnoid hemorrhage: a Phase II randomized, double-blind, placebo-controlled trial. Clinical article. J Neurosurg 111:171-180.

Villa P, Bigini P, Mennini T, Agnello D, Laragione T, Cagnotto A, Viviani B, Marinovich M, Cerami A, Coleman TR, Brines M, Ghezzi P (2003) Erythropoietin selectively attenuates cytokine production and inflammation in cerebral ischemia by targeting neuronal apoptosis. J Exp Med 198:971-975.

Yip HK, Tsai TH, Lin HS, Chen SF, Sun CK, Leu S, Yuen CM, Tan TY, Lan MY, Liou CW, Lu CH, Chang WN (2011) Effect of erythropoietin on level of circulating endothelial progenitor cells and outcome in patients after acute ischemic stroke. Crit Care 15:R40.

P-Reviewers: Navarro R, Popa-Wagner A; C-Editors: Zhao M, Yu J; T-Editor: Liu XL