

Erythropoietin in post-resuscitation neurological recovery: is there light at the end of the tunnel?

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ABSTRACT

Studies show that erythropoietin, besides its critical role in hematopoiesis, provides neuroprotection in hypoxic-ischemic cerebral injury. Antiapoptotic, anti-inflammatory, angiogenetic, and neurotrophic properties of erythropoietin could increase indications, currently restricted to anemia in chronic renal failure and cancer, to hypoxic-ischemic cerebral insult.

In the adult and neonatal animal model of hypoxic-ischemic cerebral injury, erythropoietin significantly reduces infarct size with attenuation of brain damage, and preservation of cortical integrity. The first human study on the impact of erythropoietin in stroke victims showed that erythropoietin is safe and well tolerated at high doses, and associated with improved neurological outcome. Even with intravenous application, concentrations of erythropoietin in cerebrospinal fluid of these patients were many-fold higher than in non-treated patients.

In successfully resuscitated cardiac arrest victims overall neurological recovery remains poor despite improved cardiopulmonary resuscitation strategies. Post-resuscitation care needs further advances in order to improve final outcome. Through promotion of neuroangiogenesis, inhibition of hypoxia-induced apoptosis in neurons, and thus support of the survival of neurons in the ischemic brain, erythropoietin could be used to improve functional recovery of these patients. Nevertheless, optimal molecular forms of EPO, therapeutic doses, and treatment time window have to be determined in order to lower the incidence of adverse effects and still preserve neuroprotective properties.

Key words: cardiopulmonary resuscitation, erythropoietin, post-resuscitation period, hypoxia.

Introduction

Optimal neurological recovery after successful cardiopulmonary resuscitation (CPR) is the most important aspect of post-resuscitation care because it directly affects the final outcome. Proper oxygenation, blood pressure control, normoglycemia and therapeutic hypothermia are established measures in immediate post-resuscitation care, which promote variable degrees of cerebral recovery. A brief period of cerebral hyperemia after return of spontaneous circulation

(ROSC) (1) is followed by decreased brain perfusion. Unstable, post-resuscitation hemodynamics, together with the initial hypoxic-ischemic injury, lead to further neurological damage. Despite the increased incidence of ROSC and overall survival, poor functional recovery of successfully resuscitated cardiac arrest victims demands further research in the field of post-resuscitation care. Exogenous erythropoietin (EPO) has been shown to promote neuroprotection in hypoxic-ischemic cerebral insult by regulating neurogenesis, and preventing neuronal apoptosis (2-4). These protective properties of EPO, in ischemic brain damage, could benefit patients who have survived a cardiac arrest.

Neuroprotective role of erythropoietin in cerebral ischemia

As nature proves, the simplest and yet the most important solutions lie in the capability of nature to renew itself from its own resources. EPO, a vital compound of erythroid differentiation, is also involved in non-hematopoietic tissue protective pathways with its antiapoptotic, anti-inflammatory, angiogenetic, and neurotrophic properties (5). The evidence shows that hypoxia increases production of EPO not only in kidneys, but also in brain, testis, liver, and spleen (6-8). In brain, EPO is highly expressed after neuropathological insult (6). Astrocytes produce EPO after

its upregulation due to hypoxia, and neurons express EPO receptors (3,8). It has also been shown that after systemic administration, EPO promotes neuroprotection even in severe cerebral ischemia (9), enhances neurological recovery in traumatic brain and spinal cord injury (6,10-13), and prevents the loss of autoregulation of cerebral blood flow (14). This evidence of neuroprotection and restructuring of cerebral tissue after neuropathological insult could be the rationale for using EPO in clinical practice to limit neuronal damage (15). Even though the blood-brain barrier prevents transport of systemic EPO into brain, hypoxic-ischemic brain conditions disrupt the blood-brain barrier, making brain cells accessible to intravenously administered EPO (16). There is an increasing body of evidence suggesting that EPO crosses the blood-brain barrier in human and animal models, after peripheral application (4,6,14,17-21). Usefulness of EPO as a therapeutic agent in hypoxic-ischemic cerebral conditions is thus based on its capability to cross the blood-brain barrier, upregulation of its expression in hypoxic conditions, neuroprotective and neurogenetic potential, and good clinical tolerance (14,20,22).

Erythropoietin in cardiopulmonary resuscitation: are we near?

Cerebral ischemia is inevitable during cardiac arrest and at some extent during the post-resuscitation period. During the first few hours of recovery from cardiac arrest, the brain remains hypoxic, but for no more than 2 days (23). After a hypoxic-ischemic insult, hypoxia inducible factor (HIF) isoforms, HIF-1 alpha and HIF-2 alpha, stimulate the expression of EPO in brain astrocytes (23-25). In the brain, HIF-1 alpha accumulates early during recovery from cardiac arrest, and persists for several

days after successful resuscitation (23). Intrinsic EPO, produced by the majority of brain cells after HIF stimulation (26), and exogenous EPO, protect neuronal tissue from oxygen deprivation and other noxious stimuli, promote neuroangiogenesis, inhibit hypoxia-induced apoptosis in neurons, and thus support the survival of neurons in the ischemic brain (4,16,25-29).

In the proximity of infarcted areas of the brain there is a degree of angiogenesis, axonal reorganization with neurogenesis, and neuroblast migration to areas exposed to ischemic injury, which is in part mediated by EPO (30,31). In an adult animal model of ischemic stroke, exogenous EPO reduces an infarct size (2,4,17,26,32). In the neonatal animal model, exogenous EPO improves asymmetry of forelimbs following stroke (18), reduces the infarct size with attenuation of brain injury, and preserves the integrity of cerebral cortex (33,34).

EPO induces time- and dose-dependent tolerance against oxygen and glucose deprivation in primary cortical neurons and protects from cell death. The protection is significant within minutes and lasts up to 48 hours after continued EPO exposure (27). In vivo, the protective properties are most prominent when EPO is given early after focal or global cerebral hypoxic-ischemic insult, which in turn improves long-term neurobehavioral achievements (17,32,33,35,36). The therapeutic time window for tissue protection by EPO is very wide in experimental models (37). This evidence makes EPO a potent therapeutic agent for neurological emergencies with the purpose to improve functional recovery (25,26,30), and could also present a rationale for treatment with EPO after successful CPR.

What does the future hold?

A pioneer work by Ehrenreich et al. (19), regarding the impact of EPO on hypoxic-

ischemic cerebral damage in humans, showed that intravenous high-dose recombinant EPO, a total of 100.000 units given in the first three days after acute ischemic stroke, is well tolerated and improves clinical outcome. Evidence shows that doses of at least 500 units/kg of body weight of EPO given intravenously, offer significant tissue protection, and that tissue-protecting properties of EPO start at peak serum concentrations of 50 ng/ml for central nervous system lesions (38).

In the central nervous system, tissue protecting mechanisms require doses that are higher than those for stimulation of erythropoiesis (5), while in other tissues like heart, cytoprotection could be accomplished with lower doses (39). Nevertheless, optimal recombinant EPO or its analogues, dose characteristics, and the therapeutic window for EPO use in humans have to be determined yet in order to provide appropriate tissue protection, and lower the incidence of adverse effects. The later mainly consist of increased hematocrit and platelet aggregability which significantly increases the risk of thrombosis (5), and high-risk patients are those with polycythemia-hyperviscosity syndrome, hypertension, and vascular thrombosis (40). Non-erythropoietic tissue-protective derivatives of EPO, like asialo EPO and carbamylated EPO, still provide neuroprotection, and avoid adverse effects at the same time (36,40-42).

In summary, upregulation of EPO in hypoxic-ischemic human brain, ability of exogenous EPO to cross the blood-brain barrier after peripheral application, its neuroprotective role in animal and human studies, pleiotrophism, and clinical tolerance give a strong rationale for further research of the impact of EPO on neurological recovery after successful cardiopulmonary resuscitation in humans.

REFERENCES

1. Angelos MG, Ward KR, Hobson J, Beckley PD. Organ blood flow following cardiac arrest in a swine low-flow cardiopulmonary bypass model. *Resuscitation* 1994;27:245-54.
2. Bernaudin M, Marti HH, Roussel S, Divoux D, Nouvelot A, MacKenzie ET, Petit E. A potential role for erythropoietin in focal permanent cerebral ischemia in mice. *J Cereb Blood Flow Metab* 1999;19:643-51.
3. Sakanaka M, Wen TC, Matsuda S, Masuda S, Morishita E, Nagao M, Sasaki R. In vivo evidence that erythropoietin protects neurons from ischemic damage. *Proc Natl Acad Sci USA* 1998;95:4635-40.
4. 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. Erythropoietin prevents neuronal apoptosis after cerebral ischemia and metabolic stress. *Proc Natl Acad Sci USA* 2001;98:4044-9.
5. Brines M, Cerami A. Emerging biological roles for erythropoietin in the nervous system. *Nat Rev Neurosci* 2005;6:484-94.
6. Eid T, Brines M. Recombinant human erythropoietin for neuroprotection: what is the evidence? *Clin Breast Cancer* 2002;3 Suppl 3:S109-15.
7. Tan CC, Eckardt KU, Firth JD, Ratcliffe PJ. Feedback modulation of renal and hepatic erythropoietin mRNA in response to graded anemia and hypoxia. *Am J Physiol Renal Physiol* 1992;263: F474-F481.
8. Marti HH, Wenger RH, Rivas LA, Straumann U, Digicaylioglu M, Henn V, Yonekawa Y, Bauer C, Gassmann M. Erythropoietin gene expression in human, monkey and murine brain. *Eur J Neurosci* 1996;8: 666-76.
9. Kilic E, Kilic U, Soliz J, Bassetti CL, Gassmann M, Hermann DM. Brain-derived erythropoietin protects from focal cerebral ischemia by dual activation of ERK-1/-2 and Akt pathways. *FASEB J* 2005;19:2026-8.
10. Yatsiv I, Grigoriadis N, Simeonidou C, Stahel PF, Schmidt OI, Alexandrovitch AG, Tsenter J, Shohami E. Erythropoietin is neuroprotective, improves functional recovery, and reduces neuronal apoptosis and inflammation in a rodent model of experimental closed head injury. *FASEB J* 2005;19:1701-3.
11. Boran BO, Colak A, Kutlay M. Erythropoietin enhances neurological recovery after experimental spinal cord injury. *Restor Neuro Neurosci* 2005; 23:341-5.
12. Gorio A, Gokmen N, Erbayraktar S, Yilmaz O, Madaschi L, Cichetti C, DiGiulio AM, Vardar E, Cerami A, Brines M. Recombinant human erythropoietin counteracts secondary injury and markedly enhances neurological recovery from experimental spinal cord trauma. *Proc Natl Acad Sci USA* 2002;99:9450-5.
13. Lu D, Mahmood A, Qu C, Goussev A, Schallert T, Chopp M. Erythropoietin enhances neurogenesis and restores spatial memory in rats after traumatic brain injury. *J Neurotrauma* 2005;22:1011-7.
14. Kalialis LV, Olsen NV. Erythropoietin-a new therapy in cerebral ischemia? *Ugeskr Laeger* 2003;165:2477-81.
15. Buemi M, Cavallaro E, Floccari F, Sturiale A, Aloisi C, Trimarchi M, Grasso G, Corica F, Frisina N. Erythropoietin and the brain: from neurodevelopment to neuroprotection. *Clin Sci (London)* 2002;103:275-82.
16. Marti HH, Bernaudin M, Petit E, Bauer C. Neuroprotection and angiogenesis: dual role of erythropoietin in brain ischemia. *News Physiol Sci* 2000;15:225-9.
17. Belayev L, Khoutorova L, Zhao W, Vighdorichik A, Belayev A, Busto R, Magal E, Ginsberg MD. Neuroprotective effect of darbepoetin alfa, a novel recombinant erythropoietic protein, in focal cerebral ischemia in rats. *Stroke* 2005;36:1071-6.
18. Chang YS, Mu D, Wendland M, Sheldon RA, Vexler ZS, McQuillen PS, Ferriero DM. Erythropoietin improves functional and histological outcome in neonatal stroke. *Pediatr Res* 2005;58:106-11.
19. Ehrenreich H, Hasselblatt M, Dembowski C, Cepek L, Lewczuk P, Stiefel M, Rustenbeck HH, Breiter N, Jacob S, Knerlich F, Bohn N, Poser W, Ruther E, Kochen M, Geffeler O, Gleiter C, Wessel TC, De Ryck M, Itri L, Prange H, Cerami A, Brines M, Siren AL. Erythropoietin therapy for acute stroke is both safe and beneficial. *Mol Med* 2002;8:495-505.
20. Brines ML, Ghezzi P, Keenan S, Agnello D, de Lanerolle NC, Cerami C, Itri LM, Cerami A. Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. *Proc Natl Acad Sci USA* 2000;97:10526-31.
21. Xenocostas A, Cheung WK, Farrell F, Zakszewski C, Kelley M, Lutynski A, Crump M, Lipton JH, Kiss TL, Lau CY, Messner HA. The pharmacokinetics of erythropoietin in the cerebrospinal fluid after intravenous administration of recombinant human erythropoietin. *Eur J Clin Pharmacol* 2005;61:189-95.
22. Siren AL, Ehrenreich H. Erythropoietin-a novel concept for neuroprotection. *Eur Arch Psychiatry Clin Neurosci* 2001;251:179-84.
23. Chavez JC, LaManna JC. Activation of hypoxia-inducible factor-1 in the rat cerebral cortex after transient global ischemia: potential role of insulin-like growth factor-1. *J Neurosci* 2002;22:8922-31.
24. Chavez JC, Baranova O, Lin J, Pichiule P. The transcriptional activator hypoxia inducible factor 2 (HIF-2/EPAS-1) regulates the oxygen-dependent expression of erythropoietin in cortical astrocytes. *J Neurosci* 2006;26:9471-81.
25. Liu R, Suzuki A, Guo Z, Mizuno Y, Urabe T. Intrinsic and extrinsic erythropoietin enhances neuroprotection against ischemia and reperfusion injury in vitro. *J Neurochem* 2006;96:1101-10.

26. Hasselblatt M, Ehrenreich H, Siren AL. The brain erythropoietin system and its potential for therapeutic exploitation in brain disease. *J Neurosurg Anesthesiol* 2006;18:132-8.
27. Ruscher K, Freyer D, Karsch M, Isaev N, Megow D, Sawitzki B, Priller J, Dirnagl U, Meisel A. Erythropoietin is a paracrine mediator of ischemic tolerance in the brain: evidence from an in vitro model. *J Neurosci* 2002;22:10291-301.
28. Jelkmann W, Wagner K. Beneficial and ominous aspects of the pleiotropic action of erythropoietin. *Ann Hematol* 2004;83:673-86.
29. Marti HH. Erythropoietin and the hypoxic brain. *J Exp Biol* 2004;207:3233-42.
30. Wang L, Zhang Z, Wang Y, Zhang R, Chopp M. Treatment of stroke with erythropoietin enhances neurogenesis and angiogenesis and improves neurological function in rats. *Stroke* 2004;35:1732-7.
31. Carmichael ST. Cellular and molecular mechanisms of neural repair after stroke: making waves. *Ann Neurol* 2006;59:735-42.
32. Zhang F, Signore AP, Zhou Z, Wang S, Cao G, Chen J. Erythropoietin protects CA1 neurons against global cerebral ischemia in rat: potential signaling mechanisms. *J Neurosci Res* 2006;83:1241-51.
33. Spandou E, Papadopoulou Z, Soubasi V, Karkavelas G, Simeonidou C, Pazaiti A, Guiba-Tziampiri O. Erythropoietin prevents long-term sensorimotor deficits and brain injury following neonatal hypoxia-ischemia in rats. *Brain Res* 2005;1045:22-30.
34. Sola A, Rogido M, Lee BH, Genetta T, Wen TC. Erythropoietin after focal cerebral ischemia activates the Janus kinase-signal transducer and activator of transcription signaling pathway and improves brain injury in postnatal day 7 rats. *Pediatr Res* 2005;57:481-7.
35. Kumral A, Uysal N, Tugyan K, Sonmez A, Yilmaz O, Gokmen N, Kiray M, Genc S, Duman N, Koroglu TF, Ozkan H, Genc K. Erythropoietin improves long-term spatial memory deficits and brain injury following neonatal hypoxia-ischemia in rats. *Behav Brain Res* 2004;153:77-86.
36. Grasso G, Sfacteria A, Erbayraktar S, Passalacqua M, Meli F, Gokmen N, Yilmaz O, La Torre D, Buemi M, Iacopino DG, Coleman T, Cerami A, Brines M, Tomasello A. Amelioration of spinal cord compressive injury by pharmacological preconditioning with erythropoietin and a nonerythropoietic erythropoietin derivative. *J Neurosurg Spine* 2006;4:310-8.
37. Brines M, Cerami A. Discovering erythropoietin's extra-hematopoietic functions: biology and clinical promise. *Kidney Int* 2006;70:246-50.
38. Gorio A, Madaschi L, Di Stefano B, Carelli S, Di Giulio AM, De Biasi S, Coleman T, Cerami A, Brines M. Methylprednisolone neutralizes the beneficial effects of erythropoietin in experimental spinal cord injury. *Proc Natl Acad Sci* 2005;102:16379-84.
39. Moon C, Krawczyk M, Paik D, Lakatta EG, Talan MI. Cardioprotection by recombinant human erythropoietin following acute experimental myocardial infarction: dose response and therapeutic window. *Cardiovasc Dug Ther* 2005;19:243-50.
40. Coleman TR, Westenfelder C, Tögel FE, Yang Y, Hu Z, Swenson L, Leuvenink HG, Ploeg RJ, d'Uscio LV, Katusic ZS, Ghezzi P, Zanetti A, Kaushansky K, Fox NE, Cerami A, Brines M. Cytoprotective doses of erythropoietin or carbamylated erythropoietin have markedly different procoagulant and vasoactive activities. *Proc Natl Acad Sci USA* 2006;103:5965-70.
41. Erbayraktar S, Grasso G, Sfacteria A, Xie QW, Coleman T, Kreilgaard M, Torup L, Sager T, Erbayraktar Z, Gokmen N, Yilmaz O, Ghezzi P, Villa P, Fratelli M, Casagrande S, Leist M, Helboe L, Gerwein J, Christensen S, Geist MA, Pedersen LO, Cerami-Hand C, Wuerth JP, Cerami A, Brines M. Asialoerythropoietin is a nonerythropoietic cytokine with broad neuroprotective activity in vivo. *Proc Natl Acad Sci USA* 2003;100:6741-6.
42. Erbayraktar S, de Lanerolle N, de Lotbiniere A, Knisely JP, Erbayraktar Z, Yilmaz O, Cerami A, Coleman TR, Brines M. Carbamylated erythropoietin reduces radiosurgically-induced brain injury. *Mol Med* 2006;12:74-80.