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# COMMENTARY

# Neuroprotection with or without erythropoiesis; sometimes less is more

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Erythropoietin (EPO) is a pleiotropic cytokine with a therapeutic potential that goes well beyond the treatment of anaemia. The study by Wang *et al* (2007b) examined the protective effects of EPO in a rat model of embolic stroke. The efficacy and haematological side effects of EPO were compared to those of a carbamylated EPO variant (CEPO). Treatment with EPO dose-dependently reduced infarct volume and improved long-term functional outcome. However, an increase in hematocrit was seen even for doses of EPO that did not offer neuroprotection. These data do not suggest the existence of a therapeutic window between effect and side effect for treatment with EPO. Treatment with CEPO was without haematological side effects. *British Journal of Pharmacology* (2007) **151,** 1141–1142; doi:10.1038/sj.bjp.0707287; published online 29 May 2007

**Keywords:** stroke; MCAO; EPO; CEPO

**Abbreviations:** CEPO, carbamylated erythropoietin; EPO, erythropoietin; EPO-R, erythropoietin receptor; IU, international units; MCAO, middle cerebral artery occlusion

Erythropoietin (EPO) is the primary stimulator of red blood cell formation. The human gene for this important hormone was cloned in 1983 and human recombinant EPO is now a standard treatment for anaemia due to kidney failure or cancer chemotherapy. In the 1990s, it was found that astrocytes produced EPO and neurons express the EPO receptor (EPO-R). This suggested that EPO had a paracrine and/or autocrine function in the brain (Masuda *et al*., 1993, 1994; Digicaylioglu *et al*., 1995). Indeed, EPO protected hippocampal and cortical neurons from glutamate-induced cell death *in vitro* (Morishita *et al*., 1996) and from global ischaemia in gerbils *in vivo* (Sakanaka *et al*., 1998). As infusion of EPO-neutralizing, soluble EPO-R into the ventricles increased stroke lesions, it appeared that brain EPO was an endogenous counter-regulator of ischaemic damage (Sakanaka *et al*., 1998).

Since these pivotal findings in the 1990s, this research field has exploded and EPO is now widely recognized as a pleiotropic cytokine with a therapeutic potential that goes beyond treatment of anaemia (Brines *et al*., 2000; Siren *et al*., 2001; Villa *et al*., 2003; Lu *et al*., 2005; Tsai *et al*., 2006). The first clinical trial testing the efficacy of EPO in stroke patients was conducted by Ehrenreich *et al.* (2002), which showed a significant functional and neurological improvement after three doses of 33 000 IU rhEPO over 3 days. Although EPO treatment is widely accepted as a safe treatment for anaemic patients, concerns have been raised with respect to the treatment of a non-anaemic patient population with the blood-forming hormone. For instance, a breast cancer trial on the efficacy of rhEPO on cancer-related fatigue in patients with mild anaemia was prematurely terminated because of thrombotic events (Rosenzweig *et al*., 2004). Given that post-stroke patients are at high risk of re-thrombosis (Coull and Rothwell, 2004), it would be desirable to develop compounds retaining EPO's neuroprotective properties, but without any haematological side effects. One such compound is carbamylated EPO (CEPO), which does not interact or signal through the classical EPO-R nor does it alter any haematological parameters even after chronic dosing (Leist *et al*., 2004). Both EPO and CEPO have been shown to have a long-lasting effect on functional recovery after stroke in rats with a remarkable time window for the onset of treatment after initiation of the lesion of at least 24 h (Wang *et al*., 2004; Villa *et al*., 2007).

In the current issue of *British Journal of Pharmacology,* Wang *et al*. (2007b) present a head-to-head comparison of the protective effect of EPO and CEPO in a rat model of stroke following embolic middle cerebral artery occlusion (MCAO). EPO at dose levels of 500–5000 IU $kg^{-1}$ (corresponding to 4.2–42 $\mu g\,kg^{-1}$) or CEPO at a dose level of 50 $\mu g\,kg^{-1}$ significantly reduced infarct volume and improved functional outcome when administered 6, 24 and 48 h after embolic MCAO. A dose of 50 $\mu g\,kg^{-1}$ EPO reduced the infarct volume by 28%, but this dose was also high enough to produce an increase in haematocrit compared to the vehicle-treated group. Lower doses of EPO (500 IU $kg^{-1}$) with less

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effect on the haematocrit resulted also in significantly lower neuroprotection (mean reduction of 17%). These data suggest that there is no therapeutic window for EPO between the dose needed for neuroprotective effects and the dose-inducing haematological side effects. In contrast to this, CEPO (at $50\,\mu g\,kg^{-1}$) offered the same neuroprotection as $5000\,IU\,kg^{-1}$ of EPO, but without any haematological side effects.

EPO and CEPO have previously been reported to cross the blood–brain barrier after intravenous administration. The present study shows for the first time the brain levels of EPO and CEPO after a stroke lesion. One might have expected that more cytokine would have entered the brain parenchyma in the ipsilateral (damaged) hemisphere, but no difference in cytokine levels was seen between the ipsi- and contralateral hemispheres. This might be explained by the experimental set-up where the brain is flushed with buffer before assay of EPO and this procedure might flush out cytokine preferentially in the ipsilateral hemisphere where there was damaged endothelium, induced by the lesion.

With the discontinuation of the NXY-059 drug development programme by Astra Zeneca as the most recent setback in the stroke field, new avenues need urgently to be explored. Combination therapy or multimodal drugs targeting different stages of the pathophysiological cascade after stroke have been suggested as an alternative approach. EPO and EPO derivatives have been proposed as having multiple modes of action including acute anti-apoptotic effects and facilitation of functional recovery through stimulation of neurogenesis and angiogenesis (Wang *et al*., 2004, 2007a; Tsai *et al*., 2006; Villa *et al*., 2007). The remarkably long time window in rat stroke models suggests that EPO and CEPO could indeed be a way forward for the future treatment of stroke. Further development of this concept will reveal whether these encouraging animal data can be translated into efficacy in the clinic.

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