Neuroprotective properties of epoetin alfa

Anthony Cerami¹, Michael Brines¹, Pietro Ghezzi¹,², Carla Cerami¹ and Loretta M. Itri³

¹The Kenneth S. Warren Institute, New York, USA, ²Department of Molecular Biochemistry and Pharmacology, Mario Negri Institute for Pharmacological Research, Milan, Italy and ³Genta, Inc., Berkeley Heights, New Jersey, USA

Abstract

Erythropoietin and its receptor function as primary mediators of the normal physiological response to hypoxia. Erythropoietin is recognized for its central role in erythropoiesis, but studies in which recombinant human erythropoietin (epoetin alfa) is injected directly into ischaemic rodent brain show that erythropoietin also mediates neuroprotection. Abundant expression of the erythropoietin receptor has been observed at brain capillaries, which could provide a route for circulating erythropoietin to enter the brain. In confirmation of this hypothesis, systemic administration of epoetin alfa before or up to 6 h after focal brain ischaemia reduced injury by 50–75%. Epoetin alfa also limited the extent of concussive brain injury, the immune damage in experimental autoimmune encephalomyelitis and excitotoxicity induced by kainate. Thus, systemically administered epoetin alfa in animal models has neuroprotective effects, demonstrating its potential use after brain injury, trauma and multiple sclerosis. It is evident that erythropoietin has biological activities in addition to increasing red cell mass. Given the excellent safety profile of epoetin alfa, clinical trials evaluating systemically administered epoetin alfa as a general neuroprotective treatment are warranted.

Keywords: brain injury; encephalitis; epoetin alfa; erythropoietin; ischaemia; neuroprotection

Introduction

The most well-known action of erythropoietin is its haematological effect, whereby in response to hypoxia the kidney produces erythropoietin, which targets erythroid progenitor cells to increase the number of mature red blood cells, thereby increasing oxygen delivery [1]. The mechanism of this regulation of red blood cell production is primarily an anti-apoptotic effect on committed erythrocyte precursors, rather than a stimulation of growth or development [2,3]. Apoptosis may occur at any of several points along the pathway of development from erythrocyte precursors to mature erythrocytes in the bone marrow, e.g. as erythroid colony-forming units develop into pro-erythroblasts, or as pro-erythroblasts develop into reticulocytes. In the bone marrow, erythropoietin interacts with a specific receptor to up-regulate a family of genes known as bcl-2, which has been associated with inhibition of apoptosis of erythroid cells. This anti-apoptotic mechanism of erythropoietin appears to be important in other tissues, in which erythropoietin has been shown to operate.

Effects of erythropoietin in the central nervous system

Using available methods to examine erythropoietin production and receptors in other tissues, the brain was found to have an abundance of both. For example, in response to hypoxia, astrocytes produce erythropoietin, which then interacts with specific receptors on neurones and increases their tolerance to hypoxia [4]. Neurones themselves also produce erythropoietin in response to hypoxia [4]. The mechanism of tolerance to hypoxia in these brain cells can be presumed to be similar to that in bone marrow, i.e. an anti-apoptotic effect.

For many years, it was assumed that erythropoietin, a large glycoprotein, would not cross the blood–brain barrier, which is thought to be impenetrable to molecules >500 Da. Studies of cytokines, however, suggested that preferential transport across the blood–brain barrier may exist for some types of larger molecules [5], and erythropoietin is now known to be one such molecule. Immunocytochemical techniques have shown that the erythropoietin receptor can be found within and around brain capillaries in animals and humans. Transmission electron microscopy

© 2002 European Renal Association–European Dialysis and Transplant Association
confirms that the predominance of anti-epoetin alfa receptor immunoreactivity is within astrocytic endfeet surrounding capillaries and within or on the surface of capillary endothelial cells [6]. These observations suggest an anatomical basis for direct transport of erythropoietin from the systemic circulation into the parenchyma of the brain across the blood–brain barrier in the absence of any neural insult. This hypothesis has been confirmed using biotinylated epoetin alfa in experimental animals [6]. At 5 h after systemic injection, biotinylated epoetin alfa was observed around capillaries and extending into the brain parenchyma, but not around the larger vessels, suggesting a specific transport mechanism. In addition, co-injection of excess amounts of unlabelled epoetin alfa significantly reduced the amount of biotin label, consistent with a specific and saturable transport mechanism. Also, epoetin alfa administered peripherally to rats appeared in the cerebrospinal fluid after ~1.5 h and peaks at ~1% of the peripheral concentration at 3.5 h post-administration (unpublished observations).

It has been shown that epoetin alfa injected directly into the parenchyma can prevent stroke damage in experimental models [7]. However, a more practical approach in a clinical context would be to determine whether systemically administered epoetin alfa can prevent brain damage. Four animal models of brain damage have been investigated in this way: a focal ischaemia model; a cortical impact injury model; an acute experimental allergic encephalitis model; and a kainate toxicity model [6].

Focal ischaemia (stroke) model

In the focal ischaemia (stroke) model in rats, permanent occlusion of the middle cerebral artery and homolateral carotid artery followed by a 1 h reversible occlusion of the contralateral carotid artery is used to produce an area of brain damage consisting of an ischaemic core (related to the permanent occlusion) surrounded by a penumbra of reperfusion damage (related to the reversible occlusion) [6]. The cells in the penumbra are induced to undergo apoptosis. After 24 h, the animals are sacrificed and the volume of damaged brain measured using computerized image analysis. Epoetin alfa, 5000 IU/kg intraperitoneally (i.p.), given 24 h before arterial occlusion, significantly reduced the volume of damaged brain by ~75% compared with control animals given saline [6]. The damage that remains despite pre-treatment represents primarily the ischaemic core. Furthermore, epoetin alfa could be given at any time up to 3 h after arterial occlusion and still provide protection against infarction (Figure 1) [6]. Even administration at 6 h after arterial occlusion provided ~50% protection, though by 9 h the therapeutic window was closed.

The anti-apoptotic nature of the protection afforded by epoetin alfa has been demonstrated very recently using TUNEL labelling, which identifies dead neurons. The number of TUNEL-positive neurones in the ischaemic penumbra after focal cerebral ischaemia was markedly reduced in the brains of animals who received epoetin alfa compared with control animals that received saline [8].

Cortical impact injury (blunt trauma) model

In the blunt trauma injury model, a blow is delivered to the intact calvaria by a calibrated pneumatic piston. This mechanical insult delivered to the brain elicits elements of ischaemic, excitotoxic and inflammatory injury and, if severe enough, produces a cavitory lesion after 7–10 days [9]. The brain is fixed, sectioned and stained 10 days after injury. When the mice are treated with epoetin alfa, 5000 IU/kg i.p., starting 24 h before or 0, 3 or 6 h after the blow and continuing for a further 4 days once daily, the volume of injured brain

![Fig. 1. Infarct volume after middle cerebral artery occlusion with or without epoetin alfa treatment. Male rats were given saline or epoetin alfa, 5000 IU/kg i.p., from 24 h before to 9 h after permanent middle cerebral artery occlusion with reversible carotid artery occlusion. (Reproduced with permission from [6].)](http://dx.doi.org/10.1093/ndt/9.3.729)
Fig. 2. Volume of cortical impact injury with systemic epoetin alfa treatment. Mice were given saline or epoetin alfa, 5000 IU/kg i.p., 24 h before blunt cortical impact from a calibrated piston. Epoetin alfa was continued for 4 days after injury. Extensive cavity necrosis was seen when examined 10 days after injury if treated with saline, in contrast to the minimal injury observed in the epoetin alfa-treated mice. (Reproduced with permission from [6].)
was significantly reduced by up to 90% compared with animals that received only saline (Figure 2) [6]. As in
the focal ischaemia model, the therapeutic window extended to ~6 h after injury.

At a higher magnification, the necrotic core of the injury in animals treated with saline is seen to be
surrounded by a large number of mononuclear inflammatory cells, which have migrated into the injured area
and are probably responsible for much of the damage that occurs. In mice treated with epoetin alfa, however,
very little inflammatory infiltrate was seen [6], indicating that epoetin alfa may also act as an anti-inflammatory
agent in this type of injury. Thus, these experiments demonstrate the ability of systemically administered
epoetin alfa to protect brain tissue from blunt trauma.

Acute experimental autoimmune encephalitis model

The reduction of the inflammatory response by epoetin alfa in the model of cortical impact injury suggests that
epoetin alfa might be effective in other central nervous system (CNS) diseases where an inflammatory component is observed. This hypothesis was analysed in a rat model for acute experimental allergic encephalitis, an animal model for multiple sclerosis induced by immunization of animals with guinea-pig myelin basic protein and complete Freund's adjuvant [6]. This animal model is considered a typical inflammatory pathology of the CNS, as demonstrated by the protective action of several anti-cytokine molecules.

Immunized animals develop clinical symptoms within 10 days, which peak with an increasing degree of paralysis at day 12. Daily administration of epoetin alfa, 5000 IU/kg i.p. at day 3 after immunization for a period of 15 days, delayed the appearance of symptoms, and also significantly reduced the degree of paralysis compared with saline treatment (Figure 3) [6]. Studies for 3 weeks after epoetin alfa was discontinued showed no recurrence of symptoms in these animals, as is typically observed after discontinuing treatment with glucocorticoids or interferon-β [10]. However, the clinical manifestations observed with epoetin alfa in this model are consistent with anti-inflammatory agents such as glucocorticoids. These findings, together with those from the model of cortical impact injury, suggest that epoetin alfa has an anti-inflammatory action in inflammatory pathologies of the CNS.

Kainate toxicity model

Studies in cell culture suggested that apoptosis may be induced by conditions other than hypoxia, including addition of the glutamate analogue kainate [8]. Systemic administration of kainate in experimental animals increases brain excitotoxicity, which is a prominent feature of many forms of brain injury, causing seizures and, ultimately, death [11]. The effect of epoetin alfa on kainate-induced excitotoxicity was analysed in a toxicity model, in which the severity of seizures and time to death are measured in female mice.

Fig. 3. Protective effect of epoetin alfa against acute experimental allergic encephalitis. Rats immunized with myelin basic protein and Freund's complete adjuvant received saline (PBS) or epoetin alfa, 5000 IU/kg/day i.p., starting 3 days after immunization. Epoetin alfa-treated rats show a delay in reduction of symptoms of experimental autoimmune encephalomyelitis (EAE). (Reproduced with permission from [6].)
Animals receiving epoetin alfa (5000 IU/kg, 24 h before administration of kainate) exhibited a significant reduction in mortality by ~45% compared with control animals and a significant increase in mean survival time of 42%. With lower doses of kainate that did not cause death, the severity of seizures was markedly less than in control animals. A single dose of epoetin alfa provided protection for at least 3 days (Figure 4). However, epoetin alfa did not protect against kainate toxicity if it was given 30 min before kainate or after the development of seizures. In contrast, conventional anti-epileptic agents can protect against kainate toxicity when given at the same time as or slightly after kainate administration, but protection depends on their continued presence [6]. These findings suggest that the protective effect of epoetin alfa on excitotoxicity is based on a different mode of action compared with conventional anti-epileptic agents, and presumably involves activation of gene expression that continues protection even in the absence of the cytokine [6].

Therapeutic potential of epoetin alfa in the central nervous system

The experimental findings described above suggest that epoetin alfa may have many potential therapeutic uses, particularly in the CNS. These include stroke, trauma (including surgery and radiotherapy), epilepsy and neurodegenerative diseases. Given the excellent safety profile of epoetin alfa, clinical trials are warranted to determine whether these experimental findings can be translated into therapeutic effects in humans.

References