



## Review article

### Neuroprotection by Erythropoietin – A Review

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#### ABSTRACT

Many hematopoietic growth factors are produced locally in the brain. Erythropoietin (EPO) is the principal growth factor regulating the production of red blood cells. Erythropoietin (EPO) is a glycoprotein hormone that acts as a major regulator of erythropoiesis. Erythropoietin (EPO) has a dominant role for neuroprotection, neurogenesis and acting as a neurotrophic factor in the central nervous system. The site of EPO production switches during development from fetal liver to adult kidney, with low-level expression remaining in adult liver. Originally it was believed that the role of EPO was the regulation of erythropoiesis. This role is attributed to the ability of EPO to inhibit programmed cell death (apoptosis) in erythroid cells and thus allow the maturation of erythrocytes. Erythropoietin (EPO) can act on several cell types in different ways. An independent system EPO / EPO receptor (EPOR) was detected in brain, leading to the hypothesis that this hormone could be involved in cerebral functions. EPO/EPOR expression changes during ontogenesis, thus indicating the importance of this system in neurodevelopment. Moreover, the hypoxia-induced production of EPO in the adult brain suggests that it could exert a neurotrophic and neuroprotective effect in case of brain injury. EPO could also influence neurotransmission inducing neurotransmitters (NT) release. So in the coming years, the possibility that human recombinant EPO therapy could soon be used in clinical practice to limit the neuronal damage induced by different diseases.

**KEY WORDS:** Brain injury, erythropoietin, neurotransmitter.

#### INTRODUCTION

Many hematopoietic growth factors are produced locally in the brain. Among these erythropoietin (EPO), has a dominant role for neuroprotection, neurogenesis and acting as a neurotrophic factor in the central nervous system. These functions make erythropoietin a good candidate for treating diseases, associated with neuronal cell death.

The hormone erythropoietin (EPO) is a 165 amino acid (~30 kDa) glycoprotein that belongs to the cytokine type I super family. Originally it was believed that the role of EPO was the regulation of erythropoiesis. This role is attributed to the ability of EPO to inhibit programmed cell death (apoptosis) in erythroid cells and thus allow the maturation of erythrocytes. Since blood oxygen availability is the main regulator of erythropoiesis, hypoxia induces the gene expression of EPO in the kidneys, the main site for EPO production and in the liver [1] in a negative feedback system between the kidney and the bone marrow.

Research performed in the last decade has shown that EPO and its receptors are expressed in tissues other than those involved in erythropoiesis. These include the brain, the reproductive tract, the lung, [2] the spleen and the heart. [3] Accordingly a novel cytoprotective effect of EPO was established in several organs, for example, EPO reduced injury and dysfunction after ischemia-reperfusion in the mouse kidney and it showed protection in various myocardial ischemia models. [4] EPO deficiency is the primary cause of the anemia in chronic kidney disease and a contributing factor in the anemias of chronic inflammation and cancer. In the following review, we will concentrate on the effects of EPO in the brain.

#### EPO/EPOR Expression and regulation in the brain

EPO is mainly produced in the interstitial fibroblasts in the adult kidney and the hepatocytes of the fetus, whereas EPOR is normally expressed in erythroid precursor cells in the bone marrow. [5] However, recent studies have shown

that the expression of EPO and its receptor, EPOR (both mRNA and protein) coincides in the same organ and even within the same cell. EPO and EPOR expression are widely distributed in the mammalian brain[6] albeit at lower levels than in the kidney. EPO thus has to be added in the growing list of hematopoietic growth factors found to be expressed and act in the central nervous system. EPO /EPOR, mRNA and protein were detected in several regions of the murine and primate brain, including cortex, hippocampus and amygdala, cerebellum, hypothalamus and caudate nucleus.[7]With respect to the type of cells in the brain that express, EPO, astrocytes are the main source of EPO in the brain.[8] Moreover it has been shown in vitro and in vivo that neurons express EPO, similarly EPOR is expressed on the neurons and the astrocytes.[9]In addition, primary cultures of human neurons, astrocytes and microglia express EPOR mRNA[10] and EPOR expression was also detected in primary cultures of rat oligodendrocytes.[11]

### **Production Sites of Erythropoietin Distinct From Kidney and Liver**

Ratcliffe and colleagues [12]were the first to show that erythropoietin is produced in rat organs other than the kidney and the liver. In unstimulated animals erythropoietin mRNA was detected, among other organs, in lungs, testis and brain but was not found in muscles, intestine or bone marrow. After exposure to severe hypoxia, erythropoietin mRNA was significantly increased in the spleen and mainly in the testis and the brain.[12]Subsequently erythropoietin was found in the brain of other species as well. Its mRNA was detected in biopsies from the human cortex and hippocampus and in various brain regions of rhesus monkey and the mouse.[13]

### **Epo Signaling**

EPO promotes cell survival through inhibiting apoptosis. In erythroid cells, after binding of EPO to its receptor (EPOR), Janus tyrosine kinase 2 (JAK2) is phosphorylated and thus is active. This leads to engaging secondary signaling molecules such as signal transducer and activator of transcription (STAT5), followed by activation of Ras mitogen-activated protein kinase (MAPK).[14]

### **Epo Function in the CNS**

Through its anti-apoptotic action it enables committed erythroid progenitor cells to survive and mature.[15]The important role of EPO in the CNS is also evident from many studies with EPOR knockout mice. As a result of EPOR deficiency, these mice show massive apoptosis and a reduction in the number of neuronal progenitor cells.[16]

### **Mechanism of neuroprotection by erythropoietin**

Brain derived erythropoietin could protect neurons by direct and indirect mechanisms. The direct pathway involves inhibition of hypoxia /ischemia induced apoptosis, whereas the indirect has to do with vessel growth and neovascularization induced by erythropoietin. It is commonly accepted that hypoxia/ ischemia of the brain leads to greatly enhanced release of the excitatory amino-

acid glutamate. In a large series of experiments, it was shown that the neuronal damage produced by hypoxia/ ischemia is indeed mediated by the so called N-methyl-D-aspartate (NMDA) receptor. Binding of glutamate to this receptor leads to massive entry of Ca, Na and water, subsequently to impairment of mitochondrial function, excessive free radical production, cell swelling and neuronal cell death. First cell death, induced by exposure to 1Mm glutamate of hippocampus and cortical primary neurons is prevented by pretreatment of the cells with erythropoietin before glutamate challenges. This protective effect is dose dependent.

Erythropoietin is known to increase intracellular calcium in neuronal cells, which in turn leads to a sustained increase in neuronal nitrous oxide production. Nitrous oxide in turn has recently been shown to inhibit caspase function.[17]It could also be true that erythropoietin inhibits glutamate release, increases glutamate uptake, or desensitizes glutamate receptors.

### **Indirect Neuronal Protection by Affecting Endothelial Cell Growth**

Hypoxia-ischemia-induced erythropoietin might stimulate angiogenesis in the brain. Newly formed vessels would transport more red blood cells, thereby increasing the amount of oxygen delivered to the hypoxic tissue and thus counteracting the detrimental effects of stroke on neurons (Indirect protective effect). Alternatively it has been suggested that erythropoietin acts indirectly on endothelial cells via activation of vascular endothelial growth factor (VEGF/VEGF receptor) system. VEGF is the most important specific regulator of endothelial cell growth and differentiation and the major angiogenesis factor not only during embryonic development but also in many pathological conditions such as tumor growth and ischemic disease. In addition VEGF is also a survival factor for endothelial cells. Furthermore, VEGF is able to reduce ischemic damage after transient ischemia, when applied topically on the surface of the reperfused brain [18] thereby indicating that promoting angiogenesis is indeed a strategy to protect brain tissue after stroke.

### **CONCLUSION**

Erythropoietin treatment is a promising strategy not only for erythropoiesis but also for cell survival in brain. Erythropoietin has been shown to be a promising molecule in the first clinical trials for the treatment of various neurological diseases, acute such as stroke and chronic /progressive such as Parkinson's disease. Today, Erythropoietin is a prominent member of a growing list of hematopoietic and angiogenic factors found to be expressed and acting as protective factors in the central nervous system. Attention has also been focused on the neurotropic and neuroprotective function of erythropoietin in different conditions of neuronal damage such as hypoxia, cerebral ischemia and subarachnoid hemorrhage and therefore on the possibility that human recombinant erythropoietin therapy could soon be used in clinical practice, also to limit neuronal damage induced by these diseases.

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