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Charting a course for erythropoietin in traumatic brain injury

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Abstract

Traumatic brain injury (TBI) is a severe public health problem that impacts more than four million individuals in the United States alone and is increasing in incidence on a global scale. Importantly, TBI can result in acute as well as chronic impairments for the nervous system leaving individuals with chronic disability and in instances of severe trauma, death becomes the ultimate outcome. In light of the significant negative health consequences of TBI, multiple therapeutic strategies are under investigation, but those focusing upon the cytokine and growth factor erythropoietin (EPO) have generated a great degree of enthusiasm. EPO can control cell death pathways tied to apoptosis and autophagy as well oversees processes that affect cellular longevity and aging. *In vitro* studies and experimental animal models of TBI have shown that EPO can restore axonal integrity, promote cellular proliferation, reduce brain edema, and preserve cellular energy homeostasis and mitochondrial function. Clinical studies for neurodegenerative disorders that involve loss of cognition or developmental brain injury support a positive role for EPO to prevent or reduce injury in the nervous system. However, recent clinical trials with EPO and TBI have not produced such clear conclusions. Further clinical studies are warranted to address the potential efficacy of EPO during TBI, the concerns with the onset, extent, and duration of EPO therapeutic strategies, and to focus upon the specific downstream pathways controlled by EPO such as protein kinase B (Akt), mechanistic target of rapamycin (mTOR), AMP activated protein kinase (AMPK), sirtuins, wingless pathways, and forkhead transcription factors for improved precision against the detrimental effects of TBI.

Keywords

Akt; *Alzheimer's disease*; *apoptosis*; *autophagy*; *erythropoietin*; *forkhead*; *mTOR*; *Parkinson's disease*; *neurodegeneration*; *programmed cell death*; *sirtuins*; *traumatic brain injury*; *Wnt*

Erythropoietin and traumatic brain injury: Translating experimental success into clinical efficacy

In more than 30 million individuals throughout the world, both acute and chronic neurodegenerative disorders can result in significant disability as well as eventual death [1,2]. Chronic neurodegenerative diseases, such as Parkinson's disease (PD) [3,4], can affect approximately 4 percent of individuals over the age of sixty. In addition, the number of

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individuals with PD is expected to double by the year 2030. With disorders such as Alzheimer's disease (AD), this disease also can significantly impact the global population. For the sporadic form of AD, approximately 10 percent of the global population over the age of sixty-five is affected [3,5]. Over the next two decades, it is estimated that the number of those suffering from AD will increase significantly to more than 30 million individuals [6–8].

For acute neurodegenerative injury, disorders such as stroke can impact almost 800,000 individuals every year with an annual cost of seventy-five billion United States (US) dollars [9]. Stroke results in multiple complications that can affect the economic welfare and daily function of individuals [10,11]. Cerebral ischemic disease is one of the five leading causes of death that also includes cardiac disease, cancer, chronic lower respiratory disease, and trauma [12]. As noted, trauma also significantly contributes to death and disability throughout the world and in particular, traumatic brain injury (TBI) can lead to devastating neurological disability [13,14]. TBI can result in impairments in memory and cognition, altered states of consciousness, psychiatric disturbances, and physical impairments that may be temporary or progress to permanent disability or death [15,16]. The combined rates for TBI related emergency department visits, hospitalizations, and deaths have significantly increased over the last ten years from 521 per 100,000 in 2001 to 824 per 100,000 in 2010 [17]. In young individuals of five to fourteen years of age, trauma as a result of being struck by an object accounted for almost forty percent of injuries. Falls accounted for an additional thirty-five percent of injuries. In older individuals of fifteen to forty-four years of age, assaults, falls, and motor vehicle accidents appear to account for the greatest proportion of TBI. In the age group that is sixty-five and older, falls are the predominant injury mechanism. Of significant concern is the fact that TBI can lead to both acute and chronic impairments for the nervous system [18–20]. Almost 50,000 individuals die every year as a result of TBI and approximately forty percent of all deaths from acute injuries in the United States are caused by TBI. Furthermore, more than 100,000 individuals suffer with chronic disability following TBI [21] and when severe trauma occurs, at least fifty percent of individuals die [22]. TBI continues as a severe public health problem affecting greater than four million individuals in the US alone with a continued increase in TBI rates [23].

Given the severe acute and long-standing negative health consequences that can occur following TBI, a number of interesting strategies are under investigation. These include targeting treatments with the mechanistic target of rapamycin (mTOR) [3,13], Wnt signaling pathways [24], nicotinamide [25–27], targets of oxidative stress [20,28], and sirtuins [29,30]. Yet, cytokines and growth factors offer interesting prospects for the treatment of TBI. Such agents are fostering a high level of enthusiasm for further investigation. In particular, the cytokine and growth factor erythropoietin (EPO) is under active study for the treatment of TBI [31].

At present, EPO and other erythropoiesis-stimulating agents (ESAs) have been approved by the US Food and Drug Administration for the treatment of anemia that results from chronic kidney failure, chemotherapy, human immunodeficiency virus, and to limit the number of blood transfusions for surgery [32,33]. EPO is produced and secreted throughout the body from sources that include the kidney peritubular interstitial cells, the brain, uterus, and liver

[34–38]. EPO expression can be influenced by changes in oxygen tension and not by the concentration of red blood cells [28,34,39]. Expression of EPO also may be promoted by stimuli that are not related to hypoxia [40]. For example, agents that block inflammation in cerebral microglia have been recently shown to lead to the release of EPO [41] and xenon anesthesia in cardiac surgery can result in elevated EPO serum concentrations [42].

Located on chromosome 7, the *EPO* gene is present in a 5.4 kb region of the genomic DNA [43] and encodes for a polypeptide chain that has initially 193 amino acids. Subsequently, the EPO protein is cleaved of a 27 amino acid hydrophobic secretory leader at the amino-terminal to result in a 166 amino acid peptide [44]. Additional post-translational processing occurs to result in a circulatory mature protein of 165 amino acids with a molecular weight of 30.4 kDa with the removal of a carboxy-terminal arginine^{166} in the mature human and recombinant human EPO (rhEPO) [45,46]. Structural maintenance of EPO is dependent upon the oligosaccharide side chains [37,47]. EPO contains four glycosylated chains that include three *N*-linked and one *O*-linked acidic oligosaccharide side chains [48]. The production and secretion of the mature EPO is dependent upon the *N*- and *O*-linked chains for [47]. The carbohydrates are important for the clearance of EPO [49]. In addition, the oligosaccharides may offer protection from free radical activity [50], the carbohydrate chains stabilize the EPO protein [51], and the glycosylated chains prevent EPO degradation during free radical oxygen exposure [52].

With *in-vitro* and animal models, EPO can limit the detrimental effects of both apoptosis and autophagy under specific conditions [53,54]. In relation to apoptosis, EPO can block apoptosis during advanced glycation end-product (AGE) exposure in Schwann cells [55], cerebral ischemia [56,57], β-amyloid (Aβ) toxicity [58–61], and neuronal kainate-induced oxidative stress [62]. Furthermore, EPO has been shown to protect against apoptosis during retinal disease [63,64], cerebral microglia injury [41,61,64–68], oxygen deprivation [69–71], cerebral vascular injury [66,72,73], and neurologic deterioration with diabetes mellitus [28,66,74–77].

During autophagy [78,79], EPO can block autophagy through the activation of mTOR [80–83]. EPO has been shown to modify the activity of autophagy and limit neonatal brain damage in the developing rodent during hyperoxia exposure and oxygen toxicity [84]. EPO also can protect against lipopolysaccharide-induced cell injury in other cell systems, such as renal mesangial cells, from autophagy [85]. In addition, EPO may rely upon autophagy pathways to block apoptosis. In neuronal cell line models, EPO can suppress apoptotic cell injury through the increased activity of AMP activated protein kinase (AMPK) and limited autophagy activity [86].

For models of TBI, application of recombinant human EPO and simvastatin during controlled cortical impact in a mouse model can restore axonal integrity and lead to new cellular proliferation [87]. In other animal studies, contusion volume from controlled cortical impact is reduced with EPO administration [88]. In a falling weight TBI model, EPO significantly decreases the number of apoptotic cells and brain edema [89]. During TBI, EPO also may control mitochondrial dysfunction [90] that has been previously shown with

EPO in models of vascular, neuronal, microglial, adipocyte, and cardiomyocyte injury [62,68,69,72,91–97].

Progress in identifying the protective capacity of EPO with *in vitro* and animal models in the nervous system has given way to the initiation of clinical trials with EPO for TBI [52,98]. Overall, greater than sixty current or completed clinical trials are listed on the National Institutes of Health website ClinicalTrials.gov for nervous system disorders and EPO [31]. For disorders such as sporadic AD, increased expression of the EPO receptor in temporal cortical and hippocampal astrocytes has been observed and considered to be an early neuroprotective pathway [99]. In addition, high-dose erythropoietin treatment within forty-two hours after birth in preterm infants has been associated with reduced risk of brain injury documented through magnetic resonance imaging [100,101]. However, in regards to TBI, the results with EPO have not been so clear. EPO has been evaluated in trials that assess neurological recovery following TBI. In a randomized clinical trial of 200 patients, EPO was administered daily for three days (500 IU/kg per dose) and then weekly for two additional weeks. These results were also compared with patients receiving blood transfusion. At the completion of the trial, neither the administration of EPO or maintaining hemoglobin concentration above 10 g/dL led to improvement in neurological outcome at six months [102]. In another larger clinical trial with close to six hundred patients that experienced brain injury, EPO (40,000 units administered subcutaneously) provided once per week for a maximum of three doses did not significantly affect six-month mortality, reduce severe neurological dysfunction, or increase the occurrence of deep venous thrombosis of the lower limbs [103].

Although cell culture and in vitro studies with EPO provide promising directives to translate the use of EPO into effective therapeutic strategies for disorders such as TBI, the current clinical observations with EPO and patients suffering form TBI suggest further investigations are warranted. First, although the current clinical studies with EPO and TBI did not reveal any toxicity with EPO, this may be secondary to the dosing selected for these trials. The onset, concentration, or duration of EPO administration chosen for these trials may not have been sufficient for clinical efficacy to be observed. Yet, construction of future studies that consider the dosing and exposure to EPO also must take into account that in some patients, such as in patients with diabetes mellitus and renal disease, EPO also can lead to toxic events such as a to a two-fold increase in stroke that is not attributed to any baseline characteristic or to blood pressure, hemoglobin, platelet count, or treatment dose of EPO [104]. Elevated EPO concentrations in patients with diabetes mellitus also may lead to proliferative diabetic retinopathy [105] that could be associated with excessive vascular growth. EPO may aggravate hypertension [33,53,106] and result in sustained erythrocytosis that may lead to the activation of inflammatory pathways and blood-brain barrier dysfunction [107]. EPO, as a growth factor and proliferative entity, also can promote tumor growth and lead to the progression of existing tumors [108–111].

Second, another avenue of investigation for EPO and TBI could involve targeting more specific pathways controlled by EPO such as protein kinase B (Akt) that can foster a number of protective pathways in the nervous system with EPO [112–115]. Other pathways that can modulate the protective effects of EPO include mTOR [68,80,82,83], AMPK [41,96],

sirtuins [72,116,117], wingless pathways [68,72,91,118,119], and forkhead transcription factors [57,113,120]. Focusing upon the specific exposure and concentration of EPO as well as the downstream signaling pathways of this entity may provide further precision in targeting the detrimental cellular events that ensue following TBI and concurrently limit the potential toxic effects that can develop during EPO administration.

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