ELSEVIER

Brain & Development 40 (2018) 367–375

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Original article

EPO improved neurologic outcome in rat pups late after traumatic brain injury

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Received 10 October 2017; received in revised form 11 December 2017; accepted 12 January 2018

Abstract

In adult rats, erythropoietin improved outcomes early and late after traumatic brain injury, associated with increased levels of Brain Derived Neurotrophic Factor. Using our model of pediatric traumatic brain injury, controlled cortical impact in 17-day old rats, we previously showed that erythropoietin increased hippocampal neuronal fraction in the first two days after injury. Erythropoietin also decreased activation of caspase3, an apoptotic enzyme modulated by Brain Derived Neurotrophic Factor, and improved Novel Object Recognition testing 14 days after injury. Data on long-term effects of erythropoietin on Brain Derived Neurotrophic Factor expression, histology and cognitive function after developmental traumatic brain injury are lacking. We hypothesized that erythropoietin would increase Brain Derived Neurotrophic Factor and improve long-term object recognition in rat pups after controlled cortical impact, associated with increased neuronal fraction in the hippocampus.

Methods: Rats pups received erythropoietin or vehicle at 1, 24, and 48 h and 7 days after injury or sham surgery followed by histology at 35 days, Novel Object Recognition testing at adulthood, and Brain Derived Neurotrophic Factor measurements early and late after injury.

Results: Erythropoietin improved Novel Object Recognition performance and preserved hippocampal volume, but not neuronal fraction, late after injury.

Conclusions: Improved object recognition in erythropoietin treated rats was associated with preserved hippocampal volume late after traumatic brain injury. Erythropoietin is approved to treat various pediatric conditions. Coupled with exciting experimental and clinical studies suggesting it is beneficial after neonatal hypoxic ischemic brain injury, our preliminary findings support further study of erythropoietin use after developmental traumatic brain injury.

Keywords: Controlled cortical impact; Erythropoietin; Developmental; Apoptosis; Neurocognitive impairment

1. Introduction

Pediatric traumatic brain injury (TBI) is the leading cause of traumatic death and disability in children [1]. Neurocognitive deficits in these young survivors most commonly manifest as impairments in learning and executive function domains, such as processing speed and declarative memory [2,3]. No therapies in use today directly improve neurologic outcome after pediatric TBI.

Erythropoietin (EPO) is a pleiotropic cytokine produced in the kidney and central nervous system. It mediates cell survival and proliferation in erythrocytes

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https://doi.org/10.1016/j.braindev.2018.01.003
0387-7604/

systemically and in neurons, astrocytes, and immune cells of the developing and mature central nervous system. EPO also modulates inflammation and angiogenesis. Upregulation of cerebral EPO and its receptor in various animal models led to the hypothesis that exogenous EPO could exert neuroprotection in the setting of brain injury [4]. A number of subsequent studies support EPO administration in models of adult and developmental brain injury [5,6].

In adult rats, EPO improved functional and histologic outcome at short- and long-term time points after TBI [7,8]. In our developmental TBI model using controlled cortical impact (CCI) in 17-day old rats, EPO improved performance on the Novel Object Recognition (NOR) task at post injury day (PID) 14 and decreased caspase activation, associated with increased neuronal fraction in the PID 1 and 2 hippocampus [9]. Data on EPO's effects on long-term cognitive function or histology after developmental TBI are lacking.

EPO appears to exert some of its neuroprotection via increased Brain Derived Neurotrophic Factor (BDNF) transcription in the injured brain. BDNF, a neurotrophin that regulates neuronal development, survival and function, is important for normal learning and memory throughout the lifespan [10]. Using an in vitro neurotoxicity model, Viviani et al. found that EPO time-dependently increased neuronal BDNF mRNA and greatly increased neuronal survival, the latter abrogated by pretreatment with a neutralizing anti-BDNF antibody. Viviani et al. also demonstrated that EPO administration increased BDNF transcription in vivo in the adult rodent brain [11]. In the developing rat brain, intraperitoneal (IP) co-administration of EPO reduced MK-801-induced apoptotic neurodegeneration and preserved BDNF mRNA at 24 h after injection, relative to co-administration of vehicle [12]. Finally, IP administration of EPO improved spatial learning and increased BDNF brain protein levels in the adult rat 35 days after experimental TBI using CCI [13].

Declarative memory, the conscious memory for facts and events, is an important executive function. Declarative memory is commonly impaired in adults and children after TBI [14,15]. In rodents, the Novel Object Recognition (NOR) task is the preferred method by which to test a critical component of declarative memory, object recognition [16].

We hypothesized that, in rat pups after CCI, EPO would increase hippocampal BDNF mRNA early after administration and improve object recognition at adulthood (PID 50, or age 9–10 weeks old) as shown by the NOR task. Based on published histologic outcomes in adult rats treated with EPO after CCI, we measured brain volumes, hippocampal neuronal fraction and astrocytosis at PID 35. We measured BDNF mRNA at time points after EPO dosing, namely at PID 1, 2, 3 and 7.

2. Methods

2.1. Animal model

The Animal Care and Use Committee at the University of Utah approved protocols, according to NIH guidelines. Male Sprague-Dawley rats were obtained from Charles Rivers Laboratories (Raleigh, NC) on post-natal day (P) 7–10 and housed in litters of 10 with the dam until weaning on P 21–23 in a temperature- and light-controlled environment.

Distributed evenly within litters to control for maternal rearing characteristics, rat pups were randomized to CCI or SHAM craniotomy at P17, followed by EPO or vehicle (VEH) injection to create three experimental groups: EPOCCI, VEHCCI and SHAMVEH. After weaning, rats were placed in cages without segregation by experimental group.

CCI was performed as described [9], modified only by decreased deformation depth to 1.5 mm rather than 2 mm to address the near-obliteration of injured hippocampus found late after CCI. 6–8 rats per group were used at each time point for molecular and functional studies. Rats anesthetized using a Vet Equip Bench Top Isoflurane Anesthesia System (Pleasanton, CA) were placed into a stereotaxic frame (David Kopf, Tujunga, CA). Rectal temperature was kept at 37 ± 0.5 °C via servo-controlled heating pad. A 6-mm × 6-mm craniotomy was performed over the left parietal cortex (centered 4-mm posterior and 4-mm lateral to Bregma) with care taken not to perforate the dura. Isoflurane was reduced to 1% for 5-min followed by delivery of CCI (Pittsburgh Precision Instruments, Pittsburgh, PA) to the left parietal cortex via a 5-mm rounded tip to create a 1.5-mm at 4 m/s velocity during 100 ms. Isoflurane was returned to 2–2.5%, and the bone flap replaced and secured (Patterson Dental, Salt Lake City, UT). SHAM rats underwent identical surgical craniotomy, equilibration, and closure procedures without CCI.

2.2. Drug dosing

RhEPO, recombinant human erythropoietin (Procrit®, Amgen Inc, CA) 5000 U/kg or an equal volume of vehicle was dosed intraperitoneally (IP) at 1, 24, and 48 h after CCI and again at PID 7 [9]. As described previously, the rationale for RhEPO dosing was based on its pharmacokinetics and on published dosing regimens successfully used in adult rats after TBI and in neonatal rats after hypoxic ischemic brain injury. In the 7 day old and adult rat, 5000 U/kg Rh EPO given IP reliably produces measurable brain levels of EPO [17,18]. Rh EPO takes between 3 and 9 h to peak in the brain. Therefore, we chose to give the first dose one hour after injury so it would be both clinically rele-