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Research Report

Synergistic benefits of erythropoietin and simvastatin after traumatic brain injury

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ABSTRACT

Simvastatin and recombinant human erythropoietin (rhEpo) are implicated as potential therapeutic candidates for traumatic brain injury (TBI). Prominent effects of simvastatin include its anti-inflammatory, neurotrophic and neuroregenerative actions studied in various models of neuronal injury. On the other hand, rhEpo has been shown to promote cell survival mechanisms by producing anti-apoptotic and cell proliferative actions. Beneficial effects of rhEpo and statin monotherapies have been well studied. However, there are no reports showing combined use of rhEpo and statins after TBI. This investigation examined if combined efficacy of cell proliferative ability of rhEpo along with the neuroregenerative ability of simvastatin will render maximum recovery in a controlled cortical impact (CCI) mouse model of TBI. Results showed that compared to baseline TBI, rhEpo was more effective than simvastatin in promoting cell proliferation while simvastatin was more effective than rhEpo in restoring axonal damage following TBI. Combined treatment with simvastatin and rhEpo maximally restored axonal integrity while simultaneously inducing greater proliferation of newly formed cells resulting in better functional recovery after TBI than either alone. This is the first study showing the efficacy of erythropoietin–simvastatin combinational therapeutic approach in achieving greater structural and cognitive recovery after TBI.

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Abbreviations: Akt/PKB, a serine/threonine protein kinase B; BBB, Blood Brain Barrier; BrdU, Bromodeoxyuridine; BDNF, Brain-derived neurotrophic factor; CCI, Controlled cortical impact; CREB, CyclicAMP responsive element binding protein; CNS, Central nervous system; eNOS, endothelial nitric oxide; EPO, Erythropoietin; EPOR, Erythropoietin Receptor; ERK ½, Extracellular signal related kinase 1/2; GSK-3-beta, Glycogen synthase kinase-3-beta; HCT, Hematocrit; ICAM-1, Intercellular adhesion molecule-1; IL-1beta, Interleukin-1beta; IL-6, Interleukin-6; JAK2, Janus family tyrosine kinase 2; LTP, Long-term potentiation; MIP-2, Macrophage inflammation protein-2 (MIP-2); MWM, Morris water maze; NFkB, Nuclear factor kappa B; PI3K/PI3-K, Phosphotidyl inositol-3-kinase; PTSD, Posttraumatic stress disorder; rhEpo, recombinant human erythropoietin; STAT-3, Signal transducer and activator of transcription 3; TBI, Traumatic brain injury; TLR-4, Toll-like receptor 4; TNF-alpha, Tumor necrosis factor-alpha; YM, Y maze

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1. Introduction

Traumatic brain injury (TBI) continues to remain as one of the leading causes of morbidity and mortality worldwide and hence considered a global health crisis (Christian et al., 2008; Vink and Nimmo, 2009; Wible and Laskowitz, 2010). According to the Center for Disease Control and Prevention, ~1.5 million people sustain TBI in the US alone (Nampiaparampil, 2008; Vaishnavi et al., 2009), of which ~350,000 patients suffer with long-term disability (Richardson et al., 2010), and a ~$60 billion yearly economic burden (Mammis et al., 2009; Onyszchuk et al., 2007). The magnitude of this problem has been compounded by recent combat-related head injuries of Iraq/Afghanistan wars endorsing TBI as the "Silent Epidemic" (Drake et al., 2010; Hoffman et al., 2010; Kennedy et al., 2010; Martin et al., 2008) and the "Signature Wound" of wars (Moore and Jaffee, 2010; Snell and Halter, 2010b; Warden, 2006).

TBI is a multifactorial heterogeneous type of injury that varies widely in etiology, severity, pathophysiology, neuropsychological and cognitive disabilities, involving complex interactions of anatomical, physiological, biochemical, and molecular mechanisms. TBI in humans causes short-term and long-term extensive sensomotor and cognitive dysfunctions and various degrees of neuropsychiatric problems (Kennedy et al., 2010; Vaishnavi et al., 2009). Neurodegenerative events after TBI begin with edema formation as the persistent primary causative factor that leads to a cascade of secondary degenerative events including apoptosis, axonal, synaptic and oxidative damage, inflammation, and neurotrophic deficiency (Chen et al., 2007b; DeKosky et al., 2004; Di Giovanni et al., 2005; Goss et al., 1998; Hartley et al., 2008; Li et al., 2009; Mahmood et al., 2009; Wang et al., 2007), translating into commonly observed behavioral disabilities such as deficits in cognition (thinking, memory and reasoning), and impaired mental health (posttraumatic stress disorder-PTSD, depression, anxiety) (Carlson et al., 2010; Hoffman and Harrison, 2009; Jaffee and Meyer, 2009; Sayer et al., 2009).

Current pharmacological and surgical treatments for TBI remain limited which include surgical removal of hematoma, ventricular drainage, hyperosmotic agents, and hypothermia that are known to prevent neurological deterioration to a certain extent, but not sufficient enough for full recovery after TBI (Christian et al., 2008; Nichol and Cooper, 2009 ; Wang et al., 2007; Wheaton et al., 2009). Given the heterogeneous nature of TBI involving complex primary and secondary events, TBI candidate therapies targeted towards multiple injury factors are likely to maximize successful outcome (Giles, 2009; Saatman et al., 2008). Current therapeutic limitations of TBI may be attributed to the fact that most of the therapies are "monotherapies" involving the use of a single agent targeted toward an individual injury factor (Margulies and Hicks, 2009; Vink and Nimmo, 2009). Given the multiplicity of TBI symptoms and post-TBI manifestations, maximum recovery could be achieved by combining agents with multiple targets and effects (Margulies and Hicks, 2009). Beneficial effects of recombinant human erythropoietin (rhEpo) monotherapy as a cell survival promoter with proliferative anti-apoptotic properties, as an edema-reducing and anti-oxidant agent have been widely studied (Byts and Siren, 2009; Chen et al., 2007a; Lee et al., 2006; Liao et al., 2009; Lieutaud et al., 2008; Liu et al., 2008; Matchett et al., 2006; Ozturk et al., 2008; Valable et al., 2010; Xiong et al., 2010). The effects of statin monotherapy as an anti-inflammatory agent with neurotrophic and neuroregenerative properties also have been extensively reported (Carloni et al., 2006; Chen et al., 2007b; Li et al., 2009; Lu et al., 2004a,b,c, 2007; Mahmood et al., 2009; Qu et al., 2005; Schmeer et al., 2006; Tapia-Perez et al., 2008; Wang et al., 2007; Wible and Laskowitz, 2010; Wu et al., 2008). However, there are no studies showing combined therapeutic use of statins and rhEpo in TBI. This investigation examined if combined efficacy of cell proliferative ability of rhEpo along with the neuroregenerative ability of Simvastatin will render maximum recovery after TBI. Specifically, we evaluated if treatment with rhEpo and Simvastatin together will promote new cell proliferation while maximally restoring axonal damage translating into cognitive recovery after injury in a controlled cortical impact (CCI) mouse model of TBI.

2. Results

2.1. *Systemic effects of rhEpo treatment*

The primary effect of erythropoietin is to boost erythropoiesis, however, the extra-erythropoietic neuroprotective effects of erythopoietin have begun to emerge. In order to confirm the primary systemic effects of rhEpo treatment, we analyzed hematocrit (HCT) in all control and experimental groups with the use of Stanbio H2 photometer (Stanbio Laboratory, Boerne, TX). Pre-injury HCT index before beginning TBI showed a close range HCT (45–48%), and hemoglobin (15.4–16.4 g/dl) values in all control and experimental groups (baseline, Figs. 1 and 2). After treatment with rhEpo, the HCT index (Fig. 1) and

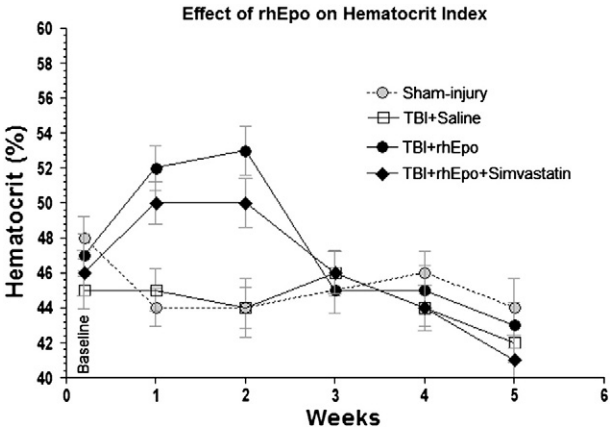

Fig. 1 – Changes in hematocrit before and after rhEpo treatment. "Baseline" represents pre-injury hematocrit levels. Note peak hematocrit levels between 1 and 2 weeks after rhEpo treatment and normalization to base levels by week 5.