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Research Report

Therapeutic window for nonerythropoietic carbamylated-erythropoietin to improve motor function following multiple infarct ischemic strokes in New Zealand white rabbits

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ABSTRACT

Carbamylated erythropoietin (CEPO) is a novel neuroprotective agent that does not bind to the classical erythropoietin receptor or affect hematocrit. Since CEPO has not been systematically studied in a fully blinded and randomized manner in an embolic stroke model, we determined if CEPO would be useful to attenuate clinical deficits associated with multiple infarct ischemia using the rabbit small clot embolic stroke model (RSCEM). Rabbits were embolized and treated with vehicle or CEPO within 6 h of embolization and behavioral analysis was conducted 48 h after embolization. Using quantal analysis, we determined the quantity of blood clot (mg) in brain that produce neurologic dysfunction in 50% of the rabbits (P_{50}), with intervention considered beneficial if it increased the P_{50} compared to controls. CEPO administered between 5 min and 3 h after embolization significantly ($p<0.05$) improved behavioral function and increased the P_{50} value by 55–216%. However, CEPO administration did not improve behavior when administered 6 h following embolization. In conclusion, in the RSCEM, CEPO had a therapeutic window of at least 3 h, where it effectively improved clinical rating scores and motor function. Our results suggest that CEPO may be useful to treat acute ischemic stroke and supports the study of CEPO in stroke patients.

1. Introduction

A substantial literature already shows that erythropoietin (EPO) is neuroprotective in a variety of animal models of disease (Ehrenreich et al., 2004; Genc et al., 2004; Sharples et al., 2006), but EPO is not an optimal drug candidate to be pursued for treating neurodegenerative diseases because of limitations such as the risk of hypertension and thromboembolic events (Finelli and Carley, 2000; Khorana et al., 2005; Singbartl, 1994). EPO is commonly known as an erythropoietic

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protein that effectively increases hemoglobin levels and is useful in patients with cancer, chronic renal failure and non-renal anemia (Hardee et al., 2006; Heuser and Ganser, 2006). One of the main pharmacological effects of EPO is an increase in hematocrit, i.e. increases in hemoglobin levels mediated via the binding of EPO to a specific EPO receptor (EPOR) located on the surface of red cell precursors in bone marrow (Richmond et al., 2005), which stimulates them to transform into mature red blood cells. In contrast to EPO, a carbamylated EPO (CEPO) derivative which, unlike EPO, does not bind to the EPOR and does not stimulate the production of red blood cells (Leist et al., 2004; Kirkeby et al., 2008) may be useful to treat neurodegenerative diseases (Leist et al., 2004; Montero et al., 2007; Villa et al., 2006).

In the rat, CEPO is effective in the treatment of ischemia induced by vascular occlusion, reperfusion-induced injury and NMDA-induced excitotoxicity; all hallmarks of acute ischemic stroke (AIS) (Lapchak and Araujo, 2007; Lapchak, 2008a; Mennini et al., 2006; Montero et al., 2007; Villa et al., 2006; Wang et al., 2007b). To date, CEPO has been studied in rodent models of stroke, but not in higher animal models of stroke (reviewed in Lapchak 2008a) that use clinically relevant outcome measures. However, to develop novel therapeutics, they should be tested in multiple animal models of stroke to ensure efficacy and include dose–response and therapeutic (time) window studies. Moreover, the studies should include tests in an animal model that mimics stroke in humans and use functional recovery as an outcome measure, because functional recovery is a major end point in clinical trials (Broderick et al., 2000; Lapchak and Araujo, 2007; Muir and Grosset, 1999).

CEPO has the potential to be a useful neuroprotectant to treat AIS patients and has previously been shown to be effective in rodent stroke models (Lapchak 2008a). In this translational study, we evaluated the effects of intravenously administered CEPO on clinical rating scores using the rabbit small clot embolic stroke model (RSCEM), an established model that uses the injection of small-sized blood clots into the cerebral vasculature causing multiple ischemic infarcts throughout the brain (Lapchak et al., 2004b; Lapchak et al., 2007). Clot injection results in both normal and abnormal animals with behavioral deficits, which can be quantitated on a simple dichotomous rating scale (Lapchak et al., 2004b; Lapchak et al., 2007). The use of clinical rating scores (a behavioral endpoint) in rabbits parallels the assessment of the modified Rankin Scale (mRS) in stroke patients, a common neurological endpoint in most clinical trials of ischemic stroke (NINDS, 1995). The present study tested the hypothesis that CEPO would be useful to attenuate embolism-induced behavioral deficits and determined the therapeutic window for neuroprotection.

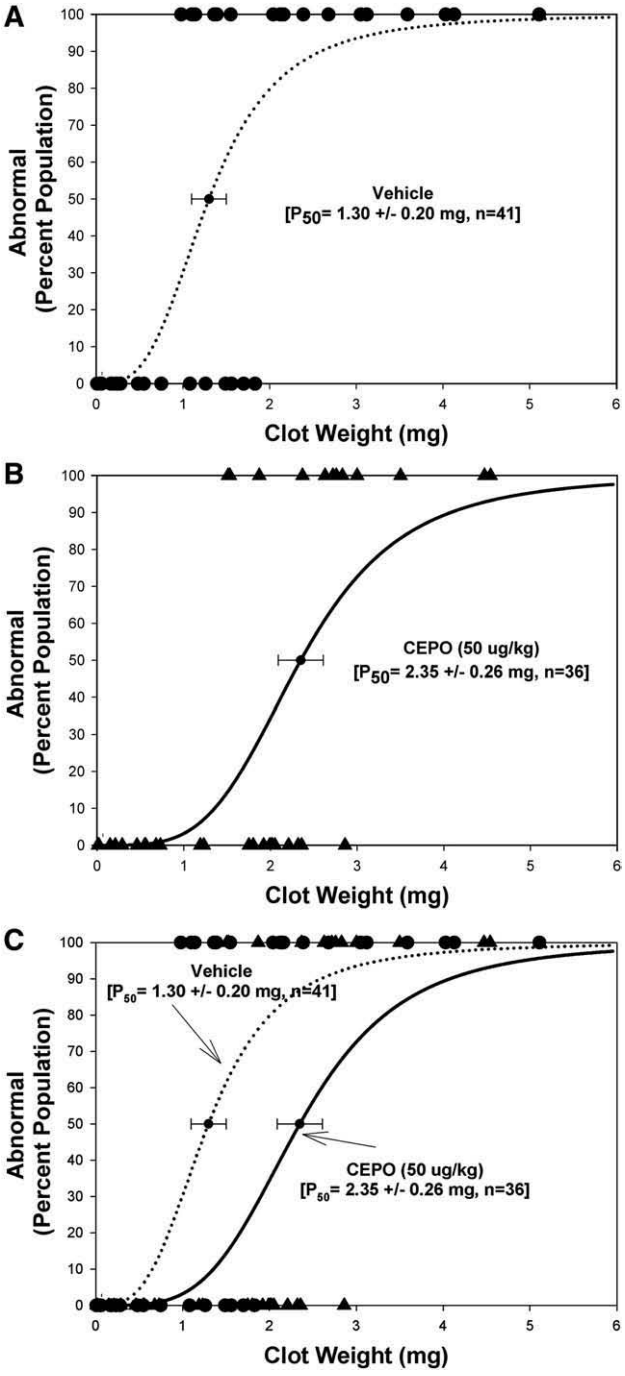

Fig. 1 – Abnormal rabbits as a function of clot weight measured in brain: 5 min post-embolization administration of 50 μg/kg CEPO. (A) The dotted line is the Vehicle Control curve (raw data from individual rabbits plotted as circles), which shows that 50% of the rabbit with a brain clot dose (P_{50} value) of 1.30 ± 0.20 mg (n = 41) are Abnormal. (B) The dark solid line is the CEPO-treated curve (raw data from individual rabbits plotted as triangles), which shows that CEPO increased the P_{50} value to 2.35 ± 0.26 mg (n = 36). (C) Composite graph showing both the Vehicle-treated curve (raw data from individual rabbits plotted as circles) and the CEPO-treated rabbit curve (raw data from individual rabbits plotted as triangles). The shift to the right is indicative of CEPO-induced neuroprotection and behavioral improvement. Results are presented as the P_{50} (in mg) mean ± SEM for the number of rabbits in each group (n). The P_{50} value is different from vehicle (*p < 0.05).