SCIENTIFIC ARTICLE

Erythropoietin Enhanced Recovery After Traumatic Nerve Injury: Myelination and Localized Effects

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Purpose We previously found that administration of erythropoietin (EPO) shortens the course of recovery after experimental crush injury to the mouse sciatic nerve. The course of recovery was more rapid than would be expected if EPO's effects were caused by axonal regeneration, which raised the question of whether recovery was instead the result of promoting remyelination and/or preserving myelin on injured neurons. This study tested the hypothesis that EPO has a direct and local effect on myelination *in vivo* and *in vitro*.

Methods Animals were treated with EPO after standard calibrated sciatic nerve crush injury; immunohistochemical analysis was performed to assay for myelinated axons. Combined *in vitro* neuron−Schwann cell co-cultures were performed to assess EPO-mediated effects directly on myelination and putative protective effects against oxidative stress. *In vivo* local administration of EPO in a fibrin glue carrier was used to demonstrate early local effects of EPO treatment well in advance of possible neuroregenerative effects.

Results Systemic Administration of EPO maintained more *in vivo* myelinated axons at the site of nerve crush injury. *In vitro*, EPO treatment promoted myelin formation and protected myelin from the effects of nitric oxide exposure in co-cultures of Schwann cells and dorsal root ganglion neurons. In a novel, surgically applicable local treatment using Food and Drug Administration−approved fibrin glue as a vehicle, EPO was as effective as systemic EPO administration at time points earlier than those explainable using standard models of neuroregeneration.

Conclusions In nerve crush injury, EPO may be exerting a primary influence on myelin status to promote functional recovery.

Clinical relevance Mixed injury to myelin and axons may allow the opportunity for the repurposing of EPO for use as a myeloprotective agent in which injuries spare a requisite number of axons to allow early functional recovery. *(J Hand Surg Am. 2016;■(■):■−■.*

Key words Erythropoietin, functional recovery, peripheral nerve injury, regeneration.

Online Only

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Received for publication January 7, 2016; accepted in revised form August 6, 2016.

No benefits in any form have been received or will be received related directly or indirectly to the subject of this article.

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0363-5023/16/■ ■-0001$36.00/0
http://dx.doi.org/10.1016/j.jhsa.2016.08.002

PERIPHERAL NERVE INJURIES FREQUENTLY result from acute traumatic compression or crushing. There are several mechanisms by which these injuries cause functional impairment, including a loss of axonal integrity, destruction to myelin, and denervation of muscle. Loss of function is variably reversible and currently no available treatments accelerate recovery from these injuries. Current management is complicated by difficulties in both the diagnosis and treatment of these disorders, with little ability to predict the injuries in which enhanced recovery is even possible. Because functional return must outpace motor end plate degeneration to prevent irreversible loss of function, enhancing this process could potentially prevent negative outcomes and allow partial or complete recovery in many settings.

One potential opportunity to enhance recovery in peripheral nerve injury is through the administration of erythropoietin (EPO). Although it is most commonly used to stimulate erythropoiesis, multiple studies have delineated the therapeutic and beneficial effects of EPO administration in multiple types of injuries and tissues.[1–5] In addition, EPO application ameliorates the loss of nerve conduction velocity in diabetic rats[6] and accelerates functional recovery after peripheral nerve injury.[7–9]

Although the cellular and molecular mechanisms by which EPO promotes repair in peripheral nerve injury are poorly understood, the speed of recovery observed in our previous studies[8] indicated that recovery is not likely caused by regeneration of transected axons alone. Whereas axonal regeneration after transection occurs at a rate of approximately 1 mm/d, we observed accelerated improvement in the deficits of injured nerves within 7 days after systemic EPO treatment.

The purpose of this study was to elucidate the mechanism by which EPO promotes neuroregeneration and functional recovery in acute nerve injuries. We hypothesized that EPO has direct effects on myelination and that these effects are mediated by interactions between EPO and local Schwann cells. Behavioral, immunohistochemical, and biochemical evaluations were employed *in vivo and in vitro* to examine this hypothesis. We also sought to develop a system for local EPO delivery that would be as efficacious as systemic administration. We hypothesized that local EPO administration would enhance recovery of nerve function comparable to that seen with systemic EPO treatment.

MATERIALS AND METHODS

Mouse model of peripheral nerve injury

The experimental design and all procedures were approved by the university committee on animal resources at our institution. Ten-week-old female C57BL/6 mice (n = 25; 20–25 g) were used in this study. All mice received a standardized sciatic nerve crush injury, as previously described.[8] Briefly, mice were anesthetized using ketamine (60 mg/kg) and xylazine (4 mg/kg). Both hind limbs were shaved, washed with 70% ethanol, and prepped with povidone–iodine. A gluteal-splitting approach was used to expose the left sciatic nerve immediately distal to the sciatic notch and proximal to the trifurcation. A smooth-tipped needle driver (Miltex, Plainsboro, NJ) was then placed around the nerve and closed to the second notch for 30 seconds to create the acute crush injury. A sham surgery was performed on the contralateral limb of each mouse to serve as the control. The same technique was used to expose the right sciatic nerve but no manipulation or injury of the nerve was performed. A multilayer closure was completed with simple interrupted 5-0 nylon sutures.

All mice tolerated the procedure well and returned to free cage activity postoperatively with unrestricted access to food and water. Buprenorphine (0.05 mg/kg) was given for postoperative analgesia immediately after surgery and every 12 hours thereafter for 3 days, at which time the mice no longer exhibited signs of pain.

Experimental design

Mice were randomized into 1 of 5 groups separated into 2 separate study arms based on the route of drug administration, with 2 groups in the systemically treated arm and 3 in the local delivery arm. Ten mice were randomized to receive either systemic erythropoietin (5,000 U/kg) (n = 5) or normal saline (as a control) (n = 5). Recombinant human EPO (PROCRIT; Amgen, Thousand Oaks, CA) was administered as a single intraperitoneal dose immediately after the surgical procedure, with the dosage of 5,000 U/kg selected based on previous studies in animals and humans.[1,6,8–10]

Local delivery of EPO through fibrin glue matrix

If EPO is acting directly on Schwann cells themselves, in contrast to stimulating a systemic healing response, localized delivery of EPO could offer a means of harnessing the potential benefits of this protein while reducing the risk of possible adverse effects of systemic administration.[11,12] To assess EPO's potential to improve functional recovery when administered locally on impaired nerves, we used a clinically applicable, Food and Drug Administration–approved delivery material, a fibrin