

● PERSPECTIVE

## Erythropoietin enhanced recovery after peripheral nerve injury

Peripheral nerve injuries are common disorders affecting the US population and represent a great economic and social burden. Acute injuries to peripheral nerves are most often due to an acute trauma or iatrogenic injury, with the resultant neurological deficit dependent on the injury type and severity. Clinical manifestations include a variable degree of motor and/or sensory deficits, with complete loss of motor function and intractable neuropathic pain seen in severe injuries. Current treatment strategies are limited and are lacking in their ability to improve outcomes. Surgical intervention is reserved for the most severe nerve injuries, leaving the mild to moderate injuries as the most challenging to treat clinically due to the lack of effective therapeutic options. In some cases, return of functional ability may occur spontaneously over time. Conversely, neurologic deficits may persist long-term with an incomplete restoration of function; complete recovery, if it occurs at all, can take months or even years. Even with surgical intervention, outcomes are variable and return to normal function is achieved in less than 50% of patients (Pfister et al., 2011).

**Erythropoietin (EPO) as neuroprotective agent in the peripheral nervous system (PNS):** EPO has typically been associated with hematopoietic properties, but recent studies have found that it is much more ubiquitous with widespread effects throughout the body. Notably, EPO has shown therapeutic benefits in a variety of disorders of the central and peripheral nervous systems (Sargin et al., 2010). Systemic EPO therapy has been shown to improve behavioral, electrophysiological, and histological outcomes in animal models of neuropathic pain (Campana et al., 2003), and diabetic neuropathy (Bianchi et al., 2004). The findings from these studies demonstrate EPO's ability to ameliorate the deleterious effects associated with peripheral nerve damage and dysfunction, which may be therapeutically exploited for clinical use in the treatment of peripheral neuropathies.

There are many questions and unknowns surrounding the mechanisms of EPO-mediated neuroprotection, and the events at the cellular and molecular levels remain poorly understood. In their description of a novel "axonoprotective" pathway, Keswani et al. (2004) demonstrated that axonal injury stimulates the local Schwann cells to upregulate production of EPO. They also showed that EPO binding to the EPO receptor (EPO-R) was a necessary step to confer EPO's axonoprotective properties. To explain this, they propose that the Schwann cell-derived EPO binds to the EPO-R expressed on the surface of neighboring neurons, initiating a signaling pathway to prevent axonal degeneration of injured neurons. In their model, EPO-R was found to be predominantly expressed by neurons, leading them to conclude that neurons were the major EPO target in the PNS (Keswani et al., 2004).

More recently, there has been evidence to support the presence of the EPO-R on Schwann cells. Li et al. (2005) discovered EPO-R expression on the surface of Schwann cells, and that injury to adjacent nerves upregulated Schwann cell expression of both EPO-R and EPO. Increased EPO in the local environment promoted Schwann cell recruitment and migration to the site of the lesion. Inoue et al. (2010) confirmed this result *in vitro*, while also demonstrating that the Schwann cell migration due to EPO occurs in a dose-dependent manner. Direct interaction of EPO with EPO-R on Schwann cells leads to Schwann cell activation of intracellular signaling pathways. Downstream effects that have been observed include Schwann cell dedifferentiation and proliferation, inhibition of neuronal apoptosis via inactivation of pro-apoptotic mediators, suppression of the inflammatory response, and amelioration of oxidative stress in the local microenvironment (Li et al., 2005; Lykissas et al., 2007). These findings suggest, through an autocrine pathway and positive feedback loop, that Schwann cells attempt to mediate the effects of the

injury by minimizing the damage to local nerve structures and to direct the process of regeneration and repair of the injured peripheral nerves. While they do not offer any direct mechanistic detail, these studies highlight EPO's significance in neuroprotection.

EPO has also been shown to give neuronal tissue anti-apoptotic and neurotrophic properties. In an *in vitro* study, Csete et al. (2004) demonstrated that not only is EPO administration responsible for decreased neuronal apoptosis, but also that it drives dopaminergic neuroblast proliferation. In EPO-treated cells there were significantly increased levels of dopaminergic neuroblasts compared to untreated cells, after both 24 and 48 hours of growth, showing EPO's neurotrophic potential.

**EPO therapy following acute peripheral nerve injury:** Our lab took an interest in EPO's role following acute peripheral nerve injury. In our introductory investigation, we looked at EPO's therapeutic potential using a validated murine model of peripheral nerve crush (Elfar et al., 2008). In injured mice, administration of EPO accelerated functional recovery and improved histologic appearance in all EPO-treated groups. Interestingly, therapeutic benefit and functional improvement was observed in each of the groups that received EPO therapy, whether treatment was initiated immediately, 24 hours, or 1 week post-injury. These findings highlight that the timing of post-injury EPO administration may be flexible with a wide window of therapeutic efficacy, although the significance of this benefit and its utility in a clinical setting has yet to be elucidated. Importantly, we observed an improvement in the EPO-treated groups greater than that which could be fully explained by axonal regeneration and repair alone, as axonal regrowth occurs at a rate of approximately 1 mm/day. This led us to posit that EPO may have additional neuromodulatory actions which have not yet been determined, but may be important for the process of nerve regeneration and repair following injury.

In our current study, we provide significant data to suggest an additional mechanism by which EPO promotes neuroregeneration and functional recovery in acute nerve injuries - by stimulating and enhancing the myelinating activity of local Schwann cells. This study demonstrates that EPO exerts direct effects on nerve fibers by promoting myelination, that these effects are mediated by interactions between EPO and local Schwann cells, and that exogenous administration of EPO potentiates these effects both *in vivo* and *in vitro* (Sundem et al., 2016). First, we reproduced the behavioral results from our original study (Elfar et al., 2008), again finding significant improvement in the functional status of injured mice treated with EPO at both 7 and 14 days following acute crush injury. Histologically, the total axon number in cut nerve specimens was comparably reduced in treated and untreated groups at day 7 post-injury, as expected in this type of injury to peripheral nerves. However, in injured nerves of EPO-treated mice, a greater number of surviving axons were myelinated compared with untreated mice, findings that support EPO-mediated protection and restoration of myelin on spared axons. We then confirmed these findings with our *in vitro* experiments. When Schwann cells were stressed with an nitric oxide (NO)-rich microenvironment, effectively recreating the pathologic state expected at the site of inflammation *in vivo*, EPO-treated primary Schwann cell cultures were able to overcome the NO-driven myelin inhibition (Sundem et al., 2016).

These results provide evidence that supports EPO having a direct influence on myelin status in the PNS, and validate previous findings of Schwann cells as a primary target of EPO-mediated neuroprotection. Taken together, these findings support the potential therapeutic role of EPO in peripheral nerve injuries with Schwann cells as a therapeutic target.

**EPO delivery:** In the previously mentioned study (Sundem et al., 2016), we reported a novel finding of therapeutic efficacy with local delivery of EPO. EPO has been extensively studied in human populations and is widely used clinically. While it is a relatively safe and well-tolerated drug, there is the possibility of adverse effects with systemic administration, potentially limiting its utility for continuous therapy. Armed with the knowledge of EPO's direct and indirect effects on Schwann cells and neurons, we developed a local



drug delivery system and conducted an experiment to compare this novel method of EPO delivery with systemic administration, the standard of care. When EPO (in a fibrin glue matrix) was placed directly on the nerve intraoperatively following the crush injury, functional recovery was enhanced when compared to control-treated mice, mimicking that seen with systemic EPO treatment (Sundem et al., 2016). Additionally, pharmacokinetic analysis demonstrated extended release of the drug, release of 90% of the drug over the course of 6 days.

Similarly, Zhang et al. (2015) demonstrated EPO's efficacy when injected in a poly(lactide-co-glycolide) microsphere. In this delivery method, EPO release was noted for as long as 14 days post-injection. The therapeutic efficacy of this delivery method was supported by their functional, electrophysiological, and histological analysis following acute peripheral nerve injury.

Given the drawbacks of systemic EPO administration, the above delivery methods could be viable alternatives and provide a gateway to clinical EPO therapy in peripheral nerve injuries.

**Limitations and questions remaining:** Numerous neuroprotective agents have been tested in large scale human trials. Despite robust theoretical background and promising experimental data from animal studies, none of these trials resulted in unequivocal success; therefore, there is no neuroprotectant currently approved for use in acute injury of peripheral nerves. While EPO's therapeutic potential as a neuroprotective agent is certainly promising, its role in the treatment of peripheral nerve injury in humans remains uncertain. Clinical trials are the next step in the investigation and will allow us to determine if the therapeutic efficacy and benefit observed in animal models translates to beneficial and clinically meaningful effects in humans. Of note, there was a retrospective study published recently which assessed the neurologic impairment in patients with postoperative nerve palsies treated acutely with combined therapy of EPO and tapered oral corticosteroids (Bernstein et al., 2016). Patients who received a combination of EPO and steroids exhibited drastic improvement in their sensory and motor function, with nearly complete resolution of the impairment. Drawbacks of this study include the retrospective nature, unclear significance of synergism from dual therapy, and an inability to determine whether EPO alone would result in the same clinical improvement. While no conclusions may be drawn from the results of this clinical investigation, these findings do add evidence to further support the necessity and utility of conducting randomized controlled human trials.

Recombinant human EPO is an FDA-approved drug that is widely used in clinical practice, so EPO's safety profile, adverse effects, tolerability, and contraindications have been well established. A limitation of drug repurposing involves the unknown pharmaceutical data, since these parameters are commonly undefined for a drug when its novel therapeutic application is under investigation. Therefore, the optimal dosage, timing and duration of therapy have yet to be clarified for the therapeutic use in nerve injury. These details will need to be investigated further in carefully designed animal studies and future clinical trials.

**Conclusions and future directions:** In summary, erythropoietin possesses many neuroprotective properties related to its multimodal mechanism of action in acute nerve injury. In our opinion, the efficacy of systemically administered EPO is very promising for therapeutic promotion of nerve regeneration. This route of drug administration has shown therapeutic benefit in animal models and is known to be a well-tolerated therapy for those seeking treatment for anemia. With wide applicability, a well-characterized safety profile, few adverse effects, and prior FDA-approval for use in humans, EPO is a great candidate for therapeutic use and application as a neuroprotective agent in peripheral nerve disorders. Encouragingly, preliminary results already indicate that early administration of EPO can accelerate the functional recovery and improve neurologic outcome in acute peripheral nerve injury. Well-designed human studies and early phase clinical trials are necessary to improve our understanding of its neuroprotective potential and delineate its clinical significance for treatment of peripheral neuropathy. Furthermore,

a local drug delivery system could prove superior to systemic EPO therapy, however further research is needed to design a safe, efficacious, and feasible mechanism as well as to characterize the pharmacodynamic and pharmacokinetic properties of that system.

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**Max Modrak, Leigh Sundem, John Elfar\***

University of Rochester School of Medicine and Dentistry, Rochester, NY, USA; Department of Orthopaedic Surgery and Rehabilitation, University of Rochester Medical Center, Rochester, NY, USA (Modrak M, Sundem L)

Department of Orthopedic Surgery and Center for Orthopedic Research and Translational Science, Pennsylvania State University College of Medicine, Hershey, PA, USA (Elfar J)

\*Correspondence to: John Elfar, Ph.D., [openelfar@gmail.com](mailto:openelfar@gmail.com).

orcid: 0000-0002-4438-7118 (John Elfar)

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