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Abstract Details:

Breakout Session: Advances in Regenerative Medicine for the Treatment of Neuromusculoskeletal Injuries from Point of Injury to Definitive Care and Beyond

Submission Category: Oral Presentation

Title: Erythropoietin is a Post-Traumatic Anti-Inflammatory Treatment that Supports Macrophage Recruitment, Transition, and Phagocytosis of Dead Cells Following Peripheral Nerve Injury in Mice.

Abstract:

Introduction:

Traumatic peripheral nerve injury (TPNI) is significant and occurs in approximately 3% of all trauma patients. Inflammation, apoptosis, and obliteration of myelin and axons are the primary pathophysiological consequences of TPNI. Infiltrating immune cells play a critical role following TPNI, and inflammation itself undergoes a transition from an initial pro-inflammatory reaction to an anti-inflammatory phase. This is critical for nerve repair and structural modification. However, a persistent and severe pro-inflammatory reaction can impair peripheral nerve recovery, which depends on macrophage infiltration and early phenotypic transitions for the phagocytosis of cellular debris in advance of Schwann-cell orchestration of nerve regeneration. There is an unmet clinical need for a therapeutic agent which can enhance functional recovery by controlling this inflammation after TPNI.

Erythropoietin (EPO), a pleiotropic hormone approved by the U.S. Food and Drug Administration (FDA) for anemia treatment, seems to have a role in guiding this transition. EPO can mitigate inflammation and may exert control of macrophage inflammation and phagocytosis. The significance of the EPO treatment on cellular debris clearance in the nerve after the injury is unknown, despite our work and the work of others motivating clinical translation of EPO for TPNI. We hypothesized that EPO may affect the critical clearance activity of M2 phenotype macrophages after TPNI, which might explain EPO's neuroregenerative potential.

Materials and Methods:

All animal experiments described conform to Institutional Animal Care and Use Committee (IACUC) approved protocols at The Pennsylvania State University College of Medicine, Hershey, PA. Ten-week-old male C57BL/6 J mice weighing 25 ± 3 g were anesthetized using intraperitoneal ketamine (100 mg/kg)/xylazine (10 mg/kg) anesthesia, under a stereo microscope, the sciatic nerve was exposed, and calibrated crush injury was performed ~3 mm proximal to the sciatic nerve trifurcation using jig-modified forceps (5 mm tip width) for 30 s. The skin was closed with surgical staples, and post-operative slow-release buprenorphine (0.05 mg/kg) was given subcutaneously to all animals as an analgesic. The experimental animals ($n = 7$ animals/group) were randomly assigned to Sham (normal saline, 0.1 ml/mouse), sciatic nerve crush injury (SNCI) (normal saline, 0.1 ml/mouse), and SNCI with EPO (5000 IU/kg; Epoetin alfa) groups. EPO was given intraperitoneally immediately after surgery and post-surgery days 1 and 2. Functional recovery following nerve injury was assessed using walking track analysis (WTA) on days 3 and 7. Mice were euthanized on post-injury days 3 and 7, and sciatic nerve was harvested from the ipsilateral hindlimbs for apoptosis, phagocytosis, and myelination analysis using immunofluorescence (IF) staining. In-vitro and ex-vivo phagocytosis of dead-Schwann cells was conducted using mouse bone marrow- and peritoneal-derived macrophages by IF and flow cytometry. The effect of EPO on the polarization of macrophages (M1 to M2 phenotypes) under lipopolysaccharide (LPS)-induced stress conditions were analyzed using qRT-PCR and flow cytometry. Data were analyzed using either one-way analysis of variance (ANOVA) and Tukey's multiple comparisons test or unpaired t-tests by GraphPad Prism Version 8.4.3.

Results:

EPO treatment significantly augmented anti-apoptosis and phagocytosis of myelin debris via CD206+ macrophages and increased myelination and sciatic functional index (SFI) compared to saline treatment after TPNI. EPO treatment showed a significant improvement in efferocytosis of apoptotic sciatic nerve-derived Schwann cells when cocultured with both bone marrow- and peritoneal-derived macrophages in-vitro. EPO also increased phagocytosis of dead Schwann cells in-vivo by peritoneal macrophages – cells totally outside the site of injury. EPO treatment significantly attenuated bone marrow-derived macrophage pro-inflammatory genes (IL1 β , iNOS, and CD68) and augmented anti-inflammatory genes (IL10 and CD163) in addition to the cell surface marker CD206 expression after LPS induction. EPO also showed anti-apoptotic (Annexin V/ 7AAD) effects under LPS conditions in bone marrow-derived macrophages. Our data demonstrate that EPO promotes the M2 phenotype macrophages to ameliorate sciatic nerve apoptosis and efferocytosis of dying Schwann cells and myelin debris and improves sciatic nerve functional recovery following SNCI.

Conclusions:

To our knowledge, this is the first study to demonstrate EPO's ameliorative effect on the advancement of nerve regeneration and functional recovery following TPNI via M2 phenotype macrophage phagocytosis of Schwann cell debris with early anti-apoptotic and anti-inflammatory effects. These data may translate to EPO's utility in the early phase post-injury to prevent destructive post-traumatic inflammation and promote recovery in the traumatized limb.

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John C. Elfar has an equity interest in a company that has licensed patents from his institution based on his work.

Learning Objectives

1. Disclose the effect of EPO on attenuation of neuronal apoptosis and the activation of macrophages for phagocytosis of myeline debris of Schwann cells following TPNI.
2. Reveal the effect of EPO on macrophage phenotypic transition (M1 to M2), where it significantly augments myelination and functional recovery following TPNI.
3. Discuss the translationally relevant properties of EPO as a systemic treatment for TPNI.

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