Impact of Erythropoietin Administration in Knockout Mice Exposed to a Repetitive Microtrauma and Neurodegeneration Protocol for a Neuropathic Murine Model

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INTRODUCTION:

Charcot neuroarthropathy (CN) is a degenerative disease that begins with peripheral neuropathy and progresses with repeated trauma. Severe foot and ankle disruption and limb-threatening consequences are often observed. Peripheral nerve research suggests that Erythropoietin's (EPO) anti-apoptotic and anti-inflammatory effects may prove to be beneficial in functional recovery of traumatized limbs. This study seeks to further explore this relationship. Our group has developed a neurodegenerative mouse model of CN by combining a high-fat diet to induce obesity (DIO), and a treadmill protocol to induce microtrauma. Using this protocol, we aim to investigate if DIO EPO knockout mice exhibit worse neurodegenerative changes than DIO genetic control mice, and if EPO administration decreases the degree of neurodegeneration when compared against those not provided EPO and those lacking EPO receptors on Schwann cells.

METHODS:

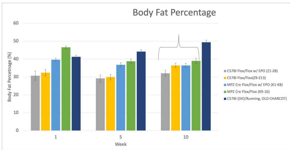
Thirty-two mice were bred in-house. Sixteen possessed EPO receptors on their Schwann cells (C57Bl); 16 lacked this receptor (MPZ Cre). At 4-weeks old, a high-fat (60% fat by kcal) was started ad libitum. This diet was continued for the study duration. At 16-weeks old, 8 C57Bl mice and 8 MPZ Cre mice completed a 3-day EPO protocol (EPO+). Then all mice began the high-intensity treadmill protocol for 10 weeks. Serum blood glucose levels were collected at week 5 of the running protocol. Bodyweight, body fat percentage, bone mineral density (BMD), and Von Frey filament sensory testing were recorded at weeks 1, 3, 5, 7, and 10. Radiographs were obtained at weeks 1, 5, and 10. After 10-weeks, the mice were sacrificed, and the hind-paws were harvested and prepared for histopathologic analysis. Repeated measures analysis of covariance for outcomes measured at multiple time points, non-parametric Wilcoxon rank sum test for outcomes measured only at 5 weeks, and two-way ANOVA for histopathology were conducted.

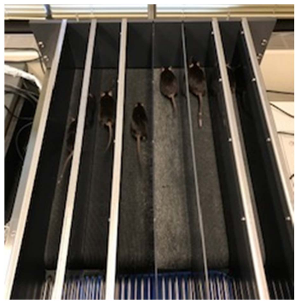
RESULTS:

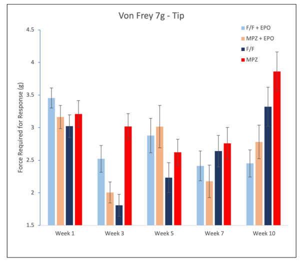
The median blood glucose for C57Bl/EPO- mice was significantly higher than MPZ Cre/EPO+ ($p=0.032$); however, none of the groups had elevated median blood glucoses. No significant changes in bodyweight were observed; a decreased trend in bodyweight was detected across all groups at week 10. MPZ Cre/EPO- mice demonstrated a marginally significant decrease in body fat percentage ($p=0.0497$), while all other groups revealed no change. Trends of increased BMD were observed, with a significant increase appreciated among MPZ Cre/EPO+ ($p=0.0004$), and a significant difference observed between C57Bl/EPO+ and C57Bl/EPO- ($p=0.0136$), and MPZ Cre/EPO+ and C57Bl/EPO+ ($p=0.0351$). Sensory testing remained unchanged, apart from C57Bl/EPO+ mice with an improved sensory response over 10 weeks ($p=0.0251$) and when compared to C57Bl/EPO- mice ($p=0.044$). Radiographic analysis demonstrated no midfoot subluxation or tarsal instability. Histopathologic analysis demonstrated no neurodegenerative destruction, hyalinized arteriolosclerosis, or intraneural vacuolization/myxoid/edema.

DISCUSSION AND CONCLUSION:

The neurodegenerative mouse model previously achieved with DIO and a treadmill protocol to induce trauma was not replicated with the current sample of C57Bl and MPZ Cre mice. While these genetically modified, knockout mice serve as suitable models for studying the impact of EPO on nerve crush injuries, the current study was unable to achieve neurodegeneration in this sample, as suggested by the lack of significant sensory deficits. BMD, radiographic, and histopathologic analyses also demonstrate a lack of support for the development of neuropathy. While the diet protocol was executed as outlined in prior studies, the mice included in this sample did not appear to intake enough of the diet to experience weight gain and/or increased body fat percentage consistent with obesity. In the absence of these metabolic changes, the treadmill protocol that previously contributed to the development of microtrauma and neurodegeneration in DIO mice, did not induce these changes in our sample. This model supports prior research by demonstrating that in the absence of DIO, repetitive traumatic insult alone does not induce neurodegenerative changes. In the absence of these changes, researchers cannot appreciate differences between groups or observe interventions' impact, such as EPO, on the development of neurodegeneration.


Body Fat Percentage
60
50
40
30
20
10
0
Body Fat Percetnage (%)
1
5
10
Week
C57Bl Flox/Flox w/ EPO (Z1-Z8)
C57Bl Flox/Flox(Z9-Z13)
MPZ Cre Flox/Flox w/ EPO (K1-K8)
MPZ Cre Flox/Flox (K9-16)
C57Bl (DIO/Running, OLD CHARCOT)




Von Frey 7g - Tip
4.5
4
3.5
3
2.5
2
1.5
Force Required for Response (g)
Week 1
Week 3
Week 5
Week 7
Week 10
F/F + EPO
MPZ + EPO
F/F
MPZ