Effects of Thrombopoietin (TPO) on Longitudinal Mouse Hind Limb Crush Injury Model

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Approximately 645 people suffer from blunt force trauma injury to the femur every day. The recovery time of such injury can last anywhere from 3-6 months. Thrombopoietin (TPO) was used as a growth factor to induce bone and muscle healing. In this study we utilized 9 separate mouse model groups (10 mice per group) were used: Crush PBS, Crush TPO, Surgery PBS, and Surgery TPO at day 3 and day 17, and controls with no surgery/crush/ treatment. Crush models were introduced to hind limb crush injury by a mechanical-gravity driven Einhorn device. Skeletal muscle was harvested from the following sites: experimental impact, experimental adjacent, and normal contralateral skeletal muscle as a control. The muscles were fixed, processed, sectioned, and stained with H&E and Masson's Trichrome stains. The slides were reviewed for skeletal muscle injury, muscle necrosis, inflammation, muscle repair, and regeneration. In addition, F4/80, an immunostain for macrophages was performed. On microscopic examination at day 3 the most common histologic changes seen were sporadic muscle fiber vacuolation, focal necrosis of varying sizes, muscle contraction bands, and infiltration of macrophages. On day 17, the skeletal muscle injury was generally healed. The main histologic lesions seen were variable sizes of muscle fibers, early fibroplasia, fat infiltration, some macrophages(less than day 3) , satellite cells, and neovascularization. A follow-up immunostain (CD206 specific for M2 double labeled with F4/80) was performed to characterize the macrophages in and around the lesions at day 3. M2 macrophages were seen around the periphery of the lesion and none in the middle of the lesion. There were very minimal differences in M2 numbers between the PBS and TPO treated groups at day 3. In conclusion, comparing the TPO treated mice versus the PBS control group with F4/80 immunostain showed the lesions at both time points were less in the TPO treated mice.

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