Selective PKC-βII Inhibition Improves Skeletal Muscle Force Generation and Gait Recovery Following Tourniquet-Induced Hindlimb Ischemia-Reperfusion Injury in Mice

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Disclosures: None

INTRODUCTION: Prolonged tourniquet application for extremity trauma in prehospital settings can lead to significant skeletal muscle damage due to ischemia-reperfusion (I/R) injury. Previous rodent studies suggest that I/R injury contributes to long-term deficits in muscle strength due to mitochondrial dysfunction and oxidative stress, resulting in inhibited satellite cell activation and delayed muscle fiber repair.[1,2] In preliminary studies, we demonstrated that Ruboxistaurin (RBX), a selective inhibitor of PKCβII-mediated mitochondrial redox protein p66Shc activity, significantly decreases mitochondrial reactive oxygen species (ROS) in murine satellite cells in vitro.[3] We hypothesized that RBX would attenuate ROS-mediated damage and restore muscle strength and function to pre-injury baseline following tourniquet-induced hindlimb I/R injury in mice.

METHODS: All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Wake Forest University Health Sciences. Hindlimb ischemia was induced using an orthodontic rubber band tourniquet applied to the left thigh of 6-month-old male (n=8) and female (n=8) C57BL/6J mice for 3h. Mice were randomly assigned to a 2-week or 9-week recovery cohort. RBX or saline was delivered via subcutaneously implanted osmotic pumps. Functional recovery of the ischemic hindlimb was assessed weekly using DigiGait software. Gait metrics were normalized to Day-0 baseline values to evaluate recovery trends (L/L baseline ratio). At study endpoint, electrodes were placed into the gastrocnemius (GC) and tibialis anterior (TA) muscles of both experimental (left) and control (right) limbs to measure muscle force (mN/g) and contraction amplitude. Nerve conduction studies on bilateral sciatic nerves were performed using electromyography. Muscle contractility was evaluated by averaging three consecutive compound muscle action potentials (CMAPs). Left limb force, CMAP, and nerve conduction velocity (NCV) were normalized (L/R ratio) to the contralateral limb to account for inter-animal variability. Independent and paired t-tests were performed using SPSS software.

RESULTS SECTION: RBX-treated mice in the 9-week cohort demonstrated significantly increased muscle force (mN*g) compared with saline controls at 40 Hz (97 ± 7 vs 26 ± 12; $p=0.01$), 60 Hz (98 ± 15 vs 23 ± 8; $p=0.01$), and 80 Hz (111 ± 23 vs 20 ± 7; $p=0.02$). When normalized to the contralateral limb, RBX-treated mice in the 9-week cohort showed significant improvement in muscle force at 60 Hz ($p=0.003$), 80 Hz ($p=0.047$), and 100 Hz ($p=0.007$) compared with RBX-treated mice in the 2-week cohort. However, RBX mice were not statistically different from saline controls within each cohort. No significant increases in muscle force were observed between the 2-week and 9-week cohorts in saline-treated mice after normalization. EMG analysis demonstrated time-dependent recovery for both saline and RBX groups, with significant improvements in CMAP and NCV at 9 weeks compared with 2 weeks ($p<0.05$). However, when normalized to the contralateral limb, only RBX-treated mice retained significant increases in tibialis anterior and gastrocnemius contractility at 9 weeks. RBX-treated mice exhibited significantly earlier recovery of gait parameters, characterized by earliest point of return to baseline (RBX vs Saline) in stance (day 28 vs 49), swing (day 49 vs 63), propulsion (day 42 vs 49), and break duration (day 28 vs 49).

DISCUSSION: RBX-treated mice exhibited significantly greater muscle contractile force and compound muscle action potentials at later recovery stages, accompanied by 1-3 weeks of earlier improvement in gait parameters compared with saline controls. These findings suggest that RBX enhances both neuromuscular conduction and contractile function, possibly through attenuation of mitochondrial ROS and preservation of satellite cell regenerative capacity. In future studies, histologic analysis of the tibialis anterior and gastrocnemius muscles from 2- and 9-week cohorts will be conducted to further characterize preservation of neuromuscular junction integrity and acceleration of myofiber regeneration as potential mechanisms of recovery.

SIGNIFICANCE/CLINICAL RELEVANCE: Tourniquet use is often essential for limb salvage in lower-extremity trauma but can induce significant I/R-mediated injury that poses a major barrier to full muscle recovery. This study suggests that pharmacologic inhibition of PKCβII with Ruboxistaurin promotes functional restoration of skeletal muscle following tourniquet-induced I/R injury.

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