Modulating Macrophage Phenotype to Decrease Muscle Fibrosis in Ischemia–Reperfusion Injury

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**OBJECTIVE:** Muscle fibrosis is a devastating sequela following ischemia–reperfusion injury that results in significant functional impairment and poor outcomes. Redirecting the response to muscle injury from a profibrotic to a regenerative pathway would be of great clinical value. We hypothesize that macrophage-specific knockout of Tgfb1 and preclinical ligand trap binding of transforming growth factor (TGF)-β1 in wild-type animals will reduce the development of muscle fibrosis and will increase regeneration of myofibers after injury with organized production of collagen by fibroadipogenic progenitor cells (FAPs).

**METHODS:** Ischemia was induced in the left hind limb of LysMCre-Tgfb1fx/fx and age and strain-matched controls with clamping of the femoral vessels for 3 hours with simultaneous injection of cardiotoxin into the left tibialis anterior muscle. Left and right tibialis anterior muscles were harvested 1 week following injury. Histologic sections were stained with hematoxylin and eosin for morphology, picrosirius red for collagen quantification, and Masson’s trichrome for fibrosis architecture. Picrosirius-stained slides were imaged and analyzed using ImageJ to measure positive collagen staining. Myovision software was used to calculate myofiber cross-sectional area and Feret diameter (n = 3 per group). Sections were stained for immunofluorescence for F4/80, PDGFR-α, and TGF-β1. Mean fluorescent area also calculated with ImageJ. Flow cytometry was performed to quantify macrophage, neutrophil, and monocyte markers (n = 4 each). Next, adaptive transfer of LysmCreα mg macrophages was performed into LysMCre-Tgfb1fx/fx mice. Separately C57BL/6J mice were treated with a TGF-βRII/Fc ligand trap (TGF-βRII-Fc) or vehicle following IR cardiotoxin (n = 3 each). Similar analyses performed as described above.

**RESULTS:** LysMCre-Tgfb1fx/fx mice demonstrated significantly less fibrosis and muscle injury compared to controls. We found significantly higher area of fibrosis by picrosirius red staining in C57BL6/J animals compared to LysMCre-Tgfb1fx/fx which appeared uninjured, grossly similar to uninjured control (52.32 versus 13.39 μm²; P < 0.0001). Immunofluorescence showed decreased macrophage infiltration (F4/80) at the injury site and organized PDGFR-α staining in LysMCre-Tgfb1fx/fx injured muscle compared to wild-type (WT) mice. Flow cytometry revealed lower number of macrophages present in injured knockout muscle compared to WT. Adoptive transfer of LysmCreα macrophages recapitulated a fibrotic phenotype. TGF-βRII-Fc treatment of WT mice produced similar results to Tgfb1 knockouts almost completely mitigating fibrosis as quantified by picrosirius red staining (57.29 versus 17.17 μm²; P < 0.0001).

**CONCLUSIONS:** Our LysMCre-Tgfb1fx/fx animals demonstrated markedly reduced muscle injury with no obvious areas of fibrosis. The presence of increased PDGFR-α interstitial staining in wild-type muscle compared to LysMCre-Tgfb1fx/fx injured muscle suggests a disorganized proliferation of FAP cells within the wild-type injury site. The decrease in FAP proliferation in the LysMCre-Tgfb1fx/fx muscle suggests that macrophage-derived TGF-β1 may induce FAP proliferation and without it, the response to injury may be more regenerative than profibrotic. Treatment with TGF-βRII-Fc ligand trap yielded similar results to knockout suggesting that it may offer a viable therapeutic agent for prevention of muscle fibrosis in ischemia–reperfusion injury.

Effects of Vasopressors on Circulation of Porcine Abdominal Island Flap Model

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**OBJECTIVES:** During reconstructive surgical procedures, systemic vasopressors are frequently used to maintain normal blood pressure. However, questions have arisen regarding the pharmacologic effects of vasopressors on flap circulation. Many plastic surgeons have expressed concern about the possibility of impaired flap circulation caused by the vasoconstrictive effect of the drugs. The opposing argument exists that the increase of mean arterial pressure from vasoactive agents may improve flap perfusion. The purpose of this study was to evaluate the effect of commonly used vasopressors on flap circulation.