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. Directing 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-Tgfb1*$^{fx/fx}$ 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 *LysmCre*^{mtmg} macrophages was performed into *LysMCre-Tgfb1*$^{fx/fx}$ mice. Separately C57BL/6J mice were treated with a TGF-β1/3 ligand trap (TGF-βRII-Fc) or vehicle following IR cardiotoxin (n = 3 each). Similar analyses performed as described above.

RESULTS: *LysMCre-Tgfb1*$^{fx/fx}$ 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-Tgfb1*$^{fx/fx}$ which appeared uninjured, grossly similar to uninjured control (52.32 versus 13.39 μm^2; $P < 0.0001$). Immunofluorescence showed decreased macrophage infiltration (F4/80) at the injury site and organized PDGFR-α staining in *LysMCre-Tgfb1*$^{fx/fx}$ 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 LysmCre^{mtmg} 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 μm^2; $P < 0.0001$).

CONCLUSIONS: Our *LysMCre-Tgfb1*$^{fx/fx}$ 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-Tgfb1*$^{fx/fx}$ injured muscle suggests a disorganized proliferation of FAP cells within the wild-type injury site. The decrease in FAP proliferation in the *LysMCre-Tgfb1*$^{fx/fx}$ 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. However, 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.

MATERIALS AND METHODS: The vertical rectus abdominis myocutaneous island flap was raised in 5 female pigs (38.2~40.7 kg). Hemodynamic parameters were measured continuously by carotid arterial catheter. A bidirectional transonic vascular Doppler flow probe and laser Doppler perfusion monitor unit were applied to record the continuous change of pedicle artery flow and microvascular perfusion following intravenous administration of dopamine (3, 5, 10 μg/kg/min), dobutamine (1.25, 2.5, 5 μg/kg/min), and norepinephrine (0.05, 0.1, 0.2 μg/kg/min).

RESULTS: Both microvascular perfusion and pedicle flow were generally proportional to mean arterial pressure, and all the 3 vasopressors improved flap perfusion and pedicle flow without deleterious effect. Norepinephrine showed that the highest microvascular perfusion and dobutamine showed the highest pedicle flow rate. Mean blood pressure was the only statistically significant factor that affects both microvascular perfusion and pedicle flow ($P < 0.0001$).

CONCLUSIONS: Our results strongly suggest that the foremost 3 vasopressors can be used for flap surgery without deterioration, and maintaining adequate systemic blood pressure is crucial for good flap circulation.

Platelet-rich Fibrin With Adipose-derived Stem Cells for the Treatment of Chronic Cutaneous Wounds: A Randomized Clinical Trial

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INTRODUCTION: Chronic wounds represent a relevant healthcare problem with enormous social and economic burden. Regenerative surgery offers innovative options for the treatment of chronic ulcers with promising results and reduced rate of complications.[1–4] The aim of this randomized clinical trial was to compare the application of platelet-rich fibrin (PRF) combined with autologous adipose-derived stem cells (ASCs) versus the application of PRF alone for the treatment of chronic cutaneous ulcers.

MATERIALS AND METHODS: To date, 33 patients with chronic skin ulcers have been randomized in 2 different groups: PRF alone (control group: 19 patients) and PRF + ASCs (experimental group: 14 patients). Patients with neoplastic or clinically infected wounds have been excluded from the study. The production and application of PRF or PRF + ASCs have been performed with Vivostat (Alleroed, Denmark). In our study, we used PRF and PRF + ASCs by local application and infiltration of wound margins. Patients were evaluated up to 12 weeks after the procedure with regular office visits. At week 4 and 12, we also preformed laser Doppler flowmetry and transcutaneous oximetry.[5]

RESULTS: Patients reported significant pain reduction in both groups. Results of laser Doppler flowmetry and transcutaneous oximetry provided promising results in terms of increased wound bed perfusion and oxygenation. Control group showed wound area reduction of 16% at 4 weeks and 49% at 12 weeks postoperatively, whereas in the experimental group, the area reduction was 49% and 81% at 4 and 12 weeks, respectively.

CONCLUSIONS: Both treatments may represent a feasible option for the treatment of chronic wounds. Our preliminary data suggest that the application of PRF + ASCs enhance the healing process more effectively than the PRF alone.

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