Optimization of Murine Hindlimb Ischemia Model for Preclinical Evaluation of Vascular Regenerative Therapeutics for Peripheral Artery Disease

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Introduction

• Peripheral arterial disease (PAD) affects 200 million patients worldwide with risks of limb amputation and mortality.
• Transplantation of autologous cells can potentially regenerate vascular tissues and restore limb perfusion.
• Current animal models of PAD only induce acute limb ischemia, which is not representative of the clinical condition, hindering therapeutic development.
• The goal of this study is to optimize the murine hindlimb ischemia model for preclinical evaluation of cell-based regenerative therapies.

Methods

• Animals: Male and female nude mice (NU/NU) at 6 or 14 weeks old
• Surgery: Double-knotted ligations on the right femoral artery to induce ischemia.
• Treatments: Intramuscular injection of PBS or Matrigel as cell delivery vehicle at the injured leg on day 1
• Imaging: Laser Doppler Imaging (LDI) to measure blood perfusion at day 0 (after surgery), 1, 4, 8, 15, 22, 29, and 36.
• Staining: Histological staining with H&E and Masson’s Trichrome; immunohistochemistry of vascular markers.

Results

• Hindlimb ischemia induced tissue necrosis and limb lost in the old but not the young mice

Age of the mice influences the surgery outcome

• Old mice exhibited significantly worse surgical outcomes, including increased limb loss due to necrosis (60% of the old mice vs 0% of the young mice), and significantly lower limb perfusion rate at various time points.
• A number of factors could have contributed to the age-related differences, including oxidative stress, senescence, inflammation, and peripheral nerve degeneration.
• An animal model that is more representative of the clinical condition is expected to improve the success rate when translating future preclinical results into clinical therapeutics.

Ongoing work

• Quantitative analysis of vascular density
• Qualitative assessment of fibrosis, inflammation, and muscle atrophy
• Analysis of sex-related differences in vascular regeneration
• Mechanistic investigation of age- and sex-related differences

Conclusions

• Femoral artery ligation in old mice induces chronic limb ischemia, tissue necrosis, and limb loss, consistent with the clinical condition of critical limb ischemia.
• This chronic PAD model with old mice could be used to evaluate cell-based regenerative therapeutics for parameters such as limb salvage and long-term vascular regeneration.

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