Microsurgical technique for locoregional delivery of extracellular vesicles in a forelimb ischemia-reperfusion injury model

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Introduction: Locoregional delivery via intra-arterial limb infusion (ILI) can present an efficient and clinically translatable approach for the targeted delivery of novel biologic therapeutics such as extracellular vesicles (EVs) to vascularized composite tissue allografts (VCA). Potential advantages of ILI include homogenous and wider distribution of the injectable substance into intact and other extremity soft tissues including skin, nerves, and vessels. Additionally, locoregional delivery can bypass the first pass effect and optimize homing to target tissues. Several studies have demonstrated the efficacy and safety of intra-arterial infusion of stem cells and recombinant adenoviral vectors for critical limb ischemia and Duchenne Muscular Dystrophy, respectively. Nevertheless, delivery of EVs using an intra-arterial method has not been reported and characterized before. In this proof-of-concept study, we describe a microsurgical approach to deliver off-the-shelf platelet-derived extracellular vesicles (pEVs) to the rat forelimb using selective brachial artery microcatheterization. We then characterized skeletal muscle EVs uptake in a tourniquet-induced forelimb ischemia-reperfusion injury (IRI) model. IRI is an inevitable consequence of upper extremity VCA due to the abundance of metabolically active skeletal muscle tissue that can potentially cause life-threatening renal failure and allograft functional impairment. Currently, the field of VCA lacks a standard donor preservation solution or paradigm effectively blunts IRI response following allograft revascularization. Strategies explored to ameliorate ischemia-reperfusion of VCA allografts include cold static storage and ex vivo machine perfusion. A recent study demonstrated that in situ pre-treatment of donor vascularized composite allografts with a complement inhibitor delivered using limb perfusion significantly reduced inflammatory infiltrates upon revascularization of the donor tissue. Thus, we also hypothesized that platelet-derived EVs can offer protection against reperfusion insult in our model following their intra-arterial limb infusion upon tourniquet release.

MATERIALS AND METHODS: Institutional IACUC approval has been granted for the in vivo experiments and procedures of this study. (1) Animals and Study Design: 13 male and female Sprague-Dawley (SD) rats were used in this pilot testing. Three rats and ten rats were tested for EVs tracking studies and biochemical analysis, respectively. (2) Selective brachial artery microcatheterization and platelet-derived EVs delivery: A silicone tourniquet was placed at the level of the shoulder joint and ischemia was induced by clamping for 15 min. No mortality was associated with this rat cohort that underwent this procedure. (3) Injection technique for therapeutic delivery: the arterial limb infusion upon tourniquet release.

RESULTS: No mortality was associated with rotational brachial artery clamping. Three clinical markers of muscle tissue damage: creatine kinase (CK), lactate dehydrogenase (LDH), and aspartate transaminase (AST) and two renal function markers: blood urea nitrogen (BUN) and creatinine (Cr) were quantified spectrophotometrically using an automated chemistry analyzer (IDEXX Bioanalytics). (4) Statistical analysis: results were presented as mean ± standard deviation.

DISCUSSION: Delivery of EVs to skeletal muscles for non-regenerative applications is challenging. Although intra-arterial administration of therapeutics is regarded as a systemic route of delivery, our technique demonstrated excellent retention of EVs by skeletal muscles up to 24 h post infusion. CK, LDH, and AST are present in the cytosol of skeletal muscles and are significantly elevated following muscle ischemia and the arteriosclerotic process. Therefore skeletal muscle is an ideal target for therapeutic efficacy of EVs delivery. Although the sample size is small, our preliminary results show diminution of these values compared to control upon administration in an IRI model. Future studies will compare outcomes and EVs uptake by skeletal muscles using other routes of delivery including intramuscular vs. intravenous injections, and timings of pEVs delivery (pre-ischemia and immediately upon limb reperfusion) to identify the therapeutic window for therapeutic efficacy of EVs delivery.

CONCLUSION: A reproducible microsurgical technique reported herein can be utilized to effectively deliver EVs in intricate rodent VCA studies using a clinically relevant paradigm. Platelet-derived EVs can be administered intrarterially to VCA grafts either in situ or ex vivo as a component of VCA preservation protocols.

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