EFFECTS OF L-SELECTIN BLOCKADE UPON SKELETAL MUSCLE FUNCTION, EDEMA FORMATION, AND LEUKOCYTE ACTIVATION ASSOCIATED WITH ISCHEMIA-REPERFUSION INJURY

Introduction: Pneumatic tourniquet application may cause significant tissue injury secondary to ischemia distal to the cuff and compression / ischemia beneath the cuff. Ischemia and reperfusion incites an inflammatory cascade, which includes neutrophil activation, migration, and exacerbation of local tissue injury. L-selectin is a leukocyte surface glycoprotein responsible for initiating neutrophil adhesion to activated endothelium and migration into injured tissue during reperfusion. Blockade of L-selectin, both with specific monoclonal antibodies and non-specific oligosaccharides may attenuate injured tissue during reperfusion. Blockade of L-selectin, both with specific monoclonal antibodies and non-specific oligosaccharides may attenuate ischemia-reperfusion injury. Yan and co-workers demonstrated some improvement in muscle function in the rat EDL, however this study tested muscle function only three hours after reperfusion. The objective of the present study is to quantify longer term effects of L-selectin blockade on tissue injury following ischemia - reperfusion.

Methods: Thirty-one NZW rabbits were separated into two groups: fourteen underwent thigh tourniquet application (leg ischemia model), and seventeen underwent leg tourniquet application (leg compression model). Rabbits were anesthetized with a combination of acepromazine, ketamine and xylazine (protocol approved by the UCSD Animal Use Committee). Prior to tourniquet inflation at 350 mm Hg, rabbits were randomized to receive either L-selectin antibody (MuDREG200 2 mg / kg, Protein Design Labs) or an equivalent volume of citrate buffer vehicle. All animals received antibody or placebo intravenously 4.5 hrs prior to cuff deflation. Tourniquets were inflated continuously for either 4 hrs (ischemia model) or 2 hrs (compression model).

SYSTEMIC BLOOD ANALYSIS: Venous blood draws were performed at seven specific time points before, during, and after cuff inflation. Blood analysis included complete blood cell counts with differential, absolute neutrophil counts, and quantitative assessment of neutrophil activation using the nitroblue tetrazolium assay (NBT).

FUNCTIONAL ANALYSIS: Tibialis anterior (TA) contractile function was evaluated 48 hours after ischemia / reperfusion. The hindlimb was stabilized by pins in the femur and tibia and the TA tendon was clamped to a force transducer. Peak tetanic tension was determined at optimal muscle length via peroneal nerve stimulation and direct muscle stimulation.

MUSCLE EDEMA FORMATION: The entire TA muscle was resected and stabilized by pins in the femur and tibia and the TA tendon was clamped to a force transducer. Peak tetanic tension was determined at optimal muscle length via peroneal nerve stimulation and direct muscle stimulation.

Results: Tetanic tension tended to be greater in the antibody group than in the placebo group after 4 hours of ischemia (p=0.07), but there was no effect of antibody administration upon TA function after two hours of direct compression (p=0.34, Fig. 1). Antibody induced marked systemic neutropenia compared to placebo, which peaked 4.5 hours after drug administration. Neutrophil activation (NBT assay) was significantly increased after four hours of tourniquet application, without a difference between antibody and control groups (Fig. 2). Muscle edema formation (reflected by lowest percent dry weight) was significantly greater in the compressed limb than in the control limb (p=0.05), without effect of antibody administration (Fig. 3).

Discussion: The present study utilizes a validated model of tourniquet compression and ischemia with a clinically-relevant assessment period. Previous studies focused upon very early evaluation after tissue ischemia. The present study suggests that there is probably a marginal beneficial effect of L-selectin blockade associated with tourniquet-induced ischemia / reperfusion injury. However, the magnitude of this effect appears to be relatively small and probably does not justify human clinical evaluation. Although L-selectin plays a role in leukocyte-mediated tissue injury, tourniquet compression and ischemia probably induces injury through other critical mechanisms.

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