Molecular Imaging of Angiogenic Therapy in Peripheral Vascular Disease with alpha$_{nu}$beta$_3$-Targeted Nanoparticles

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INTRODUCTION: In peripheral vascular disease, angiogenesis is required for revascularization following vascular occlusion. Traditional techniques, such as X-ray angiography, can reliably detect the proliferation of mature vessels following ischemic injury, but these vessels represent the last stages of revascularization. An imaging method sensitive to the early stages of angiogenesis would be invaluable for the clinical evaluation and care of peripheral vascular disease patients. Molecular imaging of angiogenesis with a targeted MRI contrast agent may offer a means for detecting angiogenesis both in native revascularization and in response to pro-angiogenic therapies, such as L-arginine treatment. This study investigates whether molecular imaging with $\alpha_v\beta_3$-integrin-targeted paramagnetic nanoparticles can detect the neovascular response to L-arginine earlier than traditional X-ray angiography.

METHODS: New Zealand White rabbits were fed a 0.25% cholesterol diet for 60 days, followed by surgical ligation of one femoral artery. After surgery, rabbits were switched to normal chow and treated with either 2.25% (wt/vol) L-arginine in drinking water (n = 10) or normal tap water (n = 8). L-arginine promotes angiogenesis by augmenting the endogenous production of nitric oxide. Ten days after ligation, 3D, T1-weighted, black blood, fat suppressed images (210 μm by 210 μm resolution, 800 μm slices, TR/TE = 37/3.6 ms, 65^o flip angle) were collected of both hindlimbs using a clinical 1.5 T scanner and a rectangular extremity surface coil. Images were collected before and two hours post intravenous injection of $\alpha_v\beta_3$-integrin-targeted paramagnetic nanoparticles (1 ml/kg) to assess angiogenesis in both hindlimbs. The $\alpha_v\beta_3$-integrin is a cellular adhesion molecule that is selectively expressed on angiogenic endothelium but not mature vasculature. MRI signal enhancement was determined by pixel-by-pixel image subtraction. The area of enhancement and the average signal increase (both in percent) were multiplied together and the ratio between the ischemic and control hindlimbs was calculated. To demonstrate targeting specificity, another group of L-arginine treated rabbits (n = 8) received non-targeted paramagnetic nanoparticles during the MRI exam. X-ray angiography was performed 40 days post-ligation in a separate cohort of L-arginine treated (n = 6) and untreated animals (n = 5). Angioscores were calculated for both hindlimbs.

RESULTS: After 60 days on the high cholesterol diet, all rabbits were hyperlipidemic with serum cholesterol ~33 times higher than normal. Ten days after ligation, L-arginine treatment had no effect on serum cholesterol (L-arginine: 1400 ± 150 mg/dl, control: 1450 ± 200 mg/dl, p = 0.85). Molecular imaging 10 days post-ligation showed L-arginine treated rabbits had significantly greater MRI signal enhancement in the ischemic hindlimb compared to the control limb. The ratio of MRI enhancement between the ischemic and control hindlimbs was significantly higher in L-arginine treated rabbits compared to either untreated rabbits or rabbits receiving non-targeted nanoparticles (Figure 1, * p < 0.05). X-ray angiography performed 40 days post-surgery confirmed successful and persistent ligation of the femoral artery. In L-arginine rabbits, a greater number of mature vessels were observed in the ischemic vs. control limbs (Figure 2, # p < 0.05). Rabbits receiving tap water showed no significant difference between the ischemic and control hindlimbs.

CONCLUSION: Targeted paramagnetic nanoparticles can be utilized to specifically detect the molecular signatures of angiogenesis in skeletal muscle. These targeted agents may allow evaluation of the response to pro-angiogenic therapies far earlier than traditional X-ray angiography techniques. These novel molecular imaging techniques could provide earlier and closer management of therapeutic interventions in peripheral vascular disease patients.

Molecular Imaging of Angiogenesis 10 Days Post-Ligation

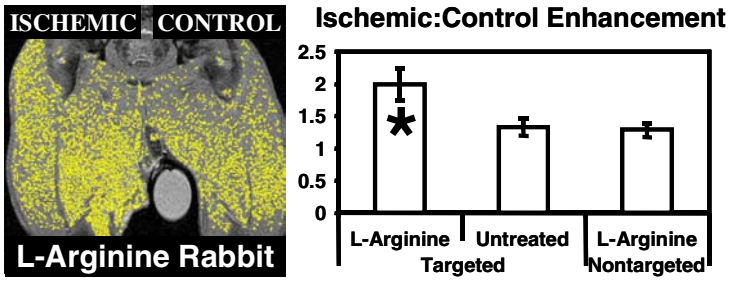

FIGURE 1: Ten days post-surgery, MRI molecular imaging (yellow overlay) shows increased angiogenesis in the ischemic hindlimb of rabbits treated with L-arginine vs. the control limb. Specificity of targeted imaging is demonstrated by lower enhancement with non-targeted nanoparticles.

X-Ray Angiography 40 Days Post-Ligation

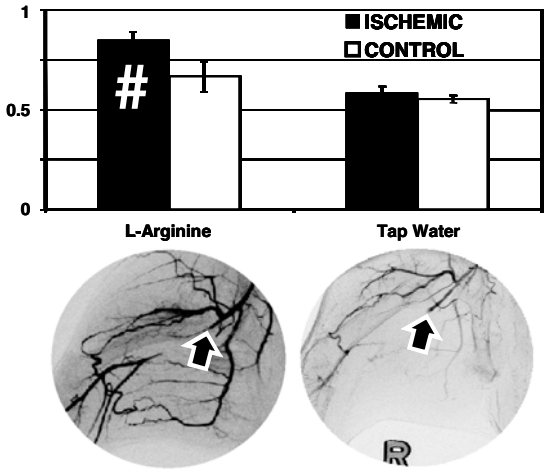

FIGURE 2: Forty days post-surgery, X-ray angiography shows higher vessel density in the ischemic hindlimb of rabbits treated with L-arginine vs. untreated rabbits and confirms ligation of the femoral artery (arrows).