$\alpha_v\beta_3$-Targeted Perfluorocarbon Nanoparticles Provide Molecular Imaging of Angiogenesis and Effective Targeted Drug Delivery

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INTRODUCTION

Molecular imaging is a new technique for the sensitive and specific detection of a wide variety of diseases. Targeted contrast agents may also serve as efficient delivery vehicles for therapeutic drugs and for monitoring drug deposition in vivo and treatment effects. Angiogenesis is integral to the development and progression of atherosclerotic disease. $\alpha_v\beta_3$-integrin is a selective molecular epitope expressed by angiogenic endothelium (1). The objective of this research is to demonstrate drug delivery and monitoring of therapeutic effect with T_1-weighted MRI after systemic injection of $\alpha_v\beta_3$-targeted paramagnetic nanoparticles.

METHODS

Nanoparticle Preparation: Paramagnetic perfluorocarbon nanoparticles were formulated by incorporating a paramagnetic lipophilic chelate into the outer lipid membrane (2). A therapeutic drug, 0.2 mol% fumagillin, was formulated into the nanoparticle membrane. $\alpha_v\beta_3$-targeted particles were coupled covalently to an $\alpha_v\beta_3$-integrin ligand. Dissociation studies showed very low release of fumagillin from the nanoparticle membrane (< 10% released over 4 days).

MRI Protocol: Male New Zealand White rabbits (n=8) were fed a 1% cholesterol diet for 80 days prior to baseline scanning. At baseline, half the animals were treated with drug laden $\alpha_v\beta_3$-targeted paramagnetic nanoparticles, while the other half received $\alpha_v\beta_3$-targeted nanoparticles without drug. Transverse black-blood MRI (TR/TE =380/11 ms) of the abdominal aorta from the diaphragm to the renal arteries was performed with a clinical 1.5 T magnet (NT Intera with Master Gradients, Philips Medical Systems, Best, Netherlands) using a quadrature birdcage neck coil (250 by 250 µm in-plane resolution and 5 mm slice thickness). Images were collected before and four hours post peripheral injection (ear vein) of 0.5 ml/kg body weight of nanoparticles. After treatment, all animals were switched to standard rabbit chow and imaged one week later with $\alpha_v\beta_3$-targeted paramagnetic nanoparticles to assess angiogenesis. Animals were then sacrificed and expression of $\alpha_v\beta_3$ in the aortic wall was corroborated by immunohistochemistry (LM609). MRI signal intensity in the aortic wall was measured before and after nanoparticle injection using a semi-automated image segmentation program. The percent enhancement in MRI signal was calculated at baseline and one week post treatment.

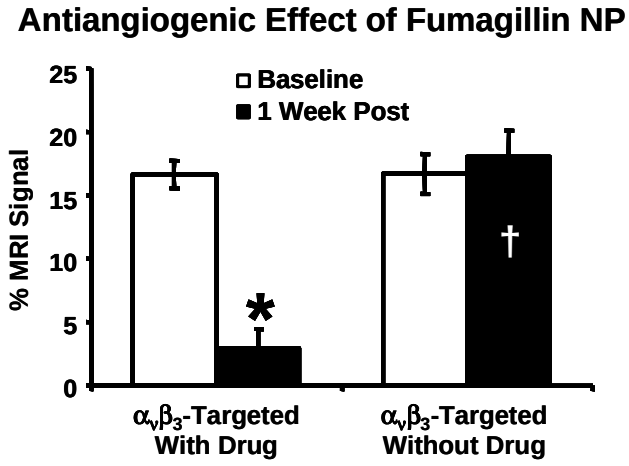

Figure 1: MRI signal enhancement of aortic wall at baseline (white bars) and one week after treatment (black bars) with drug laden $\alpha_v\beta_3$-targeted or $\alpha_v\beta_3$-targeted nanoparticles without drug. Identical levels of enhancement were measured at baseline with fumagillin and control nanoparticles. One week after treatment, $\alpha_v\beta_3$-targeted fumagillin nanoparticles significantly reduced angiogenesis compared to baseline levels (* $p < 0.05$) and compared to one week after treatment with control nanoparticles († $p < 0.05$).

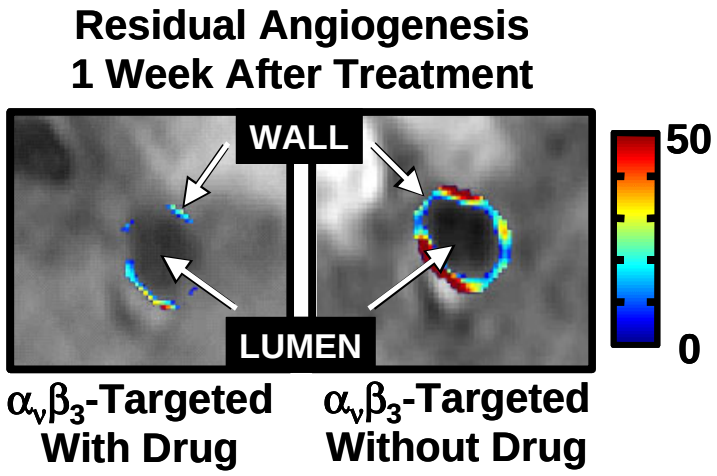

Figure 2: Assessment of residual angiogenesis with injection of $\alpha_v\beta_3$-targeted nanoparticles one week after treatment. MRI signal enhancement (false color overlay in percent) in animal treated with control nanoparticles (right) indicates active angiogenesis and no therapeutic effect. Lower enhancment is seen in animal treated with fumagillin nanoparticles (left) showing reduced angiogenesis and effective drug delivery.

RESULTS

T_1-weighted black-blood images collected before nanoparticle injection showed no gross evidence of plaque development in the aortic walls, i.e. no lumenal stenosis or wall thickening. At baseline, identical MRI enhancement was observed four hours after injection of $\alpha_v\beta_3$-targeted nanoparticles with or without fumagillin (Fig. 1). Patchy areas of the vessel wall enhanced throughout the abdominal aorta, with generally higher enhancement seen near the diaphragm. One week after treatment, residual angiogeneic activity in the aortic wall was assessed in each animal after a follow-up injection of $\alpha_v\beta_3$-targeted nanoparticles. MRI enhancement in rabbits treated with nanoparticles lacking fumagillin was unchanged from baseline, while the intensity and extent of enhancement was markedly reduced in animals treated with drug-bearing nanoparticles (Fig. 2). Enhancement one week after treatment was significantly lower in rabbits receiving fumagillin nanoparticles compared to control nanoparticles ($2.9 \pm 1.6\%$ vs. $18.1 \pm 2.1\%$, respectively, $p < 0.05$) (Fig. 1). Histology revealed less atherosclerotic plaque and lower endothelial $\alpha_v\beta_3$ expression in the aortic adventitia of rabbits treated with fumagillin nanoparticles compared with control nanoparticles.

CONCLUSIONS

Molecular imaging of angiogenesis associated with the early development of atherosclerosis was demonstrated non-invasively in cholesterol-fed rabbits using a clinical MRI scanner (1.5 T). The targeted contrast agent also served as an effective vehicle for drug delivery, allowing noninvasive confirmation of drug deposition, and provided a means to determine therapeutic effect at follow-up. Effective delivery of hydrophobic drugs, such as fumagillin, with targeted nanoparticles relies upon "contact facilitated drug delivery" (3). In solution, very little drug diffuses out of the nanoparticles prohibiting their use as delayed release agents. Instead, the drugs are only delivered when the particles are bound to cell surface. The close interactions between bound nanoparticles and the cell membrane allow lipid exchange, effectively conveying drug from the nanoparticle into the cell. These results suggest that MR molecular imaging may provide 1) a unique tool to detect the early onset and progression of atherosclerotic angiogensis, 2) an effective vehicle for targeted drug delivery and 3) a sensitive measure of atherosclerotic neovascular responsiveness to therapy.

REFERENCES

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