^{19}F MRS allows quantitative evaluation of anti-angiogenic therapy delivered with targeted perfluorocarbon nanoparticles

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Introduction Once diagnosed, aortic valve disease can progress rapidly to critical stenosis requiring surgical valve replacement. Recent data indicate some hope for medical therapy with cholesterol reducing agents if the disease is diagnosed at an early stage. In this work, we illustrate the use of molecularly targeted drug-bearing nanoparticles for both early diagnosis and therapy of experimental aortic stenosis (AS) by targeting the inflammatory angiogenic components of AS with the use of $\alpha_v\beta_3$-integrin binding perfluorocarbon nanoparticles. In this case MR spectroscopy of the unique ^{19}F signal from the nanoparticles is used to quantify the extent of angiogenesis, which expresses the $\alpha_v\beta_3$ integrin, and to measure the response to antiangiogenic drug therapy. We utilize a cholesterol-fed rabbit model of AS that exhibits gross thickening, macrophage infiltration, and angiogenesis with abundant $\alpha_v\beta_3$ endothelial biomarkers that can be quantified with ^{19}F MRS to report drug efficacy.

Methods Thirty-seven New Zealand White rabbits were maintained on a 0.25% cholesterol diet for five months, at which point they were randomized into five groups, each receiving four once-weekly treatments. The treatments were: 0.2 mole % fumagillin (delivered via targeted or nontargeted nanoparticles), 2 mole % doxorubicin (delivered via targeted or untargeted nanoparticles), and a saline control. One week after the final treatment, rabbits were injected intravenously with 2.2 mL/kg of $\alpha_v\beta_3$ integrin-targeted perfluorocarbon nanoparticles with a 15-crown-5 ether core for ^{19}F MRS. Nanoparticles were allowed to bind and circulate for two hours, at which time the rabbits were euthanized and the aortic valve leaflets excised from the aortic root and preserved in formalin. *Ex vivo* ^{19}F spectroscopy was performed on an 11.7T Varian Inova small animal scanner using a custom-built single-turn solenoid coil tuned to 470 MHz. A spin echo pulse sequence was used, with TR=2s, TE= 10ms, 512 signal averages, and a scan time of 20 minutes. Quantification of ^{19}F signal was achieved by inclusion of a reference standard containing a known quantity of nanoparticle emulsion formulated with a spectrally distinct perfluorocarbon, perfluoro-octyl-bromide (Fig. 1). A one-way analysis of variance was performed in SAS (v9.1; Cary, NC), with corrections for serum cholesterol and randomized block design as covariates. Post hoc testing and correction for multiple comparisons was performed on the calculated least squares means using the Hsu-Dunnett test for comparing treatments to a control.

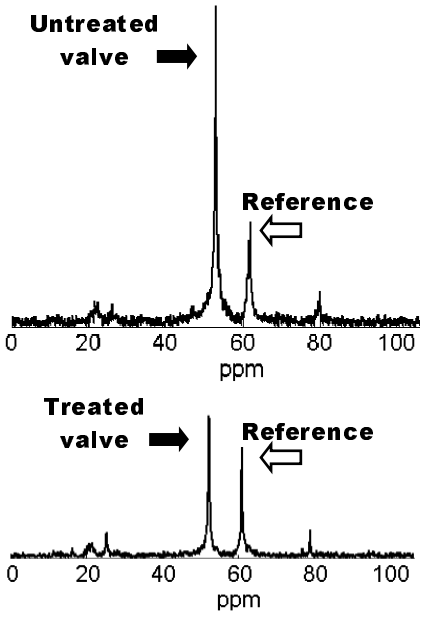

Figure 1: Representative fluorine MR spectra from an untreated valve (above) and a valve treated with $\alpha_v\beta_3$ targeted fumagillin bearing nanoparticles (below). Black arrow: nanoparticle signal; open arrow: reference standard

Results Angiogenesis was reduced by 50% as quantified by the mean ^{19}F signal, in the cohort of rabbits treated with $\alpha_v\beta_3$ integrin targeted nanoparticles containing fumagillin (Fig. 2) as compared to untreated rabbits and to rabbits treated with untargeted nanoparticles containing fumagillin (p=0.03). No significant effect was observed from treatment with doxorubicin, although a trend to reduced biomarker presence was observed. Serum cholesterol exerted a significant effect as a covariate on the amount of ^{19}F signal present, with linear regression yielding a correlation coefficient of R=0.57, significant with p=0.002.

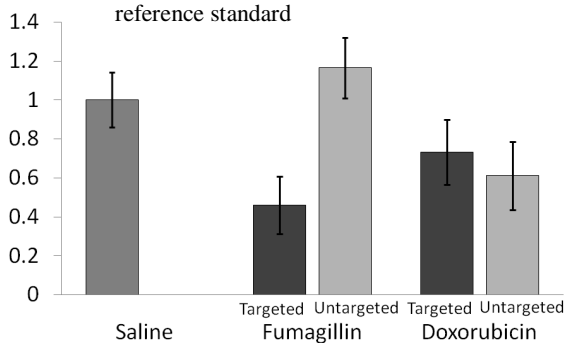

Figure 2: Average normalized ^{19}F signal, showing significant reduction in angiogenesis in animals treated with targeted fumagillin-bearing nanoparticles as compared to saline and untargeted, drug-bearing controls.

Discussion and Conclusions Angiogenesis is well correlated with inflammation in human aortic valve disease.[3] The association of serum cholesterol, a marker of disease burden, with the ^{19}F signal measured from nanoparticles bound to angiogenesis suggests that cholesterol may be a major driver for inflammation and angiogenesis in AS. Targeted drug therapy with nanoparticle carriers rapidly reduces the angiogenic burden, while minimizing toxic side effects due to the targeting approach and the drastic dose reduction enabled by this approach. The ability to image, treat, and follow with the same delivery system, using a unique MRI signature (^{19}F) of a relevant biomarker should permit the evaluation of both conventional (statins) and experimental (antiangiogenic, anti-inflammatory) agents in valve disease.

References

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