Supporting information for: 'Comparison of synthetic HDL contrast agents for MR imaging of atherosclerosis'

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Methods

Macrophage cholesterol efflux

J774A.1 murine macrophages (ATCC, Manassas, VA) were incubated overnight with 15 μg/ml cholesterol and 0.5 μCi/ml [^{3}H]cholesterol in 0.2% BSA-DMEM. After an equilibration period of 3 h with 1 μg/ml of F-1394 in 0.2% BSA-DMEM, efflux was initiated by the addition of 50 μg/ml of acceptor agent (by protein mass). 100 μl aliquots of the medium were collected after 4 h, and the [^{3}H]cholesterol measured by liquid scintillation counting (LSC). In order to measure the [^{3}H]cholesterol present in the cells, cell lipids were then extracted by incubating the cell monolayers overnight in isopropanol. After lipid extraction, the total [^{3}H]cholesterol present in lipid extract was measured by LSC and the efflux calculated as percentage of total. Such efflux experiments were carried out with 18A-Gd, 37pA-Gd, HDL and bovine serum albumin.

MRI scanning

Mice were scanned using the following protocol. The abdominal aorta was identified in a coronal section on a localizing sequence. T1-weighted MRI was performed using a black blood sequence. Twenty-two contiguous 500 μm-thick slices with a microscale in-plane resolution of 101 μm were acquired using a spin echo sequence with a 256 × 256 matrix size. The repetition time (TR) and echo time (TE) for the T1W images were 800 and 8.6 ms, respectively. An inflow saturation band of 3 mm was used with a slice gap of 3 mm for additional luminal flow suppression. Sixteen signal averages were used for a total imaging time of 55 min per scan. A saturation pulse was used to eliminate signal from fat tissue and better delineate boundary of the aortic wall and minimize chemical shift artifacts. After a preinjection, baseline MRI scan, mice were injected, via a tail-vein catheter with a 50 μmol Gd/kg dose of the synthetic HDL contrast agents. The mice were scanned again at 24 hours post-injection. n=7 apoE-KO mice were injected with each agent. Use of the medical image analysis software package eFilm allowed the MR signal intensity of various tissues from the different time points to be ascertained and the percent change in normalized enhancement ratio (% NER) post-injection to be calculated. Normalization of the MR signal intensity in the aorta wall was performed by rebasing with the signal from muscle adjacent to the aorta.

Additional figures

Figure S1 Fluorescence of macrophages incubated with Cy5.5 labeled 18A-Gd for varying times ranging from 1 to 24 hours. Error bars are +/- one standard deviation.

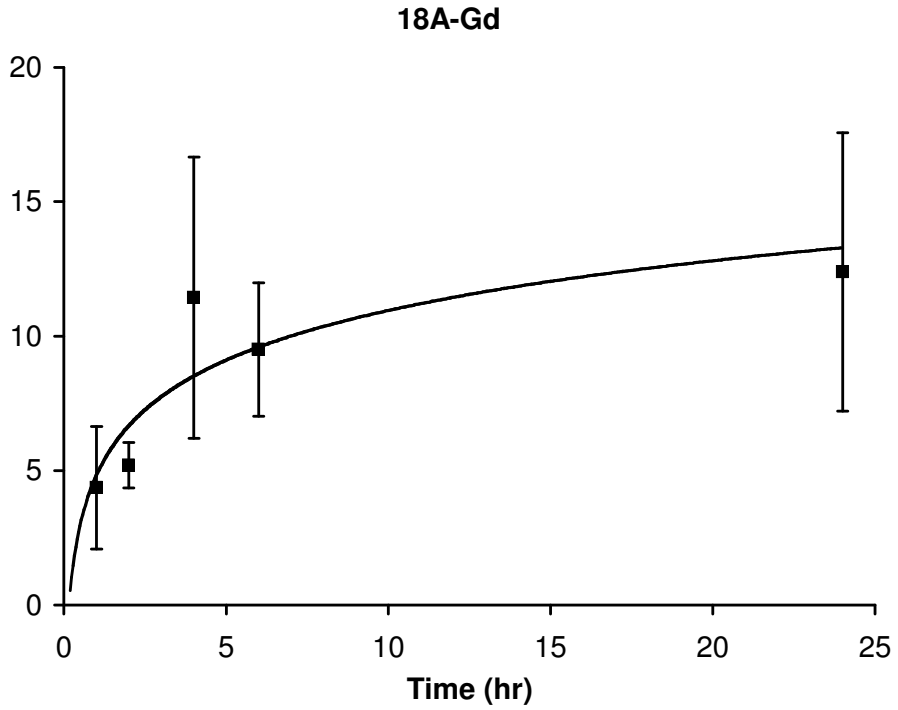


Figure S2 Fluorescence of macrophages incubated with Cy5.5 labeled 37pA-Gd for varying times ranging from 1 to 24 hours. Error bars are +/- one standard deviation.

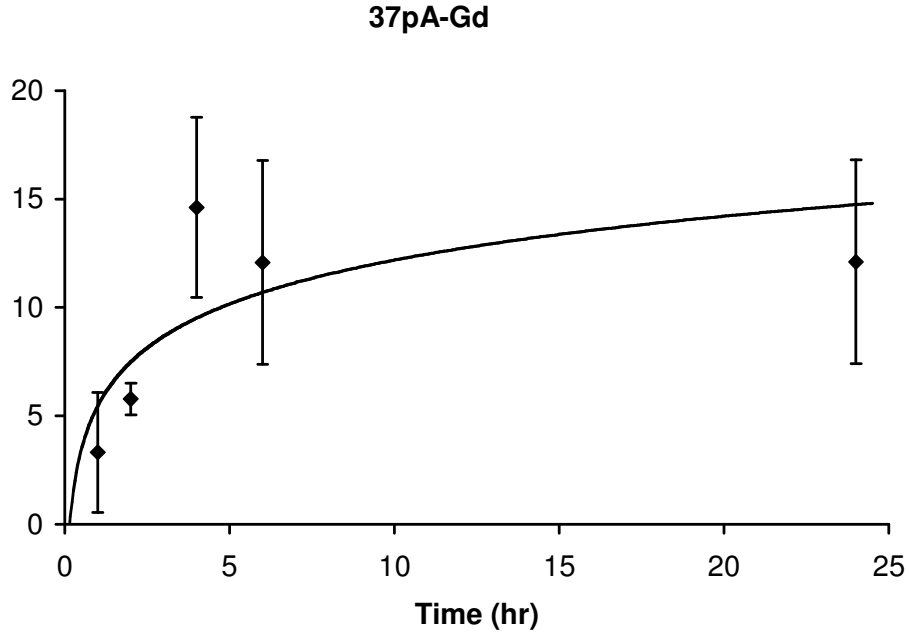


Figure S3 Uptake of cy5.5-labeled Gd-micelles in J774A.1 macrophages as determined by fluorescence imaging of cells. The data are displayed using the same scale as in Figure 2 D and E in order to allow comparison.

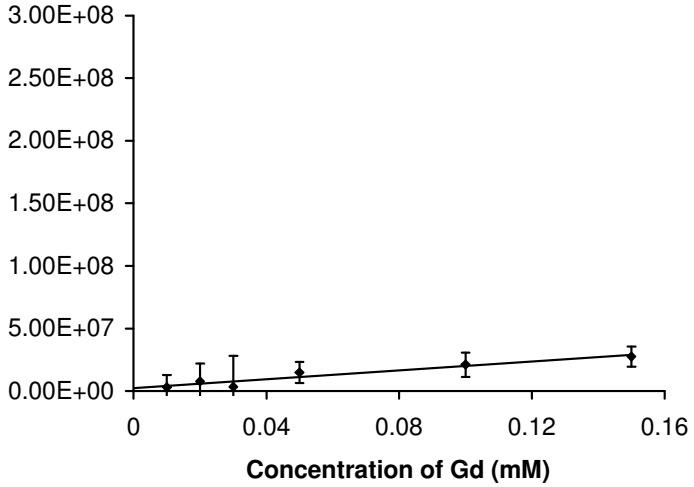
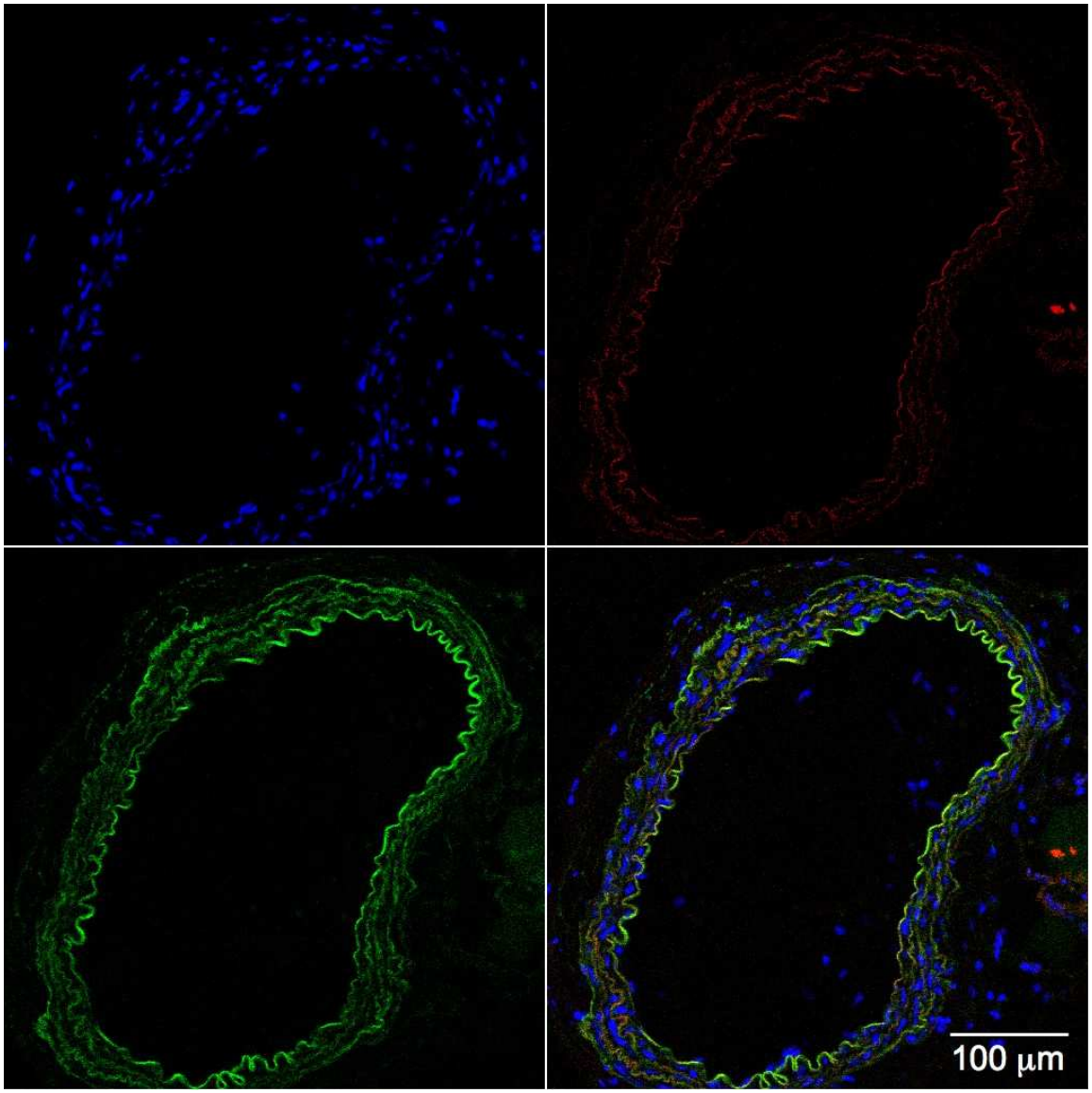


Figure S4 Confocal microscopy images of aortic tissue excised from an apoE-KO mouse injected with the 18A-Gd agent 24 hours prior to excision. A – DAPI staining for nuclei (blue), B – the rhodamine channel (red) indicating areas of 18A-Gd uptake, C – Alexa 647:CD68 staining for macrophages (green) and D – a merger of the previous 3 images. In this section there are no macrophages and no 18A-Gd uptake either.



References

1. Davidson WS, Lund-Katz S, Johnson WJ, Anantharamaiah GM, Palgunachari MN, Segrest JP, Rothblat GH, Phillips MC. The influence of apolipoprotein structure on the efflux of cellular free cholesterol to high density lipoprotein. *J. Biol. Chem.* 1994;269:22975-22982.
2. Marque V, Kieffer P, Gayraud B, Lartaud-Idjouadiene I, Ramirez F, Atkinson J. Aortic wall mechanics and composition in a transgenic mouse model of Marfan syndrome. *Arterioscler. Thromb. Vasc. Biol.* 2001;21:1184-1189.