Gd-based protein cage nanoparticles for vascular wall MRI at 3T

H. Kosuge[1], T. Kitagawa[1], M. Uchida[2], L. Liepold[2], D. Morris[3], P. E. Prevelige Jr.[3], T. Douglas[2], and M. V. McConnell[1]

[1]Cardiovascular Medicine, Stanford University, Stanford, CA, United States, [2]Chemistry and Biochemistry, Montana State University, Bozeman, MT, United States, [3]Microbiology, The University of Alabama at Birmingham, Birmingham, AL, United States

Introduction: Protein cage nanoparticles (PCNs) have shown promise for molecular/cellular imaging of cardiovascular disease and cancer. PCNs incorporating iron oxide have been used as susceptibility agents for MRI, but T1-shortening agents (e.g., Gd-DTPA) are preferred clinically. The PCN structure can incorporate targeting peptides and can also enhance the T1 effect of Gd by slowing rotational correlation time and by providing multiple Gd binding domains. Thus, Gd-based PCNs may be a potent T1-shortening MRI contrast agents at low Gd dosing.

Purpose: To evaluate Gd-containing PCNs for MRI of vascular disease.

Methods: *1) Gd-based PCNs* – Two different sized PCNs were studied - heat shock protein (Hsp,12nm in diameter) and bacteriophage P22 (60nm in diameter). A branched polymer network was synthesized within both PCNs incorporating multiple Gd-DTPA molecules. At 3T, r1 was 9.7 $mM^{-1}s^{-1}$ for Hsp-Gd and 7.6 $mM^{-1}s^{-1}$ for P22-Gd, approximately 2-3x higher than Gd-DTPA. *2) In vitro MR imaging with Hsp-Gd* – Mouse macrophage cells (RAW264.7) were incubated with Hsp or Hsp-Gd at a Gd concentration of 0, 0.025mM, 0.05mM, or 0.1mM for 24 hours. Hsp concentration was adjusted to the same protein concentration as 0.05mM Hsp-Gd that was 10.4 mg protein/ml. Two hundred million cells were scanned by a whole-body 3T MRI scanner (Signa HDx, GE Healthcare) with a 50mT/m, 150T/m/s gradient system using a fast spin echo sequence (TR=400ms, TE=14.15ms, Matrix=256x256, slice=2mm, FOV=12cm, NEX=2).

3) *Animal models* - Macrophage-rich carotid lesions: FVB mice were fed high-fat diet for one month and made diabetic by streptozotocin injection, followed by ligation of the left carotid artery. Aortic atherosclerosis: ApoE-deficient mice were fed high-fat diet for 9 weeks.

4) *In vivo MRI of Hsp-Gd and P22*-Gd – Animals (n=8) were scanned on a whole-body 3T MRI scanner with a phased array mouse coil (RAPID MR International) before and up to 24 hours after injection. The carotids (FVB) or aorta (ApoE-/-) were imaged with a fast spin echo sequence (TR=400ms, TE=15ms, Matrix=256x256, slice=1mm, FOV=3cm, NEX=6). FVB mice were intravenously injected with Hsp-Gd (n=4) or Gd-DTPA (n=2) two weeks post-ligation at a dose of 20 μmol Gd/kg (one-fifth of typical clinical dose). ApoE-deficient mice (n=2) were injected with P22-Gd at a dose of 20 or 50 μmol Gd/kg.

Results: In vitro MRI showed that macrophages incubated with Hsp-Gd had increasing signal with increasing Hsp-Gd concentration (Figure 1). In vivo MRI demonstrated that Hsp-Gd induced strong signal enhancement of the left carotid arterial wall, but no enhancement of the non-ligated right carotid (Figure 2A). In contrast, Gd-DTPA showed minimal enhancement of the carotid wall (Figure 2B). At 4 hours after injection, CNR of Hsp-Gd was about 50% higher than Gd-DTPA. Similarly, P22-Gd showed signal enhancement of the aortic arch wall in ApoE-/- mice (Figure 3).

Conclusions: Gd-based PCNs showed strong r1 values in vitro plus signal enhancement in vivo of carotid and aortic vascular disease, with low Gd dosing. PCNs allow low-dose, Gd-based vascular wall imaging, which is advantageous for clinical translation.

Reference:

1. Liepold LO, et al. Nano Lett. 2009; 9, 4520-4526

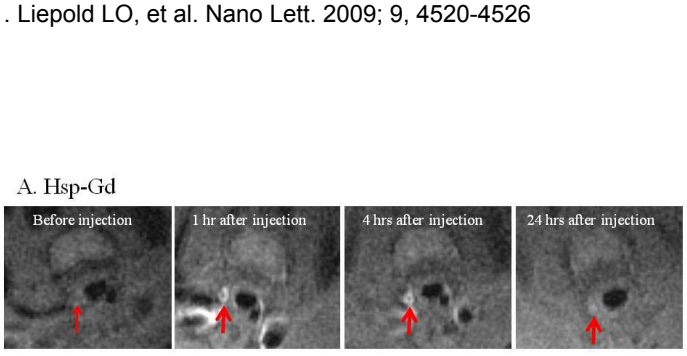

Figure 2: Enhancement of ligated carotid (arrows) after Hsp-Gd

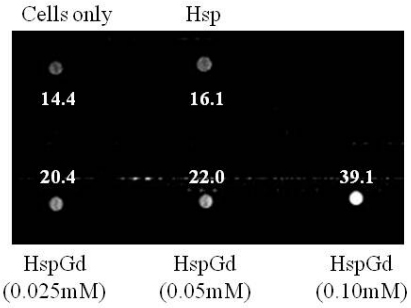

Figure 1: Macrophages incubated with and without Hsp-Gd

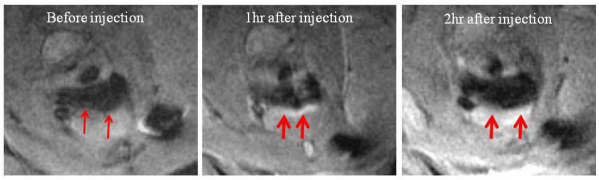

Figure 3: Enhancement of aortic arch wall (arrows) after P22-Gd