A novel contrast agent detects tropoelastin fibers in atherosclerotic plaques

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Introduction: Studies of human vessels showed increased deposition of tropoelastin fibres, the precursor of cross-linked mature elastin, in atherosclerotic vessels, and particularly in ruptured plaques, as well as aortic aneurysm [1, 2]. In addition, we found increased levels of tropoelastin (41% vs. 18%, P<0.001) in disrupted compared with stable plaques **(Figure 1)** in a rabbit model of pharmacologically induced plaque disruption. Importantly, only negligible amounts of tropoelastin were present in adjacent normal vessel wall segments. In the current work, we sought to develop a novel gadolinium-based MRI contrast agent that would selectively bind to tropoelastin and in this way differentiate the de novo synthesized fibers that are present in the vascular wall only under pathological conditions from the mature cross-linked elastin that is normally present in the vessel wall.

Materials and Methods: Animal model: Starting at 8 weeks of age, male apoE $^{-/-}$ mice were fed a high-fat diet that contained 21% fat from lard and 0.15% (wt/wt) cholesterol for 24 weeks. Peptide synthesis: The peptides *VVGSPSAQDEASPLS* and *YPDHVQYTHY* were chosen based on protein interaction and structural studies that showed their ability to selectively bind to tropoelastin fibers [3, 4]. The peptides were synthesized by Peptide Protein Research Ltd (Hampshire, UK). A DOTA-lysine amino acid was added at the amino-terminus of each peptide to facilitate the chelation of a gadolinium atom. *In vivo* MRI of the brachiocephalic and carotid arteries was performed using a 3T Philips Achieva scanner. Images were acquired before and 20 minutes after intravenous administration of 0.2 mmol/kg tropoelastin-binding peptides. Mice were placed prone on a single loop microscopy surface coil (diameter=23mm). Following a 3D GRE scout scan, time-of-flight (TOF) images were acquired for visualization of the aortic arch, the brachiocephalic and carotid arteries with a FOV=20x20x10mm, matrix=160, in-plane resolution=0.3x0.3mm (reconstructed 0.13x0.13mm), slice thickness=0.5mm, TR/TE=37/7.7 ms and flip angle=60°. The maximum intensity projection images were used to plan the subsequent delayed enhancement (DE) and T1 mapping scans. The inversion-recovery-3D-fast-gradient echo sequence was acquired 30 minutes post injection and was used for DE-MRI and visualization of contrast uptake. Imaging parameters were: FOV=30x8x30mm, matrix=300, in-plane resolution=0.1x0.1, measured slice thickness=0.25mm, slices=32, TR/TE=27/8ms, TR between subsequent IR pulses=1000ms, and flip angle=30°. T1 mapping was performed using a sequence that employs two non-selective inversion pulses with inversion times ranging from 20ms to 2000ms, followed by eight segmented readouts for eight individual images. The two imaging trains result in a set of 16 images per slice with increasing inversion times. For T1 mapping the acquisition parameters were: FOV = 22x8x36, matrix = 180x171, in plane resolution= 0.2x0.2, measured slice thickness=0.5mm, slices=16, TR/TE = 9.2/4.7 ms, flip angle = 10°. T1 values were computed on a pixel-by-pixel basis using an in-house Matlab algorithm. *Biodistribution studies: The* gadolinium concentration of different tissues was quantified using inductively coupled plasma mass spectrometry (ICP-MS).

Results and Discussion: Delayed enhancement images of the diseased brachiocephalic (BCA) and non-diseased carotid artery of apoE$^{-/-}$ mice acquired with the Lys-(Gd-DOTA)YPDHVQYTHY tropoelastin-binding peptide are illustrated in **Figure 2**. The inversion recovery (IR) and fused IR and MRA images show enhancement of the brachiocephalic artery **(Fig. 2A-2B)**, which is the region where apoE$^{-/-}$ mice form atherosclerotic plaques. Conversely, there was absence of enhancement of the carotid arteries **(Fig. 2C-2D)** that are devoid of plaques and thus tropoelastin fibers despite the presence of naturally occurring mature elastin fibers. Biodistribution experiments **(Fig. 2E)** showed that the peptide was excreted through the kidneys by1 hr and had a minimum uptake in other tissues while maintaining sufficient binding in the diseased brachiocephalic artery. Presence of tropoelastin in disease mice arteries was validated by immunohistochemistry **(Fig. 3)**. The relaxation rates and the relaxivity measurements for the unbound and bound forms are tabulated in **Table1**.

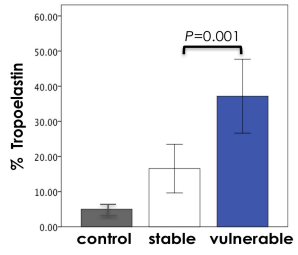

Figure 1: Expression of tropoelastin in rabbit plaques quantitated by immnohistochemistry.

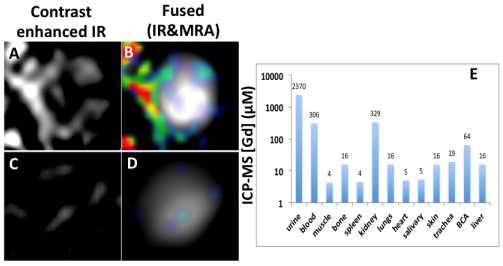

Figure 2: In vivo MRI and biodistribution of a tropoelastin-binding peptide in apoE$^{-/-}$ mice. BCA: brachiocephalic artery.

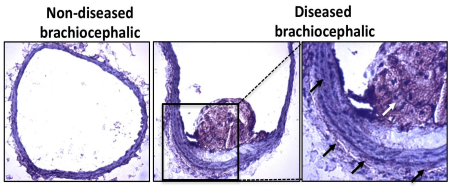

Figure 3: Detection of tropoelastin fibers (brown coloration) in disease and absence in control apoE$^{-/-}$ mice arteries immunohistochemistry.

Table 1	R1(s^{-1})	r1($mM^{-1}s^{-1}$)
Unbound (1mM)	5.0	7.7
Bound (Brachiocephallic)	2.17	20.8

Conclusions: We demonstrate the development of a new gadolinium based contrast agent that binds to tropoelastin fibers in atherosclerotic vessel walls. Both peptides showed promising pharmacokinetics, specificity and sensitivity for *in vivo* MRI imaging of disease brachiocephalic arteries in apoE$^{-/-}$ mice. Further development of such contrast agent may allow for molecular imaging of impaired elastogenesis that accompany atherosclerotic plaque development and plaque vulnerability.

References: **1.**Krettek, A., et al. ATVB 2003. **23**(4): p. 582-7. **2.** Akima, T., et al. Circulation journal : official journal of the Japanese Circulation Society, 2009. **73**(11): p. 2154-62. **3.** Moroy, G., et al. Proteins, 2009. **76**(2): p. 461-76. **4.** Finnis, M.L. and M.A. Gibson. J Biol Chem, 1997. **272**(36): p. 22817-23.