Visualizing the uptake of Combidex® (ferumoxtran-10) in a rat model of AAA using positive and negative contrast methods

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Introduction

The fact that 98% of abdominal aortic aneurysms (AAA) occur in the infrarenal aorta has led to the hypothesis that local factors contribute significantly to AAA pathogenesis. There is clinical and experimental evidence that inflammation, known to be involved in the etiology and progression of other cardiovascular diseases, may play an important role in the evolution of AAA disease as well [1, 2]. Although current data is compelling, it is limited by the fact that it is acquired invasively, is from a single timepoint, or provides global rather than spatially resolved information. The tomographic and non-invasive capabilities of MRI provide a way to overcome these limitations.

Visualizing inflammation *in vivo* using MRI is possible by intravenously administering certain ultra-small superparamagnetic iron oxide particles (USPIOs) which are phagocytized by macrophages and localize at sites of inflammation. The most common application of USPIOs, in regards to inflammation, has been to visualize atherosclerotic plaque. Typically, a gradient echo (GE) sequence is utilized to elicit signal loss in regions where USPIOs are present. Off-resonance (OR) techniques have recently been developed to exploit field inhomogeneity induced near the iron particles [3], the result being images that have little background signal and positive contrast near sites of USPIO localization.

The current work sought to assess the potential of using USPIOs to longitudinally visualize vessel wall inflammation in a rat model of AAA using MRI. Three concentrations of ferumoxtran-10, a USPIO in clinical trial, were tested. GE and OR sequences were applied *in vivo* over a 28 day period and to *ex vivo* samples.

Materials and Methods

All experiments were performed with local IACUC approval. Male 8-12 week old Sprague Dawley rats were utilized (n=3). The Anidjar/Dobrin model of AAA was used [2]. Briefly, animals were anesthetized and a segment of the infrarenal aorta was isolated while 10U/mL of porcine pancreatic elastase was infused for 1 hour. Three concentrations (250, 500, or 1000µmol/kg) of Combidex®(ferumoxtran-10) (Advanced Magnetics, Cambridge, MA), selected based on a pilot study using histology to visualize dose-dependent uptake of the USPIO into the vessel wall using the same animal model, were injected via a tail vein four days after surgery. Animals were imaged previous to ferumoxtran-10 injection and on days 0 thru 5, 7, 14, 21, and 28 after injection.

MRI was performed at 4.7T (Inova console, Varian, Inc., Palo Alto, CA) using a 6cm inner diameter RF volume coil. Animals were anesthetized using 2% isoflurane in 1L/min of O2. Body temperature was maintained between 36-37C. Spin echo, off-resonance projection images were acquired in the coronal plane (TR/TE = 200/15ms, FOV = $(8cm)^2$, 128^2, NEX = 16, -1600Hz shift) with spectrally selective RF pulses to achieve suppression of background signal from on-resonance water [3]. Standard 2D (TR/TE = 600/5ms, FOV = $(8cm)^2$, 128^2, NEX = 2, respiratory gated) and 3D (TR/TE = 15/3ms, FOV = $(8cm)^3$, 128^3, NEX = 2, visualized as maximum intensity projections (MIPs)) gradient echo data sets were also acquired.

Results

Images are displayed using standard radiological conventions. All three doses of ferumoxtran-10 were well tolerated. The presence of iron in the vessel wall at all three doses was confirmed by histology.

Signal in OR images was not apparent in a control animal; positive contrast was seen by day 1 and remained detectable through day 28in all three animals that received ferumoxtran-10 (Figure 1). The majority of signal was derived from spleen and two cohorts of lymph nodes (LNs), peritoneal and peripheral (Figure 1, #1, #2, #3, respectively). There was good correspondence between signal enhancement in OR images and signal loss in GE images as demonstrated by the red brackets positioned near positive and negative contrast derived from the same group of peripheral LNs using the OR and GE methods, respectively (Figure 1). Signal from LNs was confirmed by OR images acquired from *ex vivo* samples of the aorta with LNs present and then dissected away; no signal from the vessel wall was apparent (Figure 2).

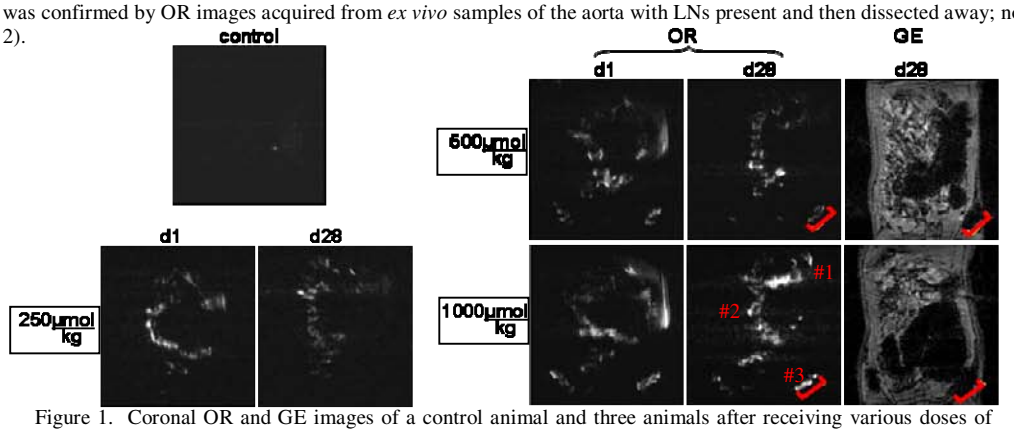

Figure 1. Coronal OR and GE images of a control animal and three animals after receiving various doses of ferumoxtran-10. Signal enhancement on OR images corresponded to signal loss on GE images (red brackets), was detectable at day 28 post injection, and was predominantly from spleen (#1), peritoneal (#2), and peripheral (#3) lymph nodes.

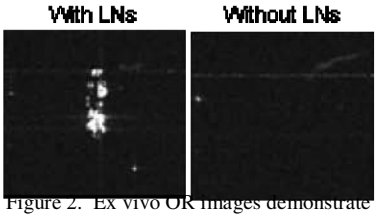

Figure 2. Ex vivo OR images demonstrate that signal is derived from ferumoxtran-10 in LNs.

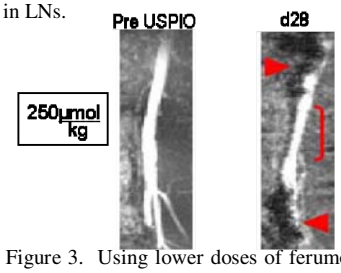

Figure 3. Using lower doses of ferumoxtran-10, sagittal MIPs from 3D GE acquisitions may provide a way to visualize inflammation of the vessel wall (red bracket) with less interference from LN's (red arrows).

Qualitative assessment demonstrated that time to peak signal in the two groups of LNs varied and was dose dependent. Similar, but less dramatic, to what has been demonstrated in experimental models of atherosclerosis [4], 3D GE images provide a pock-marked appearance to the vessel wall at the lowest dose (Figure 3) but were confounded at the higher doses due to USPIO accumulation in LNs.

Discussion

This is one of the first studies to use OR methods in a manner beyond proof-of-concept and demonstrated that the technique is reproducible and provides high-throughput (7min acquisition time). It is significant that the signal from the OR technique is specific to inhomogeneities associated with the USPIO and not other artifacts encountered in the abdominal cavity. This work provides important data: 1) *In vivo* visualization of vessel wall inflammation using MRI in this animal model may be limited by sensitivity in the case of OR methods and confounded by unwanted artifacts in the case of GE methods; 2) few if any investigations have used MRI to monitor the kinetics of IV ferumoxtran-10 in the lymph system of a small animal model for any longer than 24 hours; 3) the prolonged residence time has important consequences for visualizing inflammation in the abdominal cavity rather than in extremities [5] or more isolated organs [6], where there are fewer lymph organs present, and will require careful selection of concentration of the USPIO.

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