Fluorine-19 magnetic resonance imaging in a mouse model of atherosclerosis

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Introduction

The non-invasive characterization of atherosclerotic plaque is of great clinical interest since quantitative anatomical changes of both the coronary lumen and vessel wall measured with invasive methods (x-ray coronary angiography and intravascular ultrasound (IVUS)) have recently been established as insufficient predictors of sites that are prone to plaque rupture [1]. Several advanced molecular imaging methodologies have been developed over recent years to better characterize atherosclerosis non-invasively [2,3], but some of these promising techniques suffer from the disadvantage that the specific contrast agents are not (yet) approved for human use. One of the strategies that have successfully been used to visualize inflammation, which has been linked to plaque vulnerability [4], consists of the intravenous injection of a contrast agent that is partially absorbed by monocytes that infiltrate atherosclerotic plaques. This strategy can also be pursued using an intravenous administration of an emulsion of perfluorocarbons, which have already successfully been used to label other inflammation sites [5,6]. Perfluorocarbons are inert, non-toxic carbohydrates where the hydrogen has been replaced with fluorine, and of which several types are in phase 3 FDA trials [7]. Fluorine (^{19}F) MRI has 85% of the sensitivity of ^{1}H MRI but affords the additional advantage of not having a natural abundance in the body. Therefore, any detected ^{19}F signal is attributable to exogenous sources. The goal of this study was therefore to quantify the ^{19}F MR signal as a function of time after injection in atherosclerotic mice and in regions where atherosclerosis commonly occurs.

Materials and Methods

The animal studies were approved by the local animal ethics committee. Six apolipoprotein-E-knockout (ApoE) mice received a high-fat diet for 12 weeks to exacerbate atherosclerosis development. Every 3 days during the weeks 10-12, four animals received 100-µl tail-vein injections of a 10% perfluoro-15-crown-5-ether (PCE) emulsion, which was prepared as previously described [5]. After the 12 weeks, the animals received a large PCE emulsion injection of 750 µl. MR imaging of these mice was performed 2, 4, 6, 8 and 14 days after this last injection. All MR experiments were performed on a Varian 9.4 T horizontal bore animal spectrometer in combination with a custom-built 18 mm diameter quadrature surface coil that is tunable to both ^{1}H and ^{19}F. Animals were anesthetized with isoflurane (which has a ^{19}F signal with a ~3000Hz frequency shift), positioned prone on top of the coil in a dedicated holder. Next, ECG-triggered ^{1}H gradient echo (GRE) images (128x128 matrix, field of view 30x30x1.5 mm^3, 6 slices, 4 averages) were acquired at the level of the heart. After tuning the coil to ^{19}F, this was followed by non-triggered multi-slice ^{19}F turbo spin echo (TSE) imaging (64x64 matrix, TR/TE=1250/4.2ms, echo train length 16, 360 averages, acquisition time 5 min/slice) at the same anatomical level. The 2 animals that did not receive the PCE emulsion injections were subjected to the same imaging protocol to study the concomitant enhancement of isoflurane. All ^{19}F images were interpolated and postprocessed with a Gaussian filter, after which they were thresholded and overlaid on the ^{1}H images. Small patches of ^{19}F signal that coregistered anatomically the aortic valves or large arteries were analyzed. Due to the small size of these regions of enhancement, the signal-to-noise ratio (SNR) was defined in the unprocessed ^{19}F images as the maximum pixel intensity divided by the standard deviation of the signal of a large noise region outside the mouse. This analysis was repeated for all animals and all time-points. Unpaired two-tailed Student's t-tests were used to assess if there was a significant change in ^{19}F SNR over time. After MRI, the animals were sacrificed and Movat's pentachrome staining of the aortic valve was performed for gold-standard comparison. On histology blue refers to ground substance, blue-black to nuclei and elastic fibers, red to muscle, intense red to fibrin and green-yellow to collagen and reticulin fibers.

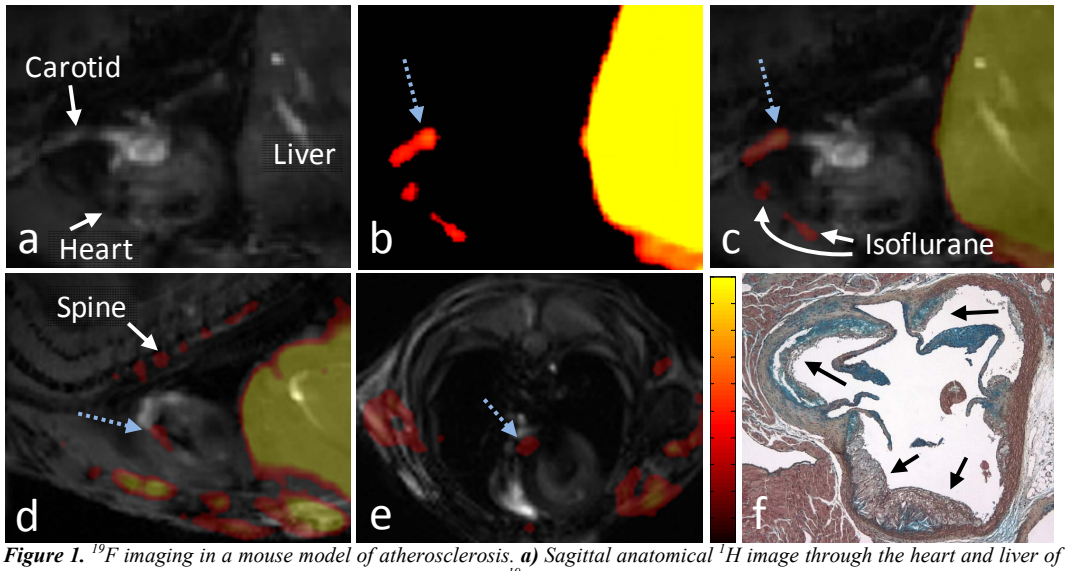

Figure 1. *^{19}F imaging in a mouse model of atherosclerosis.* ***a)*** *Sagittal anatomical ^{1}H image through the heart and liver of a mouse; a carotid artery is also visible.* ***b)*** *Unprocessed ^{19}F image at the same location at day 6 with a dominant signal from the liver and small signal from the carotid (dotted arrow).* ***c)*** *Processed fusion image of a and b, showing a clear colocalization of the ^{19}F enhancement and the carotid artery.* ***d)*** *Sagittal fusion image at day 6 in another animal, showing a significant but weak signal at the level of the aortic valve.* ***e)*** *Fusion image at day 8, showing a clear signal in the heart at the location of the aortic valve as well as shifted signals from the subcutaneous fat in the anterior chest.* ***f)*** *Movat's pentachrome staining of the aortic valve, showing significant plaque formation in different stages (arrows).*

Results and Discussion

^{19}F signal enhancement was observed in regions where atherosclerotic plaques commonly occur such as the aortic valves, aortic arch and carotids (Fig. 1a-e). Histology of the aortic valves further confirmed the presence of intermediate and advanced plaque at the locations of the ^{19}F signal, consistent with the disease model (Fig. 1f). Significant PCE signal was furthermore observed in several repositories of immune cells, such as the liver (Kupffer cells), lymph nodes and spinal cord. Signal was also observed in the subcutaneous fat, but the images of the 2 mice that did not receive PCE injections (but in whom histology showed plaque) confirmed that this was isoflurane, which accumulates exclusively in subcataneous fat. The quantitative analysis of the SNR of regions where plaque was confirmed through histology showed that a maximum of ~9 is found at 6 days after the large injection, after which it did not significantly change for 8 more days (Fig. 2). We therefore conclude that non-invasive ^{19}F MR imaging of atherosclerotic plaque may be feasible while a direct pathway for translation to the human setting exists.

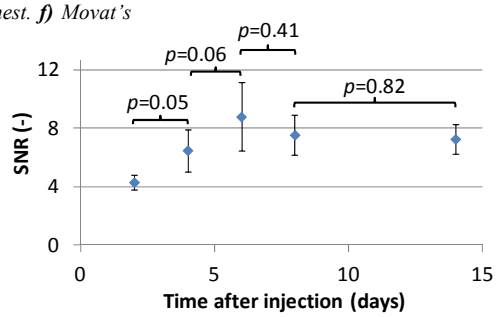

Figure 2. *Time course of the ^{19}F SNR in enhanced regions where plaque was confirmed through histology. After an initial SNR increase, there is no significant change after day 6, which suggests that this may be the optimal day of observation.*

References

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