Anti-angiotensin drug evaluation in ApoE-/- mice by USPIO-enhanced MRI at 7T

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INTRODUCTION

Studies in animal models and humans demonstrated that pharmacological blockade of the renin-angiotensin system has direct or indirect beneficial effects on atherosclerosis (1, 2). Irbesartan (SR 47436) is one of an orally active nonpeptide Angiotensin II type 1 (AT1) receptor antagonist (3). We report here the investigation of Irbesartan's effect on the development of atherosclerosis non-invasively in the apolipoprotein (apo) E-deficient mice by an MR follow up study.

MATERIALS AND METHODS

22 ApoE$^{-/-}$ female mice (C57BL/6 background, Charles-River, France) were divided in two groups (N=11 and N=11) and started on a high-fat diet (Western Diet, 0.5% cholic acid and 1.25% cholesterol) at 6 weeks of age. Irbesartan was added to the diet of one group of animals. Seven animals from each group (Treated group – TG and Not Treated group - NTG) underwent a baseline MRI session followed by the administration of an USPIO agent and by a second imaging session, 48h post contrast at 10, 24 and 38 weeks of age.

The P904, a USPIO contrast agent provided by Guerbet (Paris, France), was used (4). A dose of 250 µmolFe/kg was administered at the first imaging point and 1000 µmolFe/kg at the second and third imaging points. At each imaging time point two extra animals from each group as well as all animals at the end of the MRI follow-up were sacrificed and aorta samples were taken for histological analysis. MR imaging was performed on a 7 T Bruker small animal MRI scanner (Bruker Biospin GmbH, Rheinstetten, Germany). One slice perpendicular to the ascending aorta was imaged with cardio-respiratory gated acquisitions, a spin echo sequence for morphological information and a multi-echo gradient echo (GRE) sequence for USPIO accumulation assessment in the vessel wall. 12 echo images were acquired (TR ~ 742 ms, shortest TE = 2.1 ms) and T2* maps were then calculated in Matlab (The Mathworks, Nattick, MA) using a pixel by pixel mono-exponential fit. The goodness of the fit was also calculated and the pixels that gave a regression r < 0.7 were considered as having a T2* value lower than the detection limit (I don't think bad fit and detection limit are always related. Wouldn't detection limit mean that you don't see any signal on the shortest TE image? I think you may just say that those pixels were excluded or set to zero) and were set to 0 ms. Inner and outer contours were drawn on the MR images to measure the vessel wall area and were then used in Matlab to get the mean T2* value of the aorta.

The temporal variation of the vessel wall areas and mean T2* values of plaques for both baseline and post-USPIO was analyzed with an ANOVA test. Student's t-test was used to assess the statistical significance of the differences between vessel wall areas and mean T2* values of the two groups. Differences were considered significant when $p<0.05$. Data are expressed as mean ± standard error of the mean (SEM).

RESULTS

Vessel wall area measured by MRI increased significantly over time for both the NTG ($p=0.001$) and the TG ($p=0.05$). There was no statistically significant difference found between the two groups. Linear regression yielded a 1.8 times higher slope of vessel wall increase with time for the not treated group compared to the treated group (Figure 1). Significantly shorter mean T2* values were obtained at post-USPIO compared to baseline at every imaging point for both groups ($p<0.001$), however no differences were found between groups. Figure 2 shows examples of calculated T2* maps overlaid on the MR T2*-w GRE images (TE = 2.1 ms) acquired during the follow-up.

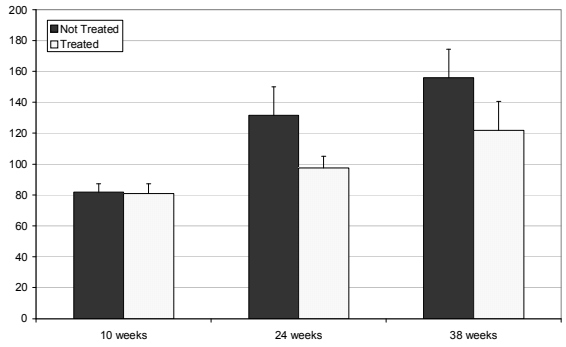

Figure 1. Temporal variation of the vessel wall surface (in number of pixels) for each investigated group. Data is presented as mean ± SEM.

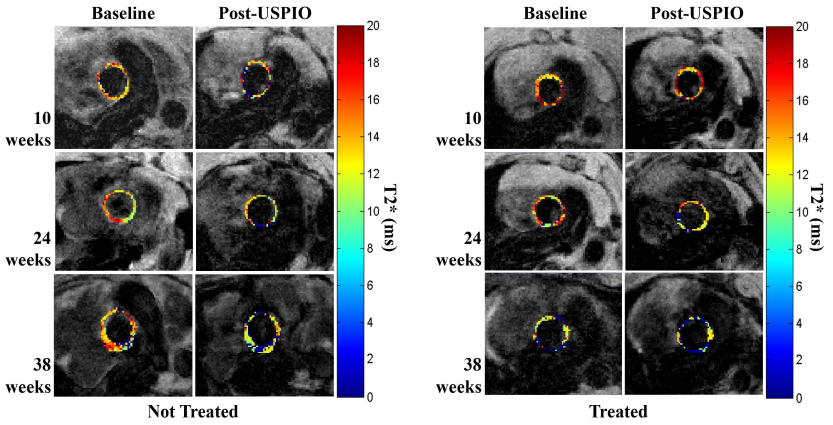

Figure 2. Overlay of T2* maps of the ascending aorta on anatomic GRE images acquired for one animal of the not treated group (NTG) (left) and one animal from the treated group (TG) (right) at every imaging time point. The colorbar represents T2* values in ms. T2* value of 0 indicates pixels that were poorly fitted, thus under the detection limit. (zoom x 3 on the MR images, pixel size 98µm, slice thickness 0.8mm).

DISCUSSION

We report here the first MRI follow-up study performed on a mouse model of atherosclerosis with the goal of following a small-molecule therapeutic effect on the development and progression of atherosclerotic lesions. Irbesartan lead to a marked reduction in the plaque formation. Concerning USPIO-enhanced MRI, the study failed to highlight differences between the two groups based on the post-contrast plaque T2* values. Literature data concerning RAS blockers seems to indicate that responses depend on age, sex, dose, duration of treatment, and site of lesion investigation (5). Thus a limitation of the current study is the fact that only one image of the ascending aorta just above the sinus was acquired, hence the obtained information may not be representative of the general effect of the drug.

References: 1. Yusuf S. et al, N Engl J Med 2000; 342:145-153 ; **2**. Fukuda D et al, Biomed Pharmacother 2009 ; **3.** Cazaubon C et al, J Pharmacol Exp Ther 1993; 265:826-834 ; **4.** Sigovan M et al, Radiology 2009; 252:401-409 ; **5**. Zhou Y el al, Am J Hypertens 2005; 18:486-492