In vivo volumetric MRI in drug discovery: a preclinical study of the ezetimibe therapeutic efficacy on atherosclerotic plaque burden in the thoracic ascending arterial tree in apoE-/- mice

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Introduction Volumetric MRI of atherosclerotic plaque in the thoracic ascending arterial tree may provide valuable insight into disease progression and therapeutic responses [1]. In this study, we established and validated an *in vivo* high resolution MRI using the Apolipoprotein E knockout (apoE$^{-/-}$) mouse model, to demonstrate the effectiveness of a potent cholesterol absorption inhibitor, ezetimibe (Zetia®) [2], on the inhibition of the progression of atherosclerosis. Atherosclerotic plaque burden changes throughout the ascending arterial tree in mouse were monitored by longitudinal MRI using a plaque targeting contrast agent, Gadofluorine M (Bayer Schering Pharma AG, Berlin, Germany) [3]. The *in vivo* MRI quantitation of plaque burden and therapeutic responses were validated by traditional biological approaches for assessment of aortic cholesterol, plasma lipoprotein content, and histology. The volumetric data were evaluated to determine the most sensitive biomarker for therapeutic response of ezetimibe through univariate analysis of total plaque burden, plaque volume, area, and thickness measurements in various arterial segments.

Methods

Animal Models Male apoE$^{-/-}$ mice (B6.129P2-*Apoe*$^{tm1\,Unc}$/J) were recruited at 4 weeks of age. Western diet containing 21% fat and 0.15% cholesterol was used to enhance atherosclerotic processes in mice. The effects of ezetimibe supplied in-feed at 50 ppm on the progression of atherosclerosis were examined by comparing serial MRI conducted at various time points within a 3~6 months treatment period. The study consisted of four experimental models (prophylactic, n=9~12/group; therapeutic early stage, n=6/group; therapeutic adult, n=7/group; and therapeutic aged, n=7/group), two groups in each model (Vehicle and Treatment), with different stages of atherosclerosis at baseline when ezetimibe treatment was initiated. The plaque burden at baseline and 12 weeks were specifically compared and analyzed.

In Vivo MRI Protocol All experiments were approved by the IACUC of research laboratories. MRI was performed on a Bruker Biospin 500WB spectrometer (Bruker NMR, Inc., Billerica, MA). The plaque targeting contrast agent Gadofluorine M was intravenously injected (50μmol Gd/kg) 24 hours prior to MRI scan. Mice were anesthetized with a 1.5% isoflurane/O_2 gas mixture during MRI and positioned within a birdcage coil of 25-mm ID. High resolution MRI protocol was optimized for detection and quantitation of atherosclerotic plaque burden in the mouse thoracic ascending arterial tree. It includes a 3-dimensional cardiac triggered T1-weighted FSE sequence (RARE=2, triger delay 50ms, TR=1 heart beat, TE=9ms, and 64 slices) with frequency selective fat saturation and proximal slab selective blood flow suppression to minimize the imaging artifacts. Images were acquired with an in-plane resolution 50μm×100μm, and through-plane resolution 300μm, which provides a broad coverage of plaque distribution in aortic root (AR), aortic arch (AA), innominate artery (IA), left carotid artery (LC), and left subclavian (LS).

Plaque Burden Measurement in Various Arterial Segments Plaque burden over time was monitored in various arterial segments throughout the thoracic ascending arterial tree: a 3-mm block of the aortic arch covering the ascending and descending aorta, 2mm left carotid artery, 1.5mm innominate artery, 1.5mm left subclavian, and 0.3mm aortic root. The 3-index is the summation of plaque volume in innominate, left carotid, and left subclavian arteries. Summation is the total plaque volume in the five segments. Volumetric MRI also allows us to evaluate the sensitivity of plaque burden by volume, area, and thickness in the innominate and left carotid arteries.

Ex vivo Imaging and Biological Measurements *Ex vivo* MRI of aortic samples of apoE-/- (with/without Gadofluorine M) and wild type (with Gadofluorine M) mice was implemented to validate the specificity of Gadofluorine M to plaque [3, 4]. At study completion, mice were euthanized with CO_2 asphyxiation, and blood was collected from the inferior vena cava for plasma cholesterol and triglyceride levels. The vasculature was then gently perfused with cold PBS and 4 mM EDTA, and aorta portions from the aortic root to right renal artery were excised for estimation of the aortic total cholesterol (TC) and cholesteryl ester (CE) content. The ascending arterial branches containing the proportion of innominate artery beginning from the aortic arch up to the bifurcation of the right carotid artery were dissected for histology.

Results ApoE$^{-/-}$ mice develop plaque in all phases of atherosclerosis throughout the arterial tree [5]. Significant inhibition of plaque progression was found in arteries of all ezetimibe treated groups. Six-month ezetimibe treatment almost completely inhibited the plaque progression in mice in the prophylactic group, as detected by both Gadofluorine M enhanced MRI and histology in Figure 1. The effects of Western diet on plaque progression in three ascending arterial branches are clearly visible in a vehicle mouse of the therapeutic adult group (Figure 2). Significant progression of atherosclerosis was found in all vehicle groups fed with Western diet, and the combination indices 3-index and summation of plaque volume show a consistent plaque progression with age (Figure 3). Statistically significant differences were also found between ezetimibe and vehicle groups in all therapeutic models after 12 weeks study period ($p<0.01$). As expected, greater sensitivity was achieved with a prolonged study period. Younger animals tend to provide better sensitivity in plaque progression, thus are more preferred for a short term study. The statistical sensitivity of plaque volume is stronger than plaque area and thickness especially in the aged group which tends to have more advanced plaque lesions. The combination indices, 3-index and summation, mitigated the plaque volume variation carried from each individual arterial segment, are most sensitive makers.

Conclusions and discussions In summary, a contrast enhanced volumetric MRI protocol was implemented at 11.7 T, to allow excellent visualization of plaque burden throughout the thoracic ascending arterial tree of the apoE$^{-/-}$ mouse. Ezetimibe significantly reduced the plasma total cholesterol and lipoprotein levels of VLDL and LDL, retarded the increase in aortic cholesterol content, and resulted in significant inhibition of plaque progression in the therapeutic models as well as the prophylactic mouse model, which were observed by MRI and confirmed by histology, aortic cholesterol, and plasma lipid measurements. High correlations were found between MRI plaque volume and aortic cholesterol ester, plasma total cholesterol and aortic cholesterol ester, and MRI plaque area and that from histology. The consistent inhibition of plaque progression by prolonged cholesterol lowering therapy confirmed that lowering of LDL cholesterol with ezetimibe is clinically beneficial, and early diagnostics and treatment could effectively prevent the evolution of atherosclerosis. Finally, the rate of plaque progression and the efficacy of ezetimibe therapy could be dependent upon the stage of plaque development and arterial locations. Sampling the stage of atherosclerosis as well as the plaque burden throughout the arterial tree using volumetric MRI could provide a more complete description of disease progression and therapeutic efficacy. Moreover, simultaneous sampling throughout the arterial tree also provides a more sensitive marker for quantifying therapeutic responses. These capabilities may prove superior to existing methodologies used in clinical medicine to track arterial plaque development, if translated for use in humans.

Reference

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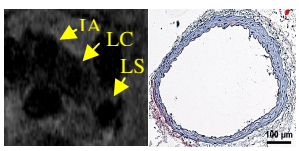

Figure 1. Ezetimibe treatment in prophylactic group completely inhibited the plaque progression

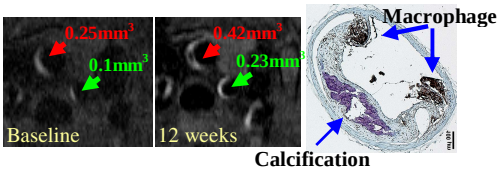

Figure 2. Plaque progression in ascending arterial branches of a mouse in the adult Vehicle group

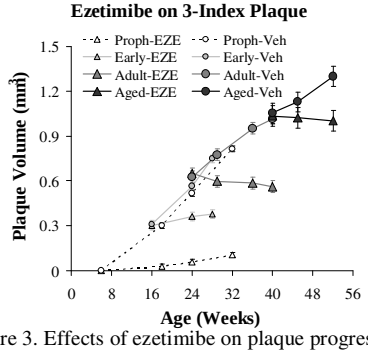

Figure 3. Effects of ezetimibe on plaque progression