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


1Imaging, Merck Research Laboratories, Rahway, NJ, United States, 2Cardiovascular Disease, Merck Research Laboratories, Rahway, NJ, United States, 3Biometrics, Merck Research Laboratories, Rahway, NJ, United States, 4TRG Diagnostic Imaging, Bayer Schering Pharma AG, Berlin, Germany, 5Applied Computer Science & Math, Merck Research Laboratories, Rahway, NJ, United States, 6LAR, Merck Research Laboratories, Rahway, NJ, United States

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 quantification of plaque burden throughout the arterial tree was 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-Apoelo/-), 2 months old, were used in the experiments. 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/O2 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 TI-weighted FSE sequence (RARE=2, trigger delay 50ms, TR=1 heart beat, TE=9ms, and 64 slices) with frequency selective fat saturation and 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).

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 the prophylactic group, as detected by both histology and MRI. 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 development 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