Molecular Imaging of Atherosclerosis using PEG-Micelles targeted by an ApoE derived Peptide

E. Leupold[1], E. Vucic[2], W. Mulder[2], M. Dathe[1], and Z. A. Fayad[2]

[1]Leibniz Institute of Molecular Pharmacology (FMP), Berlin, Germany, [2]Imaging Laboratories, Mount Sinai School of Medicine, New York, New York, United States

Introduction: Atherosclerosis is the single leading cause of death in America (statistics of the American Heart Association, 2004) and the industrialized countries. Therefore the importance of the development of accurate and sensitive diagnostic methods for this disease can not be underestimated. Due to the ability of MRI to visualize soft tissue with high resolution and contrast it is highly suitable for the non-invasive and timely diagnosis of atherosclerosis. Recently, new MRI techniques have been developed that rely on sophisticated contrast agents and allow improved and more sensitive characterization of plaque [1]. In the current study we use Gadolinium labeled PEG-micelles as contrast agents in ApoE knockout mice. Targeting is achieved by an ApoE derived peptide (A2), which consists of the residues 141-150 in tandem configuration. It is highly cationic and facilitates a rapid uptake into macrophages.

Methods: The PEG-micelles consisted of 50 % maleimide-PEG-DPPE, 49 % Gd-DTPA-BSA and 1 % rhodamine-DPPE for fluorescence microscopy and were produced by lipid-film hydration. The A2 peptide was subsequently coupled via maleimide/ sulfhydryle conjugation. Particles that were synthesized without the targeting peptide were used as a control. The particles were characterized by DLS, HPLC and relaxometry. MR scanning was performed on a Bruker small animal 9.4 T system. ApoE mice fed on a high cholesterol diet for 6 months were used as a model of atherosclerosis. Each mouse was administered a dose of 50 µmol Gd/kg of either A2-PEG-micelels or control PEG-micelles. Confocal microscopy (CLSM) was performed on sections taken from the thoracic aorta. The sections were stained with an Alexa Fluor®647 labeled anti-CD68 antibody (macrophage specific) and DAPI. To determine the blood half-life of these particles, blood samples were taken at various time points. The T1 was measured and the half-life was calculated by a mono-exponential decay algorithm.

Results: The formed micelles were characterized by a very narrow size distribution with a mean size of around 18 nm and a high stability. No change of particle size could be observed within a period of 30 days. The relaxivity was reproducible for different batches around 12 $(mM \times sec)^{-1}$. In vivo application of A2-PEG-micelles resulted in a high, long lasting (>72 h) enhancement of atherosclerotic plaque in MRI. The maximum enhancement of 129 ± 46 % (n = 6 mice) was reached at 24 h, compared to 30 ± 15 % (n = 5 mice) in mice treated with control PEG-micelles. The enhancement after 72 h was still 105 ± 50 % compared to 21 ± 15 % (see Figure 1 & 2) for the control agent. CLSM studies showed a co-localization of A2-PEG-micelles with CD68 in atherosclerotic plaques at 24 h as well as at 72 h (see Figure 3). The blood half-life of these micelles was established to be 18 hr.

Conclusion: A2 modified PEG-micelles containing Gd is a promising new contrast agent that is excellently suited for the improved MRI based diagnosis of atherosclerosis.

Reference: [1] Choudhury RP, Fuster V, Fayad ZA in Nat Rev Drug Discov. 2004; 3(11): 913-25

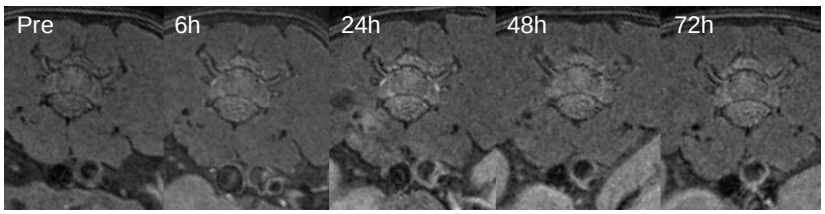

Figure 1: Representative MRI images of A2 targeted PEG-micelles obtained precontrast, 6 h, 24 h, 48 h and 72 h post injection of a 50 µmol Gd/kg dose in ApoE knock out mice

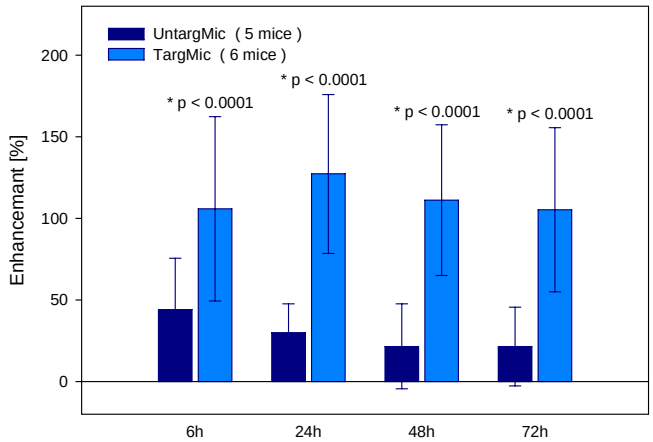

Figure 2: relative Plaque Enhancement of A2 targeted PEG-micelles at 6 h, 24 h, 48 h and 72 h post injection in ApoE knock out mice, deduced from MRI

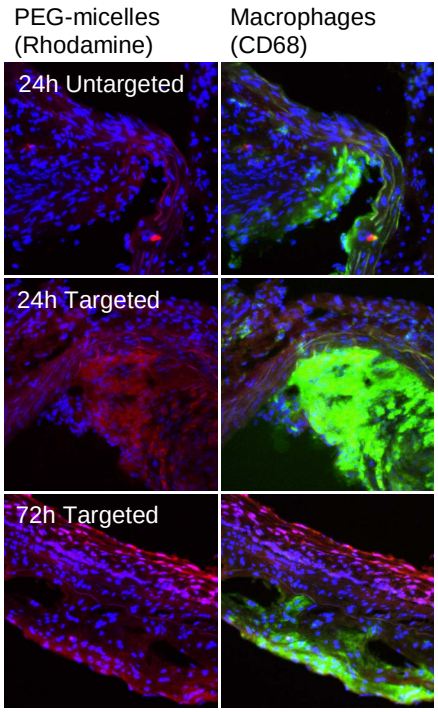

Figure 3: CLSM pictures of dissected mouse aortas showing co-localization of targeted micelles (red, left) and macrophages (green, right)