Identification of advanced atherosclerotic plaque in abdominal aorta in a murine atherosclerotic model with 24p3 (mouse homologue of neutrophil gelatinase-associated lipocalin)-targeted micelles and MRI.

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Background:
Expression of neutrophil gelatinase-associated lipocalin (NGAL) in human carotid artery plaque has been reported to correlate with the occurrence of acute cerebrovascular events (stroke and transient ischemic attack). It is believed to play a role in stabilization of matrix metalloproteinases (MMPs) and therefore to lead to a more vulnerable plaque phenotype. We tried to visualize advanced plaque with double-labeled (lissamin-rhodamin and gadolinium) 24p3-targeted micelles and T1 weighted MRI in mice.

Materials & Methods: 3 adult apoE-/-, eNOS-/- mice were fed an atherogenic diet for 8 weeks before baseline T1 weighted MRI (Inversion Recovery fast spin echo, inversion time 1300 ms) (Bruker Avance, 9.4 T) of the abdominal aorta (1 cm region around the right renal artery branch). Two mice were injected with 200 μl 24p3-antibody-conjugated and one mouse with rat isotype-conjugated micelles containing fluorophore-coupled phosphoethanolamine (lissamin-rhodamin-PE) and gadolinium-bisstearylamide. Because we have found that 24p3-targeted micelles show the highest normalized enhancement ratio (NER=signal intensity of enhanced plaque region divided by mean signal intensity of skeletal muscle (=ER after) divided by ER before) at 72 hours after administration of micelles, MRI measurements were repeated at this time. Afterwards, abdominal aortas were harvested and embedded in tissue-tek for preparation of cryosections. Sections were double-stained for macrophages (rat anti-mouse mac-3 monoclonal antibody conjugated to AlexaFluor 647) and 24p3 (biotinylated polyclonal antibody and streptavidin-FITC). Fluorescence microscopy (laser excitation/emission wavelengths: 488/520 (FITC), 543/590 (lissamin-rhodamin), and 633/670 (AlexaFluor 647)) was performed and colocalisation of 24p3, macrophages and micelles was evaluated after merging of the three fluorescence images with ImageJ and compared with corresponding MR images (figure 1). Enhancement ratios (ER) (= mean signal intensity of the enhanced region in the plaque divided by the mean signal of skeletal muscle) and normalized ER (NER) (=ER divided by ER at t=0) were calculated.

Results:
T1 weighted MR images of atherosclerotic plaques of apoE-/-, eNos-/- mice showed at 72 hours after administration of 24p3-targeted micelles positive enhancement of plaque (figure 1) (NER varied from 1.65 to 2.25), whereas MR images of the mouse at 72 hours after administration of isotype-conjugated micelles did not show positive enhancement (figure 2). FM images of mice after administration of 24p3-targeted micelles showed clear spots at excitation wavelength 543 nm (micelles: figure 1C) which colocalized with 24p3 (figure 1D), and which to a lesser extent colocalized with macrophages (figure 1E). FM images of the mouse after administration of isotype-conjugated micelles, showed only faint spots at excitation wavelength 543 nm (micelles: figure 2C), which did not colocalize with 24p3 (figure 2D), but did colocalize with macrophages (figure 2E).

Conclusion: Expression of 24p3 (mouse homologue of NGAL) in atherosclerotic plaque in murine abdominal aorta, can be visualized with 24p3-targeted micelles using in vivo molecular MRI.