Characterization of MR contrast enhancement in murine advanced atherosclerotic plaque after administration of 24p3 (NGAL)-targeted micelles.

B. C. te Boekhorst[1], S. M. Bovens[1], K. den Ouden[1], M. G. Nederhoff[1], K. W. van de Kolk[1], M. J. Cramer[1], M. ten Hove[1], P. Doevendans[1], R. E. Poelmann[2], G. Pasterkamp[1], and C. J. van Echteld[1]

[1]Dept. of Cardiology, University Medical Center Utrecht, Utrecht, Utrecht, Netherlands, [2]Anatomy and Embryology, Leiden Universitair Medisch Centrum, Leiden, Netherlands

Background:
Plaque macrophage activity, as reflected by secretion of matrix metalloproteinases (MMPs), is an important hallmark of atherosclerosis. Human neutrophil gelatinase associated lipocalin (NGAL) stabilizes MMPs. Increased expression of NGAL has been shown to correlate with vulnerability of carotid artery plaque (Hellings et al.).
Materials& methods:
24p3 (mouse analogue of NGAL)-targeting immunomicelles were tested in vivo in an old male apoE and eNOS double knockout mouse for visualization of atherosclerotic plaque in the abdominal aorta. Optimized timing of T1 weighted (inversion-recovery) MRI (9.4T) after administration of micelles was tested by repeating measurements after t=0 at t= 24, 48, 72, 96 and 120 hours after intravenous administration of rat anti mouse 24p3 (mouse analogue of human NGAL) antibody-conjugated micelles (3 mM lipid) (figure 1). Also rat isotype IgG1-conjugated micelles (3 mM) and non-conjugated micelles (10 mM) were similarly tested in two other male apoE and eNOS double knockout mice. Enhancement ratios (ER= mean signal intensity of the enhanced region in the plaque divided by the mean signal of skeletal muscle) and normalized ER (=ER divided by ER at t=0) are measured and calculated (table 1 and figure 1).
Results: NER in 24p3-targeted micelles attained the highest value (=2.65) at t= 72 hours after contrast administration. Non-conjugated and isotype-conjugated micelles showed the highest NER value at t=24-48 hours, which was lower than that of 24p3-targeted micelles.

Micelles		T=0	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Non-conjugated	$ER_{plaq/skel.\ m.}$	0.88	1.95	1.77	0.82	0.88	0.90
10 mM	$NER_{plaq/skel.\ m.}$	1	2.22	2.01	0.93	1.00	1.02
Iso-conjugated	$ER_{plaq/skel.\ m.}$	1.54	2.11	1.93	0.88	1.47	-
3mM	$NER_{plaq/skel.\ m.}$	1	1.37	1.26	0.57	0.95	-
24p3-targeted	$ER_{plaq/skel.\ m.}$	1.28	1.13	1.58	3.38	2.22	1.24
3 mM	$NER_{plaq/skel.\ m.}$	1	0.88	1.23	2.65	1.74	0.97

Table 1. (Normalized) Enhancement Ratios (N)ER (= signal intensity of the enhanced region in the plaque divided by the mean signal of skeletal muscle) at baseline and at several times after administration of non-conjugated, isotype antibody-conjugated (iso-conjugated) (3mM) and 24p3 antibody-conjugated (3 mM) micelles.

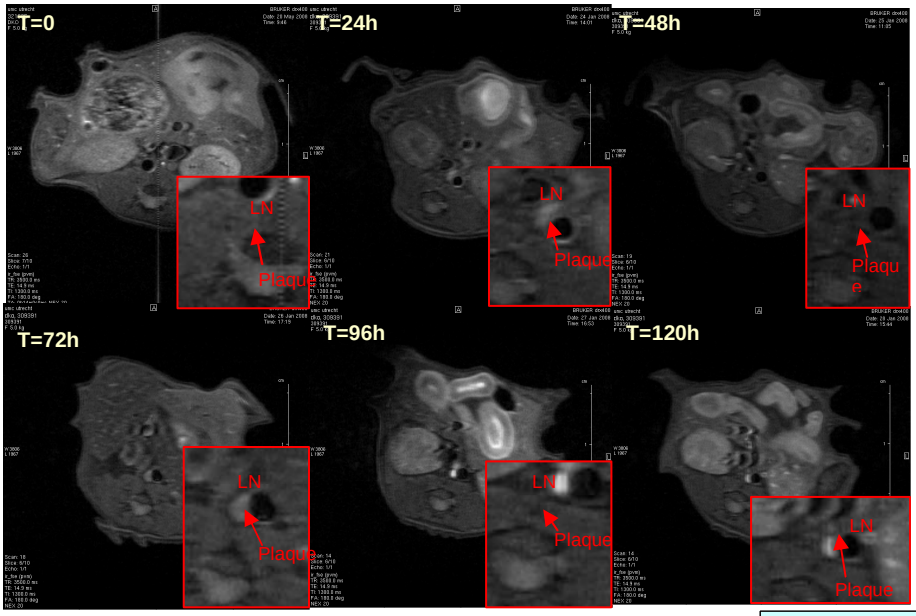

Figure 1. T1-weighted MRI of murine abdominal aorta at different times after administration of 200 ul 24p3-targeted micelles (3 mM).

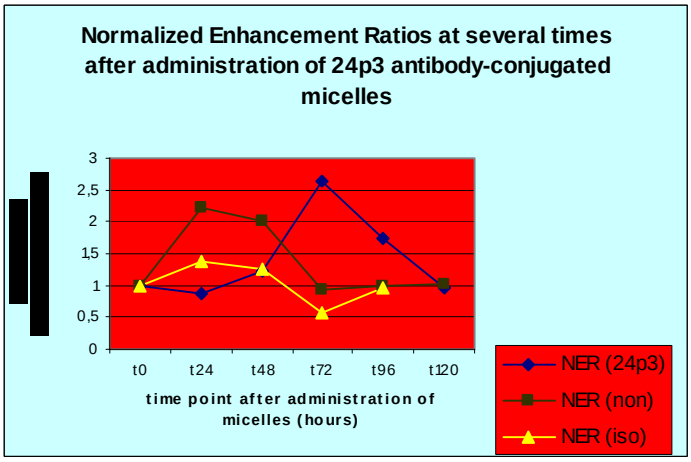

Figure 2. Time-course of NER after administration of 24p3-targeted (3 mM), isotype-conjugated (3 mM) and non-conjugated (10 mM) micelles.

Conclusion:
Advanced plaque in murine abdominal aorta of apoE-/- and eNOS-/- mice could be visualized with MRI within the timing window of 72-96 hours after administration of 24p3-targeted micelles. The (false) positive contrast raised by the non-conjugated micelles after 24 – 48 hours shows that care must be taken in identifying the right time to asses the effect of molecular contrast agents like these.