Ultrasmall Superparamagnetic Particles of Iron Oxide-enhanced *in vivo* MRI of human atherosclerotic plaques.

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Abstract
Magnetic Resonance Images of carotid atherosclerotic plaques of ten patients scheduled to undergo endarterectomy, were acquired before and both 24 and 72 hours after application of Ultrasmall Superparamagnetic Particles of Iron Oxide (USPIO). The USPIO induced a decrease of the signal intensity of the vessel wall in both the T1 and T2 weighted gradient-echo MR Images. These signal decreases were confined to parts containing macrophages. Histology and electron microscopy showed uptake of USPIO by activated macrophages. Therefore, USPIO-enhanced plaque imaging may be a promising method for *in vivo* plaque characterization.

Introduction
Plaque instability is responsible for most of the complications of atherosclerotic vessel disease. Up to now, it has been impossible to reliably differentiate stable from unstable plaques *in vivo*. One of the morphological characterizations of an unstable plaque is a preponderance of macrophages. Recently, it was shown in animal models that uptake of Ultrasmall Superparamagnetic Particles of Iron Oxide (USPIO) by macrophages in atherosclerotic plaques induces signal changes in MR images of those plaques [1,2]. With the present project we intended to investigate the possibility of *in vivo* MR imaging of activated macrophages in human carotid atherosclerotic plaques using USPIO.

Material & Methods
The carotid atherosclerotic plaques of ten symptomatic patients scheduled to undergo a carotid endarterectomy were imaged *in vivo* on a 1.5 T whole body scanner (Intera, Philips Medical Systems) using a small diameter surface coil as described in Ref. [3]. The following pulse sequences were used: T1w gradient-recalled-echo, TR/TE: 43/9.2 ms; α=25°; T2*w gradient-echo, TR/TE: 1 heart beat/20 ms; α=40°; and PDw FSE, TR/TE: 2-3 beats/20 ms; echo train length 5. Other parameters were: transversal slices; FOV: 70 mm; matrix size: 208-256x208-256; and slice thickness: 4.5 mm. Then, the USPIO (Sinerem®, Guerbet) was administered at 2.6 mg Fe/kg. The patients were examined again 24 hours after administration using the same MRI sequences. Further, five patients were examined additionally 72 after administration. Within 12 hours after surgery, the excised carotid artery segments were imaged *ex vivo* in a saline solution at room temperature using the same MR sequences. In addition, the following additional pulse sequences were used: proton density weighted (PDw), TR/TE: 2000/20; T2w, TR/TE: 2000/85; and T1w, TR/TE: 500/20. Following MR Imaging, the specimens were formalin fixed and embedded in paraffin. Subsequently, 4-μm sections were subjected to histo-immunological analysis of plaque phenotype (HE staining), macrophage content (CD68 immuno-staining) and USPIO uptake (PERL's iron staining). In addition, for two of the specimens electron microscopical analysis of PERL's positive regions was performed.

Results
PERL's staining showed that USPIO was taken up in the plaque of all patients. Double staining for iron (PERL's) and macrophages (CD68) indicated USPIO uptake in a subset of macrophages, which was verified by electron microscopy. In the *ex vivo* as well as the *in vivo* gradient-echo MR Images the uptake of USPIO induced a decrease in signal intensity. From a comparison with histology it was shown that the decrease in relative signal intensity was confined to parts of the vessel wall containing macrophages. *Ex vivo*, the changes were most clearly visible on the T1w GE MR Images. *In vivo*, the images acquired 24 hours after administration showed more changes compared to pre-administration than those acquired after 72 hours for both sequences. In addition, 24 hours after administration, the signal of the lumen is still hyperintense in the *in vivo* T1w GE MR images. In some of the images a narrow band of low signal intensity adjacent to the hyperintense region was observed. This "ring phenomenon" has been observed as well by Schmitz *et al.*[1] in the animal model. Due to this phenomenon the images acquired after 72 hours were easier to interpret. In the PDw MR images no signal changes were observed. An example of a corresponding *in vivo* MRI, *ex vivo* MRI and histological section is presented in Fig. 1 and Fig. 2.

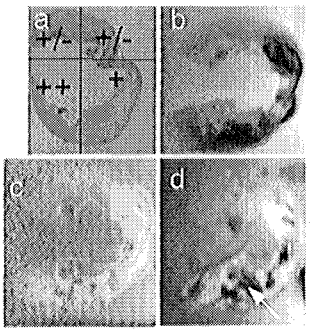

Figure 1. Corresponding a) histological section of internal carotid artery (HE staining) +/- hardly any USPIO uptake; + some USPIO uptake; ++ many USPIO uptake, b) *ex vivo* T2w SE MRI, c) *ex vivo* T1w SE MRI, and d) *ex vivo* T1w GE MRI. A signal decrease can be observed on the T1w GE MR image in the part of the vessel wall which showed a large number of Fe positive cells in the histological section (arrow).

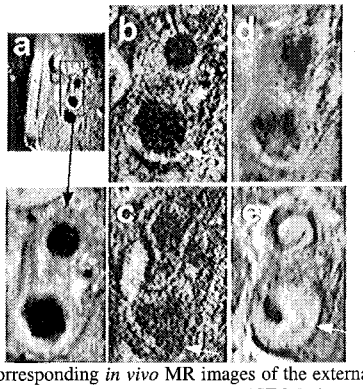

Figure 2. Corresponding *in vivo* MR images of the external (above) and internal (below) carotid artery. a) PDw FSE MR image, b) T2*w GE MR image before and c) 24 hours after application of USPIO, d) T1w GE MR image before and e) 24 hours after application. A signal decrease can be observed on the post-contrast T2*w MR image in the part of the vessel wall which showed a large number of Fe positive cells in the histological section presented in Fig. 1 (arrow). On the post-contrast T1w GE MR image a "ring phenomenon" is observed (arrow).

Discussion and Conclusion
Histological and electron microscopy analysis showed USPIO uptake in activated macrophages in the human atherosclerotic plaque. The uptake of USPIO induces signal decreases in the *in vivo* and *ex vivo* gradient-echo MR images. In the future, MR imaging of atherosclerotic plaque using USPIO may be a suitable method for the *in vivo* differentiation between stable and unstable plaques.

References
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