MULTIMODALITY INVESTIGATION OF HIGH DENSITY LIPOPROTEIN ACTION IN ATHEROSCLEROSIS

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Introduction

Lipoproteins are endogenous nanoparticles and the primary vehicles of lipid transportation within the body. It is well recognized that lipoproteins have an important role in cardiovascular disease and the smallest in the lipoprotein family, high density lipoprotein (HDL), is pivotal for reverse cholesterol efflux, thereby having the ability to regress atherosclerosis. However, the mechanisms of cholesterol efflux and interaction with macrophages (in the plaques) are still not fully understood. We recently presented a novel HDL-like nanoparticle platform that allows for multimodality imaging of atherosclerosis. In the current study we apply fluorescent and superparamagnetic iron oxide HDL (FeO-HDL, Figure 1) to investigate the uptake mechanism and metabolism of HDL in atherosclerosis. FeO-HDL is a sophisticated nanoparticle platform that mimics native HDL and is detectable by MRI, optical imaging and TEM, thereby enabling its visualization at the anatomical, cellular and sub-cellular level. The aforementioned platform and imaging techniques were applied to a macrophage cell line *in vitro* while MRI and liver biopsies on atherosclerotic apoE-KO as well as wild-type mice were done *in vivo* after intravenous administration of FeO-HDL.

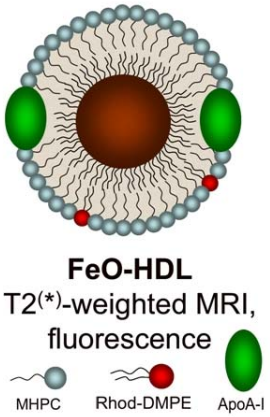

Figure 1

Methods

The nanoparticles were synthesised as recently described and extensively characterized by TEM, relaxometry, protein and phosphorus analyses.

In vitro experiments were performed on J774A1 murine macrophage cells and FeO-HDL association was investigated using confocal laser scanning microscopy, TEM and cell pellet MRI. Uptake mechanistics and metabolism were investigated at sub-cellular detail using TEM and was verified by element analysis. Time and temperature dependency, the effect of inhibitors and competitive inhibition was investigated. In addition cholesterol efflux was measured.

In vivo experiments included high resolution MRI of the abdominal aorta and liver of apoE-KO and wild type mice. Liver biopsies as well as aortic sections of sacrificed animals were further studied with fluorescence imaging, confocal microscopy and TEM.

Results

Characterization of FeO-HDL revealed this platform to closely resemble native HDL, in terms of both physical properties and cholesterol efflux capacity.

In vitro experiments showed that the particles were avidly taken up by macrophages, as evidenced by confocal microscopy (Figure 2A), T2-weighted MR cell pellet imaging (Figure 2B) and TEM performed on the cells. TEM on the cells revealed clathrin coated receptor mediated uptake as well as cellular localization of the HDL particles.

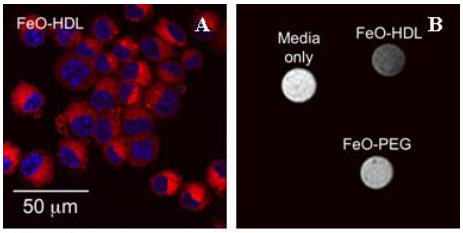

Figure 2

In vivo experiments on apoE-KO mice demonstrated the affinity of FeO-HDL for macrophages in the liver and revealed a substantial signal decrease on the lesioned vessel wall on T2* - weighted MR images (Figure 3A). *Ex vivo* results confirmed the presence of FeO-HDL in atherosclerotic plaques using confocal laser scanning microscopy (Figure 3B) and by Perl's staining, depicted in Figure 3C.

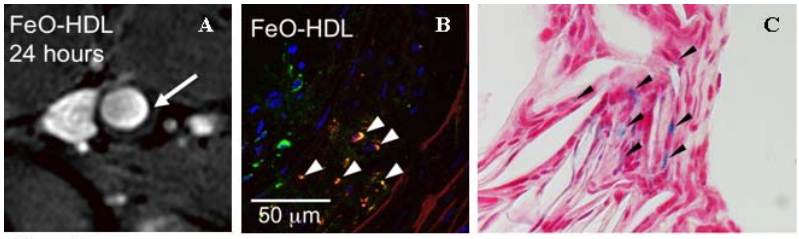

Figure 3

Conclusion

We here report the application of a HDL mimicking iron oxide nanoparticle to enable the multimodality investigation of receptor mediated interactions and metabolism of high density lipoproteins in cardiovascular disease.