A multimodality investigation of the dynamics, trafficking and properties of iron oxide core high-density lipoprotein in experimental 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 high-density lipoprotein (HDL) has an important role in atherosclerosis and is pivotal for reverse cholesterol transport, thereby contributing to plaque regression. In the current study we exploit fluorescent and superparamagnetic iron oxide HDL (FeO-HDL, Figure 1A) to investigate the uptake mechanism, trafficking and *in vivo* behaviour of HDL in atherosclerosis. FeO-HDL is a multimodal nanoparticle platform detectable by MRI, optical imaging and TEM, thereby enabling its visualization at the anatomical, cellular and sub-cellular level. FeO-HDL and various imaging techniques were applied to macrophage and hepatocyte cell lines *in vitro*, while MRI, liver biopsies and lipid dynamics analyses in atherosclerotic apoE-KO as well as wild-type mice were done *in vivo* after intravenous administration of FeO-HDL.

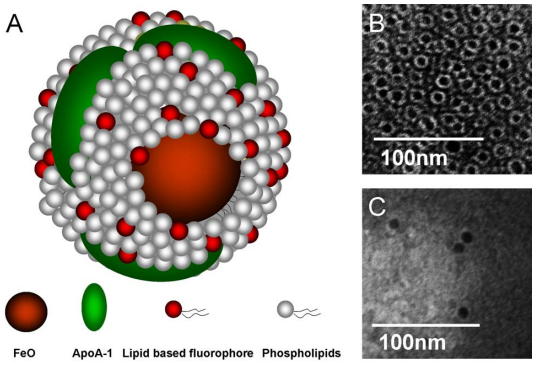

Figure 1

Methods

The nanoparticles were synthesized as recently described[1] and extensively characterized by TEM, relaxometry, protein and phosphorus analyses. The uptake of FeO-HDL in various cell lines *in vitro* was visualized with confocal laser scanning microscopy, TEM and cell pellet MRI, while the uptake mechanism was investigated by a competitive inhibition and concentration dependent experiment, as well as with TEM to visualize sub-cellular details. In addition, cholesterol efflux and cell viability assays were performed. *In vivo* experiments included high resolution MRI of the abdominal aorta of apoE-KO mice as well as the investigation of lipid exchange dynamics between FeO-HDL and natural lipoproteins using FPLC and fluorescent imaging on blood from apoE-KO and wild type mice. Aorta and liver sections were examined *ex vivo* using fluorescent imaging, confocal microscopy and TEM.

Results

Characterization of FeO-HDL revealed this platform to closely resemble native HDL. Negative staining TEM of FeO-HDL in solution showed monodisperse particles with a monolayer of phospholipids (Figure 1B). Negative staining TEM of blood revealed that FeO-HDL preserved its morphology *in vivo* (Figure 1C), i.e. no aggregation and intact lipid coating after intravenous administration. Cholesterol efflux capacity (Figure 2A) was found to be similar to that of native HDL. *In vitro* competitive inhibition, with native HDL (Figure 2B) and concentration dependent uptake experiments revealed a receptor-like uptake of FeO-HDL, while experiments with fluorescent imaging, confocal microscopy, T_2-weighted MR cell pellet imaging (Figure 2C) and TEM showed that the nanoparticles exhibited strong affinities for macrophages and hepatocytes, two of the main cell types HDL interacts with *in vivo*. *In vivo* experiments on apoE-KO mice revealed a substantial signal decrease in the lesioned vessel wall on T_2*- weighted MR images (Figure 3A) 24 hours after administration. Lipid dynamics measurements showed transfer of lipids in apoE KO and wild type mice from FeO-HDL to other lipoprotein fractions, confirming the HDL like nature of FeO-HDL. *Ex vivo* optical imaging and TEM confirmed the presence of individually dispersed FeO-HDL in atherosclerotic plaques (Figure 3B, C and D), while TEM on liver tissue revealed trafficking of the HDL within the hepatocyte and macrophage cells.

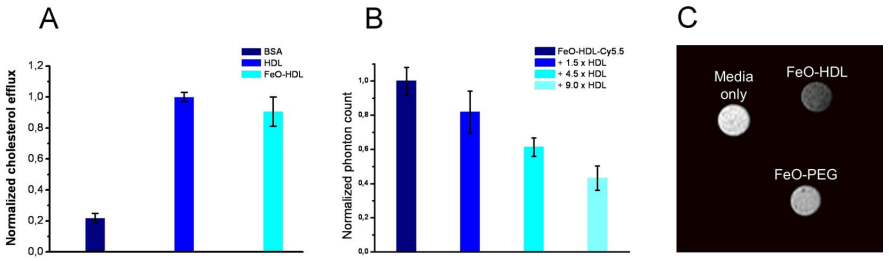

Figure 2

Conclusion

HDL mimicking iron oxide nanoparticles enable the multimodality investigation of receptor mediated interactions, trafficking and lipid dynamics of high-density lipoproteins. In the present study we investigated its behaviour in a cardiovascular disease model, but it may also be applied to other pathologies in which lipoproteins play a pivotal role.

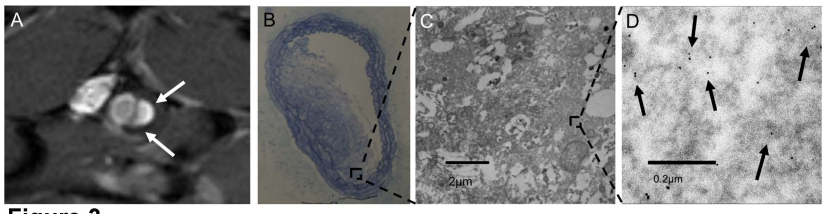

Figure 3

1. Cormode, D. P. et al. Nanocrystal Core High-Density Lipoproteins: A Multimodality Contrast Agent Platform. Nano Lett. (2008). 3715–3723