Longitudinal high resolution MRI to evaluate a novel statin loaded HDL nanoparticle therapy in experimental atherosclerosis.

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Introduction

In addition to their serum cholesterol lowering effect, statins have been shown to exert anti-inflammatory effects in the atherosclerotic disease process, a pleiotropic effect that has been suggested to retard plaque development. However, the limited bioavailability of even the highest dose of oral statin therapy prohibits full exploitation of this anti-inflammatory effect. Therefore, we hypothesize that targeted statin delivery to atherosclerotic plaque can more potently inhibit plaque inflammation and development. For this purpose we developed a reconstituted high density lipoprotein (rHDL) like nanoparticle loaded with simvastatin ($rHDL_{simva}$, Figure 1). We evaluated its efficacy on aortic plaque progression in an atherosclerotic mouse model using longitudinal 9.4 Tesla MRI studies and extensive histology. Control groups were treated either with oral statin therapy or intravenous (i.v.) rHDL (without simvastatin) for comparison.

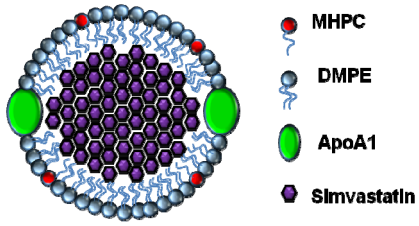

Figure 1. $rHDL_{simva}$ nanoparticle.

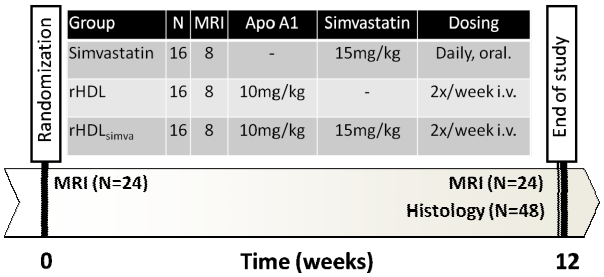

Figure 2. Study design.

Methods and Results

ApoE-KO mice were used for the current study, since statins are known not to alter serum lipid levels in this mouse model, which allows us to investigate the anti-inflamatory properties of statins exclusively. 48 apoE-KO mice were included, and all mice were fed a high cholesterol diet from 4 weeks of age onwards. At an age of 14 weeks, all mice were randomized to recieve either oral dose simvastatin 15mg/kg (n=16), twice a week i.v. rHDL containing 10mg/kg ApoA1 (n=16), or twice a week i.v. $rHDL_{simva}$ containing 10mg/kg ApoA1 and 15 mg/kg simvastatin (n=16). After 12 weeks of treatment all mice were sacrificed and of each mouse 63 transverse cross sections of the aortic valve area and a longitudinal cross section of the abdominal aorta were obtained for histology. Histological sections were stained with hematoxylin phloxine saffron and Oil Red O to assess plaque size and lipid content. Immunostaining of CD68 was used to quantify plaque macrophage content. We also assessed plaque accumulation of gadolinium and rhodamine labeled $rHDL_{simva}$ nanoparticles with in vivo MRI and immunofluorescence in a separate group. Histology image analysis was performed with automated image analysis software written in Matlab.

In vivo MR imaging was performed on 8 mice per group at a 9.4 Tesla MRI scanner (Bruker, Germany). T1-weighted image stacks (20 slices) were acquired of the abdominal aorta of 24 mice (8 in each of the other groups) at baseline and after 12 weeks of follow up. Scan parameters: TE 8.4 ms, TR 800 ms, 16 averages, matrix size 256 x 256, FOV 3 x 3 cm, and slice thickness of 0.5 mm,. Active fat suppression was applied. In Figure 2 the study design is schematically depicted and in Figure 3 typical MR images of the abdominal aortas of the different groups at baseline and after 12 weeks of treatment are shown.

Image analysis was done with semiautomatic software (VesselMass, Leiden) to delineate the aortic lumen area (LA) and outer wall area (OWA). The difference between LA and OWA was defined as the mean wall area (MWA). The primary outcome parameter was the normalized wall index (NWI), which was calculated as: NWI = MWA / OWA. Multiple linear regression analysis was used to assess the association between end of study NWI and treatment group, with NWI as the response variable and treatment group as the explanatory variable. We adjusted for baseline NWI and serum total cholesterol levels. In the oral dose simvastatin group, NWI increased from 0.33 (SD 0.03) to 0.41 (SD 0.03, p=0.001), in the rHDL group NWI increased from 0.35 (SD 0.04) to 0.42 (SD 0.02, p=0.007), and in the $rHDL_{simva}$ group NWI remained unchanged, 0.35 (SD 0.04) at baseline and 0.36 (SD 0.03, p=0.58) at 12 (Figure 4).

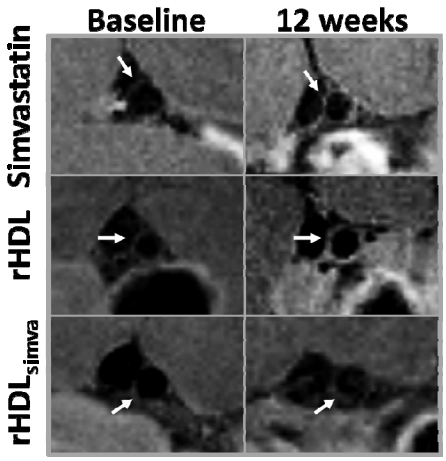

Figure 3. Typical MR images of the abdominal aorta of ApoE-KO mice, before and after 12 weeks of therapy. The artery walls are indicated by the white arrows.

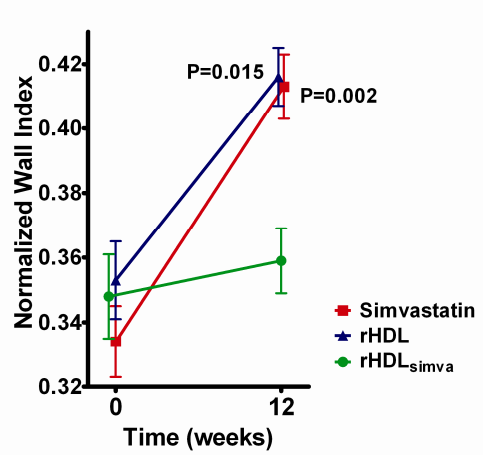

Figure 4. Progression of aortic atherosclerosis in ApoE-KO mice as assessed by 9.4 Tesla MRI. P-values are for comparison of progression to the $rHDL_{simva}$ treatment group. Bars represent SEM.

Conclusions

Targeted statin delivery to atherosclerotic plaque with rHDL like nanoparticles showed considerable efficacy in the treatment of atherosclerosis. In fact, while strong progression of atherosclerosis was observed in the oral simvastatin treatment or rHDL infusion groups, the plaque growth was halted in the group treated with the simvastatin loaded rHDL like nanoparticle. Extensive histological examination of the aortas will be used to corroborate the in vivo MRI data and to monitor changes in plaque composition and phenotype.