Lyp-1 Liposomes Encapsulating a Liver X Receptor Agonist Target to Atherosclerotic Plaques and Reduce Macrophage Content

Aims

Atherosclerosis is the predominant underlying pathology of cardiovascular disease and is one of the leading causes of death worldwide. It is characterized by the retention of lipids such as cholesterol in macrophages (foam cells) in the intima of arteries [1]. Liver X receptor (LXR) agonists, such as GW3965, are promising compounds for treating atherosclerosis since they induce reverse cholesterol transport in foam cells. However, as accumulation of LXR agonists in the liver can lead to high levels of lipids in the plasma or liver [2]. We, therefore, encapsulated GW3965 in liposomes to attempt to reduce the side effects, and functionalized these liposomes with a peptide Lyp-1 (CGNKRTRGC) [3] to target the drug to atherosclerotic lesions and enhance their therapeutic effectiveness.

Methods

Lyp-1 was synthesized and conjugated to PEG2000-DSPE. Synthesis was confirmed by LC-MS. Liposomes composed of DOPC:DOPS:DSPE-PEG:DSPE-PEG-Lyp-1:DOPC-Cy5 in mol% 75.9:19:4.3:0.7:0.1 were prepared using the dehydration-rehydration method. GW3965 was loaded in the bilayer of the liposomes. Liposomes were extruded to a size of around 80 nm (PDI < 0.1), and the zeta-potential of liposomes was around -20 mV as measured by DLS. Lyp-1 and drug content were measured by UPL. Drug loading was around 80%. For *in vitro* experiments, macrophages were cultured from bone marrow of $LDLr^{-/-}$ mice. Foam cells were made by incubating these macrophages with oxLDL. Liposome uptake was measured by flow cytometry. *In vivo* targeting experiments were carried out in male $LDLr^{-/-}$ mice on western type diet (WTD) for 13 weeks. Liposomes were injected i.v. and organs were harvested after 3 hours. Detection of fluorescent label in organs was performed using an in vivo imaging system, and specific uptake of liposomes was measured by flow cytometry. The effect of treatment with GW3965-loaded Lyp-1 liposomes or controls on atherosclerotic plaque development was performed in male $LDLr^{-/-}$ mice on WTD for 8 weeks. Mice subsequently received i.v. injections twice per week for 5 weeks while maintaining WTD. GW3965 dose was 0.2 mg/mouse, and Lyp-1 dose was 35 μg/mouse. Mice were sacrificed and lipid analyses were performed on the plasma and livers. Atherosclerotic burden was assessed in the heart of mice by oil-red-O staining (lipid content) and MOMA2 staining (macrophage content).

Results/Conclusion

Lyp-1 liposomes specifically targeted to foam cells *in vitro*, showing 15x more uptake in foam cells compared to macrophages. While a large percentage of liposomes was taken up in the livers, kidneys and spleens of atherosclerotic mice, the Lyp-1-conjugated liposomes showed significant retention in the aortas, which was confirmed to be due to macrophage uptake. Plaque size was unchanged for all groups, but Lyp-1 liposomes significantly reduced macrophage content compared to PBS, free drug, and empty liposomes. None of the groups led to changes in plasma or hepatic lipid content, while lipogenetic genes were significantly upregulated in the livers of mice receiving drug-containing treatments compared to PBS control, indicating that the liposomes do not fully protect against lipogenesis. In conclusion, Lyp-1 liposomes successfully target foam cells in atherosclerotic plaques for the delivery of GW3965, and decrease plaque macrophage content compared to free drug by almost 50%.

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3. Seo, J.W., et al., *(64)Cu-labeled LyP-1-dendrimer for PET-CT imaging of atherosclerotic plaque.* Bioconjug Chem, 2014. **25**(2): p. 231-9.