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The inherent dynamics of HDL lipids challenge the interpretation of rHDL-based uptake studies that rely on the fluorescence from lipid-anchored fluorophores

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Introduction

The dynamics of the lipids in lipoproteins, including high-density lipoproteins (HDL), are widely acknowledged [1-2]. This dynamics could challenge the interpretation of reconstituted HDL (rHDL)-based uptake studies that depend on the fluorescence from lipid-anchored fluorophores incorporated into rHDL, e.g. using flow cytometry or confocal microscopy. The uptake studies rely on the assumption that the fluorophore-label and rHDL are associated throughout the experiment.

This assumption is tested by quantifying the degree of desorption of lipid-anchored fluorophores from discoidal rHDL (containing 1 mol% fluorophore) into serum components after incubation in heat-treated FBS for 2 hours at 37 °C. Size-exclusion chromatography (SEC) was used to separate rHDL from non-rHDL serum components and quantification of fluorophore desorption was obtained by calculating the ratio between fluorescence intensity from the non-rHDL fractions and the total intensity (Fig. 1).

Desorption of lipid-anchored fluorophore from rHDL

The degree of fluorophore desorption from DPPC rHDL into serum components was evaluated using several commonly used lipid-anchored fluorophores (Fig. 2). The effect of lipid composition of rHDL on the fluorophore desorption was studied (Fig. 3), as well as fluorophore desorption from rHDL based on the apoA-I mimicking peptide 4F instead of the full-length apoA-I (Fig. 4).

Effluent of rHDL remodeling

Remodeling of both DPPC rHDL (Fig. 5A) and POPC rHDL (Fig. 5B) was observed in FBS, in each case resulting in two distinct populations of possibly different sized rHDL. Interestingly, no such remodeling was observed for DMPC rHDL (Fig. 5C). How come?

Conclusions

• We quantified desorption of several lipid-anchored fluorophores from different types of rHDL formulations.
• What are we looking at? Fluorophore desorption from rHDL could challenge the interpretation of uptake studies based on fluorescence readout.
• Be aware (!) of the lipid dynamics in lipoproteins which could also lead to desorption of therapeutic agents when using rHDL for drug delivery.

References


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