Introduction

High plasma cholesterol levels are positively correlated with the risk of cardiovascular diseases (CVD), the leading cause of death in developed countries. Low Density Lipoprotein (LDL) are known as the "bad cholesterol" as they deliver cholesterol to peripheral tissues. On the other hand, High Density Lipoprotein (HDL) are known as the "good cholesterol" as they promote Reverse Cholesterol Transport (RCT), whereby they remove excess cholesterol from peripheral tissues and transport it back to the liver for elimination [1,2]. Therefore, HDL is known to reduce the risk of CVD.

A critical step in HDL biogenesis involves the interaction between lipid poor apolipoprotein A1 (apo-A1), a major protein constituent of HDL and the transporter ATP-Binding Cassette A1 (ABCA1) [3]. Apo-A1 interacts with ABCA1 to trigger a signal transduction pathway that mediates efflux, where intracellular cholesterol is released (effluxed) out of the cells [1,2].

Effluxed lipids bind to apo-A1 to form nascent discoidal HDL (nHDL). Further efflux and modifications by enzymes such as LCAT result in esterification of cholesterol and the formation of spherical particles [1]. Mature HDL particles become larger, and cholesterol esters are delivered back to the liver for elimination [2].

Ganglioside GM1 is a glycosphingolipid on the plasma membrane that modulates cell signal transduction [4]. It is the binding site for E.coli heat-labile enterotoxin and Cholera toxin, and abnormal accumulation of GM1 correlates with diseases such as Alzheimer’s and GM1 gangliosidosis [4]. Some studies suggest that accumulation of gangliosides causes "trapping" of cholesterol [5].

Sucralose (splenda) is a synthetic, non-caloric sweetener widely used to reduce calorie intake. Although it is approved by FDA and the World Health Organization as a safe additive, its effects on CVDs are not fully known.

Objective

To determine whether:
1) Cholesterol loading results in formation of larger HDL particles
2) Additives (sucralose) negatively affect HDL biogenesis

Methods

1. Effects of Cholesterol Loading on HDL Biogenesis
   - Day 0: Mouse macrophage-derived cells (J747) were plated in a 24-well plate
   - Day II: Cells were incubated in media lacking apoA-1 or CPT served as negative controls.
   - Day III: Aliquots of filtered media and solubilized cells were harvested for further analysis.

2. Effects of Sucralose on Efflux and HDL Biogenesis
   - Day III: Cells were incubated with sucralose for 24 hrs.
   - Day IV: Cells were incubated with CTB to detect GM1.

Fluorescence Measurements to Determine % Efflux
- Allophycocyanin media and solubilized cells were transferred into a 96-well plate to measure fluorescence.

Western Blotting
- HDL biogenesis (apo-A1 lipoprotein) was analyzed by native PAGE followed by immunoblotting using antibodies to apo-A1. Levels of GM1 associated with nascent HDL was measured by incubating cells with a specific monoclonal antibody, while GM1 was visualized using a monoclonal antibody against the C8 subunit B [CTB], which has a high affinity to GM1.
- The signal on the blots were detected using chemiluminescence imagers and quantified using the program GelAnalyzer 2010a.

Results

EFFECTS OF CHOLESTEROL LOADING ON HDL BIOGENESIS

Fig. 6. Analysis of particles secreted by cells as a function of increased cholesterol loading.
B. Quantification of apo-A1 signal from panel A. Band 5 is the top band, representing larger particles.
C. Quantiﬁcation of GM1 signal from panel B. As opposed to apo-A1 in 6C, GM1 signal is decreasing substantially as cells are loaded with cholesterol.

SUCRALOSE NEGATIVELY AFFECTS HDL BIOGENESIS

Fig. 8. % cholesterol efflux as a function of sucralose concentration.
A. Immunoblot probed with antibodies to apo-A1. Compared to control (no sucralose), there is far less signal in cells exposed to sucralose. Therefore, loading cells with sucralose decreases cholesterol efflux and thus formation of HDL particles.
B. Membrane incubated with CTB to detect GM1. Trends are consistent with 9A.

Discussion/Conclusion

1. ABCA1 is absolutely essential for cholesterol efflux.
   - This project studies the factors involved in the RCT pathway, one of which is the transporter protein ABCA1. From Fig. 6, it is clear that efflux is signiﬁcantly reduced when ABCA1 is not upregulated (No CPT). An explanation for this is that ABCA1 interacts with apo-A1 and vice versa, so efflux is signiﬁcantly impaired without one or the other. The exact mechanisms by which apo-A1 and ABCA1 interact to trigger signal transduction pathways to HDL formation are to be further investigated.

2. Increasing cholesterol loading increases HDL biogenesis but decreases the content of GM1 in HDL (nascent HDL).
   - Increased cholesterol loading resulted in increased lipoprotein and thus HDL biogenesis, presumably due to increased efflux.
   - This is supported by Fig. 6C. Further understanding on the mechanisms by which HDL is made could lead to practical applications to accelerate RCT and HDL biogenesis and thus reduce the risk of CVD.
   - As predicted, GM1 decreases as a function of cholesterol efflux.
   - GM1 levels are lower in cells without ABCA1 upregulation, but higher with upregulation.
   - This implicates that ABCA1 may be involved in recruiting GM1 to the membrane.

3. Sucralose signiﬁcantly inhibits cholesterol efflux, and its effects are irreversible.
   - An explanation for this conclusion is that sucralose could be affecting the level of ABCA1. Previous experiments in the lab showed that the level of ABCA1 when exposed to sucralose was significantly reduced. This may be because of failure to upregulate ABCA1 or enhanced degradation of ABCA1.
   - Even more problematic is that despite the negative effects of sucralose on HDL biogenesis, the concentrations of sucralose used in this project were much higher than the levels found in the blood of people consuming sucralose. Thus, sucralose seems to be safe as a sweeter at least with regards to cardiovascular disease. At the same time, it gives us more leverage to explore RCT and HDL biogenesis.

References


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