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Lysosomal Cholesterol and Disease: Pharma Target or Decoy?

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Lysosomal storage disorders are characterized by excess accumulation of material in lysosomes. The accumulation occurs because of innate or acquired defects in the process of lysosomal hydrolysis. The disorders are most pronounced in cells of the mononuclear phagocytic system, such as macrophages, because these cells are specialized for taking up and degrading foreign material within lysosomes. In the best characterized lysosomal disorders the link between defect and dysfunction is related directly to defective lysosomal enzymes. More recently, however, it has been recognized that accumulation can occur as the result of secondary effects creating imbalances in cell homeostatic mechanisms [1; 2]. These acquired lysosomal defects are becoming recognized as mediators of a number of diseases. Most importantly, in many case the abnormal lysosomal accumulation eventually produces severe cell dysfunction followed by cell death. Recent work from our laboratory and others have highlighted the importance of lysosomes in diseases of cholesterol metabolism, such as Niemann-Pick type C (NPC) and atherosclerosis and suggest that excess cholesterol can produce an acquired lysosomal disorder [3].

To further study the effects of abnormal lipid accumulation on lysosome function and overall cell health, we studied macrophages maintained in tissue culture and incubated with various cholesterol-containing particles that mimic those found in pathologic conditions. Excess cholesterol accumulation within lysosomes inhibited lipolysis and proteolysis as evidenced by a lack of cholesteryl ester hydrolysis and the build up of undegraded cholesteryl ester and apolipoprotein B within lysosomes (Figure 1A). Using the dye lysososensor yellow-blue we found that cholesterol-engorged lysosomes failed to maintain the necessary acidic pH to support normal hydrolytic activity. We further discovered that the cholesterol content of the lysosome membrane modulates the activity of the vacuolar-type membrane ATPases (v-ATPase). These proton pumps are responsible for maintaining the acidic environment of lysosomes. We report that, in the presence of excess cholesterol, the v-ATPases become nonfunctional. The loss of pump activity corresponded to an increase in lysosomal membrane stiffness produced by excess cholesterol, suggesting that cholesterol-mediated alterations in membrane physical properties contributed to the loss of v-ATPase activity.

To further investigate both the mechanism of cholesterol-driven lysosome dysfunction and the consequences of the dysfunction, we treated cells with compounds that normally promote cholesterol clearance from cells. All the compounds tested promoted the rapid clearance of cytoplasmic cholesterol stores. However, the lysosomally sequestered cholesterol was not reduced even under the most extreme conditions. In contrast to the therapeutic compounds, triglyceride-containing particles (TRP) such as VLDL produced a 50% reduction in lysosomal cholesterol stores. Moreover, the

liberated cholesterol was channeled into cellular efflux pathways and was rapidly cleared from cells when appropriate cholesterol acceptors, such as HDL, were present in the culture medium (Figure 1B).

Our studies highlight the role of membrane cholesterol levels in modulating lysosome function and suggest a possible therapeutic target for patients with atherosclerosis, Niemann-Pick type C disease, Wolman's disease and other pathologies related to excess cellular sterol accumulation. Alternate therapies such as TRP-treatment could be particularly useful for patients who are refractory to current treatments for sterol accumulation diseases. However, hypertriglyceridemia is also risk factor for a number of diseases, so it remains to be seen whether specific strategies can be developed to modulate specific cellular triglyceride pools related to lysosome function without adversely increasing overall circulating levels of triglyceride.

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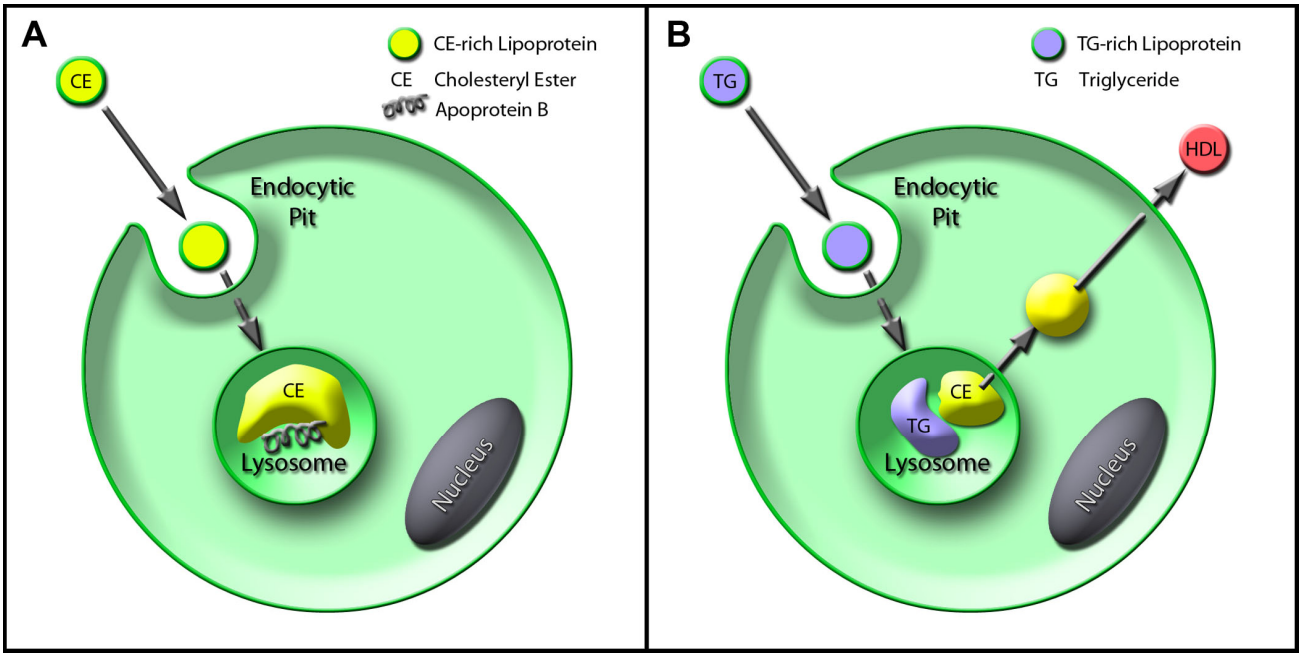

Figure 1 Summary of results: A) In the presence of cholesteryl ester-rich lipoproteins, excess unesterified cholesterol build up in lysosomes inhibits lysosomal hydrolysis and cholesteryl esters and protein accumulate. B) Incubation of cholesterol-loaded cells with triglyceride-rich lipoproteins results in a clearance of cellular stores of cholesterol; including the cholesteryl esters from lysosomes.

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