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LDL Isolation and Copper-Catalysed Oxidation

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Introduction

A characteristic feature of atherosclerotic lesions is macrophage-derived foam cells, filled with cholesteryl esters, in the intima. Recent studies have shown that macrophages take-up oxidatively-modified low density lipoprotein (LDL) (oxidized LDL) by means of the scavenger receptor and are converted into foam cells. Thus, it is important to understand how oxidized LDL is produced in the body.

Although there is increasing evidence that oxidized LDL occurs in the body, little is known about how the lipoproteins are oxidatively modified. ln this protocol we describe a method of preparation of oxidatively modified LDL using a copper salt *ex vivo*. Probucol, a lipid-soluble antioxidant which protects LDL from copper-induced modification, has been shown to inhibit atherosclerosis in hypercholesterolaemic animals. The sensitivity of the LDL towards copper-induced modification is, therefore, Iikely to correlate with its atherogenicity.

Protocol

Isolation of LDL [1].

1. Add EDTA.Na2 (pH 7.4, 0.25 M, 7.5 mL) to blood (500 mL).
2. Obtain 80 ml plasma fraction by low-speed centrifugation.
3. Add potassium bromide (density 1.019 9 mL^{-1}, 3.81 g).
4. Ultracentrifuge in Beckman Polyallomer centrifuge tubes at 100,000 g for 20 h at 4°C.
5. Slice tube and discard top fraction. Save bottom fraction (80 mL) and add KBr (5.27 g).
6. Ultracentrifuge in a Beckman Polyallomer centrifuge tube at 100,000 g for 20 h at 4°C.
7. Slice tube and save top fraction (10 mL; density 1.040 g mL^{-1}). Add KBr (density 1.063, 0.34 g). 8. Ultracentrifuge in a Beckman Polyallomer centrifuge tube at 100,000 g for 20 h at 4°C.
8. Slice tube and save top fraction (10 mL).
9. Dialyse against 12 L P8S for 48 h at 4°C (two changes of 6 L each).
10. Sterilize through a 0.45 µm filter.
11. Determine protein content by the Lowry method.

Preparation of Oxidized LDL

1. Dissolve LDL (5 mg) in P8S (1 mL).
2. Add P8S (1 mL) containing $CuSO_4$ (10 µM).
3. Transfer to 3.5 cm sterile plastic culture dish and incubate for up to 48 h at 37°C.
4. Dialyse against 12 L P8S for 48 h at 4°C (two changes of 6 L each).
5. Determine protein content by the Lowry method [2].

Measurement of LDL Thiobarbituric Acid-Reactive Substances (TBARS) in Oxidized LDL

1. Add LDL (50 µg) to NaCI (150 mM, 1.5 mL) in a 13×10 cm culture tube.
2. Add TCA (20% w/v, 0.5 mL) and T8A reagent (0.5 mL).
3. Boil at 95°C for 1 h.
4. Cool with tap water.
5. Add butan-1-ol (2.0 mL) and vortex mix vigorously.
6. Centrifuge at 4,000 g for 15 min.
7. Remove top layer and measure fluorescence (excitation at 515 nm, emission at 550 nm) [2].

References

1. Goldstein JL, Basu SK, Brown MS (1983) Receptor-mediated endocytosis of low-density lipoprotein in cultured cells. Methods Enzymol 98: 241-260.
2. Yokode M, Kita T, Kikawa Y, Ogorochi T, Narumiya S, et al. (1988) Stimulated arachidonate metabolism during foam cell transformation of mouse peritoneal macrophages with oxidized low density lipoprotein. J Clin Invest 81: 720-729.

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