KALEN Human OxLDL™

Technical Note 1

Evaluation of the Stability of KALEN Human OxLDL

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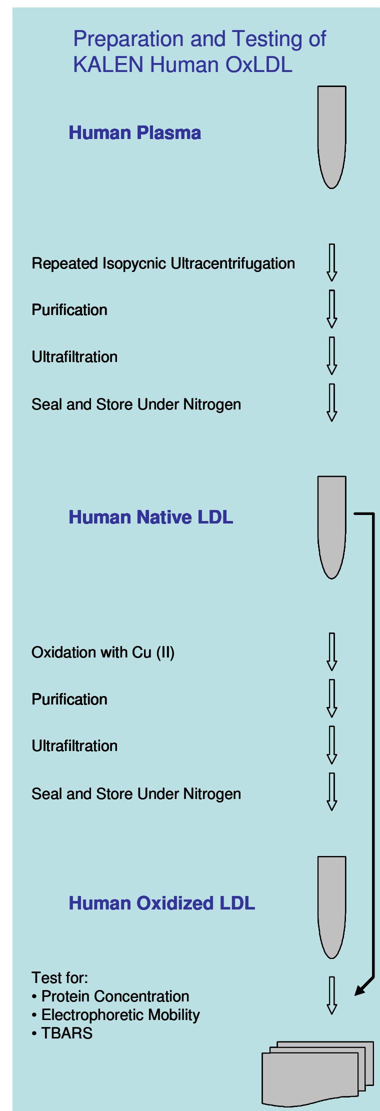
Evaluation of Stability

Introduction

For years, the importance of oxidized low density lipoprotein (LDL) as a factor in the development of atherogenesis has been recognized (1, 2, 3, and 4), and the presence of oxidized LDL has been described in artherosclerotic lesions of humans and rabbits (5). The oxidized form of LDL binds to arterial wall smooth muscle extracellular matrix with 15-45 fold greater binding capacity than native LDL (6). When stimulated with oxidized LDL, monocytes are induced to differentiate, become adherent, and mature into macrophages (7). Macrophages interact with oxidized lipoproteins, becoming cholesterol-filled foam cells leading to atherosclerotic plaque formation (8, 9).

Although of significant research interest, oxidized LDL is a relatively labile particle, a key factor in its commercial availability. Therefore, research into the role of oxidized LDL in atherosclerosis would be facilitated by the development of a more stable reagent for cardiovascular research. With a stable reagent, research laboratories would be free to schedule experiments according to their needs, not those dictated by a supplier's production schedule. Furthermore, multi-month stability would allow for multiple experiments over time from the same lot of material.

We report herein on the stability of oxidized LDL produced by Kalen Biomedical, LLC. Kalen has optimized the production of LDL and oxidized LDL to reduce the number of required manipulations and minimize handling of the material. This has resulted in a reduced production time and a gentler treatment of the lipoprotein. The purified product, stored aseptically, has been found to be stable as shown by agarose gel electrophoresis, protein concentration, and content of oxidized lipids.



Methods

LDL with a density of 1.019 – 1.063 g/mL was prepared from human plasma by serial isopycnic ultracentrifugation in potassium bromide (10-12). Purity was assessed by agarose gel electrophoresis. All products are produced from human plasma tested negative for Hepatitis C, HIV-I and HIV–II antibodies as well as Hepatitis B surface antigen. Native LDL was oxidized with 20 µM copper (II) sulfate at 37°C in the presence of oxygen (13). The protein concentration of the oxidized material, in a solution with 0.9% NaCl and 0.01% EDTA at pH 7.2, was then adjusted to 1.2 mg/mL. Subsequently, the material was ultrafiltered, aliquoted into vials, and stored under nitrogen at 4°C. The purity of oxidized LDL was assessed by agarose gel electrophoresis. The level of oxidation was determined by the thiobarbituric acid reactive substance (TBARS) assay. See figure at right.

The electrophoretic mobility of oxidized LDL was determined by agarose gel electrophoresis in sodium barbital buffer. (Titan Gel Lipoprotein Kit 3045, Helena Laboratories, Beaumont TX). Lipoprotein was visualized by staining with Fat Red 7B. The electrophoretic mobility of oxidized LDL relative to native LDL (REM) was calculated by comparing the migration of oxidized LDL (in mm) to that of a native LDL reference; the distance that oxidized LDL migrated was divided by the migration of the reference to calculate the REM.

Protein concentration was determined with the BCA Protein Assay Kit (Cat. 23227, Pierce, Rockford, IL) with bovine serum albumin as the reference. Lipid peroxidation has commonly been estimated by the

measurement of thiobarbituric acid reactive substances (TBARS) (14, 15). These reactive substances are produced by the oxidation and subsequent breakdown of poly-unsaturated fatty acids in the lipid moiety of the LDL particle. TBARS were measured using the TBARS Assay Kit (Cat No. 10009055, Cayman Chemical Company, Ann Arbor MI).

Results

The data presented below were derived using a single preparation of KALEN Human OxLDL studied over a period of 15 weeks. Initial characterization of the sample was performed with a set of three assays: REM, protein concentration, and TBARS. Assays were not performed on a predetermined time schedule but were carried out as the preparation was used as a reference control for the assays of other samples.

The results of studies assessing the stability of KALEN Human OxLDL using REM are outlined in Table 1. The data indicate that the REM of oxidized LDL stored for up to 15 weeks did not change significantly. In each gel, the sample of oxidized LDL appeared as a single band with a REM to native LDL of 3.1-3.2, indicating that the oxidized material had significantly greater mobility in the gel. Spiking experiments indicated that contamination with another component would be readily apparent at about 2%.

Table 1. Relative electrophoretic mobility of KALEN Human OxLDL stored at 4°C.

Weeks of Storage	REM
1	3.2
3	3.1
6	3.2
8	3.2
15	3.2

The data in Figure 1 depict the results of determination of protein concentration in KALEN Human OxLDL stored at 4°C. The concentration of protein in the material ranged from 1.0 to 1.4 mg/mL with the protein concentration at week one being 1.2 mg/mL.

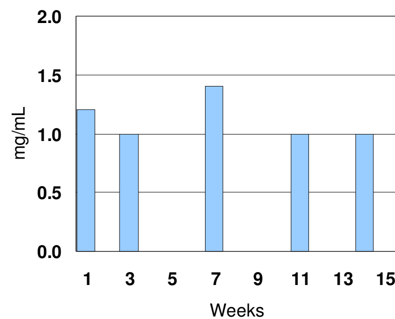


Figure 1. Protein concentration of KALEN Human OxLDL stored at 4°C.

As shown in Figure 2, the results of the TBARS assay for KALEN OxLDL stored at 4°C for up to 14 weeks confirm the stability of the material. TBARS values ranged from 87% to 114% of the mean with the lowest value at week 9 and the highest at week 12.

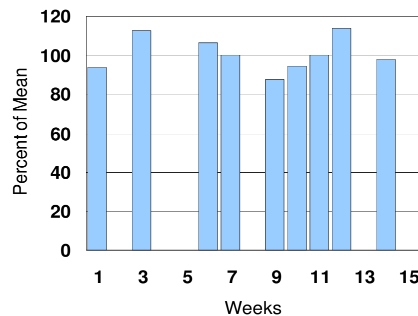


Figure 2. TBARS assay results for KALEN Human OxLDL stored at 4°C.

Discussion

The results presented herein indicate that KALEN Human OxLDL is stabile for at least three months, a significant improvement over other commercially available oxidized LDL preparations. When subjected to agarose gel electrophoresis the material, stored at 4°C for up to 14 weeks, migrates as a single band with an electrophoretic mobility greater than native LDL as has been previously reported (16). This indicates a reduction in the overall positive charge of the LDL particle caused by the oxidation and breakdown of the lipid and protein components of LDL. Subsequent alterations of the single band in the electrophoretic pattern or changes in the REM would be indicative of further alterations and instability of the oxidized LDL (17). No breakdown of KALEN Human OxLDL was observed during storage for over 3 months as determined by agarose gel electrophoresis.

Studies of protein concentration and TBARS assays confirm the stability of KALEN Human OxLDL. The protein concentration was stable with no loss of material during the storage period. Further, very little aggregation or precipitation of the KALEN Human OxLDL was seen, in contrast with some competitive products. Rather, the stability of TBARS level indicates that the oxidation and subsequent lipid breakdown was completed during the manufacturing process. During storage, there is no further breakdown of oxidized lipids to change the composition of atherogenic compounds or the chemical properties of the product.

The greater stability of KALEN Human OxLDL relative to that of other commercially available material is likely attributable to gentle processing, reduced handling, optimized production procedures, purity of the material used, and storage conditions for the final product. In conclusion, the availability of this material should facilitate research into the cardiovascular pathogenesis of oxidized LDL by providing a reliable source of stable material.

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Ordering Information

Catalog Number	Description	Size
770202-7	KALEN OxLDL	1 mg
770202-4	KALEN OxLDL	5 mg

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