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Original Article

Effect of Ubiquinol-10 on the Affinity of LDL to Its Receptor: A Model for Prevention of Atherogenesis

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Abstract

The affinity of low density lipoprotein(LDL) to its receptor is very important, because most of LDL-uptake pathway is done by the LDL receptor and the change in size of LDL particle and the modification in its components may affect the LDL affinity for its receptor.

In this study, the effects of a powerful lipid-soluble antioxidant "ubiquinol-10" have been investigated on the affinity of LDL to its receptor. LDL receptor was purified of bovine adrenal tissue. LDL was isolated by sequential density ultracentrifugation from normolipidemic human plasma. Then, LDL was labeled with fluoresein isothiocyanate (FITC) at 4°C for 24 h. Native LDL was incubated with various concentrations of ubiquinol-10 for 2 h. Finally, native LDL(treated with ubiquinol-10) was incubatd with the LDL receptor in the presence of labeled-LDL at 37°C for 30 min. After incubation, the medium was centrifuged at 4000×g for 20 min and the fluorescence intensity(FI) of supernatant from each sample was determined at excitation=495 nm and emission=515 nm. The elevation of FI in each fraction demonstrates increasing the affinity of non-labeled-LDL to its receptor.

Our results showed that ubiquinol-10 increased the affinity of LDL to its receptor, and at the concentration of 200 μM it had the greatest effect.

These finding raise the possibility that ubiquinol-10 may decrease the effect of LDL in formation of atherosclerotic lesions.

Keywords: LDL; LDL receptor; Ubiquinol-10.

Introduction

It is now well established from previous studies that the majority of serum LDL is catabolized through the LDL receptor (1, 2). Studies have shown that the tissues such as adrenal and liver have numerous of this receptor (3). The LDL receptor regulates the concentration of plasma LDL and cholesterol. The role of LDL receptor is to provide cholesterol to cells throughout the body and deliver excess cholesterol to liver for recycling or excretion.

The modification of the lipid and apoprotein components of LDL occur in the arterial walls. The LDL particles differ in size and composition. The larger particles have the greater receptor affinity than the smaller and dense particles (4). On the other hand, the dense LDL particles have higher susceptibility to the modificative reaction such as oxidation and glycation (5).

We postulated that incubation of lypophilic agent with LDL may increase the affinity of LDL to interact with the LDL receptor. To test this hypothesis, I used ubiquinol-10. This compound is highly lipophilic and operative in membranes

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or lipoproteins. It can enter the LDL particles and probably increase their size (6). Ubiquinol-10, the reduced form of ubiquinone-10 (coenzyme Q_{10}), is a well-known proton-electron carrier in inner mitochondria membrane and a potent lipophilic antioxidant in different cell membranes and LDL. It is well established that both uboquinols and ubiquinones are active against lipid peroxidation in mitochondria and liposomes but that the quinols are much more powerful antioxidants than the corresponding quinines (7). Recently, we reported that ubiquinol-10 can protect human LDL more efficiently against lipid peroxidation than vitamin E (8). The prominent antioxidant activity of ubiquinol-10 in human LDL was first reported by Stocker *et al* (9). Also, it has been already suggested, that the ubiquinol-10 level may represent a surrogate measure of some other LDL property affecting its oxidizability, for example its initial lipid hydroperoxide content (10).

Much less is known about the action of lipophilic antioxidants on the affinity of LDL to its receptor since most previous have been performed on the LDL oxidation. Taber *et al* reported (6) that vitamin E increased the uptake of LDL via the high affinity receptor. In other study, we also showed that some antioxidants increased affinity of LDL to its receptor (11).

In this study, we investigated the effect of ubiquinol-10 on the affinity of native LDL to its receptor.

Experimental

Animals

The fresh adrenal glands were obtained from male and female bovine (200-300 kg). Immediately after steers were slaughtered, 30 adrenal glands were placed in ice-cold 0.15 M NaCl.

Chemicals

Ubiquinol-10 and other reagents were purchased from Sigma (Deisenhofen, Germany) or Merck (Darmstadt, Germany).

LDL isolation

LDL (density 1.019-1.063 g/ml) was isolated from fresh normolipidemic human plasma by sequential ultracentrifugation, and dialyzed extensively against phosphate-buffered saline (PBS) with 0.01% ethylenediamidinetetraacetic acid (EDTA) (12). The purified LDL was examined by electrophoresis carried out at pH 8.6 in 0.05 M barbital buffer on 0.8% agarose gel. Gels were stained with Sudan Black B stain (13). The LDL suspension was stored at 4 °C under argon in the dark within 4 weeks. LDL was used for experiments within 10 days.

Bovine LDL receptor purification

Procedures for isolation and purification the bovine LDL receptor have been established by Schneider *et al* (14). Purification of LDL receptor was carried out from bovine adrenal glands using a combination of DEAE-cellulose (DE 52, Whatman) chromatography, LDL-Sepharose 4B (Pharmacia) chromatography and preparative SDS/polyacrylamide-gel electrophoresis.

Labeled-LDL preparation

The LDL suspension was made fluorescent by labeling with FITC. First, 0.1% solution of FITC in 50 mM bicarbonate sodium buffer (pH = 8.5) was prepared. Then, aliquots of LDL (at a protein concentration of 1 mg/ml) was incubated with 1 vol of FITC-buffer for one day at 4 °C, and loaded onto a (Sephadex G-25) column (PD-10, Pharmacia Uppsala, Sweden) and eluted with 0.15 M NaCl. In alkaloid medium, FITC was covalently coupled to lysine and arginine residues of the LDL particle (15). FITC-LDL subfractions (1 ml) were collected and stored at 4 °C in the dark.

Incubation of ubiquinol-10 with native LDL

Ubiquinol-10 was added to LDL prior to incubation as concentrated stock solutions in dimethylsulfoxide (DMSO). Concentrations of this compound in the stock solutions were 50, 100 and 200 μM. Immediately before addition of them, EDTA was removed from the LDL suspension by gel chromatography on Sephadex PD-10 columns (Phamacia Uppsala, Sweden). The suspension was incubated for 2 h at 37 °C to achieve an incorporation of the compound into LDL (16). No special plastic or glass was used to avoid adsorption of the compound on the

Table 1. The effects of various concentrations of ubiquinol-10 on affinity of LDL to its receptor.

Concentration (μM)	Fluorescence intensity (mean ± SD)[a]
0	350 ± 12
50	422 ± 7.1*
100	507 ± 8.7*
200	602 ± 10*

*P < 0.05 (n=5) , significantly differ from control (Fisher's test).
[a] Values are given as mean ± SD.

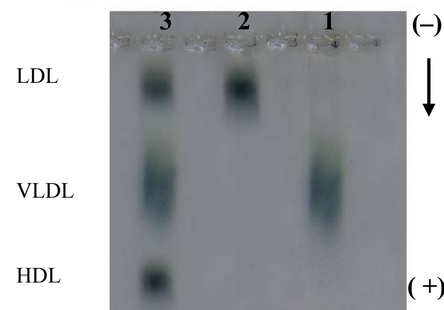

Figure 1. Electrophortic analysis of VLDL (Lane 1), LDL fraction (Lane 2) and plasma (Lane 3) on 0.8% agarose gel. Values are given as mean ± SD of triplicated determinations.

vessels. No LDL precipitation was observed as a result of its addition.

Affinity of LDL to its receptor

Competitive studies between LDL subfractions for binding to the LDL receptor were performed according to the Goldstein and Brown (17). After incubation of native LDL with various concentration of ubiquinol-10, the LDL suspension was added to the LDL receptor and labeled-LDL at 37°C for 30 min, and then the medium was centrifuged at 4000×g for 20 min and supernatant was isolated and intensity of the fluorescence was measured with an excitation of 495 nm and emission at 515 nm. The fluorescence intensity is directly related to non-labeled LDL binding to the receptor.

Statistical analysis

All results were presented as mean ± standard deviations (n=5). All p-values of less than 0.05 were considered statistically significant.

Results and Discussion

The isolation of LDL was confirmed by agarose gel electrophoresis (Figure 1B). This figureure shows that separated fractions (Lane 2 and Lane 3) according to LDL and VLDL bands in plasma, as control (Figure 1B, Lane 1).Incubation of the LDL suspension with ubiquinol-10 for 2 h at 37 °C resulted in LDL antioxidant enrichment and increased the affinity of LDL to its receptor. Of course, all these depended on agent concentration in medium. The effects of various concentrations of this compound on the affinity of native LDL to its receptor are shown in Table 1. As shown in this table, 200 μM had the greatest effect on the increasing affinity of LDL to its receptor (72%). It is clear that the intensity of the fluorescence is proportional to non-labeled LDL binding to the receptor. This means that the more LDL binding to the receptor than the labeled LDL is excluded in the medium and participates in the measurement. The concentrations of volatile oils chosen for this study was obtained from our previous dose-response studies and published data and is equivalent to values in the upper end of the reference range (8, 11).

Numerous studies reported that plasma LDL is a heterogeneous collection of particles that vary in size (17), density (18), composition (19), and electrical charge (20). Some LDL forms are more atherogenic than the others (21). Cohn *et al* (22) repoted that in the presence of lipophilic antioxidants such as vitamin E the uptake of LDL was increased by LDL receptor. Also, Sakuma *et al* (23) showed that alpha-tocopherol and ubiquinol-10 protected the modified LDL to be recognized by LDL receptor. Ubiquinol-10 is known to be a highly efficient lipid-soluble antioxidant in protection of lipids in a variety of biological and model systems including cell membranes, LDL and liposomes. It is generally assumed that the quinol may exhibit its protective effect by preventing a formation of lipid free radicals and/or by eliminating them. On the other hand, the possibility that the increasing affinity of LDL to its receptor by ubiquinol-10 is related to its interactions on the lipid structure of the LDL particle needs further investigation (24). Comparing with other lipophilic antioxidants,

ubiquinol-10 is one of the most efficients. It contains shielding methyl groups adjacent to the phenolic hydroxyl group and it is optimally positioned in the LDL particle by its phytyl side-chain. In addition, it protects LDL against oxidative modification, maintaining its ability to act as a ligand for LDL receptors (25).

Moreover, in vivo supplementation of LDL with ubiquinol-10 is known to decrease LDL susceptibility to modification and to increase LDL clearance in the plasma (8, 22). A decreased level of total ubiquinol is found in plasma of patients with atherosclerosis as well as numerous examples of successful treatment with ubiquinol-10 of some pathologies raise the possibility that ubiquinol-10 may decrease the effect of LDL in formation of atherosclerotic lesions (16).

Incubation of LDL suspension with ubiquinol-10 for 2 h may enrich the LDL particles sufficiently to increase their affinity to their receptors.

In summary, we have demonstrated that ubiquinol-10 has the positive effect on the affinity of native LDL to its receptor. Our findings are important because the uptake of LDL by its receptor decreases the modification of LDL and prevents formation of atherosclerosis.

References

(1) Feilding J and Christopher J. Lipoprotein receptors, plasma cholesterol metabolism and the regulation of cellular free cholesterol concentration. *FASEB J.* (1992) 6: 3162-3168

(2) Brown MS, Kovanen PT and Goldstein JL. How LDL receptor influence cholesterol and atherosclerosis. *Sci. Am.* (1984) 251: 58-65

(3) Saxena K and Shipley GG. Structural studies of detergent solubilized and vesicle reconstituted LDL receptor. *Biochemistry* (1997) 36: 15940-15948

(4) Krauss RM. Heterogeneity of plasma low density lipoproteins and atherosclerosis risk. *Curr. Opin. Lipidol.* (1994) 5: 339-349

(5) Leake DS. Oxidized low density lipoproteins and atherogenesis. *Br. Heart J.* (1993) 69: 476-478

(6) Traber MG and Kayden HJ. Vitamin E is delivered to cells via the high affinity receptor for low density lipoprotein. *Am. J. Clin. Nutr.* (1984) 40: 747-751

(7) Fremant L, Belguendouz L and Delpal S. Antioxidant activity of resveratrol and alcohol-free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids. *Life Sci.* (1999) 64: 2511-2521

(8) Safari MR, Rezaei M and Taherkhani H. Effects of ubiquinol-10 and β-carotene on the *in vitro* susceptibility of low-density lipoprotein to copper-induced oxidation. *Med. J. Islamic Rep. Iran* (2005) 19: 169-174

(9) Stocker R, Bowry VW and Frei B. Ubiquinol-10 protects human LDL more efficiently against lipid peroxidation than does α-tocopherol. *Proc. Natl. Acad. Sci. USA* (1991) 88: 1646-1651

(10) Tribble DL, Vander-Berg JJM, Motchnik PA, Ames B, Lewis DM and Chait A. Oxidative susceptibility of LDL subfractions is related to their ubiquinol-10 and α-tocopherol content. *Proc. Natl. Acad. Sci. USA* (1994) 91: 1183-1187

(11) Safari MR, Taherkhani H, Ani M, Naderi GA and Asgary S. The effects of lipophilic antioxidants on the affinity of LDL to its receptor. *Iranian Biomed. J.* (2002) 6: 111-116

(12) Murakami M, Horiuchi S, Takata K and Morino Y. Distinction in the mode of receptor-mediated endocytosis between high density lipoprotein and acetylated high density lipoprotein: evidence for high density lipoprotein receptor-mediated cholesterol transfer. *J. Biochem. (Tokyo)* (1987) 1729-1741

(13) Noble RP. Electrophoretic separation of plasma lipoproteins in agarose gel. *J. Lipid Res.* (1968) 9: 963-701

(14) Schneider WJ, Goldstein JL and Brown MS. Purification of the LDL receptor. *Meth. Enzymol.* (1982) 109: 405-417

(15) Feurstein DL and Sellek RE. The chemical stability of fluoresein under normal fluorometric condition. *J. Sanit. Eng. Div. Am. Soc.* (1965) 89: 1-11

(16) Kontush A, Hubner C, Finckh B, Kohlschutter A and Beisiegel U. Antioxidative activity of ubiquinol-10 at physiologic concentrations in human low density lipoprotein. *Biochem. Biophys. Acta* (1995) 1258: 177-187

(17) Goldstein JL and Brown MS. Binding and degradation of low density lipoproteins by cultured human fibroblasts. *J. Biol. Chem.* (1974) 249: 5153-5162

(18) Shen MS, Krauss RM, Lindgren FT and Forte TM. Heterogeneity of serum low density lipoproteins in normal human subjects. *J. Lipid Res.* (1981) 22: 236-244

(19) Nelson CA and Morris MD. The ultracentrifugal heterogeneity of serum low density lipoproteins in normal humans. *Biochem. Med.* (1977) 18: 1-8

(20) Kirchhausen TG, Fless G and Scanu AM. The structure of plasma low density lipoproteins: experimental facts and interpretation-a minireview. *Lipids* (1980) 15: 464-467

(21) Rudel LL, Parks JS, Johnson FL and Babiak J. Low density lipoproteins in atherosclerosis. *J. Lipid Res.* (1986) 27: 465-474

(22) Cohn W and Kuhn H. The role of the low density lipoprotein receptor for alpha-tocopherol delivery to tissues. *Ann. N. Y. Acad. Sci.* (1989) 570: 61-71

(23) Sakuma N, Yosikawa M, Hibino T, Okada M, Jinno Y and Tamai N. Alpha-tocopherol and ubiquinol-10

protected the peroxidative modification of LDL to be recognized by LDL receptors. *J. Nutr. Sci. Vitaminol (Tokyo)* (1998) 44: 697-703
(24) Giugliano D. Dietary antioxidants for cardiovascular prevention. *Nutr. Metab. Cardiovasc. Dis.* (2000) 10: 38-44
(25) Thomas SR, Leichtweis SB, Pottersson K, Crft KD, Mori TA and Brown AJ. Dietary cosupplementation with vitamin E and coenzyme Q10 inhibit atherosclerosis in apoprotein E gene knockout mice. *Aterioscler. Thromb. Vasc. Biol.* (2001) 21: 585-593

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