The Effects of Lipophilic Antioxidants 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 affects the LDL affinity for its receptor. In this study, the effects of lipophilic agents such as vitamin E and seven volatile oils: anethol, eugenol, geraniol, limonene, linalool, pulegone and thymol 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 each of the volatile oils and vitamin E for 2 h. Finally, the native LDL treated with volatile oils and vitamin E 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. We showed that vitamin E and volatile oils increased the affinity of LDL to its receptor, and among these compounds, vitamin E and thymol are the best agents that increase the affinity of native LDL to its receptor. The effects of these compounds are as follows: vitamin E > thymol > eugenol > anethol > geraniol > linalool > limonene > pulegone. These findings raise the possibility that vitamin E and some of the volatile oils may decrease the effect of LDL in formation of atherosclerotic lesions. *Iran. Biomed. J. 6 (4): 111-115, 2002*

INTRODUCTION

It is now well-established from clearance studies that the majority of serum LDL is catabolized through the LDL receptor [1-3]. Tissues such as adrenal and liver have numerous LDL receptor [4] that 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 occurs in the arterial walls [5]. The LDL particles differ in size and composition. The larger particles have the greater receptor affinity than the smaller and dense particles [6, 7]. Also, the dense LDL particles have been displayed the modificative reaction such as oxidation and glycation [8, 9].

We postulated that the incubation of lypophilic agents with LDL might increase the affinity of LDL to interact with the LDL receptor. To test this hypothesis, we used vitamin E and volatile oils. All these compounds are highly lipophilic and operative in membranes or lipoproteins. They can enter the LDL particles and probably increase their size [10].

Vitamin E is thought to be the major non-enzymatic antioxidant present in the lipid structures of cells and lipoproteins. It is a donor anti-oxidant (reductant) that appears to react with peroxyl radicals to inhibit the propagation cycle of lipid peroxidation. In lipid solutions and dispersions, vitamin E inhibits radical formation linearly with time until consumed in the process [11]. Volatile

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oils are bioactive substances in plants and therefore potentially present in human plasma in a diet-dependent concentration. A number of these compounds acts as antioxidants with mechanisms involving both free radical scavenging and metal chelation [12, 13].

Much less is known about the action of vitamin E and volatile oils on the affinity of LDL to its receptor, since most previous studies have been performed on the LDL oxidation. Traber and Kayden [10] reported that vitamin E increased the uptake of LDL via the high affinity receptor. Also, Naidu and Thippeswamy [14] and Rajalakshmi *et al.* [15] reported that some of the volatiole oils such as eugenol were effective anti-oxidants and protected the oxidation of human LDL to be recognized by LDL receptors.

In this study, we investigated and comprised the effects of vitamin E and seven volatile oils on the affinity of native LDL to its receptor.

MATERIALS AND METHODS

Adrenal glands. 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. Vitamin E, volatile oils and other reagents were purchased from Sigma (Deisenhofen, Germany) or Merck (Darmstadt, Germany).

Isolation of LDL. LDL (density 1.019-1.063 g/ml) was isolated from fresh normolipidemic human plasma by sequential ultracentrifugation, and dialyzed extensively against PBS containing 0.01% Ethylenediamidinetetraacetic acid (EDTA) [16]. The LDL suspension was stored at 4°C under argon in the dark. LDL was used for experiments within 10 days.

Purification of bovine LDL receptor. Procedures for isolating and purifying the bovine LDL receptor have been established by Schneider *et al.* [17]. Purification of LDL receptor was carried out from bovine adrenal glands using a combination of DEAE-Cellulose (DE 52, Whatman) chromato-graphy, LDL-Sepharose 4B (Pharmacia) chromato-graphy and preparative SDS-PAGE.

The labeled-LDL preparation. The LDL suspension was made fluorescent by labeling with FITC. First, 0.1% 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 volume of FITC-buffer for a day at 4°C, and loaded onto a column of Sephadex G-25 (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 [18]. FITC-LDL subfractions (1 ml) were collected and stored in the dark at 4°C.

Incubation of vitamin E and volatile oils with the native LDL. Vitamin E and seven volatile oils including "anethol, eugenol, geraniol, limonene, linalool, pulegone and thymol" were added to LDL prior to incubation as concentrated stock solutions in DMSO. Concentrations of these compounds in the stock solutions were 50, 100 and 200 µM. Immediately before addition, EDTA was removed from the LDL suspension by gel chromatography on Sephadex PD-10 columns. The suspension was incubated at 37°C for 2 h to achieve an incorporation of the compounds into LDL [19]. No special plastic or glass was used to avoid adsorption of the compounds on the vessels. No LDL precipitation was observed.

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 [20]. After the incubation of native LDL with various concentrations of vitamin E and volatile oils, the LDL suspension was added to the LDL receptor and labeled LDL at 37°C for 30 min. Then the medium was centrifuged at 4000 ×g for 20 min and the supernatant was isolated and the intensity of the fluorescence was measured with an excitation of 495nm and emission at 515nm. The fluorescence intensity is directly related to non-labeled LDL binding.

Statistical analysis. All results are presented as mean ± standard deviations (n = 5). *P<0.05* was considered statistically significant.

RESULTS

Incubation of the LDL suspension with vitamin E and volatiole oils at 37°C for 2 h resulted in LDL antioxidants enrichment and increased the affinity of LDL to its receptor. Of course, all these depended on agent concentration in medium.

Table 1. The effects of various concentrations of vitamin E, anethol, eugenol, geraniol, limonene, linalool, pulegone and thymol on affinity of LDL to its receptor.

Concentration (μM)	FI (Mean ± SD)							
	Vitamin E	Anethol	Eugenol	Geraniol	Limonene	Linalool	Pulegone	Thymol
0	350 ± 12.0	350.0 ± 12.0	350 ± 12.0	350.0 ± 12.0	350 ± 12.0	350 ± 12.0	350 ± 12.0	350 ± 12.0
50	395 ± 15.5*	381.6 ± 11.0*	390 ± 17.0*	391.2 ± 9.5*	369 ± 11.0	380 ± 18.0*	352 ± 6.2	400 ± 11.0*
100	507 ± 8.7*	418.6 ± 13.0*	500 ± 5.5*	402.5 ± 10.0*	389 ± 9.6*	404 ± 10.0*	358 ± 2.2	504 ± 3.3*
200	602 ± 10.0*	514.0 ± 9.8*	558 ± 13.0*	459.0 ± 16.0*	422 ± 7.7*	420 ± 5.5*	388 ± 5.9*	570 ± 10.5*

*$P<0.05$ (n = 5)

The effects of these agents on the affinity native LDL to its receptor are shown in Table 1. As shown in this Table, vitamin E had the greatest effect on the increasing affinity of LDL to its receptor (72%). Among the volatile oils used, "Thymol" had the highest affinity effect (63%) and the rest are as follow: vitamin E > thymol > eugenol > anethol > geraniol > linalool > limonene > pulegone.

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.

DISCUSSION

Numerous studies have demonstrated that plasma LDL is a heterogeneous collection of particles that varies in size [20], density [21], composition [22], and electrical charge [23]. Some LDL forms are more atherogenic than other [24, 25]. Our results show that vitamin E and volatile oils added to the suspension of human LDL increased the affinity of LDL to its receptor and the uptake of LDL. The effects of these compounds depend on concentration. Cohn and Kuhn [26] repoted that in the presence of vitamin E, the uptake of LDL was increased by LDL receptor. Also, Sakuma *et al.* [27] showed that alpha-tocopherol protects the modification of LDL to be recognized by LDL receptor.

Vitamin E is known to be a highly efficient lipid-soluble antioxidant in the protection of lipids in a variety of biological and model systems including cell membranes, LDL and liposomes [28, 29]. It is generally assumed that vitamin E 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 inhibition of LDL modification such as oxidation or glycation by vitamin E is related to its interactions on the lipid structure of the LDL particle needs further investigation. Although vitamin E is the most abundant antioxidant in LDL, its concentration in LDL does not appear to be the sole determinant of the oxidative modification of LDL [30]. Hence, the present study was not undertaken to test the inhibitory effect of vitamin E against LDL oxidation, since it is also present in the LDL particle and the concentrations could easily manipulated by dietary supplementation. In comparison with other lipophilic antioxidants, vitamin E is the most efficient anti-oxidants in the lipid phase. 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, vitamin E protects LDL against peroxidative modification, maintaining its ability to act as a ligand for LDL receptors [27]. Also, LDL is delivered to cells via the high affinity receptor for vitamin E [10].

Like vitamin E, the antioxidant properties of volatile oils have been investigated *in vivo* and *in vitro* in various studies. Crawford *et al.* [31] reported that dietary lipophilic antioxidants inhibited both LDL oxidation and atherogenesis in animals. Teissedre and Waterhouse [32] also showed that phenolic substances in different essential oils varieties inhibited oxidation of LDL. The findings of Grassmann *et al.* [33] showed that lemon oil and its components inhibited the oxidation of LDL by copper ions. Wilcox *et al.* [34] reported that the citrus volatile oils had the hypocholesterolemic properties and enhanced the expression of the LDL receptor and the LDL clearance. Volatile oils, contain a number of phenolic substances, are present in fruits and vegetables. These agents have highly lipophilic properties and are incorporated into hydrophobic region of the membrane and the lipoprotein particle. All volatile oils used, have phenolic hydroxyl groups, which are considered to be necessary for antioxidant activity. Among them, thymol was the best compound that increases the affinity of LDL to its receptor. It contains two phenolic hydroxyl groups. This may be one reason for the more marked effects of this volatile oil. The incubation

of the LDL suspension with volatile oil for 2 h may enrich the LDL particles sufficiently to increase their affinity to the receptors.

In summary, we have demonstrated that vitamin E and some of the volatile oils have the positive effect on the affinity of native LDL to its receptor. These findings are important because of the uptake of LDL by its receptor decreases the modification of LDL and prevents the formation of atherosclerosis.

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