Antioxidative Role of Geraniin in Lipid Peroxidation of Human LDL

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Growing evidence indicates that oxidized low density lipoprotein (LDL) may promote atherogenesis. Therefore, inhibition of LDL oxidation may impede this process. The effect of geraniin on the susceptibility of human low density lipoprotein (LDL) to macrophages-induced oxidation was investigated by monitoring a thiobarbiruric acid reactive substrance (TBARS). The antioxidative activity of geraniin was higher than that of α-tocopherol on low density lipoprotein (LDL) oxidation by thiobarbituric acid reactive substance (TBARS). Geraniin inhibited the Cu^{2+} mediated oxidation of human LDL in a dose dependent manner at concentration of 50 and 100 μg/mL. Geraniin, almost completely inhibited the macrophages mediated LDL oxidation in electrophoretic mobility and conjugate diene of LDL oxidation. Also, geraniin almost completely inhibited O_2^- at concentration of 100 μg/mL. The physiological relevance of the antioxidative activity was validated at the cellular level where geraniin inhibited endothelial cell mediated LDL oxidation, When compound with several other antioxidants geraniin showed a high activity equal to natural antioxidants, α-tocopherol and ascorbic acid, and the synthetic antioxidant, probucol. These results indicate that geraniin might play a protective antioxidant effects on LDL, probably affecting both the structural properties of macrophage and endothelial cell for the LDL oxidation.

Key words – Low density lipoprotein (LDL), antioxidant, geraniin

An increased concentration of low density lipoprotein (LDL) cholesterol is considered a key event in the early development of atherosclerosis[23].

There are several lines of evidence for the existence of oxidatively modified LDL in early atherosclerotic lesions [21,28]. LDL is known to contain various antioxidative factors such as α-tocopherol, β-carotene and lycopene[15]. It has been reported that α-tocopherol in LDL can preserve endogenous antioxidants in LDL and delay the onset of lipid peroxidation of LDL *in vitro*[27]. It may be possible that other naturally occurring antioxidants directly or indirectly inhibit the oxidation of LDL. Hence, the importance of LDL associated antioxidants in the prevention of LDL oxidation and thereby the possible prevention of the formation of atherosclerotic plaques becomes rather evident[24,25]. The role of antioxidants would therefore be significant, if oxidatively modified LDL contributes to atherogenesis. Therefore, inhibition of LDL oxidation has been suggested as an approach to impede atherogenesis.

In fact, the French paradox has been attributed to the regular consumption of red wine, and more specifically, to the high phenolic compound content of the wine[6,22]. Furthermore, a high dietary intake of phenolic compound has been associated with a decreased risk of developing cardiovascular diseases[11]. If oxidized LDL is crucial to atherogenesis, the potential role of antioxidants in the prevention of the oxidative modification of LDL assumes great importance. A class of compounds has recently been identified that appears to work by either mimicking or enhancing lipid oxidation action. There is growing interest in understanding the role and mechanisms of phenolic compounds as inhibitors of deterious oxidative processes, particularly cancer and atherosclerosis[14]. Phenolic compounds are also present in human diet in representative amounts. Particular attention has been given to the antioxidant or oxygen free radical scavenging action of these phenolic compounds accounting for most physiological activities. Some have already been found *in vivo* and additional evidence suggests that they act as antioxidant *in vivo* suppression of lipid peroxide levels, inhibition of LDL modification.[I]

Therefore, the intake of vegetable foods and beverages that contain polyphenols as antioxidant potentially protects against atherosclerosis[14]. It has been postulated that the high content of flavonoid in red wine might be responsible for the beneficial effects of moderate drinking on coronary heart disease[10]. The main natural polyphenols of wine

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are flavonoids produced by healthy plants. Some have already been found *in vivo* and additional evidence suggests that they act as antioxidants[4]. Higher intake of flavonoid or α-tocopherol in food has also been associated with decreased incidence of vascular diseases[16].

Geraniin was isolated from a higher flavonoid content *Geranium Sanguineum* and has been reported to provide a strong resistence to oxidation[19].

Following these considerations, the present study was undertaken to characterize the antioxidative activity of flavonoid after its incorporation *in vitro* in physiologic concentrations into human LDL. The antioxidative properties of geraniin and α-tocopherol in this system were also compared.

Materials and Methods

Materials

Geraniin was obtained from Dr. Greenspan, College of Pharmacy, University of Georgia. 2,2'-azobis (2-midinopropane) dihydrochloride (AAPH) was from Wako Chemical Co. (Tokyo, Japan). α-Tocopherol and other chemicals were purchased from Sigma Co. (St. Louis, U.S.A).

Cell culture

Mouse resident peritoneal macorphages were obtained from male CD mice (20-30 g) (The University of Georgia, Altens, Georgia). Ca^{2+}, Mg^{2+} -free phosphate-buffered saline (pH 7.4) was injected into the peritoneal cavity, then macrophages were collected by peritoneal lavage and pelleted by centrifugation (1000 rev./min for 5 min). The cells were resuspended in Ham's F-10 cell medium containing 100 μg/mL of gentamicin, plated onto 35-mm dishes at 4×10^6 cells/mL (1 ml per dish) and allowed to adhere for 2 hrs in a 37℃ humidified 5% CO_2 incubator. Non- adherent cells were removed with 2 washes of Ham's F-10 medium oxidation of LDL.

Lipoproteins

Human LDL was isolated through ultracentrifugation[9] and dialyzed extensively against 0.9% (w/v) NaCl and 0.004% (w/v) EDTA, pH 7.4. Prior to oxidation, LDL was dialyzed against phosphate-buffered saline, pH 7.4, to remove the EDTA.

Oxidation of LDL

Oxidation was made by exposing LDL (10 μg ml^{-1}) to 10 μM Cu^{2+} in 2 mM phosphate buffer, pH 7.5 containing 20 μM hydrogen peroxide or 2 mM AAPH, at 37℃. At intervals, aliquots of the reaction mixture were taken to measure the extent of lipid peroxidation evaluating the thiobarbituric acid reactive substances (TBARS) and hydroperoxides. The entity of oxidation was expressed as malondialdehyde equivalents (MDA) using standard MDA obtained by acid hydrolysis of tetraethoxypropane[2]. Hydroperoxides (LOOH) were measured by the method of Cramer[3] on chloroform : methanol (2:1) extracts of 100 μL aliquots of the LDL solution diluted with 300 μL saline and acidified to pH 3.5 with citric acid.

Detection of conjugated dienes

The formation of conjugated dienes associated with oxidized LDL was measured by monitoring the absorption at 234 nm using a UV-VIS spectrophotometer[5]. Briefly, 1 ml LDL solution (100 μg LDL, protein/ml) in phosphate-buffered saline, pH 7.4, was incubated with 5 μM $CuSO_4$ at 37℃ in both the presence and absence of samples, and the absorbance at 234 nm was measured every 30 min. The formation of conjugated dienes in control solutions containing antioxidant in the absence of LDL and 5 μM $CuSO_4$ was also determined.

LDL gel electrophoresis

Electrophresis of oxidized and native LDL was carried out on agarose gel in barbital buffer, pH 8.6. The agarose plates were then stained with Nile red. Result are expressed as relative electrophoretic mobilities compared to the migration of native LDL[7].

Assay of thiobarbituric acid-reactive substances (TBARS)

TBARS levels were determined spectrophotometrically [29]. 1 ml of 20% trichloroacetic acid and 1 ml of 1% thiobarbituric acid containing EDTA were added to 0.1 ml aliquots of post-incubation mixture and tetramethoxypropane standards. Tubes were placed in a boiling water bath for 30 min. After cooling, tubes were centrifuged at 1,500 ×g for 15 min. Absorbance of the supernatant was measured at 532 nm.

LDL oxidation by endothelial cells

Primary cultures of human umbilical vein endothelial cells were obtained from cord vein, after 15 min digestion

by 0.2% collagenase solution[13,20]. Cells were plated into a 75 cm^2 tissue culture flask and allowed to grow to confluence in RPMI 1460 containing 20% foetal calf serum, 10m penicillin, 10 μg ml^{-1} streptomycin and 2 mM L-glutamine at 37℃ in a humidified atmosphere of 95% air and 5% CO_2. Confluent human endothelial cell cultures in multiwell clusters (1.5×10^5 cells cm^{-2}) were washed three times with serum-free medium, supplemented with 5 μM $CuSO_4$, and incubated with LDL (200 μg, ml^{-1}) in serum-free medium containing 1% human serum albumin. Before addition to endothelial cells LDL was loaded (30 min at 37℃) with vitamin E, geraniin or their vehicles (DMSO or ethanol respectively), and sterilzed by passage through 0.22 μm Millipore filters. After 18 h incubation at 37℃ the medium was aspirated, centrifuged to remove cell debris and processed for lipid peroxidation assay[8].

Measurement of superoxide (O_2^-) released from mouse macrophages

Superoxide (O_2^-) was measured by the method of Yue, et al,[31]. Briefly, macrophages (1×10^6) were seeded in microtiter wells (96-well plate, Nunc, high affinity) and solutions containing phorbol myristate acetate (PMA) (10^{-7} M) and Cyto c (0.32 mM) were applied to each of three wells. Three wells received Cyto c and HEPES buffer to measure basal, unstimulated release of O_2^-. One well served as an assay blank which contained Cyto c, buffer and 700 unit SOD to confirm that Cyto c reduction was inhibitable by SOD. Changes in the optical density were measured intermittently on a MAX microplate reader at 550 nm. O_2^- relese was calculated using the following conversion: nmol O_2^- = [(mean O.D. of three test wells) - (mean O.D. of three regent blank)]×15.9

Determination of cellular protein

Cell protein was measured by Lowry method using bovine serum albumin as standrd[18].

Statistics

Data in text and figures are mean±S.E.M. values[26]. Statistical analysis was performed as indicated in the figure legends; significant difference was accepted at $P<0.05$.

Results and Discussion

Antioxidative activities of geraniin

Antioxidative activities of geraniin were evaluated by comparison with *t*-butyl-4-hydroxyanisole (BHA) and α-tocopherol. As shown in Fig. 1. the order of antioxidant activity was BHA〉 α-tocopherol〉 .geraniin, Antioxidants activity of geraniin have been investigated, although the antioxidant on LDL in these studies were not always clear. The role of nutrition and diet in the development of atherosclerosis is becoming increasingly recognized. This apparent cardioprotective action of firuits is currently believed to be at least partly attributable to the antioxidant activity of ascorbic acid, β-carotene, α-tocopherols and flavonoids abundant in fruits and other palnt foods. Comparatively little is known about the antioxidant and potential biological activities of polyphenol compounds.

Antioxidative effect on human LDL

The antioxidative effect of geraniin on the oxidation of LDL, as measured by the production of TBARS, was initially examined at various concentrations of geraniin, As shown in Fig. 2, geraniin demonstrated a concentration-dependent inhibition of the production of oxidized LDL by Cu^{2+}-mediated LDL oxidation. Geraniin showed a dose-dependent inhibition of Cu^{2+}-mediated LDL oxidation after 6 and 24 h of incubation. At a concentration of 50 μg/mL geraniin, the oxidation of LDL was approximately 40% of that observed in the absence of geraniin. Although research supports the *in vivo* existence of oxidized LDL, the most persuasive data on the role of oxidized LDL in atherogenesis derives from studies showing that antiox-

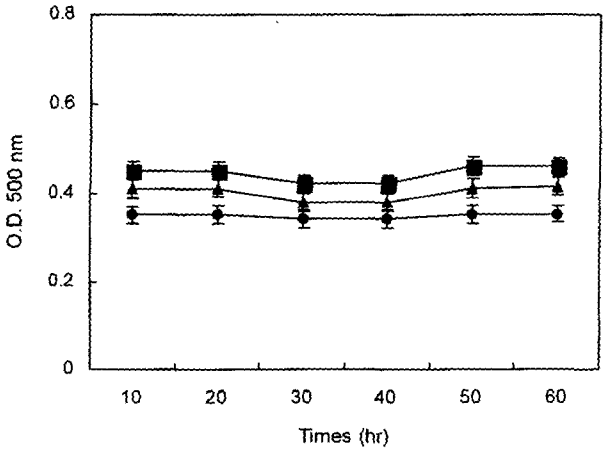

Fig. 1. Antioxidative activity of geraniin compared with that of BHA and α-tocopherol. (●—●: α-tocopherol, ▲—▲ : geraniin, ■—■ : BHA). Antioxdative activity was measured by the thiocyanate method. The concentration of each sample was tested at a dose of 100 μg/ml. Result represent average of two seperate experiments.

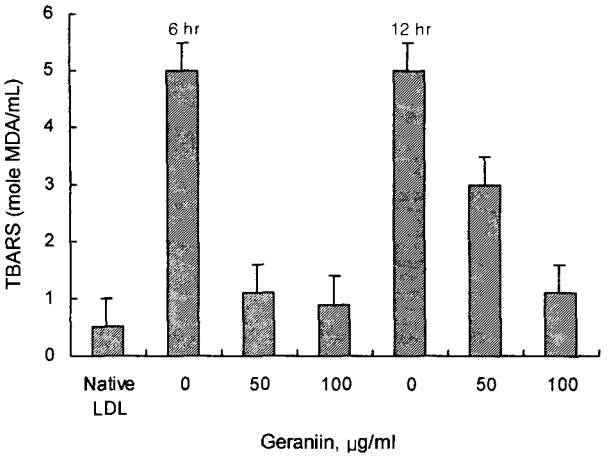

Fig. 2. Concentration-dependent inhibition of Cu^{2+} mediated LDL oxidation by geraniin. LDL (100 μg protein/ml) was incubated for 6 and 24 hr at 37℃ in phosphate-buffered saline containign 5 μM $CuSO_4$ in the presence or absence of increasing concentration of geraniin. Results are presented as means±SEM of there to five independent experiments.

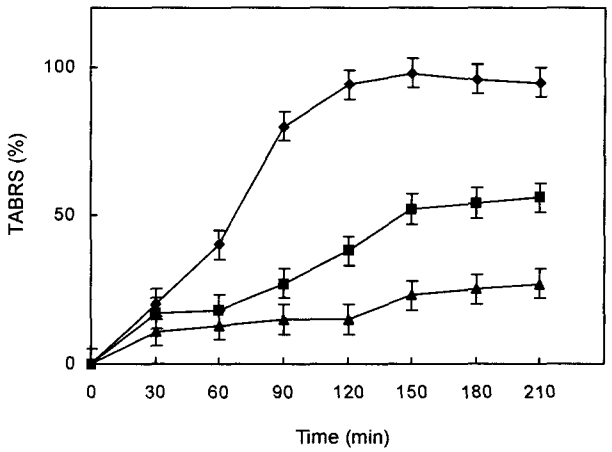

Fig. 3. Inhibition of LDL lipid peroxidation by geraniin and α-tocopherol. LDL (100 μg protein ml^{-1})was subjected to oxidation with 2 mM AAPH in PBS in the absence (●) or present (■) of geraniin (100 μg/ml) and α-tocopherol 50 μg/ml (▲). TBARS contents were expressed as a percent of maximum content observed at 12 hr of incubation with 2 mM AAPH and vehicle alone : Data are presented as the mean±SD of 5 independent experiments.

dants prevent atherosclerosis in animal models while some antioxidants such as BHA and BHT prevented atherosclerosis in animals[1], but their side effects preclude their use in human subjects[12]. Oxidation of LDL in the presence of copper was maximal between 2 and 3 h of incubation; oxidation for 24 h of incubation was almost four fold greater than at 4 h[14]. This may reflect the level of endogenous antioxidants present in the LDL preparation, which may vary with individual donors. For example, vitamin E as dietary antioxidant protects against LDL oxidation, but LDL of donors from smokers were more susceptible to oxidation than that from non-smoker[17].

To further examine this relationship, this study first investigated the affinity of geraniin for the lipoprotein and the corresponding antioxidant effect. Geraniin inhibited to LDL oxidation but did not stop the oxidation of lipoprotein.

Fig. 3 shows inhibitory potencies of geraniin and α-tocophenol on AAPH-induced lipid peroxidation of LDL. LDL oxidation was induced chemically by copper ions in the absence or presence of hydrogen peroxide.

The LDL was then incubated with 2 mM AAPH and various concentrations of α-tocopherol (5-20 μM) or geraniin (5-50 μM), and TBARS was measured at 12 h of incubation. Under these experimental conditions, both geraniin and α-tocopherol inhibited AAPH induced LDL oxidation in a dose dependent manner. The 50% inhibitory concentrations of geraniin and α-tocopherol were 100 μg/ml and 50 μg/ml, respectively. This experiment proposed an antioxidative effect on geraniin, which has a hindered phenol and is similar to flavonoids in structure. This study demonstrated that geraniin exhibited an antioxidative effect on the oxidative modification of human plasma LDL, and that its antioxidative potency was to that of with α-tocopherol. Peroxy radicals derived from AAPH abstract hydrogen atoms from polyunsaturated fatty acids in LDL and initiate the chain reaction[21].

The hindered phenol of α-tocopherol acts as a chain breaking antioxidant, whereas the major function of the phytyl side chain is to retain the molecule in LDL. Although the mechanism of enhanced antioxidative activity of geraniin is not clear, the phenol group of geraniin may effect the stability of the molecule and the affinity for LDL.

In all cases, geraniin inhibited LDL oxidation with the characteristic induction of a concentration-dependent lag time, similar to classic antioxidants.

Effects of geraniin on conjugated diene formation

Macrophages induced oxidation of human LDL was followed by measurement of the LDL oxidative process of diene conjugation.

In the presence of geraniin, the increase of time in the reaction mixture coupled with a increase of the value of lag time, was determined graphically by the interception of

the tangents to the slow and fast increase of the diene absorption. As shown in Fig. 4. geraniin did not modify the maximum formation of conjugated dienes, but decreased significantly. When higher amounts of geraniin were employed, the conjugated dienes formed were significantly lower in the presence of geraniin than that of the control. The Cu^{2+} concentration was coupled with the decrease of conjugated diene formation, which is an index of a lipid propagation phase and dependent only on the lipid composition LDL. The presence of 50-100 μg/ml geraniin in the incubation mixture delayed the reaching of high absorbance and this delays was higher at a concentration of 100 μg/ml.

Effects of geraniin on LDL oxidation and assay electrophoretic mobility

Table. 1 shows the effect of geraniin on the electrophoretic mobility of LDL submitted to oxidative modification by macrophages. Geraniin reduced the relative electrophoretic mobility of LDL at dose dependently. LDL oxidized by $CuSO_4$ displayed a greater electrophoretic mobility in agarose gels compared to native LDL. When LDL was incubated with various concentration of geraniin,

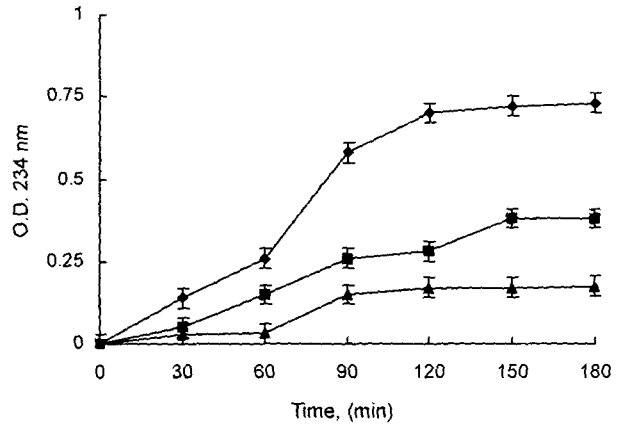

Fig. 4. Antioxidative effects of geraniin on the formation of conjugated diene observed during the oxidation of LDL.
LDL (100 μg protein/mL) was incubated in the prosence or abcence of 50 or 100 μg/ml geraniin. Oxidation was initiated by the addition of 5 μM $CuSO_4$. The formation of conjugated dienes was measured by LDL oxidation.
▲—▲: Control (LDL + 5 μM $CuSO_4$).
○—○: LDL + 5 μM $CuSO_4$ + 50 μg/ml geraniin.
●—●: LDL + 5 μM $CuSO_4$ + 100 μg/ml geraniin.
Results are presented as means±S.E.M. of 3-5 independent experiments.

Table 1. Effects of geraniin as assessed by electrophoresis mobility

Incubation conditions	Relative electrophoretic mobility	*P*
Native LDL	1.0	
LDL + cell + vehicle (control)	1.86±0.02	
LDL + cell + geraniin 20 μg/ml	1.56±0.02	
LDL + cell + geraniin 40 μg/ml	1.23±0.03	<0.05
LDL + cell + geraniin 60 μg/ml	1.42±0.01	<0.01
LDL + cell + geraniin 100 μg/ml	1.12±0.01	<0.01

LDL (100 ug/ml) was incubated for 24 hr in Hams F-10 medium in 35-min dishes containing macrophages in the presence or absence of geraniin. The electrophoretic mobility of LDL was determined in agarose gel as described in the text. Result are means±SEM of three to five independent experiments.

the electrophoretic mobility of oxidized LDL was only slightly greater than that of native LDL. Geraniin inhibited the cell-induced oxidation of LDL as measured by lipoperoxide content of the electrophoretic mobility of LDL in agarose gels. Steinbrecher, *et al*[24] demonstrated that LDL could be modified by the addition of fatty acid peroxidation in the absence of cells. This modified LDL possesses an enhanced electrophoretic mobility without the lipid consitituents of LDL being oxidized. It is possible that oxidation of LDL mediated by macrophages in Ham's F-10 culture medium can also contribute to the modification of the LDL protein as determined by the enhanced electrophoretic mobility.

Effects of geraniin on endothelial cells mediated LDL oxidation

With the aim of substantiating whether the antioxidant drug is also acting under more physiological conditions, experiments at cellular level were undertaken using human endothelial cells to oxidize LDL. Geraniin successfully inhibited endothelial cell-mediated LDL oxidation in a dose-dependent manner (Fig. 5), and the 50% inhibition was calculated to require about 50 μg/ml. The antioxidant and vehicle concentrations applied did not induce any toxic effect to endothelial cells, they kept the characteristic cobblestone morphology. Furthermore, no dettachment was observed after the incubation period and cells washed at the end of the incubation period. Accordingly, geraniin can inhibited LDL oxidation at the cellular level. Endothelial cells have been proposed as one of the sources responsible

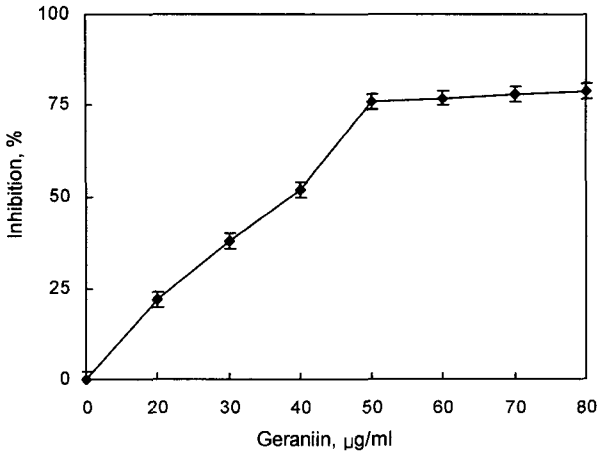

Fig. 5. Concentration-dependent inhibition of endothelial cell mediated oxidation by geraniin. Each data point represents mean±SD of three separate experiments.

for LDL modification *in vivo*[10,20], by a free radical mediated mechanism. Inhibition of the oxidative modification of LDL is a crucial event in the suggested mechanism of atherosclerosis. Among several biological characteristic of oxidized LDL is the uptake and degradation by the macrophage scavenger receptor, which causes the formation of foam cells, and cytoxicity to most cells[24].

Protective activity of geraniin, probucol, ascorbic acid, α-tocopherol and probucol

The protective activity towards LDL oxidation was compared to the activity exerted by the synthetic antioxidant, probucol and the physiological antioxidants, ascorbic acid and α-tocopherol. Inhibitory activity was studied in the LDL oxidation by copper, copper/hydrogen peroxide and by endothelial cells. Cumulative data, shown in Table 2, are based on analysis of MDA and hydroperoxide formation. The order of increasing activity was probucol > geraniin > α-tocopherol. The magnitude of antioxidant geraniin was higher than α-tocopherol. In the endothelial cell-mediated LDL oxidation, the IC_{50} was 16 and 20 μM for geraniin and vitamin E, respectively. Inhibition of MDA and hydroperoxide formation required similar between vitamin E and geraniin. Compared to ascorbic acid, the activity of geraniin was higher, apart from the diene system where the difference is reduced to a factor. Finally, geraniin is more potent than vitamin C by a factor ranging from 0.2 to 0.4 times, depending on the system.

Table 2. Relative antioxidative activity of geraniin and the synthetic antioxidant probucol and the natural occuring antioxidants, ascorbic acid and α- tocopherol.

	Lipid peroxidation (Cu^{2+}/H_2O_2)		Endothelial cell mediated oxidation
	MDA	LOOH	
Geraniin	15.6	16.0	16.7
Ascorbate	14.6	17.0	25.6
Probucol	15.3	15.0	10.2
α-tocopherol	16.7	16.7	14.4

For each antioxidant data are obtained from concentration dependent inhibition experiments

To further support the physiological relevance of these results, geraniin was compared to ascorbic acid and vitamin E, which can be considered reference antioxidants in biological systems, and with probucol. Vitamin E, a normal constituent of LDL, is generally thought to function as a major lipid-soluble antioxidant, and ascorbic acid is considered the most important aqueous phase antioxidant in plasma[25]. Probucol was selected because most research into LDL oxidation has concentrated on the protection by this synthetic compound[I]. In all instances, geraniin produced the most potent inhibition of LDL oxidation. The LDL oxidation is a complex mechanism involving initiator radicals generated by Fenton-like chemistry, and propagating carbon-oxygen radicals, like peroxyl radicals. Thus, geraniin should operate at two main levels, the initiation and propagation, of the lipid peroxidation. In addition, the superiority of geraniin can be explained in terms of partitioning in the LDL lipids/aqueous phase and by better accessibility to sites of free radical attack. Geraniin, as demonstrated in this study, strongly associates with LDL and is sufficiently water soluble enough to be able to intercept both the oxidation initiating radicals coming from the aqueous phase and the lipid radicals generated during the chain reaction.

These data show that geraniin is a inhibitor of LDL oxidation *in vitro* and suggest evaluation of this activity *in vitro*.

Effects of geraniin on superoxide released from macrophage

Phorbol myristate acetate (PMA, 0.1 μm) induced a time-dependent increase in O_2^- production by mouse macrophages and the production of O_2^- reached a plateau at 60 min. As such, all data were obtained at 60 min incubation following stimulation. As shown in Fig. 6 the production of O_2^- increased from a basal value of 0 to 1.84±0.02 nmol.

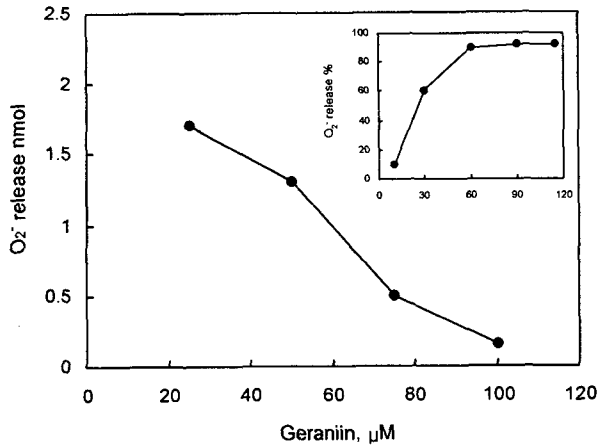

Fig. 6. Effect of geraniin on PMA-induced O_2^- release from mouse macrophages.
Cells were treated with geraniin for 20 min at 37℃ and then activated by addition of PMA (0.1 μM). O_2^- formation was monitored at 550 nm by measurement of ferricytochrome reduction and the incubation time was 60 min as described in Methods. Each point represents the mean±S.E.M. (n=3-5). The inset is the time course of PMA (0.1 μM)-stimulated prodution of O_2^- from mouse macrophages. Each point is the average of duplicate samples.

Geraniin protected PMA-induced O_2^- production in a dose independent manner at a concentration of 30 μg/ml to 100 μg/ml. It has been reported that O_2^- is an important oxygen radical in mediating LDL oxidation by smooth muscle cells[16] and human monocytes[30] and that O_2^- dismutase (SOD) inhibits LDL oxidation by smooth muscle cells[21]. Our previous study demostrated that flavonoids scavenge O_2^- in human endothelium cells[28]. In order to examine whether geraniin inhibits LDL oxidation by inhibiting O_2^-, this studies the effect of geraniin on O_2^- released from macrophages. The results show that geraniin almost completely inhibits O_2^- at a concentration of 100 μM. Geraniin is also likely to effect the amount of maupphages available for the oxidation, as suggested by the marked increase of the constant in the presence of geraniin. Under our experimental conditions, geraniin could be inhibited on LDL oxidation which employed at a low concentration. This phenomenon could be explained on the basis of two different mechanisms : (1) a partial esquestration of free radical by the polyanion chain of chitin sulfate; (2) the possible changes in the ability of LDL to bind free radical, the structural modification of the particle following the interaction with geraniin. Our study emphasizes that the antioxidant effect of geraniin protects not only a free fatty acid, but also a biological at structure, such as human LDL, against cells induced oxidation.

This model of oxidation was demonstrated to produce LDL sharing many structural and functional properties with LDL oxidized by cells[28].

On the basis, the results suggest that geraniin might play a regulatory role in the process of LDL oxidation in vivo, which may change the metabolic fate of the particle, increasing its atherogenic potential.

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초록 : 사람 LDL의 지질과산화에 의한 geraniin의 항산화 효과

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본 연구는 동맥 경화의 원인으로 알려진 사람 oxidized low density lipoprotein (LDL)에 대한 geraniin의 산화 억제 효과에 대하여 실험하였다. 사람 LDL을 Cu^{2+}유도 LDL로 산화 시킬 때 50와 100 ug/ml 농도의 geraniin를 첨가하여 TBARS을 측정한 결과 LDL에 대한 항산화가 높았으며 용량 의존형으로 나타났다. Geraniin를 20-100 μg/ml의 농도를 조절하여 전기 영동에 의한 이동상을 조사한 결과 100 μg/ml geraniin의 농도에서 거의 완전한 억제 효과를 보였다. 사람 LDL에 Cu^{2+}로 유도하여 LDL를 산화시킬때 conjugated diene를 보면 geraniin를 100 ug/ml 첨가하였을 때 억제 효과가 높았다. 또한 geraniin은 동맥의 내피세포에서도 그 농도에 따라 억제효과를 나타내었다. 그리고 phorbol myristate acetate를 처리한 macrophage 유도 활성 산소의 소거 효과는 geraniin의 농도가 100 μg/ml일때 거의 소거하였다. 이상의 결과로 보아 geraniin는 α-tocopherol, ascorbic acid 및 합성 항산화제인 probucol과 거의 비슷한 항산화 활성이 있어 동맥 경화의 예방에 효과적이라는 결론을 얻었다.