©2004, Acta Pharmacologica Sinica
Chinese Pharmacological Society
Shanghai Institute of Materia Medica
Chinese Academy of Sciences
http://www.ChinaPhar.com

Antioxidative effect of schisanhenol on human low density lipoprotein and its quantum chemical calculation[1]

Ling-hong YU, Geng-tao LIU[2], You-min SUN[3], Hong-yu ZHANG[4]

Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100050, China;
[3]Institute of Theoretical Chemistry, Shandong University, Ji-nan 250100, China;
[4]Laboratory for Computational Biology, Shandong University of Technology, Zibo 255049, China

KEY WORDS LDL lipoproteins; free radical scavengers; molecular structure; quantum theory; structure-activity relationship; xanthenes; schisanhenol

ABSTRACT

AIM: To investigate the effect of schisanhenol (Sal) on copper ion-induced oxidative modulation of human low density lipoprotein (LDL). **METHODS:** The antioxidative activity of eight schisandrins (DCL) on microsome lipid peroxidation induced by Vit C/NADPH system was first observed, and then, the effect of Sal on Cu^{2+}-induced human LDL oxidation was studied. The generation of malondialdehyde (MDA), lipofuscin, reactive oxygen species (ROS), consumption of α-tocopherol as well as electrophoretic mobility of LDL were determined as criteria of LDL oxidation. Finally, the quantum chemical method was used to calculate the theoretical parameters of eight DCL for elucidating the difference of their antioxidant ability. **RESULTS:** Sal was shown to be the most active one among eight schizandrins in inhibiting microsome lipid oxidation induced by Vit C/NADPH. Sal 100, 50, and 10 μmol/L inhibited production of MDA, lipofuscin and ROS as well as the consumption of α-tocopherol in Cu^{2+}-induced oxidation of human LDL in a dose-dependent manner. Sal also reduced electrophoretic mobility of the oxidized human LDL. Further study of quantum chemistry found that Sal was the strongest one among eight DCL to scavenge $O_2^{\cdot-}$, $R^{\cdot}$, $RO^{\cdot}$, and $ROO^{\cdot}$ radicals. **CONCLUSION:** Sal has antioxidative effect on human LDL oxidation. The mechanism of Sal against LDL oxidation may be through scavenging free radicals.

INTRODUCTION

Oxidative modulation of low-density lipoprotein (LDL) has been recognized as one of the main atherogenic forms of LDL[1]. Oxidized low density lipoprotein (ox-LDL) can be uptaken by macrophages via the scavenger receptor, then the macrophages become foam cells and cellular cholesterol accumulated in vascular endothelial cells. Ox-LDL is cytotoxic to arterial wall cells, stimulates haemostatic and thrombotic process and secretion of cytokines and growth factors from cells of the arterial wall[2]. It has been reported that antioxidants such as β-carotene and α-tocopherol could prevent LDL oxidation and delay the development of atherosclerotic plaques in animals[3]. Eight schisandrins were isolated from *Schizandracea* belong to dibenzocyclooctene lignans (DCL, Fig 1). Our previous study found that several of DCL have antioxidant activity[4]. Of the eight DCL, the antioxidant activity of Schisanhenol (Sal) was more active than others. The aim of this article was to investigate whether Sal could inhibit

[1] Project supported by the National Natural Science Foundation of China (No 39870865).
[2] Correspondence to Prof Geng-tao LIU. Phn 86-10-6316-5178. Fax 86-10-6301-7757. E-mail liugt@imm.ac.cn
Received 2003-06-13　　Accepted 2004-04-01

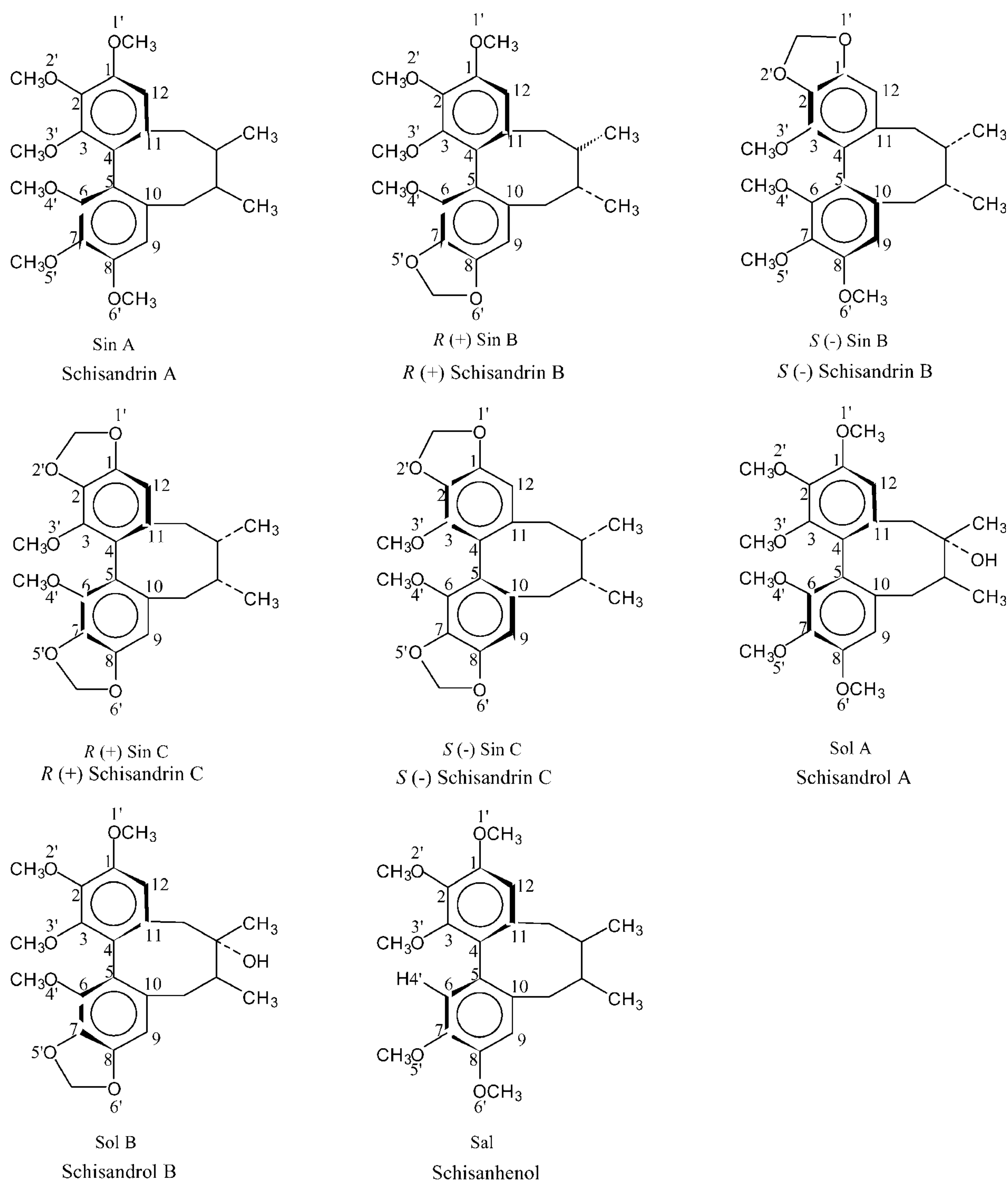

Fig 1. Chemical structures of 8 schisandrins (DCL).

oxidative modulation of human LDL induced by copper ion. In order to elucidate the difference in the antioxidant activity of eight DCL, quantum chemical method was used to calculate the theoretical parameters of eight DCL.

MATERIALS AND METHODS

Compounds and reagents Eight DCL samples were provided by Prof Yan-yong CHEN and Lian-niang LI. DCL compounds are white crystals and their purity is over 98 %. The compounds were dissolved in dimethyleneformamide (DMF) before use as they are not water soluble substances. Human blood serum was obtained from Blood Station of Shijingshan District in Beijing. 1,1,3,3-Tetramethoxypropane (TEP), α-tocopherol, and agarose were purchased from Sigma

Co. α-Tocopherol was dissolved in DMF before use. Dialysis pocket (M_r: 8000-10 000) was purchased from Tianxiangren Biochemistry Company. Oil-red "O" was purchased from Fluka. Other reagents were all chemical pure from local market.

Measurement of malondialdehyde (MDA) formation of liver microsomes induced by Vit C/NADPH system Liver microsomes from adult male Wistar rats were prepared by the ultra-centrifugation method[5]. Protein was determined by method of Lowry[6]. Microsomal protein 1.4 mg in 1 mL of 0.1 mol/L phosphate buffer (pH 7.4) was incubated with DMF 10 μL as control or with tested schisandrins and α-tocopherol (1 mmol/L) 10 μL, respectively, for 15 min at 37 ºC water bath. Lipid peroxidation was then initiated by the addition of Vit C (4.4 μg) and NADPH (0.16 mg) and incubated at 37 ºC for 15 min again. Ice-cold 20 % trichloroacetic acid 0.5 mL was added to stop the reaction. The formation of MDA in the supernatant after centrifugation of the incubation mixture was determined by the thiobarbituric acid method[6]. Percentage of inhibition of MDA formation was used to indicate the potency of the tested schisandrins in comparison with the control.

Separation and identification of human LDL Human serum LDL was prepared by discontinuous density gradient ultra centrifugation[7]. The separated LDL was dialyzed against phosphate-buffered saline containing 0.01 % EDTA (pH 7.4), then sterile-filtered and stored at 4 ºC. The identification of LDL was performed by agarose electrophoresis[8]. The electrophoresis was carried out under the condition of 200 V, 80 mA in 0.05 mol/L barbital buffer for nearly 3 h, and then the gel plate was fixed in methanol for 15 min and stained with oil-red for 16 h. After staining, the gel was washed with bleaching solution (95 % ethanol:water=5:3). The position of LDL was between VLDL and HDL layers.

LDL oxidation induced by copper ion[9] The LDL was dialyzed against PBS, EDTA-free buffer (pH7.4) for 40 h before oxidative modulation. The concentration of LDL was expressed on the bases of protein and adjusted to 1 g/L. After pre-incubation with various concentrations of Sal for 1 h, LDL oxidation was initiated with $CuSO_4$ 10 μmol/L at 37 ºC for 20 h. The oxidative modulation was stopped by the addition of 200 μmol/L EDTA. Native-LDL (*n*-LDL) was incubated in the same condition without $CuSO_4$ and served as control.

Assay of lipid peroxidation The MDA formation was determined to indicate the extent of lipid peroxidation[10]. Ox-LDL or *n*-LDL 0.1 mg was mixed with 150 μL methanol, 3 mL 0.17 mol/L sulfuric acid and 0.5 mL 10 % phosphatic tungstic acid. The mixture was kept at room temperature for 10 min. Then the mixture was incubated with 1 mL 0.67 % thiobarbituric acid-acetic acid (v/v=1/1) at 100 ºC for 10 min. After cooling with tap water, the reactant was extracted with 4 mL *n*-butanol and then centrifuged at 2000×*g* for 10 min. The fluorescent intensity of *n*-butanol extract was recorded at emission wavelength of 553 nm and excitation wavelength of 515 nm on a spectrofluorometer. The amount of MDA was calculated from the standard curve. The extent of lipid peroxidation was expressed as MDA (μmol)/g protein.

Determination of α-tocopherol content of LDL[11] Ox-LDL or *n*-LDL 0.7 mg was incubated with 1 mL anhydrous alcohol and 0.5 mL 25 % acetic acid at 70 ºC for 5 min, then 1 mL 10 mol/L KOH was added and incubated for 30 min at 70 ºC. The mixture was extracted with 4 mL skellysolve B and then centrifuged at 2000×*g* for 10 min. The fluorescent intensity of skellysolve B extract was measured at emission wavelength of 330 nm and excitation wavelength of 280 nm on a spectrofluorometer. The content of α-tocopherol was calculated according to the standard curve and expressed as α-tocopherol (μmol)/g LDL.

Determination of lipofuscin products of LDL[12] After 50 μg ox-LDL or *n*-LDL was diluted with 2.5 mL PBS, the lipofuscin products were determined as the fluorescent intensity at emission wavelength of 410 nm and excitation wavelength of 360 nm on a spectrofluorometer.

Determination of electrophoretic mobility of LDL The electrophoretic mobility of *n*-LDL and ox-LDL was determined by agarose gel electrophoresis (0.5 % agarose and 0.1 mol/L barbital buffer containing 4 % bovine serum albumin) under the condition of 200 V, 80 mA in 0.05 mol/L barbital buffer for nearly 3 h, then the gel was fixed in methanol for 15 min and stained with oil-red for 16 h. After staining, the gel was washed by bleaching solution (95 % ethanol:water=5:3). The mobility was expressed as the distance moved by the samples in 3 h.

Measurement of reactive oxygen species (ROS) generation during Cu^{2+}-induced LDL oxidation by ultra weak chemiluminescence analyzer[13] After pre-incubation with various concentrations of Sal for 1 h, LDL (1.0 mg) oxidation was initiated with 10 μmol/L

$CuSO_4$ at 37 °C for 20 h. The generation of ROS during this period was monitored by the spectrum of low level-chemiluminescence (LL-CL) on ultra weak chemiluminescence analyzer. The signal was recorded every 90 s and the illuminative intensity was indicated as count/s.

Quantum chemistry method The molecular geometries of DCL were optimized firstly by molecular mechanic method MMX[14] in program PCMODEL, and then, by semiempirical quantum chemistry method Austin Model 1 (AM1)[15]. Finally, by single point electronic energy calculation using density functional theory B3LYP[16-18] on 6-31G** level was performed to obtain the theoretical parameters including charge density, proton dissociation enthalpy and O-H bond dissociation enthalpy. For large molecules, this method is commonly believed to be economical and accurate[19,20].

Statistical analysis Data were expressed as mean±SD and *t*-test was used to judge statistical differences between groups. $P<0.05$ was considered significant.

RESULTS

Comparison of eight DCL against liver microsomal lipid peroxidation induced by Vit C/NADPH system All eight schisandrins at the concentration of 100 μmol/L inhibited liver microsomal MDA formation induced by Vit C/NADPH to different degree. Sal, *S*(-) Sin C, and *S*(-) Sin B were more potent than the other schisandrins and α-tocopherol, particularly Sal and *S*(-) Sin C were the most active (Tab 1). Both compounds inhibited MDA formation by almost 100 %.

Tab 1. Effect of eight schisandrins on liver microsomal MDA formation induced by Vit C/NADPH system *in vitro*. *n*=3. Mean±SD.

Group	Concentration/ μmol·L-1	MDA/ μmol·g-1 protein	Inhibition/%
Control		3.200±0.234	
Vit E	100	1.223±0.398	61.8
SinA	100	1.717±0.069	46.4
R(+)SinB	100	1.360±0.165	57.5
S(-)SinB	100	0.343±0.206	89.3
R(+)SinC	100	1.126±0.069	64.8
S(-)SinC	100	0.041±0.096	99.0
SolA	100	1.950±0.371	39.1
SolB	100	2.527±0.247	21.0
Sal	100	-0.687±0.165	100.0

The tested schisandrins such as Sal and *S*(-)SinC were not found to interfere with the colour development of MDA assay. As Sal was shown to be the most active compound in inhibiting MDA formation, so Sal was selected in the following study of its effect on human LDL oxidation.

Effect of Sal on MDA production during oxidative modulation of LDL induced by Cu^{2+} Incubation of LDL with 10 μmol/L $CuSO_4$ resulted in significant increase of MDA formation as compared with *n*-LDL ($P<0.01$). Pretreatment of LDL with various concentrations of Sal (100, 50, and 10 μmol/L) inhibited Cu^{2+}-induced MDA formation in a concentration-dependent manner (Tab 2).

Tab 2. Effect of Sal on LDL oxidation induced by copper ion. *n*=4. Mean±SD. $^bP<0.05$, $^cP<0.01$ *vs* ox-LDL.

Group	MDA/ μmol·g-1 protein	α-Tocopherol/ μmol·g-1 protein	Lipofuscin (fluorescent intensity)
n-LDL	0.58±0.17^c	34.49±6.73^c	1.7±0.26^c
ox-LDL	43.76±0.27	3.30±0.61	4.85±0.1
Sal 100 μmol/L	5.91±0.11^c	15.66±3.67^c	2.88±0.34^c
Sal 50 μmol/L	10.80±0.92^c	13.45±1.71^c	3.38±0.46^b
Sal 10 μmol/L	14.76±4.73^c	7.34±2.45^b	4.22±0.24^b

Effect of Sal on α-tocopherol content in ox-LDL The content of α-tocopherol in LDL was nearly exhausted by Cu^{2+}-induced oxidation. Sal dose-dependently prevented depletion of α-tocopherol content in LDL after oxidation induced by Cu^{2+} (Tab 2).

Effect of Sal on lipofuscin production for ox-LDL After the oxidation of LDL by Cu^{2+}, the lipofuscin content in ox-LDL increased about 3 times in comparison with *n*-LDL. Various concentrations of Sal markedly decreased the production of lipofuscin in ox-LDL (Tab 2).

Effect of Sal on electrophoretic mobility of LDL After LDL oxidized by Cu^{2+}, the ox-LDL moved faster than *n*-LDL on agarose gel electrophoresis. Pre-incubation of LDL with various concentrations of Sal significantly slowed down the electrophoretic mobility of LDL oxidized by Cu^{2+} (Fig 2).

Effect of Sal on ROS generation during LDL oxidation induced by Cu^{2+} Incubation of LDL with Cu^{2+} increased the chemiluminescence intensity that

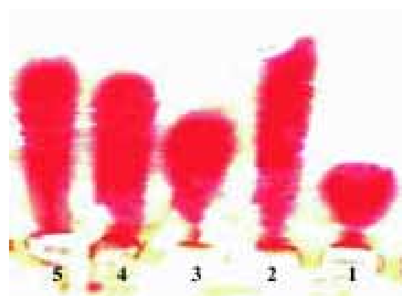

Fig 2. Effect of Sal on electrophoretic mobility of LDL oxidized by Cu^{2+}. (1) *n*-LDL; (2) Ox-LDL; (3)-(5) Ox-LDL preincubated with different concentrations of Sal (100, 50, 10 μmol/L).

indicated the production of ROS. Pre-incubation of LDL with Sal 100 μmol/L completely inhibited the generation of ROS (Fig 3).

Scavenging activity on superoxide anion radical calculated by quantum chemistry methods The carbon atoms of dioxo-methylene groups possess fairly large net charge, so, DCL with dioxo-metheylene group such as *S*(-) SinC, *R*(+)SinC, *S*(-)SinB, *R*(+)SinB, SolB, and Sal had high activity to scavenge $O_2^{\cdot -}$. In addition, the average charge density of the carbon atom showed the order of scavenging activity on superoxide anion radical: Sol B>S(-) Sin C~S(-)Sin B>R(+)Sin C>Sin A>R(+)Sin B>Sol A>Sal (Tab 3).

Calculations showed that the proton dissociation energy of Sal was lower than that of α-tocopherol. Hence, the $O_2^{\cdot -}$ scavenging activity of Sal was higher than that of α-tocopherol (Tab 4).

Scavenging activity on alkyl ($R^{\cdot}$), alkoxyl ($RO^{\cdot}$) and alkyl peroxyl ($ROO^{\cdot}$) radicals O-H bond dissociation enthalpy of Sal was higher than that of α-tocopherol (Tab 4), suggesting that α-tocopherol had

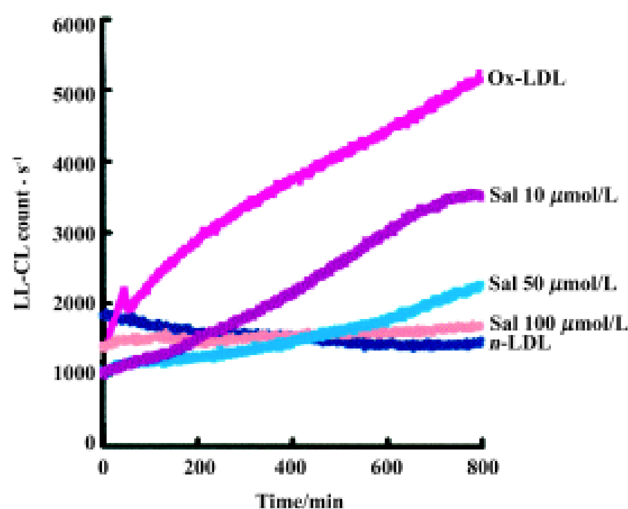

Fig 3. Effect of Sal on reactive oxygen species (ROS) generation during Cu^{2+}-induced LDL oxidation by ultra weak chemiluminescence analyzer.

higher scavenging activity than Sal. As other DCL molecules had no phenolic hydroxyl group and the C-H bond dissociation enthalpy was much higher than the O-H bond dissociation enthalpy (data omitted), other DCL molecules had no activity to scavenge alkyl, alkoxyl, and alkyl peroxyl radicals.

In summary, the molecules of DCL possessing dioxo-methylene groups had high scavenging activity on $O_2^{\cdot -}$ and there existed quantity-activity relationships. The more the dioxo-methylene moiety of DCL has, the more active the DCL antioxidant is. For the different isomers, the activity of *S*(-) form was higher than that of *R*(+) form. Sal possessed the activity to scavenge $O_2^{\cdot -}$, $R^{\cdot}$, $RO^{\cdot}$, and $ROO^{\cdot}$. Considering superoxide anion radical plays a key role in biological system, the

Tab 3. Net charge of carbon for 8 schisandrins (DCL).

Atom Number	SinA	*R*(+)SinB	*S*(-)SinB	*R*(+)SinC	*S*(-)SinC	SolA	SolB	Sal
1	0.300650	0.349830	0.348758	0.332769	0.330093	0.346236	0.312608	0.299760
2	0.285531	0.267542	0.250538	0.263637	0.261414	0.273588	0.308996	0.299267
3	0.294413	0.295724	0.327043	0.338061	0.334523	0.285584	0.297840	0.241306
6	0.333921	0.308066	0.330176	0.332952	0.340331	0.305078	0.331242	0.281165
7	0.248614	0.275354	0.260077	0.261169	0.261038	0.263828	0.287846	0.262101
8	0.352346	0.330176	0.329534	0.329569	0.334547	0.349951	0.342206	0.348039
10	0.119632	0.115701	0.118268	0.092478	0.102915	0.104903	0.108363	0.099009
11	0.095132	0.085460	0.092425	0.103323	0.091942	0.092998	0.087220	0.083344
Average	0.254440	0.253482	0.257102	0.256745	0.257100	0.252771	0.259540	0.239249

Tab 4. Proton dissociation enthalpy[1)] and O-H bond dissociation enthalpy[2)] for schisanhenol and α-tocopherol.

	Schisanhenol	α-tocopherol
Total energy for parent molecule (TE_p, in hartree)	-1345.80020	-696.02176
Thermal correction to enthalpy for parent molecule (TCE_p, in hartree)	0.53843	0.33504
Total energy for anion (TE_a, in hartree)	-1345.22750	-695.43868
Thermal correction to enthalpy for anion (TCE_a, in hartree)	0.52674	0.32289
Total energy for free radical (TE_f, in hartree)	-1345.15788	-695.39678
Thermal correction to enthalpy for free radical (TCE_f, in hartree)	0.52441	0.32146
Proton dissociation enthalpy (kcal/mol)	352.83	359.06
O-H bond dissociation enthalpy (kcal/mol)	82.22	71.61

[1)] Proton dissociation enthalpy=$[(TE_a+TCE_a\times0.973)-(TE_p+TCE_p\times0.973)+H_p]\times627.5095$, in which, H_p is the enthalpy for proton, 0.000944 hartree.
[2)] O-H bond dissociation enthalpy=$[(TE_f+TCE_f\times0.973)-(TE_p+TCE_p\times0.973)+H_h]\times627.5095$, in which, H_h is the enthalpy for hydrogen atom, -0.49765 hartree.

total activity of Sal to scavenge $O_2^{\bar{\cdot}}$, R$^\cdot$, RO$^\cdot$, and ROO$^\cdot$ radicals may be higher than that of α-tocopherol.

DISCUSSION

The oxidative modulation of LDL induced by copper ion is related to free radical reaction, though the mechanism of the oxidant production has not been elucidated yet. LDL oxidation may require the generation of superoxide anion and probably the ultimate generation of hydroxyl radicals by the Fenton reaction[24]. After the oxidation by copper ion, polyunsaturated fatty acid of LDL was oxidized which resulted in an elevation of lipid peroxides and depletion of α-tocopherol in ox-LDL. Both the binding of aldehydic lipid peroxidation product to the ε-amino groups of lysine residues in apo B during oxidation and the conversion of histidine and proline residues to negatively charged aspartic acid and glutamic acid by reactive oxygen species all increased the negativity of LDL, thereby leading to increase of electrophoretic mobility of LDL. The derivation of apo B is a prerequisite for scavenger-receptor recognition of macrophages[3]. Our biochemical results clearly showed that Sal had strong anti-oxidative effects on human LDL oxidation induced by Cu^{2+} as indicated in the inhibition of the generation of ROS, MDA, lipofuscin as well as decrease of α-tocopherol consumption and the retardation of electrophoretic mobility of LDL.

To further elucidate the structure-antioxidant activity relationship of the eight DCL theoretically by quantum chemistry calculation, appropriate theoretical parameters for characterizing the free radical scavenging activity according to different radicals were calculated. $^\cdot$OH, $O_2^{\bar{\cdot}}$, and as well as R$^\cdot$, RO$^\cdot$, and ROO$^\cdot$ are main three types of radicals in biological system. $^\cdot$OH is the most active radical, but once it is produced, it promptly reacts with other biological molecules. So, directly scavenging ·OH by antioxidant is unimportant. Although $O_2^{\bar{\cdot}}$ is far less active than ·OH, it is the source of generating ·OH by superoxide dismutase in the biological system. The mechanisms of scavenging superoxide anion radical are nucleophilic addition, nucleophilic substitution, and a coupled reaction including proton transfer and electron transfer. The theoretical indexes corresponding to these reactions are pK_a values for phenolic hydroxyl and the charge of carbon. DCL compounds (Fig 1) have no proton readily abstractable except Sal. Hence, scavenging $O_2^{\bar{\cdot}}$ is mainly through nucleophilic addition and nucleophilic substitution reactions. The larger the charge of carbon, the higher the $O_2^{\bar{\cdot}}$ scavenging activity for DCL antioxidants. In addition, R$^\cdot$, RO$^\cdot$, and ROO$^\cdot$ are not very active, while they are also very harmful due to their long lifetime. The mechanism for antioxidant to scavenge these radicals is hydrogen abstraction reaction of the phenolic hydroxyl group. The theoretical index characterizing this process is O-H bond dissociation enthalpy [21-24]. Therefore, the activity of eight DCL compounds to scavenge $O_2^{\bar{\cdot}}$, R$^\cdot$, RO$^\cdot$, and ROO$^\cdot$ were theoretically calculated with quantum chemistry method. As a result, Sal could scavenge superoxide anion through nucleophilic addition, nucleophilic substitution, and through proton transfer reaction. Sal is the only one with the phenolic hydroxyl group in eight DCL that can scavenge alkyl,

alkoxyl, and alkyl peroxyl radicals. The results illustrated Sal was the most active among DCL compounds theoretically which is coincided with the biochemical results of our present and previous studies.

REFERENCES

1 Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: modulations of low-density lipoprotein that increase atherogenicity. New Engl J Med 1989; 320: 915-24.

2 Aviram M, Rosenblat M. Macrophage-mediated oxidation of extracellular low density lipoprotein requires an initial binding of the lipoprotein to its receptor. J Lipid Res 1994; 35: 385-98.

3 Yang TTC, Koo MWL. Inhibitory effect of Chinese green tea on endothelial cell-induced LDL oxidation. Atherosclerosis 2000; 148: 67-73.

4 Liu GT, Zhang TM. Protective action of seven natural phenolic compounds against peroxidative damage to biomembranes. Biochem Pharmacol 1992; 43: 147-52.

5 Lesca P, Lecointe C, Paoletti C, Mansuy D. Induction of rat hepatic mono-oxygenases by ellipticine: formation of cytochrome p-448. Hydroxylating activity. C R Acad Sci Hebd Seances Acad Sci D 1976; 282: 1457-61.

6 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265-75.

7 Heval RJ, Eder HA, Beragdon JH. The distribution and chemical composition of altracentrifugally separated lipoproteins in human serum. J Clin Invest 1955; 34: 1345-53.

8 Noble RP. Electrophoretic separation of plasma lipoproteins in agarose gels. J Lipid Res 1968; 9: 693-7.

9 Ziouzenkova O, Gieseg SP, Ramos P, Esterbauer H. Factors affecting resistance of low density lipoproteins to oxidation. Lipid 1996; 31 Suppl: S71-76.

10 Yagi K. A simple fluorometric assay for lipoperoxide in blood plasma. Biochem Med 1976; 15: 212-8.

11 Tayor SL. Sensitive fluorometric method for tissue tocopherol analysis. Lipid 1976; 11: 7-15.

12 Cominacini L, Garbin U, Davoli A, Micciolo R, Bosello O, Gaviraghi G, *et al*. A simple test for predisposition to LDL oxidation based on the fluorescence development during copper-catalyzed oxidative modulation. J Lipid Res 1991; 32: 349-58.

13 Riccardo A, Giancarlo DL, Giuseppina P. Mechanistic aspects of the relationship between low-level chemiluminescence and lipid peroxides in oxidation of low-density lipoprotein. FEBS Lett 1999; 459: 47-50.

14 Gajewski JJ, Gillbert KE, McKelvey JM. An enhanced version of MM2. Adv Mol Model 1990; 2: 65-92.

15 Dewar MJS, Zoebisch EG, Healy EF, Stewart JJP. AM1: a new general purpose quantum mechanical molecular model. J Am Chem Soc 1985; 107: 3902-9.

16 Stevenes PJ, Devlin FJ, Chabalowski CF, Frisch MJ. Ab initio calculation of vibrational absorption and circular dichroism spectra using density functional force fields. J Phys Chem 1994; 98: 11623-7.

17 Lee C, Yang W, Parr RG. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Phys Rev B Condens Matter 1988; 37: 785-9.

18 Becke AD. A new mixing of Hartree-Fock and local density-functional theories. J Chem Phys 1993; 98: 1372-7.

19 Wright JS, Carpenter DJ, McKay DJ, Ingold KU. Theoretical calculation of substituent effects on the O-H bond strength of phenolic antioxidants related to vitamin E. J Am Chem Soc 1997; 119: 4245-52.

20 Sun YM, Zhang HY, Chen DZ. Evaluation of theoretical methods used in calculating O-H bond dissociation enthalpy. Chin J Chem 2001; 19: 657-61.

21 Tomiyama S, Sakai S, Nishiyama T, Yamada F. Factors influencing the antioxidant activities of phenols by an abinitio study. Bull Chem Soc Jpn 1993; 66: 299-304.

22 Migliavacca E, Carrupt PA, Testa B. Theoretical parameters to characterize antioxidants. part 1. The case of vitamin E and analogs. Helv Chim Acta 1997; 80: 1613-26.

23 Zhang HY. Selection of theoretical parameter characterizing scavenging activity of antioxidants on free radicals. J Am Oil Chem Soc 1998; 75: 1705-9.

24 Zhang HY. Theoretical methods used in elucidating activity differences of phenolic antioxidants. J Am Oil Chem Soc 1999; 76: 745-8.

25 Parthasarathy S, Fong LG, Quinn MT, Steinberg D. Oxidative modulation of LDL: comparison between cell-mediated and copper-mediated modulation. Eur Heart J 1990; 11 (Suppl E): 83-7.