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Original article

Antioxidant effects of 14 Chinese traditional medicinal herbs against human low-density lipoprotein oxidation

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ABSTRACT

The relationship between the antioxidant activities and inhibitory effect of 14 Chinese medicinal herbs against oxidized low-density lipoprotein (LDL) formation was evaluated. Prolongation of the lag phase of LDL oxidation depended on the concentration of the herbs. The concentration of each herb that was able to prolong the lag time by about two-fold was calculated and expressed as doubling-time concentration. The lower the doubling-time concentration, the stronger the inhibitory effect exhibited toward LDL oxidation. Among them, Chrysanthemi Flos (*Chrysanthemum morifolium* ramat; 甘菊花 gān jú huā), Crataegi Fructus (*Crataegus pinnatifida* Bge. var. *major* N.E.Br.; 山楂 shān zhā), and Roselle (*Hibiscus sabdariffa* Linn.; 洛神 luò shén) showed significant inhibitory effects. Correlation coefficients between doubling-time concentration and radical-scavenging activities were high; the total phenolic content was also high. In conclusion, phenolic compounds contributed not only to antioxidant activities, but also to the inhibitory effect against LDL oxidation. Chrysanthemi Flos, Crataegi Fructus, and *H. sabdariffa*, with lower doubling-time concentrations, could be potent phytochemical agents to reduce LDL oxidation and prevent the progression of atherosclerosis.

1. Introduction

Cancer, heart disease, diabetes, and brain infarction are the leading causes of death in developed countries, and their impact is steadily growing.[1] Chronic diseases, despite being the most serious health problems, are also preventable. A report by the World Health Organization[1] stated that avoiding unhealthy diet, practicing sufficient exercise, and stopping tobacco use are important ways of prevention of such diseases.

Several epidemiological findings revealed that high-fat and -sugar diets increase the risk of obesity and adult chronic diseases.[2,3] In addition, high-calorie and -fat foods significantly elevate cardiovascular risk factors, including low-density lipoprotein (LDL), total cholesterol, and apolipoprotein-B.[4] A recent study indicated that atherogenesis was affected greatly by the formation of oxidized LDL.[5] Because atherosclerosis accounts mostly for cardiovascular disease[6] and brain infarction, prevention of LDL oxidation should be placed on the front line of the prophylaxis. Intake of dietary antioxidants may be a useful preventive treatment to suppress the formation of oxidized LDL and progression of atherosclerosis. Therefore, red wine, teas, soy foods, coffee, vegetables, and fruits, which contain polyphenols, may reduce the formation of oxidized LDL.[7–11]

Chinese medicinal herbs such as Roselle (*Hibiscus sabdariffa* Linn.; 洛神 luò shén) and *Salvia miltiorrhiza* Bunge (丹參 dān shēn) Bunge received a lot of attention, since they have been proved to have inhibitory effects against LDL oxidation, *in vitro*[12] and *in vivo*.[13] Moreover, a popular formulation of Chinese herbal medicine "Da Chai Hu Tang (大柴胡湯 dà chái hú tāng)" had been used for antihyperlipidemic treatment in ancient times.[14]

A recent study showed that the 25 types of Chinese medicinal herbs containing phenolic compounds exhibited potent antioxidant activities.[15] Steinberg et al[16] indicated that phenolic compounds in foods could promote the stability of LDL to oxidation. However, a comparative study of Chinese medicinal herbs on the

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relationship between their antioxidant activity and LDL oxidation has not yet been attempted. In our previous study, using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2′-deoxyguanosine oxidation methods, we have proved the linear correlations between antioxidant activity and phenolic content.[17]

Because the traditional Chinese belief in the medicinal values of food is based on the concept that food and medicine share the same origin, this view can be considered a forerunner of nutritional science in the world.[18] Many treasured Chinese herbs can be taken as part of a medicinal diet, which is referred to as the homology of medicine and food.[19] Screening of the antioxidant activities of these Chinese medicinal herbs has been reported in our previous work.[17] The aim of this study is to evaluate the antioxidant effects of Chinese medicinal herbs on LDL oxidation.

2. Materials and methods

2.1. Chemicals and reagents

LDL isolated from human plasma (in 0.1% EDTA, pH 7.4) was purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). The Bio-Rad protein assay kit used for determining the concentration of solubilized protein was a product of Bio-Rad Laboratories, Inc. (Hercules, CA, USA). Bovine serum albumin was obtained from Nacalai Tesque Inc. (Kyoto, Japan). Disodium hydrogen phosphate, sodium bromide, sodium chloride, sodium hydroxide, potassium dihydrogen phosphate, and 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) were obtained from Wako Pure Chemical Industries (Osaka, Japan). All the other reagents used were of analytical grade.

2.2. Oxidation of LDL

EDTA and salt from the density gradient were removed from the LDL solution using a prepacked column (Econo-Pac 10DG; Bio-Rad, Richmond, CA, USA), as described by Puhl et al.[20] The concentration of LDL, free of EDTA, was adjusted to 50 μg/mL of protein with 10mM phosphate-buffered saline (pH 7.4), and it was transferred into a quartz cell for spectrophotometric analysis. An aliquot of the sample (100 μL) was then added to the cell. Oxidation was started at 37°C by the addition of AAPH to a final concentration of 1mM. Trolox, as control, was dissolved in ethanol, dried under nitrogen, and incubated with LDL solution (final Trolox concentrations of 0.625 μg/mL, 1.25 μg/mL, and 2.5 μg/mL). The kinetics of LDL oxidation was determined from the change in conjugated diene formation by monitoring the change in absorbance at 234 nm using a Shimadzu UV-3100 spectrophotometer (UVeVISeNIR scanning; Shimadzu Corp., Kyoto, Japan). Absorbance was recorded every 10 minutes for 4 hours. The changes in absorbance at 234 nm and time were divided into three phases: lag, propagation, and decomposition.

2.3. Preparation of sample

Dried Chinese traditional herbs were purchased from local oriental herbal stores in Kaohsiung, Taiwan. The herbal samples were lyophilized in liquid nitrogen. The lyophilized samples were then ground into a fine powder with a food processor and stored at −80°C until analysis.

2.4. Preparation of herbal sample extracts

The lyophilized herbal samples (0.05–0.2 g) were extracted with 2 mL of methanol and acetic acid mixture (methanol:5% acetic acid = 9:1, v/v). The extraction was centrifuged at 1500 × *g* for 10 minutes. The extraction step was repeated three times, and the resulting supernatants were combined and dried under nitrogen. Various concentrations of sample were prepared by dissolving the sample residue in 2–5 mL of 10mM phosphate-buffered saline.

2.5. Inhibitory effect of herbal sample extracts on LDL oxidation

The inhibitory effects of selected herb sample extracts on LDL oxidation were measured, according to the method of oxidation of LDL.

3. Results and discussion

3.1. Optimizing the concentration of radical initiator AAPH

The LDL concentration of 50 μg/mL was adopted because this concentration was sufficiently sensitive to evaluate the effects on LDL oxidizability.[21,22] To optimize the hydrophilic AAPH concentration, concentrations ranging from 0.5mM to 2.0mM were examined to evaluate their initial lag, propagation, and decomposition phases. As shown in Fig. 1, the lag times for 0.5mM, 1.0mM, and 2.0mM were 100 minutes, 60 minutes, and 40 minutes, respectively. Conjugated-diene formation depended on AAPH concentration, showing that the higher the concentration, the shorter the lag phase. However, an unclear or an unsteady decomposition phase was observed at 0.5mM and 2.0mM, respectively. In addition, the propagation phases for 1.0mM and 2.0mM showed almost the same gradient ratio. Therefore, 1.0mM was considered to be the most optimum concentration of AAPH to oxidize 50 μg/mL LDL.

3.2. Identification of inhibitory parameter (doubling-time concentration)

The lag phase in lipid peroxidation processes reflects the antioxidant status of membranes and lipoproteins, and, as a corollary, their resistance to oxidation.[23] Fig. 2 shows the relationship between the concentration of Chrysanthemi Flos [(Ju Hua); *Chrysanthemum morifolium* ramat] and the lag time of LDL oxidation. This result shows that the lag phase of LDL oxidation is concentration dependent at concentrations ranging from 6.7 μg/mL to 20 μg/mL. As shown in Fig. 2, the lag phase was lengthened up to 150 minutes

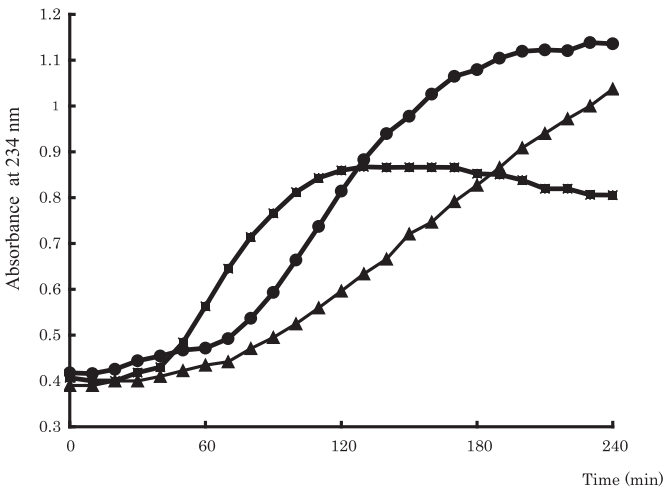

Fig. 1. Time course of LDL oxidation at different concentrations of AAPH. Key: ▲, 0.5mM; ●, 1.0mM; and ■, 2.0mM. AAPH = 2,2′-azobis(2-amidinopropane) dihydrochloride; LDL = low-density lipoprotein.