Arch Pharm Res Vol 26, No 1, 43-46, 2003

Archives of Pharmacal Research
http://apr.psk.or.kr

In Vivo Anti-Oxidant Activities of Tectochrysin

Sanghyun Lee[1,2], Kyoung Soon Kim[1], Youmie Park[1], Kuk Hyun Shin[3], and Bak-Kwang Kim[1]

[1]College of Pharmacy, Seoul National University, Seoul 151-742, Korea, [2]Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea, and [3]Seokwon Life Science Research Institute, Paju 413-832, Korea

(Received October 1, 2002)

The anti-oxidant activities of tectochrysin, a major compound of propolis, were investigated. Tectochrysin exhibited a significant decrease in serum transaminase activities elevated by hepatic damage induced by CCl_4-intoxication in rats. Tectochrysin tested exhibited a lipid peroxidation causing a significant decrease in MDA production in TBA-reactant assay. Tectochrysin was strong in the increase in the anti-oxidant enzymes such as hepatic cytosolic superoxide dismutase, catalase and glutathione peroxidase activities in CCl_4-intoxicated rats. These results suggest that tectochrysin possess not only the anti-oxidant, but also the activities in CCl_4-intoxicated rats. Especially, tectochrysin was found to cause significant increases in the rat liver cytosolic SOD, catalase, GSH-px activities as well as a significant decrease in the MDA production.

Key words: Tectochrysin, Anti-oxidant enzymes, Transaminase TBA-reactant assay

INTRODUCTION

There is extensive evidence to implicate free radicals in the development of degenerative diseases (Cross, 1987). It is suggested that free radical damage to cells leads to the pathological changes associated with aging (Beckman and Ames, 1998). Free radicals may also be a contributory factor in a progressive decline in the function of the immune system (Pike and Chandra, 1995). Cooperative defense systems that protect the body from free radical damage include the anti-oxidant nutrients and enzymes. The anti-oxidant enzymes include superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-px) and indirectly glutathione reductase. Their roles as protective enzymes are well known and have been investigated extensively both *in vivo* and *in vitro* in model systems. The first three enzymes directly catalyze the transformation of peroxides and superoxides to nontoxic species. Glutathione reductase reduces oxidized glutathione to glutathione, a substrate for glutathione peroxidase.

The consequences of oxidative stress are serious, and in many cases are manifested by increased activities of enzymes involved in oxygen detoxification. Identification of new anti-oxidants remains a highly active research area because anti-oxidants may reduce the risk of various chronic diseases caused by free radicals.

The ultimate purpose of this investigation is focused on revealing its biologically active principles effective both on free radical scavenging enzymes and lipid peroxidation. For this purpose, effects of tectochrysin on free radical scavenging enzymes and lipid peroxidation were investigated. Tectochrysin were found to have the anti-oxidant activities.

In the previous paper, we reported the anti-oxidant effects of some propolis (Kim *et al.*, 2002b) and the quantification of tectochrysin from propolis (Kim *et al.*, 2002a).

This paper describes the anti-oxidant activities of tectochrysin that is one of the major compounds of propolis.

MATERIALS AND METHODS

Chemicals

Chrysin, dicyclohexylcarbodiimide (DCC) and diethylphosphoryl cyanide (DEPC) were purchased from Sigma Chemical Co. (St. Louis, MO). Other derivatizing reagents were obtained from Aldrich Chemical Co. (Milwaukee, WI). Sodium azide, ethylenediamine tetraacetic acid (EDTA), β-nicotinamide adenine dinucleotide phosphate, reduced form (NADPH), cumene hydroperoxide, glutathione reductase, DL-α-tocopherol acetate, carbon tetrachloride (CCl_4), xanthine, potassium cyanide (KCN), sodium dodecylsulfate, trichloroacetic acid (TCA), cytochrome C, thiobarbituric acid, *n*-butanol and pyridine were purchased from Sigma

Correspondence to: Bak-Kwang Kim, College of Pharmacy, Seoul National University, Seoul 151-742, Korea
E-mail: kmbk2@snu.ac.kr

Chem. Co. All other chemicals and reagents were analytical grade.

Materials

Tectochrysin, one of the major flavonoids of propolis, was synthesized. To a solution of chrysin in THF, $KHCO_3$ (or K_2CO_3, 2.5 eq) and alkyl bromide (or alkylchloride 5.0-10.0 eq) were added. The resulting mixture was stirred at an ambient temperature for 36-48 h. The reaction mixture was washed with H_2O, 5% HCl, H_2O, and brine, successively. Then, the organic extracts were dried, evaporated, and concentrated *in vacuo*. Next, the resulting residue was chromatographed on silica gel by using $CHCl_3$-MeOH to afford a white or pale yellow solid. A product was recrystallized and purified by using the proper solvent ($CHCl_3$, ethyl acetate or MeOH etc.).

Anti-oxidant assay *in vivo*

Male Sprague-Dawley rats weighing 200-250 g were used in all experiments. Animals were maintained on 12 h light/dark cycle at approximately 22°C and allowed food and water *ad libitum*. All treatments were conducted between 9:00 and 10:00 h to minimize variations in animal response due to circadian rhythm. Rats were injected i.p. with a mixture of CCl_4 in olive oil (1 : 1) at a dose of 0.6 ml/kg to induce hepatotoxicity. Control animals were given the vehicle alone. Rats were pretreated once with DL-α-tocopherol acetate s.c. at a dose of 400 mg/kg and test samples were given i.p. at a dose of 100 mg/kg/day for seven consecutive days prior to the administration of CCl_4. Animals were sacrified 24 h after CCl_4 dosing and blood was collected by decapitation for the determination of serum transaminases.

Hepatic tissues were carefully excised and homogenized in cold 1.15% KCl-10 mM phosphate buffer with EDTA (pH 7.4) and centrifuged at 10,000 rpm for 10 min. The supernatant was further centrifuged at 40,000 rpm for 60 min to obtain cytosolic extract for the measurement of liver cytosolic SOD, catalase, GSH-px activities and malondialdehyde (MDA) content. The protein content was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard.

Determination of serum GOT and GPT activities

Hepatocellular damage was estimated by measuring sGOT and sGPT activities (Reitman and Frankel, 1957). Blood was collected from the abdominal aorta of each rat. The blood was centrifuged at 3,000 rpm at 4°C for 10 min to separate the serum. sGOT and sGPT activities were expressed as Karmen unit.

Determination of anti-oxidant enzyme activities

SOD was assayed by the method of McCord and Fridovich (1969). The reaction mixture containing 0.5 mM xanthine as substrate (300 μl), 0.05 mM KCN (100 μl), 1% sodium deoxycholate (100 μl), xanthine oxidase (20 μl), cytosolic extract (20 μl) and 0.1 mM cytochrome C (300 μl) was placed in a 1 cm cuvette and the rate of increase in absorbance at 550 nm was recorded for 5 min. SOD activity was expressed as unit/mg protein.

Catalase was assayed by the method of Rigo and Rotilio (1977). The cytosolic extract of liver (40 μl) diluted 10 times was added with 0.13 mM phosphate buffer (pH 7.0, 500 μl), distilled water (660 μl) and 15 mM H_2O_2 (1800 μl), and thoroughly mixed. The rate of changes in the absorbance at 240 nm for 5 min was recorded. Catalase activity was expressed as unit/mg protein.

GSH-px was assayed by the method of Burk *et al.* (1978). The reaction mixture containing 0.3 mM phosphate buffer with 4.0 mM EDTA (pH 7.2, 1000 μl), 26.56 mM sodium azide (500 μl), 294.37 mM GSH (60 μl), 8.4 mM NADPH (110 μl), 1 mM cumene hydroperoxide (320 μl), glutathione reductase (5 μl) and cytosolic solution (30 μl) was placed in 1 cm cuvette and the rate of changes in absorbance was recorded at 340 nm for 5 min. GSH-px activity was expressed as unit/mg protein.

Determination of lipid peroxidation

Lipid peroxidation in rat liver microsomes was evaluated by the TBA method (Buege and Aust, 1978). The reaction mixture of liver microsome, 8.1% sodium dodecylsulfate (0.2 ml) and TCA-thiobarbituric acid in 20% acetate buffer (2 ml, pH 3.5) was heated for 1 h in boiling water bath. After cooling, *n*-butanol : pyridine (15 : 1) solution was added and centrifuged to obtain *n*-butanol:pyridine layer. The absorbance of the sample was determined at 532 nm. The level of lipid peroxides is expressed as MDA nmol/mg protein.

Statistical analysis

Multiple comparison test was applied for detecting the significance of difference between different groups.

RESULTS AND DISCUSSION

Tectochrysin was tested for their effects on lipid peroxidation as well as on hepatocellular damage in CCl_4-intoxicated rats. The effects of tectochrysin on serum transaminases in CCl_4-intoxicated rats were estimated and the results are shown in Table I. In the CCl_4-intoxicated control group, the sGOT and sGPT activities increased significantly when compared with the control group. In contrast, the groups treated with tectochrysin decreased significantly these elevated transaminase activities.

Lipid peroxidation was assessed by means of an assay that determines the production of MDA in rat liver microsome. MDA production in the CCl_4-intoxicated rats decreas-

Table I. Effect of tectochrysin on sGOT and sGPT activities

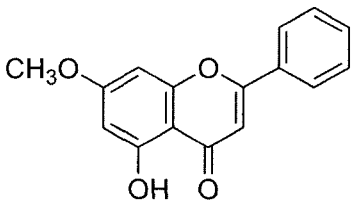
Treatments	GOT (Karmen unit)	GPT (Karmen unit)
Control	108.69 ± 10.34	120.88 ± 8.13
Tocopherol acetate	70.61 ± 5.98	72.81 ± 4.98
Tectochrysin	79.60 ± 5.18*	79.02 ± 5.47**

Rats were preintoxicated with DL-α-tocopherol acetate given subcutaneously at a dose of 400 mg/kg tectochrysin given orally at a dose of 100 mg/kg, 24 h or 7 days, respectively, prior to the administration of CCl_4. Rats were sacrified 24 h after CCl_4. Heparinized blood sample was collected. Hepatocellular damage was estimated by measuring sGOT and sGPT activities. Significantly the different from the control; *p<0.05, **p<0.01.

ed when compared with the control group. Tectochrysin, when administered i.p. with a dry weight equivalent dosage of 100 mg/kg/day of total extract for seven consecutive days in the CCl_4-intoxicated rats, was shown to exhibit significant inhibition of MDA production (Table II) and cause a significant elevation of free radical scavenging enzyme activities such as SOD, catalase and GSH-px. As shown in Table II, tectochrysin caused significant elevation of SOD activity. Tectochrysin was almost equipotent to that of DL-α-tocopherol acetate. Similar results were obtained in case of the catalase and the GSH-px activities as shown in Table II.

The extracts from *Culcitum reflexum* have been reported to possess anti-oxidant and photo-protective activities and their major compounds were flavonoids (Aquino *et al.*, 2002). Many researchers have demonstrated the *in vitro* anti-oxidant and free radical scavenging activities of flavonoids (Brown *et al.*, 1998; Cao *et al.*, 1997; Plumb *et al.*, 1999; Rice-Evans *et al.*, 1997).

In conclusion, the present study demonstrated that tectochrysin possess not only the anti-oxidant, but also the activities in CCl_4-intoxicated rats. Especially, tectochrysin was found to cause significant increases in the rat liver cytosolic SOD, catalase, GSH-px activities as well as a significant decrease in the MDA production. The results of the present study suggest, therefore, that free radical-mediated damage can be controlled with adequate anti-oxidant defenses.



ACKNOWLEDGEMENT

This research was supported by Brain Korea 21 and Research Institute of Pharmaceutical Science, College of Pharmacy, Seoul National University, Korea.

REFERENCES

Aquino, R., Morelli, S., Tomaino, A., Pellegrino, M., Saija, A., Grumetto, L., Puglia, C., Ventura, D., Bonina, F. Antioxidant and photoprotective activity of a crude extract of *Culcitum reflexum* H. B. K. leaves and their major flavonoids. *J. Ethnophamacol.*, 79, 183-191 (2002).

Beckman, K. B., Ames, B. N. The free radical theory of aging matures. *Physio. Rev.*, 78, 547-581 (1998).

Blois, M. S. Antioxidant determinations by the use of a stable free radical. *Nature*, 26, 1199-1200 (1958).

Brown, J. E., Khodr, H., Hider, R. C., Rice-Evans, C. A. Structural dependence of flavonoids interactions with Cu^{2+} ions: implications for their antioxidant properties. *Biochem, J.*, 15, 1173-1178 (1998).

Buege, J. A., Aust, S. D. Microsomal lipid peroxidation. *Methods in Enzymology*, 52, 302-306 (1978).

Burk, R. F., Nishiki, K., Lawrence, R. A., Chance, B. Peroxide removal by selenium-dependent and selenium-independent glutathione peroxidases in hemoglobin free perfused rat liver. *J. Biol. Chem.*, 253, 43-46 (1978).

Cao, G., Sofic, E., Prior, R. L. Antioxidant and prooxidant behaviour of flavonoids: structure-activity relationships. *Free Radical Biology & Medicine*, 22, 749-760 (1997).

Cross, C. E. Oxygen radicals and human disease. *Ann. Inter. Med.*, 107, 526-545 (1987).

Kim, K. S., Lee, S., Kim, M. B., Park, Y., Kim, B-K. Quantification of tectochrysin from propolis. (Manuscript submitted for publication, 2002a).

Kim, K. S., Lee, S., Park, Y., Lee, J. S., Kim, B-K. Anti-oxidant effects of some propolis. (Manuscript submitted for publication, 2002b).

Lowry, O. H., Posenbrough, N. N., Farr, A. L., Randall, R. J. Protein measurement with the folin phenol reagent. *J. Biol.*

Table I. Effect of tectochrysin on the liver cytosolic SOD, the liver cytosolic GSH-px, the liver cytosolic catalase activities and the hepatic MDA production

Treatments	SOD (unit/mg protein)	GHS-px (unit/mg protein)	Catalase (unit/mg protein)	MDA (nmol/mg protein)
Control	5.29 ± 0.24	0.23 ± 0.01	215.11 ± 11.39	7.56 ± 0.65
Tocopherol acetate	7.42 ± 0.50	0.30 ± 0.02	294.20 ± 10.22	5.30 ± 0.40**
Tectochrysin	6.77 ± 0.28*	0.29 ± 0.02*	279.26 ± 17.11*	5.33 ± 0.33**

Each value represents mean ± S.E.M. Significantly the different from the control; *p<0.05, **p<0.01.

Chem., 193, 265-275 (1951).

McCord, J. M., Fridovich, I. Superoxide dismutase: An enzymatic function for erythrocuprein (hemocoprein). *J. Biol Chem.*, 244, 6049-6055 (1969).

Pike, J., Chandra, R. K. Effect of vitamin and trace element supplementation on immune indices in healthy elderly. *Int. J. Vitamin Nutr. Res.*, 65, 117-120 (1995).

Plumb, G. W., Price, K. R., Williamson, G. Antioxidant properties of flavonol glycosides from green beans. *Redox Report*, 4, 123-127 (1999).

Reitman, S., Frankel, S. A chlorotrimetric method for the determination of sGOT and sGPT. *Am. J. Clinic. Patho.*, 28, 56-63 (1957).

Rice-Evans, C. A., Miller, N. J., Paganga, G. Antioxidant properties of phenolic compounds. *Trends in Plant Sciences Reviews*, 2, 152-159 (1997).

Rigo, A., Rotilio, G. Simultaneous determination of superoxide dismutase and catalase in biological materials by polarography. *Anal. Biochem.*, 81, 157-166 (1977).