Galangin protects hydrogen peroxide induced oxidative stress via the scavenging of reactive oxygen species

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Abstract

Flavonoids are a class of secondary metabolites abundantly found in fruits and vegetables. In addition, flavonoids have been reported as potent antioxidants with beneficial effects against oxidative stress related diseases such as cancer, aging, and diabetes. Galangin (3,5,7-trihydroxyflavone), a member of the flavonol class of flavonoid, is present in high concentrations in medicinal plants (e.g. *Alpinia officinarum*) and propolis, a natural beehive product. The present study was carried out to investigate the protective effects of galangin against hydrogen peroxide (H_2O_2)-induced oxidative stress. Galanginwas found to quench the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and intracellular reactive oxygen species generated by H_2O_2 treatment in cells, which is detected by a spectrofluorometer after staining of 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA). These results suggest that galangin protected V79-4 lung fibroblast cells against H_2O_2-induced oxidative stress via the scavenging of reactive oxygen species (ROS). (J Med Life Sci 2009;6:362-364)

Key Words : Galangin, Reactive oxygen species.

Introduction

Galangin (3,5,7-trihydroxyflavone) (Fig. 1), a member of flavonol class of flavonoid, is present in high concentrations in honey and *Alpinia officinarum*, a plant which has been used as spice and as a herbal medicine for a variety of ailments in Asia for centuries. From the ethanol extract of *A. officinarum* root, galangin makes up approximately 10% of the extract[1]. Galangin is also present in high concentrations in propolis, which is a natural composite balsam produced by honeybees from the gum of various plants, with the following components: galangin (9%), chrysin (4%), and quercetin (2%)[2]. Galangin possesses certain biological activities, including anti-mutagenic[3], anti-clastogenic[4], anti-oxidative and radical scavenging[5, 6], and metabolic enzyme modulating activities[7].

Reactive oxygen species, including the superoxide anion, hydroxyl radical, single oxygen, and hydrogen peroxide, are oxygen containing molecules with unpaired electrons or abstract electrons from other molecules. These reactive oxygen species can lead to functional damage in lipid, proteins and DNA, which can eventually result in cell death[8]. Furthermore, the oxidative stress induced by the overproduction of ROS plays an important role in various lung pathologies, including bronchial asthma, cystic fibrosis, pulmonary sarcoidosis, and lung cancer[9, 10, 11] and lung fibroblast is very sensitive to oxidative stress[12].

This study focused on evaluating the protective effect of galangin on H_2O_2-induced oxidative stress in V79-4 lung fibroblast cells.

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Materials and methods

1. Reagents

Galangin (Fig. 1) was obtained from Professor Sam Sik Kang of SeoulNational University, Republic of Korea. Galangin was freshly dissolved in dimethyl sulfoxide (DMSO), yielding a final concentration, which did not exceed 0.1%. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) were purchased from Sigma Chemical Company (St. Louis, MO). All other chemicals and reagents used were of analytical grade.

2. Cell culture

Previous reports have shown that the lung is an organ which is sensitive to oxidative stress[12, 13]. To study the effect of galangin on oxidative stress, we used Chinese

hamster lung fibroblasts (V79-4 cells). The V79-4 cells were obtained from the American Type Culture Collection and maintained at 37 ℃ in an incubator at a humidified atmosphere of 5% CO_2 and cultured in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal calf serum, streptomycin (100 μg/ml) and penicillin (100 units/ml).

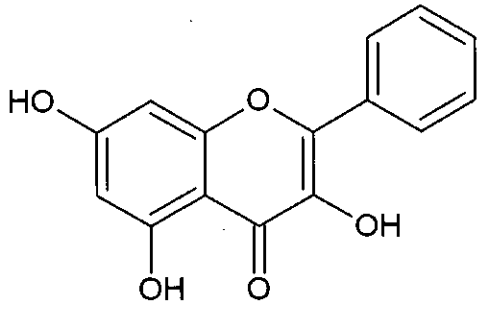
3. DPPH radical scavenging activity

Galangin at concentrations of 0.1,1 and 10 μg/ml were added to a 1 × 10^{-4} M solution of DPPH in methanol, and the reaction mixture was shaken vigorously. After 30 min, the amount of DPPH remaining was determined at 520 nm[14]. The DPPH radical-scavenging activity (%) was calculated as 100 × [(optical density of DPPH radical treatment) − (optical density of galangin with DPPH radical treatment)]/(optical density of DPPH radical treatment).

4. Intracellular ROS measurement

To detect intracellular ROS, the DCF-DA method was used. DCF-DA diffuses into cells, where it is hydrolysed by intracellular esterase to polar 2',7'-dichlorodihydrofluorescein. This non-fluorescent fluorescein analogue is trapped in cells and can be oxidised to the highly fluorescent 2',7'-dichlorofluorescein by intracellular oxidants[15]. The V79-4 cells were seeded in a 96-well plate at 2 × 10^4 cells/well. Sixteen hours after plating, the cells were treated with galangin at concentrations of 0.1, 1 and 10 μg/ml. After 30 min, 1 mM H_2O_2 was added to the plate. The cells were incubated for an additional 30 min at 37 ℃. After the addition of 25 μM DCF-DA solution for 10 min, the fluorescence of 2',7'-dichlorofluorescein was detected using a Perkin-Elmer LS-5B spectrofluorometer. The intracellular ROS scavenging activity (%) was calculated as 100 × [(optical density of H_2O_2 treatment) − (optical density of galangin with H_2O_2 treatment)] / (optical density of H_2O_2 treatment).

Figure 1. Chemical structure of galangin.



5. Statistical analysis

All measurements were made in triplicate and all values were expressed as the means ± standard error. The results were subjected to an analysis of variance (ANOVA) using the Tukey test to analyze the difference. $P < 0.05$ was considered significantly.

Results and Discussion

The radical-scavenging effects of galangin on the DPPH radical and intracellular ROS were measured. The DPPH radical-scavenging activity of galangin showed dose dependent manner, 3.3% at 0.1 μg/ml, 7% at 1 μg/ml, and 35% at 10 μg/ ml. The DPPH radical-scavenging activity of

Figure 2. Effect of galangin on the scavenging of DPPH radical. Measurements were made in triplicate and values are expressed as means ± standard error. *Significantly different from control ($P < 0.05$).

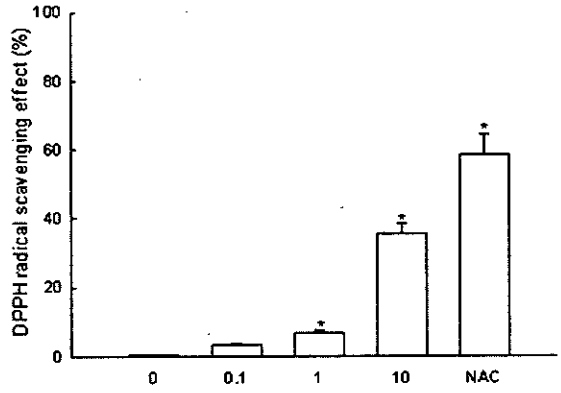
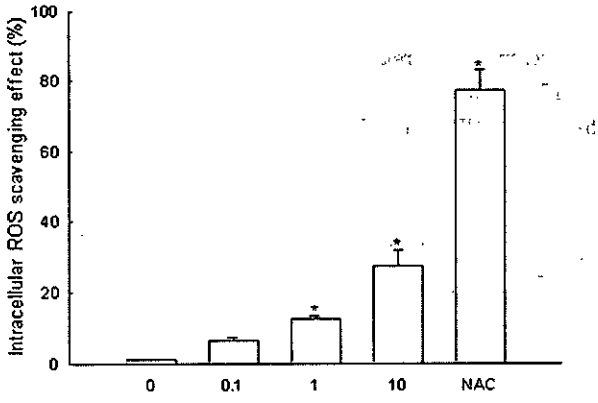


Figure 3. Effect of galangin on the scavenging intracellular ROS. The intracellular ROS generated were detected with spectrofluorometer after DCF-DA treatment. Measurements were made in triplicate and values are expressed as means ± standard error. *Significantly different from control ($P < 0.05$).



N-acetylcystein (NAC), a major antioxidant used as a positive control, showed 58% at 2 mM (Fig. 2). The intracellular ROS scavenging activity of galangin showed dose dependent manner, 6% at 0.1 μg/ml, 13% at 1 μg/ml, and 28% at 10 μg/ml; The intracellular ROS scavenging activity of NAC was 77% at 2 mM (Fig. 3). These results indicate a reduction of ROS by galangin treatment, and suggest that galangin possesses antioxidant properties. Flavonoids are polyphenolic compounds present ubiquitously in fruits, vegetables, and beverages such as tea and red wine[16, 17]. They have been shown to possess a variety of biological activities at non-toxic concentrations in organisms. Polyphenols have an ideal and intrinsic structure for capturing free radicals and electron delocalization, causing higher antioxidant activity[18].The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet-oxygen quenchers[19]. Galangin, a member of the flavonol class of flavonoid, is present in high concentrations in medicinal plants (e.g. *Alpinia officinarum*) and propolis, a natural beehive product[1]. Results from V79-4 cells studies indicate that galangin with anti-oxidative and free radical scavenging activities. The antioxidant effect of galangin is attributed to this polyphenolic structure.

References

1) Heo MY, Sohn SJ, Au WW. Anti-genotoxicity of galangin as a cancer chemopreventive agent candidate. Mutat Res 2001 May;488(2):135-50.
2) Park YK, Koo MH, Sato HH, Contado JL. Survey of some components of propolis which were collected by *Apis mellifera in Brazil*. Arch Biol Technol 1995;38:1253-9.
3) Wall ME, Wani MC, Manikumar G, Abraham P, Taylor H, Hughes TJ, Warner J, McGivney R. Plant antimutagenic agents, 2. Flavonoids. J Nat Prod 1988 Nov-Dec;51(6): 1084-91.
4) Heo MY, Jae LH, Jung SS, Au WW. Anticlastogenic effects of galangin against mitomycin C-induced micronuclei in reticulocytes of mice. Mutat Res 1996 May 17;360(1):37-41.
5) Cholbi MR, Paya M, Alcaraz MJ. Inhibitory effects of phenolic compounds on CCl4-induced microsomal lipid peroxidation. Experientia 1991 Feb 15;47(2):195-9.
6) Imamura Y, Migita T, Uriu Y, Otagiri M, Okawara T. Inhibitory effects of flavonoids on rabbit heart carbonyl reductase. J Biochem 2000 Apr;127(4):653-8.
7) Shih H, Pickwell GV, Quattrochi LC. Differential effects of flavonoid compounds on tumor promoter-induced activation of the human CYP1A2 enhancer. Arch Biochem Biophys 2000 Jan 1;373(1):287-94.
8) Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol. 1990;186:1-85.
9) Koura T, Gon Y, Hashimoto S, Azuma A, Kudoh S, Fukuda Y, Sugawara I, Yodoi J, Horie T. Expression of thioredoxin in granulomas of sarcoidosis: possible role in the development of T lymphocyte activation. Thorax 2000 Sep;55(9):755-61.
10) Langley SC, Brown RK, Kelly FJ. Reduced free-radical-trapping capacity and altered plasma antioxidant status in cystic fibrosis. Pediatr Res 1993 Mar;33(3):247-50.
11) Petruzzelli S, Hietanen E, Bartsch H, Camus AM, Mussi A, Angeletti CA, Saracci R, Giuntini C. Pulmonary lipid peroxidation in cigarette smokers and lung cancer patients. Chest 1990 Oct;98(4):930-5.
12) Murray JI, Whitfield ML, Trinklein ND, Myers RM, Brown PO, Botstein D. Diverse and specific gene expression responses to stresses in cultured human cells. Mol Biol Cell 2004 May;15(5):2361-74.
13) Pryor WA, Stone K, Zang LY, Bermúdez E. Fractionation of aqueous cigarette tar extracts: fractions that contain the tar radical cause DNA damage. Chem Res Toxicol 1998 May;11(5):441-8.
14) Soong YY, Barlow PJ. Quantification of gallic acid and ellagic acid from longan (Dimocarpus longan Lour) seed and mango (Mangifera indica L.) kernel and their effects on antioxidant activity. Food Chemistry 2006 Aug 97(3):524-30.
15) Rosenkranz AR, Schmaldienst S, Stuhlmeier KM, Chen W, Knapp W, Zlabinger GJ. A microplateassay for the detection of oxidative products using 2',7'-dichlorofluorescin-diacetate. J Immunol Methods 1992 Nov 25;156(1):39-45.
16) Hertog MGL, Hollman PCH, and Katan MB. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J Agric Food Chem* 199240(12):2379-83.
17) Hertog MGL, Hollman PCH, and van de Putte B. Content of potentially anticarcinogenic flavonoids of tea infusions, wines and fruit juices. *J Agr Food Chem* 199341(8):1242-6.
18) Sangeetha P, Das UN, Koratkar R, Suryaprabha P. Increase in free radical generation and lipid peroxidation following chemotherapy in patients with cancer. Free Radic Biol Med 1990;8(1):15-9.
19) Lenton KJ, Greenstock CL. Ability of human plasma to protect against ionising radiation is inversely correlated with age. Mech Ageing Dev 1999 Feb 1;107(1):15-20.