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Inhibitory Effects of Isoflavones in *Sophora mooracrotiana* on Lipid Peroxidation by Superoxide

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Abstract

The possible inhibitory effects were investigated for three isoflavones: sophoraisoflavone A, and licoisoflavones A and B, isolated from *Sophora mooracrotiana* Benth *ex* Baker, on lipid peroxidation by superoxide anion. They inhibited the production of lipid peroxidation each by superoxide anion and the generation of superoxide anion by the xanthine-xanthine oxidase system. Their effects were similar to superoxide dismutase as a superoxide anion scavenger. These results demonstrate that these isoflavones have inhibitory effects on oxidative stress.

Keywords: Licoisoflavone A, licoisoflavone B, lipid peroxidation; sophoraisoflavone A, *Sophora mooracrotiana*, superoxide anion.

Introduction

Oxygen free radicals, such as the superoxide anion, induce lipid peroxidation (LPO), which injures cell membranes (Kappus & Sies, 1981). The diseases induced by LPO involve arteriosclerosis (Evensen et al., 1983), halothane hepatoxicity (de Groot and Noll, 1996) and liver disease (Lewis & Paton, 1982). Recently, natural antioxidants have been found in many plants, such as spices, vegetables and herbs (Nakatani et al., 1988). Kaempferol and quercetin are flavonoids that scavenge superoxide anion, and inhibit LPO (Erben-Russ et al., 1987). We showed that some isoflavones, such as biochanin A, formononetin and genistein, from the crude drugs, inhibit LPO by superoxide anion (Toda & Shirataki, 1999).

In this paper, we investigated the inhibitory effects of sophoraisoflavonee A, and licoisoflavones A and B isolated from *Sophora mooracrotiana*, a crude drug used in China, on production of LPO by superoxide anion and generation of superoxide anion by the xanthine-xanthine oxidase system (Fig. 1).

Materials and methods

Materials

Adenosine-5-diphosphate monopotassium (ADP), bovine serum albumin (BSA), butylated hydroxytoluene (BHT), ethylenediaminetetraacetic acid disodium salts (EDTA), ferric chloride ($FeCl_3$), genistein, lecithin (from egg yolk), nitroblue tetrazolium (NBT), superoxide dismutase (SOD; EC 1,15.1.1, from butter milk), thiobarbituric acid (TBA), trichloroacetic acid (TCA), xanthine and xanthine oxidase (XOD; EC 1.2.3.2, from butter milk, Grade I), were obtained from Wako Pure Chemicals (Osaka, Japan). Other reagents were of analytical grade.

Extraction and isolation of sophoraisoflavone A, licoisoflavone A and B from *Sophora moorcroftiana* Benth *ex* Baker

The dried roots of *Sophora mooracrotiana* Benth *ex* Baker, which were collected in Jomsom, Nepal in September, 1986, were first extracted with methanol and then with ether. The ether extracts were fractioned repeatedly by silica gel column chromatography, with benzene-ethyl acetate mixture as the eluent. They afforded sophoraisoflavone A and licoisoflavones A and B, identified by Dr. Yoshiaki Shirataki, Department of Pharmaceutical Science, Josai University, Japan (Shirataki et al., 1988). A voucher specimen was deposited in the Department of Pharmaceutical Science of the Josai University in Japan, by Dr. Yoshiaki Shirataki.

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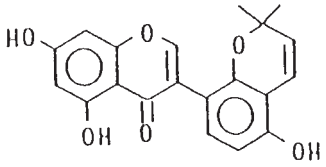
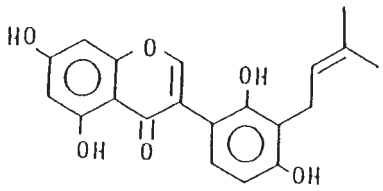
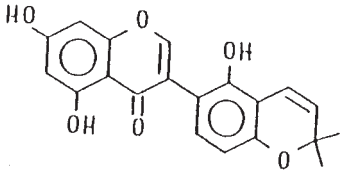
Sophoraisoflavone A



Licoisoflavone A



Licoisoflavone B



Genistein

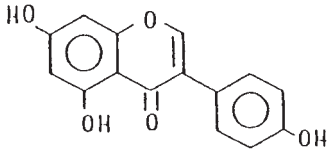


Figure 1. Chemical structures of Sophoraisoflavone A, licoisoflavones A and B, and genistein.

Assay of inhibitory effect on production of LPO induced by superoxide anion

One ml of the reaction mixture, containing 1 µmol/ml of lecithin, 0.33 mM xanthine, 1.7 mM ADP-0.1 mM $FeCl_3$, 0.11 mM EDTA-0.1 mM $FeCl_3$, 0.1 U/ml of XOD and the test sample in the 50 mM Tris-HCl buffer (pH 7.4), was incubated at 37 °C for 10 min. For incubation, the mixture was added to 2 ml of TBA reagent which contained 0.37% TBA, 15% TCA and 15% BHT, and this was heated at 100 °C for 15 min. The solution was centrifuged at 3000 rpm for 10 min. The absorbance of the supernatant at 535 nm was determined as malonedialdehyde (MDA) (Tien & Aust, 1982).

The inhibitory ratio of the test sample was also evaluated by the following equation:

$$\text{Inhibitory ratio (\%)} = \frac{\text{MDA formed in the absence of test sample} - \text{MDA formed in the presence of test sample}}{\text{MDA formed in the absence of test sample}} \times 100$$

Assay of inhibitory effects on generation of superoxide anion by xanthine-xanthine oxidase system

The activities of the test samples were measured according to the method of Yamanaka et al. (1979). Three ml of the reaction mixture, containing 0.1 mM xanthine, 0.1 mM EDTA, BSA (50 µg protein/ml), 40 mM sodium carbonate (pH 10.2), 25 mM NBT, test sample and 7×10^{-9} XOD, was incubated at 25 °C for 20 min. The reaction was terminated after incubation by addition of 0.1 ml of 6 mM $CuCl_2$ solution. The absorbance of formazan, produced in the absence of test sample solution, was measured. Inhibitory effects of the test samples on the generation of superoxide anion were estimated by the following equation:

$$\text{Inhibitory ratio} = \frac{\text{Absorbance with no addition of test sample} - \text{Absorbance with addition of test sample}}{\text{Absorbance with no addition of sample}} \times 100$$

Caluculation of 50% inhibitory concentration (IC_{50})

The inhibitory ratio was plotted against the log concentration, and these dose-response data were used to calculate the IC_{50} value.

Statistical analysis

Values were expressed as the mean ± standard error of 5 experiments. The Student's *t*-test was used for statistical treatment of the results, setting $p < 0.001$ for the significance of differences.

Results

The inhibitory ratios of sophoraisoflavone A, and licoisoflavones A and B on lipid peroxidation by superoxide anion, were 65.8 ± 3.4, 85.0 ± 1.7 and 92.5 ± 4.8% respectively, at a concentration of 100 µM. Those of genistein as a positive control and SOD as a superoxide anion scavenger, were 74.3 ± 3.6% at concentration of 100 µM and 65.1 ± 1.1% at concentration of 1 U/ml. They increased in a concentration-dependent manner. IC_{50} values of sophoraisoflavone A, licoisoflavones A and B were 27.0, 7.2 and 2.7 µM, respectively. Those of genistein and SOD were 6.3 µM and 6.0×10^{-2} U/ml, respectively (Table 1).

The inhibitory ratios of sophoraisoflavone A, and licoisoflavones A and B on generation of superoxide anion

Table 1. IC_{50} (μM) of sophoraisoflavone A, licoisoflavones A and B on lipid peroxidation by superoxide anion and generation of superoxide anion by the xanthine-xanthine oxidase system.

Test sample	Lipid peroxidation	Generation of superoxide anion
sophoraisoflavone A	27.0	–
licoisoflavone A	7.2	18.0
licoisoflavone B	2.7	31.2
genistein	6.3	–
SOD (U/ml)	6.0×10^{-2}	10.0×10^{-3}

by xanthine-xanthine oxidase were 40.9 ± 6.2, 58.3 ± 0.6 and 58.3 ± 3.1% respectively, at a concentration of 100 μM. Those of genistein as a positive control and SOD as a superoxide anion scavenger, were 13.8 ± 2.8% at the concentration of 100 μM and 98.6 ± 1.1% at the concentration of 1 U/ml. licoisoflavone A and B, and SOD increased in a concentration-dependent manner. IC_{50} values of licoisoflavones A and B, and SOD were 18.0, 31.2 μM and 10.0×10^{-3} U/ml, respectively (Table 1).

Discussion

Rice-Evans et al. (1997) demonstrated that 5,7-dihydroxy or *ortho* 3′,4′-dihydroxy groups of flavonoids increase antioxidative activity. There is a 5,7-dihydroxy group in sophoraisoflavone A, licoisoflavones A and B, but there is not an *ortho* 3′,4′-dihydroy group in these isoflavones. Hu et al. (1995) demonstrated that the superoxide anion-scavenging activities of isoflavonids increase with an increasing number of hydroxy groups in the B ring. However, there is a hydroxy group in the B ring of sophoraisoflavone A or licoisoflavone B. There is the 2′-hydroxy group in licoisoflavone A or B, which had stronger effects than sophoraisoflavone A on the production of LPO by the superoxide anion and the generation of superoxide anion by xanthine-xanthine oxidase system. From these results it can be speculated that the 2′-hydroxy group of isoflavone increases the inhibitory effect on the production of LPO by superoxide anion and the generation of superoxide anion.

These results demonstrate that these isoflavones have inhibitory effects on LPO by superoxide anion, and that the differences of inhibitory effects depend on the functional groups in the B ring of isoflavone.

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