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The prooxidant, rather than antioxidant, acts of daidzein in vivo and in vitro: Daidzein suppresses glutathione metabolism

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Abstract

The present study was performed to investigate the effects of chronic administration of daidzein on lipid peroxidation and glutathione concentration in the livers and brains of rats. Male Sprague-Dawley rats were divided into two groups, one of which was fed a normal diet and the other a vitamin E-free diet. Each of these groups was divided further into three subgroups and treated either with vehicle or with daidzein administered orally at either 2 or 20 mg/day for 4 weeks. The concentrations of α-tocopherol in the serum and the brain increased following daidzein treatment, and these increases were significantly greater in rats maintained on a vitamin E-free diet. Daidzein significantly decreased the concentration of malondialdehyde in the organs, and this decrease was more pronounced in vitamin E-deprived rats than in those maintained on a normal diet. Although the liver glutathione concentration was not affected, daidzein treatment (20 mg/day) decreased the glutathione concentration in the brain significantly and to a similar extent in vitamin E-deprived rats and those fed normal diet. In addition the daidzein metabolite, equol, severely decreased the ratio of GSH and GSSG in primary cortical neuron cells exposed to it. Collectively, these results suggest that daidzein may act not only as an antioxidant, but also a prooxidant in brain rats, this should be in the brains of rats.

Keywords: Antioxidant; Daidzein; Equol; Glutathion; Prooxidant

1. Introduction

Cells in the body continuously produce free radicals and reactive oxygen species (ROS) as a result of metabolic processes. These free radicals are neutralized by an antioxidant defense system consisting of enzymes such as catalase, superoxide dismutase, and glutathione peroxidase, as well as nonenzymatic antioxidants, including vitamins (e.g., ascorbic acid, tocopherol), glutathione, and flavonoids. Recently, a great deal of attention has focused on "natural antioxidants" that purportedly possess specific medically and physiologically beneficial activities.

Among natural antioxidants, flavonoids are most abundant in our daily foods. The common structure of flavonoids is the flavan nucleus, which consists of 15 carbon atoms arranged in three rings (phenylchromanone structure, C6-C3-C6). Rings A and B are benzene rings and ring C is a heterocyclic pyran or pyrone. Daidzein (7,4-dihydroxyisoflavone) has a flavone nucleus composed of two benzene rings linked through a heterocyclic pyrone ring (Rice-Evans and Miller, 1996). Daidzein belongs to the isoflavone subclass of flavonoids and is found in fruits, nuts, soybeans, and soy-based products (Liggins et al., 2000a,b).

Several epidemiological studies have supported the hypothesis that the antioxidant action of flavonoids may reduce the risk of developing cancer and cardiovascular disease. For example, the incidence of chronic diseases is significantly lower in populations in Asia, where isoflavone consumption is high, than in Western countries, where isoflavone consumption is low (Wu et al., 1996; Ziegler et al., 1993). It is also known that daidzein inhibits LDL oxidation, which plays a potential role in atherosclerosis (Zhu et al., 1999; Hwang et al., 2000; Van Acker et al., 2000), by scavenging reactive oxygen species

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(ROS) or blocking generation of ROS involved in numerous pathological events.

Increasing evidence, however, suggests that high concentrations of daidzein may induce harmful effects. Rohrdanz et al. (2002) reported that daidzein affected the antioxidant enzyme defense system in rat hepatoma H4IIE cells, and these changes added to the oxidant rather than antioxidant properties of daidzein. It has also been reported that daidzein was readily metabolized to monohydroxylated compounds such as a equol, 3′-hydroxy-isoflavones, 6-hydroxy-isoflavones, 3′,4′,7-trihydroxy-isoflavones, 4′,6,7-trihydroxyisoflavone, and 4′,7,8-trihydroxyisoflavone. These metabolites have been identified in man. In addition, some of these metabolites have been formed by oxidative processes (Kulling et al., 2000, 2001, 2002).

However, few in vivo studies have assessed whether chronically administering high doses of daidzein produces prooxidant effects, and no data are available. Therefore, the present study was performed to investigate the effects of chronic daidzein administration on lipid peroxidation and glutathione-related metabolism in the liver and brain of rats. The effects of daidzein treatment on the condition of oxidative stress were compared in rats maintained on normal and vitamin E-free diets. The findings were then confirmed by an in vitro study with primary cultures of cortical neurons.

2. Materials and methods

2.1. Preparation of liver and brain homogenate

Male Sprague-Dawley rats (250–260 g; Daehan Biolink, Chungbuk, Korea) were housed individually in cages (22 ±2 °C, 40–50% relative humidity) under controlled lighting (12-h light/dark cycle). Rats were fed a standard diet [AIN 93M containing 75 IU of vitamin E (all-rac-α-tocopheryl acetate)/kg diet; Dyets, Bethlehem, PA, USA] and allowed free access to water. After an adaptation period, the rats were divided into two groups, one of which was maintained on a normal diet and another that was fed a vitamin E-free diet (Dyets). Subsequently, each group was subdivided randomly into three treatment groups. Daidzein (TCI Tokyo Kasei Kogyo Co., Tokyo, Japan) was dissolved in corn oil and administered orally to two of the three groups at 2 and 20 mg/day for 4 weeks, respectively. Rats in the remaining (control) group were given the vehicle alone. Animal care in this study conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health. After 4 weeks, rats were anesthetized by intravenous injection of ketamine (50 mg/kg) and xylazine (5 mg/kg) at 6.5 h after final administration of daidzein. Their livers and brains were isolated, blotted, weighed, frozen in liquid nitrogen and stored at −70 °C until assayed. Tissue was homogenized in 0.1 M phosphate-EDTA buffer (pH 7.4) with 25% HPO_3 at 4 °C.

2.2. Culture of primary cortical neuron cells

The medium, fetal calf serum and horse serum used for cell culture were purchased from Gibco-BRL. Primary culture of embryonic cortical neurons was performed as follows; 6-well plates were coated with poly-L-lysine solution, supplemented with laminin. Cortical neurons removed from E-18 Sprague-Dawley rats were dissociated and triturated with a Pasteur pipette. Cortical neurons were plated at a density of 1.0 $\sim 1.5 \times 10^6$ cells/well and $1.5–2.0 \times 10^5$ cells/well on 6-well plates. Cells were cultured with MEM solution supplemented with 2 mM L-glutamate, 10% (v/v) fetal calf serum and 10% horse serum at 37 °C in 95% air/5% CO_2. After 3 days, the medium was replaced with culture medium containing either daidzein or equol (0.3–100 μM, dissolved in DMSO) or 0.1% dimethyl sulfoxide (DMSO), and cells were incubated for various time.

2.3. Measurement of daidzein, its metabolite equol and α-tocopherol concentrations

The concentrations of daidzein, its metabolite equol and α-tocopherol were analyzed by high-performance liquid chromatography (HPLC). Determination of daidzein and equol concentration in serum was performed accordingly to a modified method of King and Bursill (1998). Briefly, each 200-μl aliquot of serum was mixed with 400 μl of 0.17 M ammonium acetate (pH 4.6) containing 1000 units of β-glucuronidase, and hydrolyzed by incubation overnight (16 h) at 37 °C. The sample solution was extracted twice with 500 μl of diethyl ether. The aqueous and organic phases were separated by centrifugation and the upper (organic) phase was dried under nitrogen gas. The dried residue was re-dissolved in 200 μl of methanol and injected into the HPLC system. The mobile phase consisted of methanol, 0.1 M ammonium acetate (pH 4.6) and 25 mM EDTA (55:50:1, v/v/v), at a flow rate of 1.0 ml/min and a potential of 700 mV.

To quantify α-tocopherol concentrations in serum and liver homogenates, sample was deproteinized with ethanol containing 0.01% ascorbic acid. Extraction was carried out twice with 1 ml of *n*-hexane. The separation and injection procedures were the same as described above for daidzein. The mobile phase (methanol/water, 95:5, v/v) was processed at a flow rate of 1.5 ml/min at 290 nm.

The HPLC system consisted of a Waters 501 pump (Waters, Watford, UK) fitted with a UV spectrophotometer (Shimadzu, Kyoto, Japan). Samples were analyzed on a 4.6 × 250 mm RP C-18 Mightysil column (5 μm) (Kanto Chemical, Tokyo, Japan) and HPLC-grade daidzein, equol and α-tocopherol (Sigma) were used as standards.

2.4. Measurement of lipid peroxidation

Lipid peroxidation was determined by measuring the concentration of malondialdehyde in the liver homogenates according to the method of Ohkawa et al. (1979). Liver homogenates were mixed with 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid (pH adjusted to 3.5 with NaOH), and 1.5 ml of a 0.8% aqueous solution of thiobarbituric acid. The volume of the resultant solution was made up to 4.0 ml with distilled water and heated for 60 min