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Comparison between daidzein and genistein antioxidant activity in primary and cancer lymphocytes

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Abstract

The main objective of this study was to compare the protective effect of daidzein and genistein against induced oxidative damage in Jurkat T-cell line and in peripheral blood lymphocytes of healthy subjects. After supplementation of cells with isoflavones (from 2.5 to 20 μmol/L in Jurkat T-cell and from 0.01 to 2.5 μmol/L in primary lymphocytes, 24 h), we determined DNA damage induced by hydrogen peroxide using the comet assay and lipid peroxidation evaluating malondialdehyde (MDA) production after ferrous ion treatment. Supplementation of Jurkat cells and primary lymphocytes with both isoflavones significantly increased DNA protection from oxidative damage at concentrations between 0.1 and 5 μmol/L ($P < 0.05$), and with just daidzein, at concentrations higher than 2.5 μmol/L, there was a decrease in the production of MDA ($P < 0.05$). Our results seem to support that daidzein is just as effective as genistein in protecting cells against oxidative damage especially with respect to DNA. Moreover, since the protective effect was found at concentrations reachable in plasma after soy consumption (less than 2 μmol/L), it can be assumed that the antioxidant activity of isoflavones could really contribute to the healthy properties of soy.

Keywords: Isoflavones; Lipid peroxidation; DNA damage; Jurkat T cells; Peripheral blood lymphocytes

Epidemiological studies have shown that a regular intake of soy foods is associated with a reduced risk of several chronic pathologies, such as coronary heart disease, cancer, and osteoporosis [1]. Different hypotheses have been suggested to explain these health benefits, such as the involvement of the two major isoflavones in soy foods, genistein and daidzein, whose different biological activities have been documented in vitro and in vivo studies. For example, it was demonstrated that genistein, and to a much lesser extent daidzein, inhibits tyrosine kinase [2–4], and malignant angiogenesis at physiological concentrations in vitro [5]. In addition, genistein was demonstrated to prevent activation of the redox-sensitive transcription factor, NF-κB in cancer cells in vitro [6], and in human blood lymphocytes [7]. This isoflavone also inhibits DNA topoisomerase I and II [8,9], and ribosomal S6 kinase [10], which may lead to protein-linked DNA strand breaks. Since it is believed that anticancer [11], anti-inflammatory [12], cardioprotective [13], and enzyme-inhibitory [9] effects of isoflavones might be related to their antioxidant activities, several studies have been undertaken to evaluate these properties. However, most of the research on isoflavones' antioxidant activity has been focused on genistein [14,15]. For example, it has been demonstrated that genistein inhibits hydrogen peroxide (H_2O_2) production due to tumour promoter in mouse skin cells [16] and the formation of 8-hydroxy-2′-deoxyguanosine following UV light irradiation [17,18]. Record et al. [19] in their in vitro study provided evidence that genistein is an effective scavenger of H_2O_2 but it is less effective against other peroxidative systems. Recent studies have considered the radical scavenging activity of isoflavones [19,20] demonstrating that genistein is more effective than daidzein in inhibiting oxidation of β-carotene linoleate in vitro [21], while

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significant inhibition of lipoprotein oxidation was found only for daidzein metabolites [15].

Daidzein was specifically studied in relation to the estrogenic property of its main mammalian metabolite, equol [22]. However, the high bioavailability of daidzein and the resulting reliable plasma concentration of this isoflavone [23–25] could suggest the occurrence of biological and antioxidant activities of daidzein independently from its conversion in equol.

The aim of this study was to compare the protective effects of daidzein with respect to those of genistein against oxidative damage to DNA and lipid membranes in Jurkat T cells and in primary human lymphocytes subjected ex vivo to oxidative stress.

Materials and methods

Chemicals and reagents

All chemicals and reagents were purchased from Sigma Chemical (St. Louis, MO) and Merck (Merck KgaA, Darmstadt, Germany). Standards of isoflavones (genistein and daidzein) were obtained from Extrasynthèse (Genay, France).

Cells

Immortalised and primary lymphocytes were used as cellular models. In fact lymphocytes, being involved in immune response, are normally subjected in vivo to oxidative stress [26–28]. Moreover, since lymphocytes are exposed immediately to variations of antioxidants in blood due to modification of dietary habits, they represent a reliable cellular line to study the effect of dietary antioxidants on cell protection [27,28].

Jurkat cell line

The human leukaemia T-cell line (Jurkat, clone E6-1), used as cellular model, was purchased from American tissue culture collection (ATCC) (Rockville, MD). Jurkat T cells were considered because they have membranes with markers similar to normal lymphocytes [29].

Peripheral blood lymphocyte isolation

Human lymphocytes were isolated from the blood of four healthy subjects from the Blood Donors Center of the Istituto Nazionale Per lo Studio e la Cura dei Tumori. Blood was collected into tubes containing Histopaque-1077 and centrifuged at 400*g* at room temperature for 30 min. Cells, recovered from the gradient, were washed twice with phosphate-buffered saline (PBS) and suspended in culture medium.

Cell handling

Both Jurkat and primary lymphocytes were cultured in RPMI 1640 medium containing 100 ml/L heat inactivated newborn calf serum, 2 mmol/L L-glutamine, 1×10^5 IU/L penicillin, and 100 mg/L streptomycin. Moreover, 4 μg/ml phytohemoagglutinin (PHA) as mitogen was added to primary lymphocyte flasks.

Lymphocytes were grown in 275 ml flasks at 37 °C in a humidified atmosphere of 5% carbon dioxide/95% air and the medium was changed every 48 h, adjusting the cell number to 5×10^5 cells/ml after hemocytometer counts.

Experimental design

We first compared the effect of daidzein and genistein on Jurkat cells by using concentrations ranging from 2.5 to 20 μmol/L. Afterwards, trying to study a more physiological condition, we further confronted the protective effect of daidzein and genistein against oxidative damage in human primary lymphocytes supplemented with low concentrations of isoflavones (ranging between 0.01 and 2.5 μmol/L). In both trials, the DNA oxidative damage and membrane lipid peroxidation in control and supplemented cells were measured by the comet assay and the quantification of MDA[1] production, respectively.

Isoflavone supplementation

Genistein and daidzein were dissolved in tetrahydrofuran (THF) stabilised with 0.025% butylated hydroxytoluene (BHT) and added separately to the cell cultures to reach 2.5, 5, 10, and 20 μmol/L in Jurkat cells and 0.01, 0.05, 0.1, 0.25, 0.5, 1, and 2.5 μmol/L in primary lymphocytes. Control cells were similarly treated with the same amount of THF stabilised with 0.025% BHT (0-THF group). The supplementation was carried out at 37 °C in a humidified atmosphere of 5% carbon dioxide/95% air for 24 h in triplicate.

Oxidative treatments

After supplementation, the cells, washed with PBS, were embedded in agarose gel (see determination) and treated with 500 μmol/L H_2O_2 in PBS for 5 min to induce DNA oxidative damage [30]. To induce oxidative lipid damage, 100 μmol/L of ferrous ions (as $FeSO_4 \cdot 7H_2O$) in PBS for 15 min was used. Treated cells were then counted, centrifuged, resuspended in PBS, and stored at −80 °C for further analysis. We chose these concentrations of Fe^{2+} and H_2O_2 to produce detectable oxidative damage consistent with cell integrity as previously dem-

[1] *Abbreviation used:* D, daidzein; G, genistein; MDA, malondialdehyde.