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Radioprotection by two phenolic compounds: Chlorogenic and quinic acid, on X-ray induced DNA damage in human blood lymphocytes in vitro

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ABSTRACT

The present study was designed to determine the radioprotective effect of two phytochemicals, namely, quinic acid and chlorogenic acid, against X-ray irradiation-induced genomic instability in non-tumorigenic human blood lymphocytes. The protective ability of two phenolic acids against radiation-induced DNA damage was assessed using the alkaline comet assay in human blood lymphocytes isolated from two healthy human donors. A Siemens Mevatron MD2 (Siemens AG, USA, 1994) linear accelerator was used for irradiation. The results of the alkaline comet assay revealed that quinic acid and chlorogenic acid decreased the DNA damage induced by X-ray irradiation and provided a significant radioprotective effect. Quinic acid decreased the presence of irradiation-induced DNA damage by 5.99–53.57% and chlorogenic acid by 4.49–48.15%, as determined by the alkaline comet assay. The results show that quinic acid and chlorogenic acid may act as radioprotective compounds. Future studies should focus on determining the mechanism by which these phenolic acids provide radioprotection.

1. Introduction

Ionizing radiation is often given as a form of therapy in cancer treatment with the intent of destroying cancer cells, thereby curing the disease. However, radiation treatment also injures or destroys normal cells by damaging their genetic material, often making it impossible for these healthy cells to continue to grow and divide (Hall, 2000). Therefore, several attempts have been made to minimize the damage to normal tissue with synthetic compounds, such as cysteine, cysteamine and amifostine (Tiwari et al., 2009). However, due to the side effects and toxicity of these compounds, the use of these drugs is limited in clinical practice. The potential use of flavonoid compounds as radioprotectors is gaining much interest (Mauryaa et al., 2007). Much of the attention given to flavonoid compounds comes from the results of epidemiological studies that suggest high fruit and vegetable consumption is associated with a decreased risk of several types of cancer, including breast, colon, lung, larynx, pancreas, oral and prostate cancer (Middleton et al., 2000; Kok et al., 2012). Thus, it is necessary to assess the potential of common phytochemicals and phenolics as non-toxic radioprotectants for cancer patients undergoing radiation therapy.

Chlorogenic acid (5-*O*-caffeoylquinic acid) is an important plant polyphenol that is widely distributed in the leaves and fruits of dicotyledonous plants, such as coffee beans. This acid is the ester of caffeic acid with quinic acid (Fig. 1) and belongs to the hydroxycinnamic acid group. (Clifford, 1999; Shahidi and Chandrasekara, 2010). Chlorogenic acid is hydrolized by intestinal microflora into various aromatic acid metabolites including caffeic acid and quinic acid (Gonthier et al., 2003). Quinic acid (1,3,4,5-tetrahydroxycyclohexane carboxylic acid) is a naturally occurring polyphenol distributed in fruits, coffee, cocoa beans, wine and chinchona. Quinic acid can also be formed synthetically by hydrolysis of chlorogenic acid.

Coffee beans are a major source of chlorogenic acid and quinic acid in many people's diets. Daily intake of chlorogenic/quinic acid in coffee drinkers is approximately 1 g (Clifford, 1999). Quinic acid has been reported as an anti-inflammatory and antimutagenic agent in prior studies (Boyer and Liu, 2004; Bonita et al., 2007). In vitro, chlorogenic acid and quinic acid have vicinal hydroxyl groups on an aromatic residue, and they react with radicals generated in the aqueous phase (Rice-Evans et al., 1996) and inhibit DNA damage (Kasai et al., 2000; Cheng et al., 2007). Chlorogenic acid and quinic acid, being inhibitors of the N-nitrosation reaction in vitro (Da Cunha et al., 2004), might inhibit the formation of mutagenic and carcinogenic N-nitroso compounds. Chen and Ho (1997) found that

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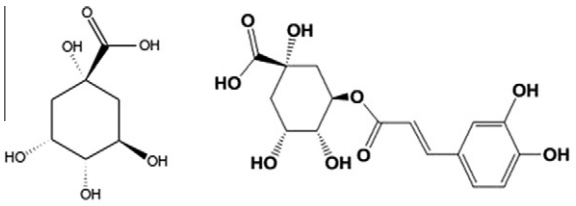

Fig. 1. The chemical structures of quinic and chlorogenic acid.

among quinic acid and its derivatives, chlorogenic acid had greater 1,1-diphenyl 2-picrylhydrazil (DPPH) radical scavenging activity than other hydroxycinnamates such as ferulic acid and its phenethyl ester. Quinic acid derivatives also have neuroprotective effects on PC12 and C6 glioma cells in vitro (Hur et al., 2001; Soh et al., 2003). In vivo, when added to the diet, chlorogenic acid inhibits chemically induced carcinogenesis of the large intestine, liver and tongue in rats and hamsters (Tanaka et al., 1990, 1993; Tsuchiya et al., 1996; Kasai et al., 2000).

Natural hydroxyl cinnamates (chlorogenic, ferrulic, caffeic and cinnamic acids) have been shown to possess antitumorigenic and antioxidant properties (Cheng et al., 2007; Epifano et al., 2007; Fiuza et al., 2004; Gomes et al., 2003); however, no studies exist investigating the use of these compounds in the defense against radiation-induced cellular damage. The present study attempts to evaluate the effect of chlorogenic acid and quinic acid as protective agents against X-ray-mediated DNA damage in human blood lymphocytes.

2. Materials and methods

2.1. Chemicals

Heat-inactivated fetal calf serum (FCS), colcemid, ethidium bromide, Ficoll-Histopaque, low-melting agarose (LMA), normal-melting agarose (NMA), chlorogenic acid (Fig. 1), quinic acid (Fig. 1), RPMI-1640 medium, penicillin–streptomycin, dimethyl sulfoxide (DMSO), L-glutamine, triton X-100, ethylene diamine tetraacetic acid (EDTA), trypan blue dye and sodium sarcosinate were purchased from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals and phytohemagglutinin M were purchased from GIBCO-BRL, USA.

2.2. Lymphocyte isolation and culture conditions

The experimental design was approved by the Uludag University Faculty of Medicine Human Ethics Committee. Blood samples were collected from two healthy donors, specifically, one male and one female. The donors were 24–36-year-old non-smokers with no history of radiotherapy, no alcohol or medication consumption and no disease at the time of blood collection. For cytogenetic studies, preservative-free heparin was used as an anticoagulant. Written consent was obtained from each blood donor. Lymphocytes were isolated from the blood using a Ficoll-Histopaque gradient (Sigma, MO, USA) and were cultured as previously described (Boyum, 1968). Briefly, blood samples were diluted 1:1 with phosphate-buffered saline (PBS) and were subsequently layered onto the Ficoll-Histopaque with a 4:3 ratio of [blood + PBS]/Histopaque. The samples were centrifuged at 400*g* for 35 min, and the lymphocyte-enriched layer was removed, washed twice with PBS by centrifugation at 350*g* for 10 min and finally washed with RPMI-1640 medium. The number of viable cells was assessed by staining the cells with trypan blue and counting the cells by hemocytometer. Viable cells were suspended in RPMI-1640 supplemented with 15% FCS, 200 mM L-glutamine, penicillin (100 Units/ml) and streptomycin (100 μg/mL). Phytohemagglutinin (0.2 mL) was added to cultured lymphocytes to initiate cell division. Cells were incubated at 37 °C in a humidified incubator maintained with 5% CO_2.

2.3. Study design

To determine the concentration-dependent effect of quinic acid and chlorogenic acid, lymphocytes were incubated with graded drug concentrations of both compounds (0.5, 1, 2 and 4 μg/ml). For X-ray radiation, five doses, 0.05, 0.1, 0.5, 1 and 2 Gy, were used. Quinic acid and chlorogenic acid were dissolved in 2% DMSO and lymphocytes treated with 2% DMSO used as a negative control group of our study. The final concentration of DMSO was 0.02% in 2 ml culture medium. As a sham control we used untreated blood lymphocytes. All dose treatments were duplicated.

The cross combination of phenolic concentrations are as follows:

Quinic acid (QA) + radiation	Chlorogenic acid (CA) + radiation
0.5 μg/ml QA + 1 Gy irradiation	0.5 μg/ml CA + 1 Gy irradiation
0.5 μg/ml QA + 2 Gy irradiation	0.5 μg/ml CA + 2 Gy irradiation
1 μg/ml QA + 1 Gy irradiation	1 μg/ml CA + 1 Gy irradiation
1 μg/ml QA + 2 Gy irradiation	1 μg/ml CA + 2 Gy irradiation
2 μg/ml QA + 1 Gy irradiation	2 μg/ml CA + 1 Gy irradiation
2 μg/ml QA + 2 Gy irradiation	2 μg/ml CA + 2 Gy irradiation
4 μg/ml QA + 1 Gy irradiation	4 μg/ml CA + 1 Gy irradiation
4 μg/ml QA + 2 Gy irradiation	4 μg/ml CA + 2 Gy irradiation

0.05, 0.1, 0.5 Gy X-ray irradiation doses were not selected for combinations because those were observed with the alkaline comet assay not to be cytotoxic or genotoxic in dose trials.

2.4. Irradiation of the cells

Thirty minutes prior to sample irradiation, four test concentrations (0.5, 1, 2 and 4 μg/ml) of chlorogenic and quinic acid dissolved in 2% DMSO separately were added to cultured lymphocytes. The final concentration of DMSO was 0.02% in 5 ml of culture medium. Preliminary studies were conducted to determine whether these concentrations showed toxicity by trypan blue dye exclusion prior to X-ray irradiation. Chlorogenic and quinic acid treatments did not affect lymphocyte viability. The cell viability of all dose-samples was >85%.

Next, 6-MV X-rays were used to irradiate the cells contained in 40 mm covered Petri dishes. The treatment doses were administered with a 200 cGy per minute of dose rate using a linear accelerator (Siemens Mevatron MD2, Erlangen, Germany) in the Uludag University, Faculty of Medicine, Radiotherapy Center Bursa, Turkey. The total administered dose was calculated by a radiotherapy planning system (CMS-XiO, Freiburg, Germany) using the computerized tomography images of the petri dishes in the treatment position. A gel bolus was used to eliminate the dose reduction due to build up regions of 6 MV X-rays.

After irradiation, the samples were incubated for 1 h at 37 °C to allow time for DNA repair that would normally occur in vivo. Samples were then transported to the laboratory on ice. Cultures were equilibrated at room temperature and were subsequently subjected to an alkaline comet assay.

2.5. Alkaline comet assay and scoring methodology

The comet assay was performed according to Singh et al. (1988) with several modifications. Roughened slides were cleaned with 100% methanol and air-dried.

For each sample, two slides were prepared, and 200 cells were scored from each slide. Observations were made at 400× magnification using a fluorescence microscope equipped with a 530-nm excitation filter and a 590-nm barrier filter. The genetic damage index (GDI) was visually determined based on the size and intensity of the comet tail. The following five categories (0–4) were used: Class 0 (no damage); Class 1 (little damage with a tail length that is shorter than the diameter of the nucleus); Class 2 (medium damage with a tail length one to two times the diameter of the nucleus); Class 3 (significant damage with a tail length between two-and-a-half and three times the diameter of the nucleus); and Class 4 (significant damage with a tail longer than three times the diameter of the nucleus). Categories based on those of Collins (2004) were used. We used this categorization to obtain a quantitative measurement of DNA damage based on a score average that is weighted according to the number of cells with each grade of damage as follows: genetic damage index (GDI) = (Class 1 + 2 × Class 2 + 3 × Class 3 + 4 × Class 4)/(Class 0 + Class 1 + Class 2 + Class 3 + Class 4). DNA damage was expressed as the mean percentage of cells with medium, high and complete DNA damage and was calculated as the sum of the cells with Class 2, 3 and 4 damage (Palus et al., 2003).

Percentage of damaged cells (% DC) = [Class 2 + 3 + 4/sum of cells in all classes including 0 and 1] × 100.

2.6. Magnitude of protection by chlorogenic and quinic acid

The degrees of protection offered by both compounds were calculated according to the following equation:

$$\text{Protection magnitude } (\%) = (M_{\text{cont irrad}} - M_{\text{treat irrad}})/M_{\text{cont irrad}} \times 100$$

$M_{\text{cont irrad}}$: mean GDI of irradiated lymphocytes, $M_{\text{treat irrad}}$: mean GDI of chlorogenic/quinic acid-pre-treated irradiated lymphocytes.

2.7. Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA), as well as post hoc Turkey tests. Values are represented as the mean ± the standard deviation for the samples in each group. *p*-Values <0.05 were considered to be significant.