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Antioxidant and genoprotective effects of spent coffee extracts in human cells

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ABSTRACT

Spent coffee has been shown as a good source of hydrophilic antioxidant compounds. The ability of two spent coffee extracts rich in caffeoylquinic acids, mainly dicaffeoylquinic acids, and caffeine (Arabica filter and Robusta espresso) to protect against oxidation and DNA damage in human cells (HeLa) was evaluated at short (2 h) and long (24 h) exposure times. Cell viability (MTT) was not affected by spent coffee extracts (>80%) up to 1000 μg/mL after 2 h. Both spent coffee extracts significantly reduced the increase of ROS level and DNA strand breaks (29–73% protection by comet assay) induced by H_2O_2. Pretreatment of cells with robusta spent coffee extract also decreased Ro photosensitizer-induced oxidative DNA damage after 24 h exposure. The higher effectiveness of Robusta spent coffee extract, with less caffeoylquinic acids and melanoidins, might be due to other antioxidant compounds, such as caffeine and other Maillard reaction products. This work evidences the potential antioxidant and genoprotective properties of spent coffee in human cells.

1. Introduction

Global health policies promote increase consumption of plant foods, such as fruits and vegetables to contribute to the prevention of several chronic diseases related with oxidative stress, such as cancer, diabetes, cardiovascular and neurodegenerative diseases (World Health Organization, 2004). Plant foods, including beverages and extracts, are rich sources of bioactive compounds like antioxidants. A possible mechanism of antioxidants action in the prevention of chronic diseases is the decrease of oxidative stress, a condition that appears when an imbalance between the production of free radicals and antioxidant defences occurs. Free radicals, such as reactive oxygen species (ROS), damage macromolecules as proteins, lipids and DNA. DNA damage by ROS can contribute to the formation of single and double strand breaks (SBs), as well as to the oxidation of purine and pyrimidine bases, leading to genome instability and subsequent potential cancer development (Chobotova, 2009). The formation of 8-oxoguanine (8-oxoGua) by ROS oxidation of guanine is one of the most common DNA lesions. This is a potential biomarker of carcinogenesis because it is relatively easily formed and is mutagenic (Valko et al., 2007). The comet assay is one of the most useful approaches for the quantification of this oxidative DNA damage (SBs and 8-oxoGua). For example, several authors have reported, employing the comet assay, that supplementation of the diet with food antioxidants decreases endogenous oxidative DNA damage in human lymphocytes (Duthie et al., 1996; Porrini and Riso, 2000).

Coffee is well-known as a rich source of antioxidants in human diet that may contribute to the prevention of oxidative stress related diseases (Dorea and da Costa, 2005; Pulido et al., 2003; Svilaas et al., 2004). The preparation of instant coffee in industries and coffee beverages in restaurants, cafeterias and also at domestic levels generates tons of coffee residues. It has been proposed that spent coffee grounds could be valuable by-products because of their antioxidant properties due to the presence of phenolic and nonphenolic bioactive compounds (Bravo et al., 2013, 2012; Murthy and Madhava Naidu, 2012; Mussatto et al., 2011; Ramalakshmi et al., 2009; Yen et al., 2005). However, the chemical-based assays used for the antioxidant activity evaluation of spent coffee are suitable for the initial antioxidant screening but do not reflect the cellular physiological conditions. Therefore, there is a need for applying cell cultures models to support antioxidant research (Liu and Finley, 2005). To our best knowledge, only the cell

Abbreviations: 8-oxoguanine, 8-oxoGua; CQAs, caffeoylquinic acids; diCQAs, dicaffeoylquinic acids; DAPI, 4,6-diamidino-2-phenylindole; FPG, formamidopyrimidine DNA glycosylase; H_2DCF-DA, 2′,7′-dichlorfluorescein-diacetate; H_2O_2, hydrogen peroxide; MRPs, Maillard reaction products; MTT, (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide); ROS, reactive oxygen species; SBs, strand breaks.

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viability in rodent cell culture systems has been evaluated to report the anti-tumor, anti-allergic and anti-inflammatory activities of spent coffee extracts obtained from instant coffee (Ramalakshmi et al., 2009). However, this is the first time that direct effect and prevention against ROS formation and DNA damage of spent coffee extracts in human cells has been reported. In a previous study, we have reported that spent coffee grounds obtained from the most common coffeemakers used at domestic and cafeterias levels (filter and espresso), and in less proportion from plunger (French press) ones, have antioxidant capacity because of the presence of relevant amounts of hydrophilic bioactive compounds, such as caffeoylquinic acids, mainly dicaffeoylquinic acids, and caffeine (Bravo et al., 2012). Because some of these coffee compounds have shown protective effects against oxidation (ROS formation) and DNA damage in human cell models when they were evaluated individually (Bakuradze et al., 2010; Cho et al., 2009; Faustmann et al., 2009; Pavlica and Gebhardt, 2005) or in coffee matrices (instant or coffee brew) in human cell models both directly or in interventional studies (Bakuradze et al., 2010; Bichler et al., 2007; Del Pino-García et al., 2012; Hoelzl et al., 2010); we hypothesize that aqueous spent coffee extracts might have antioxidant and genoprotective effects in human cells. However, up to now, the effect of these coffee by-products on oxidation and DNA damage in cells is still unknown.

Therefore, the aim of the present work was to evaluate the ability of two of the most antioxidant spent coffee extracts (from Arabica filter and Robusta espresso) (Bravo et al., 2012) to protect against oxidation and DNA damage in a cancer human cell model system. For this purpose, firstly, cytotoxicity was measured in order to choose the adequate extract concentration to be evaluated. Afterwards, the direct effects of the extracts on intracellular ROS level using the diclorofluorescein assay, and on DNA oxidation damage using the comet assay were determined. And last, the protection ability of the spent coffee extracts against H_2O_2-induced intracellular ROS level and DNA strand breaks and Ro-induced oxidative DNA damage (as FPG-sensitive sites) increase was assessed.

2. Materials and methods

2.1. Chemicals

Dulbeccós Modified Eaglés Medium (DMEM), fetal bovine serum (FBS), antibiotic solution, and trypsin solution were purchased from Gibco (Prat de Llobregat, Barcelona, Spain). Hydrogen peroxide (H_2O_2), 3-(4,5-methyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) and 4,6-diamidino-2-phenylindole (DAPI) were obtained from Sigma–Aldrich (Steinheim, Germany). Dichlorodihydrofluorescein diacetate (H_2DCF-DA) was from Invitrogen Molecular Probes (Eugene, Oregon, USA). DMSO was purchased from Panreac Quimica SAU (Barcelona, Spain). Ro (photosensitizer 19-8022) and formamidopyrimidine DNA glycosylase (FPG) were kindly supplied by Dr. A. Collins (Institute for Nutrition Research, University of Oslo, Norway).

2.2. Preparation of Spent Coffee Extracts

Roasted coffee (without defective beans) from Guatemala (*Coffea arabica*, named Arabica, 3.03% water content, L^* = 25.40 ± 0.69, roasted at 219 °C for 905 s) and Vietnam (*Coffea canephora* var. robusta, named Robusta, 1.59% water content, L^* = 24.92 ± 0.01, roasted at 228 °C for 859 s) was provided by a local factory. Coffee beans were ground for 20 s using a grinder (model Moulinex super junior "s", París, France). The L^* value was analyzed by means of a tristimulus colorimeter (Chromameter-2 CR-200, Minolta, Osaka, Japan) using the D65 illuminant and CIE 1931 standard observer. The instrument was standardized against a white tile before sample measurements. Ground roasted coffee was spread out in a 1 cm Petri plate, and the L^* value was measured in triplicate on the CIELab scale. Water content was measured by weight loss after drying for 2 h at 102 ± 3 °C in an oven JP SELECTA (Barcelona, Spain).

Arabica and Robusta spent coffee grounds were obtained as coffee by-products after the preparation of coffee brews with filter (24 g/400 mL water, model Avantis 70 Inox, Ufesa, Spain) and espresso coffeemakers (7 g/40 mL water, model Saeco Aroma, Italy), respectively. Then, spent coffee extracts were prepared according to the method described by Bravo et al. (2013). Briefly, first, spent coffee grounds were dried to a constant weight at 102 ± 3 °C in an oven JP SELECTA (Barcelona, Spain) and defatted with petroleum ether (1:11, w/v) for 3 h at 60 °C in a Soxhlet extraction system (Extraction Unit B-811 Standard Büchi, Flawil, Switzerland). Then, 24 g of spent coffee was extracted with a volume of 400 mL of water using a filter coffeemaker (model AVANTIS 70 Inox, Ufesa, Spain). Extraction took approximately 6 min at 90 °C. Aqueous spent coffee extracts were lyophilized using a CRYODOS Telstar (Terrassa, Spain) and stored at −20 °C until sample analysis.

2.3. Cell culture

HeLa cells (derived from human cervical cancer) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were maintained as monolayer cultures in DMEM supplemented with 10% FBS and 1% antibiotic (10,000 U/mL penicillin and 10,000 μg/mL streptomycin, Gibco), under an atmosphere of 5% CO_2 at 37 °C. Cells were trypsinized when nearly confluent.

2.4. Cytotoxicity

Cell viability was determined by assessing the reduction of MTT to formazan by the mitochondrial enzyme, succinate dehydrogenase, as described by Mosmann (1983). Cells were seeded in 96-well plates at 2×10^4 cells/well and maintained for 24 h until confluence. Arabica and Robusta spent coffee extracts were then added to medium at 37, 111, 333, 1000 and 3000 μg/mL. After 2 and 24 h treatments, cells were washed with phosphate-buffered saline (PBS), and 25 μL MTT (5 mg/mL) in PBS was added to 225 μL of fresh medium to each well. After an incubation of 2 h and 30 min at 37 °C, the supernatant was removed, and the insoluble formazan crystals were dissolved with 100 μL of DMSO. The absorbance was measured at 540 nm using a spectrophotometer reader (Spectra MR, Dinex Technologies). Results were expressed as the percentage of viability (%) with respect to the control (medium treated cells) according to the following formula: [(absorbance treated cells-absorbance blank)/(absorbance control cells-absorbance blank)] × 100.

2.5. Intracellular ROS level

Intracellular ROS level was determined by using fluorescent probe dichlorodihydrofluorescein diacetate (H_2DCF-DA), according to Wang and Joseph (1999). H_2DCF-DA is enzimatically hydrolyzed by intracellular esterases to originate non-fluorescent H_2-DCF, which is then rapidly oxidized to originate highly fluorescent DCF in the presence of ROS. The DCF fluorescence intensity parallels the amount of intracellular ROS. Two different types of experiments were carried out: (1) treatment of cells with spent coffee extracts to test their direct effect on intracellular ROS level and (2) pretreatment of cells with spent coffee extracts before submitting the cells to an oxidative stress to test their protective effect against ROS level increase induced by hydrogen peroxide. HeLa cells were seeded in 96-well plates at 2×10^4 cells/well. In experiment (1), 24 h after seeding, 200 μL of H_2DCF-DA (100 μM) in serum- and phenol red-free DMEM was added to each well for 30 min at 37 °C. Afterwards, cells were washed once with PBS and exposed for 2 and 24 h to Arabica and Robusta spent coffee extracts in phenol red-free DMEM at 37, 111, 333 and 1000 μg/mL. For experiment (2), cells were pretreated during 2 and 24 h with Arabica and Robusta spent coffee extracts at 37, 111, 333 and 1000 μg/mL, the H_2DCF-DA (100 μM) probe was added to each well for 30 min at 37 °C, and the cells were washed once with PBS and fresh phenol red-free DMEM containing 500 μM H_2O_2 was added to all cultures except controls for 10 min at 37 °C. In both experiments, intracellular ROS were measured using a microplate fluorometer Fluoroskan Ascent (Thermo Labsystems) at an emission wavelength of 538 nm and an excitation wavelength of 485 nm. ROS level was expressed as the fluorescence of the treated samples (spent coffee treated cells) compared to the fluorescence of the control samples (medium treated cells) = [(fluorescence treated cells/fluorescence control cells) × 100].

2.6. DNA damage

DNA damage was determined by comet assay able to detect SBs and oxidative DNA damage in culture cells. The inclusion of FPG digestion allowed the detection of the main purine oxidation product 8-oxoguanine as well as other altered purines. Two different types of experiments were carried out: (1) treatment of cells with spent coffee extracts to rule out their ability to cause SBs and oxidative DNA damage and (2) pretreatment of cells with spent coffee extracts before submitting the cells to an oxidative damage to evaluate their genoprotective effect against induced DNA damage. HeLa cells were seeded in 24-well plates at 8×10^4 cells/well. A negative control with cells treated with medium and two positive controls were also included: cells treated with a solution of 500 μM H_2O_2 for 10 min (on ice) to induce SBs and cells treated with 1 μM Ro plus visible light from a 500 W tungsten-halogen source at 33 cm (10 min on ice) to induce 8-oxoGua. In experiment (1), 24 h after seeding, cells were exposed for 2 and 24 h to spent coffee extracts in medium at 111 and 333 μg/mL. For experiment (2), cells were preincubated for 2 and 24 h at 37 °C with spent coffee extracts in medium and then were washed with PBS and treated on ice with H_2O_2 (500 μM) for 10 min or with 1 μM Ro plus visible light