Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 18 (2007) 457–466
Journal of Nutritional Biochemistry

Phenolic-rich juice prevents DNA single-strand breakage and cytotoxicity caused by *tert*-butylhydroperoxide in U937 cells: the role of iron chelation

Francisco J. Garcia-Alonso[a,*], Andrea Guidarelli[b], María J. Periago[a]

[a]*Food Science and Nutrition Department, Faculty of Veterinary, University of Murcia, Campus de Espinardo, 30071 Murcia, Spain*
[b]*Institute of Pharmacology and Pharmacognosy, University of Urbino "Carlo Bo", 61029 Urbino (PU), Italy*

Received 27 April 2006; received in revised form 16 August 2006; accepted 22 August 2006

Abstract

The antioxidant potential of phenolic compounds is generally linked to their ability to scavenge free radicals. However, in addition to their radical-scavenging activity, phenolic compounds can chelate metal ions, such as iron, to prevent their participation in Fenton-type reactions, which lead to the formation of free radicals. The aim of the present study was to evaluate the ability of a phenolic-rich juice made from grapes, cherries and berries to protect human myeloid leukemia (U937) cells from oxidative stress caused by *tert*-butylhydroperoxide (tB-OOH). Preincubation of cells with extracts of the phenolic-rich juice at different concentrations (0–200 μM ferulic acid equivalents) for 3 h partially prevented cell death and abolished the DNA cleavage induced by tB-OOH. Moreover, when preincubating cells with the 100-μM juice extract (the dose that diminished cell death by around 50%), the partial prevention of tB-OOH-induced formation of reactive oxygen species (ROS) and mitochondrial permeability transition pore opening was observed. The radical scavenger antioxidant *N*,*N*′-diphenyl-1,4-phenylene-diamine (DPPD) and the intracellular iron chelator *o*-phenanthroline (*o*-Phe) were also tested to know whether protective effects depended on radical-scavenging or iron-chelating activities. *o*-Phe prevented cell death, DNA cleavage and ROS generation, whereas DPPD only prevented cell death, suggesting that phenolics in the juice afforded protection against induced oxidative stress, most probably by means of an iron-chelating mechanism.

Keywords: Phenolics; Iron chelators; Radical scavengers; DNA single-strand breakage; Cytotoxicity; *tert*-Butylhydroperoxide

1. Introduction

Several human chronic disease states have been associated with oxidative stress, which occurs in a cell or in a tissue when the concentration of the reactive oxygen species (ROS) generated exceeds the antioxidant capability of that cell [1]. Since the 1990s, different world organizations have recommended an increase in the intake of dietary antioxidants (carotenoids, polyphenols and vitamins C, A and E), with the aim of preventing cancer and many chronic diseases [2,3]. Nowadays, there is growing interest among scientists, food manufacturers and consumers in the study of food properties for maintaining human health [4] — a fact that gives rise to the development of antioxidant-rich functional foods with potential health benefits for consumers. However, any beneficial effect has to be scientifically demonstrated with human intervention studies [5], and functional foods need to be evaluated from a toxicological point of view. For this, in vitro cell culture systems can be used to assess cytotoxicity and cellular responses and to perform toxic kinetic modeling, since they are a valuable tool for elucidating the mechanisms of action of natural antioxidants present in fruit-derived functional foods [6,7]. An experimental in vitro analysis of biological activities of whole foods will therefore serve several purposes. On one hand, the determination of toxicity can be used as a tool to define the concentrations at which chemoprotective effects can be further characterized; on the other hand, cells can be treated with subtoxic concentrations of the compounds to identify new cellular responses, among them mechanisms of potential chemoprevention [7].

Phytochemicals, especially the phenolics found in fruits and vegetables, have been proposed as major bioactive

* Corresponding author. Departamento de Tecnología de los Alimentos, Nutrición y Bromatología, Facultad de Veterinaria, Universidad de Murcia, Espinardo (Murcia) 30071, Spain. Tel.: +34 968 364798.
E-mail address: fjgarcia@um.es (F.J. Garcia-Alonso).

0955-2863/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2006.08.003

compounds that provide health benefits associated with diets rich in plant foods. In line with this, several plant phenolics have been found to show strong antioxidant activity [8–10] and to protect against oxidant-induced damage in cell culture models [11–18].

The principal hypothesis associated with the biological effects of phenolics is linked to their radical-scavenging properties. However, in addition to directly quenching ROS and free radicals, phenolic compounds can chelate metal ions such as iron to prevent their participation in Fenton-type reactions, which lead to the formation of free radicals [8,19]. Although iron chelation has generally been regarded as playing a minor role in the antioxidant activity of polyphenols, the presence of iron-chelating groups in foods and their efficiency in iron chelation may partly explain the health-protective role of specific phenolics in the human diet [20,21]. Nevertheless, it is not easy to establish the relative contribution of the radical-scavenging properties versus the iron-chelating properties of polyphenols to their antioxidant properties.

To address this issue, Sestili et al. [11,15] developed a U937-cell-based approach, which, in a relevant biological setting, discriminates free-radical-scavenging versus iron-chelating mechanisms. The approach is based on the observation that DNA cleavage evoked by the oxidant *tert*-butylhydroperoxide (tB-OOH) is abolished by iron chelators and is insensitive to radical scavenger antioxidants [22–25], whereas the cell death induced by tB-OOH is abolished by both iron chelators and antioxidants [22,25]. Using this experimental approach, they have demonstrated that the most prominent activity of selected flavonoids and hydroxycinnamic acids that affords protection against tB-OOH-induced cell death and DNA damage resides in their ability to chelate iron [11,15]. This fact has been further confirmed by other researchers in a Caco-2 cell model [12].

In the present study, we evaluated the cytoprotective activity of a phenolic-rich juice made from pigmented fruits against tB-OOH-induced oxidative stress in U937 cells. The aim was to find out whether the abovementioned experimental approach is suitable to elucidate the most prominent antioxidant mechanism displayed by a whole phenolic-rich food.

2. Materials and methods

2.1. Chemicals

tB-OOH, *N*,*N*'-diphenyl-1,4-phenylene-diamine (DPPD) and *o*-phenanthroline (*o*-Phe), as well as most reagent-grade chemicals, were obtained from Sigma-Aldrich (Milan, Italy). Dihydrorhodamine 123 (DHR) and calcein acetoxymethylester (calcein-AM) were from Molecular Probes Europe (Leiden, The Netherlands). Cyclosporin A (CsA) was obtained from Sandoz (Bern, Switzerland). Folin–Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl assay (DPPH), 2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonate) (ABTS), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich (Madrid, Spain). 2,4,6-Tri-pyridil-*s*-triazine (TPTZ) and ferulic acid were from Fluka (Buchs, Switzerland). Ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$) was from Panreac (Barcelona, Spain).

2.2. Description and analysis of the juice

The experimental juice was prepared by the Research and Development Department of Hero Spain (Alcantarilla, Murcia, Spain). The major ingredient was water, which was mixed with commercially available concentrated juices of grape (26%), cherry (2%), raspberry (1%), blackberry (0.6%) and blackcurrant (0.6%). The total phenolic compounds were analyzed by a colorimetric assay using Folin–Ciocalteu's phenol reagent [26]. Ferulic acid was used as standard, and the total phenolic content is expressed as milligrams per liter or as micromolars of ferulic acid equivalents for cell culture assays.

The main groups of phenolic compounds were analyzed by high-performance liquid chromatography (HPLC) according to Cantos et al. [27]. Briefly, phenolics were extracted with HPLC-grade methanol plus 3% formic acid and analyzed on an L-7100 liquid chromatograph equipped with a Merck-Hitachi 7455 UV diode array detector and a 25×0.4-cm Licrochart RP-18 column with a particle size of 5 mm (Merck, Darmstadt, Germany). The solvents used were water plus 5% formic acid (Solvent A) and HPLC-grade methanol (Solvent B) at a flow rate of 1 ml/min. Elution was performed with a gradient starting with 2% Solvent B to reach 32% Solvent B at 30 min, 40% Solvent B at 40 min and 95% Solvent B at 50 min, which was then isocratic for 5 min. Anthocyanins were quantified at 510 nm, stilbenoids at 320 nm, hydroxycinnamic acid derivatives (caffeic acid derivatives) at 320 nm and catechins at 280 nm.

The total antioxidant activity of the juice was evaluated using four common tests: the Trolox equivalent antioxidant capacity (TEAC; I and II) assay, the DPPH assay and the ferric-reducing/antioxidant power (FRAP) assay. The colorimetric TEAC assay measures the ability of antioxidants to scavenge the ABTS radical cation. The TEAC I assay [28] was carried out using a kit manufactured by Randox Laboratories (Ardmore, UK; cat. no. NX2332). This method is based on antioxidants' inhibition of the absorbance of the radical cations of ABTS at 600 nm. ABTS radical cations are formed by the incubation of ABTS with metmyoglobin (MetMb) and H_2O_2. The TEAC II assay [29] is based on the reduction of the radical cation of ABTS, which is generated by filtering an ABTS solution through manganese dioxide powder. The antioxidant activity of the samples is calculated by determining the decrease in absorbance at 734 nm. In the DPPH assay [30], antioxidants reduce the free radical DPPH, and the decrease in absorbance is measured at 515 nm. In all these tests, Trolox was used as standard, and results are expressed as millimoles of Trolox equivalents per liter. The FRAP assay [31] measures the iron-reducing