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Effect of polyphenols from coffee and grape on gene expression in myoblasts

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ABSTRACT

Coffee and grape contain various bioactive compounds like polyphenols that may exert beneficial effects, especially antioxidant activity, on human health upon consumption. However, the molecular mechanisms through which these effects are achieved are not fully elucidated. Thus, in the present study in order to investigate these mechanisms, a whole genome expression DNA microarray analysis was carried out in myoblasts treated with polyphenols of coffee and grape pomace at concentrations that improved the redox status. Grape was composed of catechin, epicatechin, cyanidin, malvidin, delphinidin, petunidin, myrtillin, kuromanin, oenin, peonidin, quercetin, gallic acid and caftaric acid as LC–MS revealed, with a total polyphenolic content (TPC) of 648 mg of gallic acid equivalents/g of dry matter. Coffee had a TPC of 42.61 mg GAE/g coffee and was composed of 3-chlorogenic acid (16.61 mg/g), 4- and 5-chlorogenic acids (13.62 mg/g), as UHPLC-HRMS revealed. According to the results, grape polyphenols altered mainly the expression of cytoskeleton and differentiation-associated genes, while coffee compounds had a more profound effect, on the expression levels of many metabolic and antioxidant genes possibly through the nuclear factor (erythroid-derived 2) like-2 (Nrf2) pathway.

1. Introduction

Reactive Oxygen Species (ROS) are produced in all aerobic organisms. They are the result of normal functions like cellular respiration or macrophagal 'oxidative burst', however there are also exogenous factors that induce ROS production, including smoking, air pollution and UV radiation (Schieber and Chandel, 2014). Normally, ROS partake in various physiological functions including redox cell signaling and gene expression on top of being essential for the immune system (Ray et al., 2012). However, when they accumulate, they may cause damage to cellular macromolecules, namely proteins, lipids and DNA (Halliwell, 2001). In order to neutralize excess free radicals or non-radical oxidants, aerobic organisms possess a variety of antioxidant mechanisms including both enzymes and non-enzymatic compounds (Birben et al., 2012). The condition where the balance between oxidants and antioxidants favors the former is known as oxidative stress. Oxidative stress is associated with many pathological conditions (Rochette et al., 2014; Wang et al., 2014).

Despite having a complex arsenal of endogenous antioxidants, all organisms may absorb dietary antioxidants, especially from food plant products (Landete, 2013). For instance, polyphenols are an abundant class of molecules found in plants, as a part of their secondary metabolism, that exert significant bioactivity (Landete, 2012). Upon consumption, a fraction of the aforementioned polyphenols is absorbed, possibly eliciting beneficial effects on human health. (Pandey and Rizvi, 2009). Plant polyphenols may act as direct antioxidants by scavenging free radicals at low concentrations through hydrogen donation, but due to their mechanism of action they become radical species themselves (albeit less interactive than typical ROS) (Bouayed and Bohn, 2010). Hypothetically, there is a threshold in polyphenol concentration, over which their 'prooxidant' nature predominates, and thus they may interact with the main free radical-induced molecular mechanism in cells, the Keap1/Nrf2 pathway (Bayele et al., 2016). More specifically, polyphenols may oxidize Keap1 cysteines and consequently Nrf2 is released and allowed to accumulate in the nucleus where it regulates the expression of genes possessing the antioxidant response element (ARE) in their promoter regions (Bayele et al., 2016; Scapagnini et al., 2011).

Coffee beans and grapes are two plant products rich in polyphenols (Henry-Vitrac et al., 2010; Goutzourelas et al., 2015b).

Abbreviations: GSH, glutathione; ROS, reactive oxygen species; TPC, Total polyphenolic content; DMEM, Dulbecco's modified eagle medium; FBS, fetal bovine serum; PBS, Phosphate buffer saline; Nrf2, Nuclear factor (erythroid-derived 2) like-2; AhR, Aryl Hydrocarbon Receptor

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Coffee beverage in particular is rich in chlorogenic acids (CGAs), polyphenolic compounds that belong to the phenolic acid group (Henry-Vitrac et al., 2010; Murthy and Naidu, 2010). These species have been the focus of various studies concerning their bioactivity and especially their antioxidant properties (Park, 2013; Sato et al., 2011; Xu et al., 2012).

Grapes and their product wine contain high levels of polyphenols like *trans*-resveratrol, a stilbene (Goutzourelas et al., 2015b). Although grape seeds are known for their abundant polyphenolic content, grape pomace (a by-product of the winemaking process) is rich in polyphenols including *trans*-resveratrol. In our previous studies, grape pomace extracts displayed potent antioxidant activity (Goutzourelas et al., 2015a, 2015b, 2014).

Many studies have been performed concerning the effects of coffee or wine consumption on human health. However, their molecular mechanism of action remains unknown. Thus, for the investigation of these mechanisms the effects of a roasted coffee extract (from *Coffea arabica*) and a grape pomace extract (from *Vitis vinifera*) on the whole genome expression levels of a mouse myoblast cell line (C2C12) were examined. The myoblasts derive from satellite cells which have the ability to differentiate into myotubes (Yin et al., 2013). Being able to affect their redox status and differentiation process might allow their fine-tuned utilization in cell-based therapies in pathological conditions including muscular dystrophies, such as Duchenne's Muscular Dystrophy (DMD) (Sienkiewicz et al., 2015). In muscular dystrophies, muscle degeneration occurs due to a prolonged inflammatory response along with oxidative stress (Choi et al., 2016). The constant activated status of satellite cells leads to their senescence. Some of the hallmarks of senescence include dysregulated proteostasis network, altered epigenetic profile, genetic instability and mitochondrial dysfunctions among others (Lopez-Otin et al., 2013).

Therefore, compounds or extracts with the ability to reverse or slowdown some of the aforementioned characteristics are sought and in this context, coffee and grape pomace extracts were administered on myoblast cells. We have already displayed previously that these extracts improved the redox status of C2C12 cells, especially through enhancement of the glutathione (GSH) system (Goutzourelas et al., 2014; Priftis et al., 2015).

2. Materials and methods

2.1. Coffee and grape extracts

The grape extract examined was obtained from Batiki Tyrnavou variety (grown in Central Greece) of the *Vitis vinifera* species. The isolation of the extract was carried out as previously described (Veskoukis et al., 2012). Briefly, the raw material was dried in a shady, well-ventilated environment and extracted using ethanol (96%) at 50 °C for 4 h. Following filtration, the solvent was evaporated under reduced pressure, and the residue was kept at −20 °C until further use.

The polyphenolic composition of the extract was identified by a liquid chromatography/high resolution mass spectrometry (LC-HRMS) method in positive and negative mode has been reported previously (Veskoukis et al., 2012). Thus, the extract was composed of flavan-3-ols (catechin and epicatechin), anthocyanidins, (cyanidin, malvidin, delphinidin and petunidin), anthocyanins (myrtillin, kuromanin, oenin and peonidin-3-O-glucoside) and flavonols (quercetin), phenolic acids (gallic acid and caftaric acid). Moreover, the total polyphenolic content (TPC) of the extract was evaluated and found equal to 648 mg of gallic acid per g of extract (Veskoukis et al., 2012).

For the preparation of the coffee extract, the beans were ground using mortar and pestle. Then, they were dissolved in distilled water (10% w/v) followed by a brief sonication treatment applied for 20 min (70% amplitude, 0.7 s cycle). Then, the solution was stirred for 20 min in moderate heat (35 °C). After centrifugation (7000*g*, 10 min, 10 °C) the supernatant was stored in aliquots and kept at −80 °C for further analysis. The total polyphenolic content TPC was 42.61 mg GAE/g extract and was determined using the Folin-Ciocalteu assay as previously described (Priftis et al., 2015).

2.2. UHPLC-HRMS analysis of coffee extracts

2.2.1. Reagents and materials

Chlorogenic acid with purity > 95% was purchased from Sigma-Aldrich (Shanghai, China). Methanol, acetonitrile (ACN), and formic acid (FA; ~98%, for mass spectrometry) were of HPLC grade and purchased from Sigma-Aldrich. The analytical column Ascentis Express Fused-Core™ C18 (100 × 2.1 mm i.d., 2.7 mm) was purchased from Supelco, PA, USA.

2.2.2. Preparation of standard stock solutions

Stock solutions of chlorogenic acid were prepared by dissolving the analyte in H_2O/FA (0.1%), 60:40 v/v in a final concentration of 200 μg/mL and stored at 4 °C until analysis. An aliquot of the stock solutions was diluted using H_2O/FA (0.1%) to achieve a series of working solutions of 40, 30, 20, 15, 5 and 1 μg/ml for the calibration curve of chlorogenic acid. In addition, reserpine was used as internal standard (IS) in a concentration of 1.6 μg/mL dissolved in 0.1% formic acid in both cases.

2.2.3. UHPLC-ESI(±)MS/MS conditions

UHPLC−HRMS/MS analyses were performed using an Accela High Speed LC System hyphenated to a hybrid LTQ Orbitrap XL discovery mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with an electrospray ionization (ESI) probe.

An optimum UHPLC separation gradient was developed in order to efficiently resolve the compounds. The flow rate was set to 0.4 mL/ min and the solvent system was (A) water 0.1% formic acid and (B) acetonitrile. The elution program was: 5% B for 3 min; 95% B in 37 min; and hold for 7 min; return back to 5% B in 1 min, column equilibration was performed for 2 min at the end of the run. The injection volume was 10 μL and samples were injected at 0.4 mg/mL in water-acetonitrile solution (1:1). The HRMS & MS/MS data were acquired in negative mode over a *m/z* range of 100–1000. The MS profile was recorded in full scan mode (scan time = 1 micro scans and maximum inject time = 500 ms) in the form of TIC (Total Ion Current) chromatogram. The ESI conditions were as follow: capillary temperature 320 °C; capillary voltage −40 V; tube lens −120 V; ESI voltage 2.7 kV. Nitrogen was used as sheath gas (40 Au) and auxiliary gas (8 Au). For the MS/MS acquisitions, a data-dependent method including the detection (full scan) and fragmentation of the 3 most intense peaks per scan was used. The mass resolving power was 30,000 for both levels and the normalized collision energy in the ion trap was set to 35.0% (q = 0.25) for the MS/MS experiments. The raw data were acquired and processed with Xcalibur 2.2.4 software from Thermo Scientific.

For the quantification of chlorogenic acid a rapid UHPLC-HRMS separation gradient was developed in order to efficiently resolve the internal standard from each of the standards that were to be quantified. Negative ionization mode was used for chlorogenic acids due to sensitivity aspect. The flow rate was set at 0.4 mL/ min and the solvent system was (A) water 0.1% formic Acid and (B) acetonitrile. The elution program in both quantifications was: 15% B as the initial condition 30% B in 2.8 min; 100% B in 7.0 min; return back to 15% B in 0.2 min, while column equilibration was performed for 1.0 min at the end of the run. The injection volume was 10 μL and the 9 samples were injected at 10 μg/mL in water 0.1% formic acid. The ESI conditions were the aforementioned and the data dependent method was carried out to identify accurately the isomers of chlorogenic acid according to their MS/MS fragmentation patterns.

2.2.4. Chlorogenic acid calibration curve

Chlorogenic acid was quantified by UHPLC-HRMS via external

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