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Low-molecular procyanidin rich grape seed extract exerts antihypertensive effect in males spontaneously hypertensive rats

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ABSTRACT

Grapes are a good source of flavonoids, which have been previously demonstrated to exert beneficial healthy effects on cardiovascular diseases. The aims of this study were to extensively characterise a grape seed procyanidin extract (GSPE) (total phenolic content, antioxidant capacity and HPLC–MS phenolic profile) and, to assess its antihypertensive effect in spontaneously hypertensive rats (SHR) which is a model of genetically hypertensive rat analogue to the essential hypertension in humans. The hypotensive effect of GSPE was also proved in normotensive Wistar–Kyoto rats. Chromatographic analysis of the extract showed that the most abundant polyphenols are monomers and dimers, in their free forms and linked to a gallate. GSPE produced a significant decrease in systolic and diastolic blood pressure of SHR dose-dependently up to 375 mg/kg (maximum decrease 6 h post-administration) and did not affect blood pressure of Wistar–Kyoto rats. GSPE increased the activity of an antioxidant endogen system, but did not affect plasma ACE activity in these animals.

1. Introduction

Hypertension (HTN) is a major risk factor for stroke and is the most common disease found in patients in primary care (Chobanian et al., 2003). It is estimated that by 2025, the incidence of hypertension will increase to 24% in developed countries and to 80% in developing countries (Messerli, Williams, & Ritz, 2007). The current and common method for controlling hypertension is the use of long-term drug therapy. However, it is well known that drugs have many side effects, which may complicate the patient's medical condition. New strategies for treating hypertension based on natural products could greatly benefit hypertensive patients. In this context, there is evidence that a diet rich in vegetables and fruits, which are rich in flavonoids and phenolic compounds, helps to control arterial blood pressure. In fact, increased fruit and vegetable intake has been included recently in the guidelines for the management of arterial hypertension (Mancia et al., 2007).

Grapes and wine are well known as significant sources of flavonoids (Aherne & O'Brien, 2002), which exhibit several pharmacological properties, including vasodilator (Andriambeloson et al., 1997; Diebolt, Bucher, & Andriantsitohaina, 2001; Moura et al., 2002; Zenebe, Pechanova, & Andriantsitohaina, 2003), antihypertensive (Diebolt et al., 2001; Jang & Lee, 2011) and antioxidant (Frankel, German, Kinsella, Parks, & Kanner, 1993; Jang & Lee, 2011; Moura et al., 2002) activities. These activities have led to grapes and wine being considered as functional food candidates (Barreiro-Hurlé; Colombo, & Cantos-Villar, 2008; Gollucke, 2010; Schieber, Stintzing, & Carle, 2001; Shrikhande, 2000). Nevertheless, different grape products often widely vary in both the type and content of flavonoids, and the characterisation of the types of polyphenols present in a grape variety or grape-derived product is important for understanding the possible health-promoting effects associated with its consumption. In fact, grape botanical variety/species, cultivation area, harvesting season, cultural practice, sun exposure, environmental factors, grape maturity, and manufacturing factors may affect the flavonoid content of grapes, grape extracts or wine (Aherne & O'Brien, 2002; Downey, Dokoozlian, & Krstic, 2006; Yang, Martinson, & Liu, 2009). In addition, the phenolic distribution in the juice, pulp, skins and seeds is very different; the phenolic contents of these components are approximately 5%, 1%, 30% and 64%, respectively (Singleton, 1981; Singleton & Esau, 1969). Moreover, in the grape fruit, flavonoids, such as anthocyanins and resveratrol, are mainly localised in the skins, whereas the procyanidins or flavanols are principally located in the seeds (Yang et al., 2009).

Grape seeds are a by-product of the grape/wine industry, but they are one of the richest sources of procyanidins (Nakamura, Tsuji, & Tonogai, 2003), and their beneficial effects have been extensively investigated. Our research group has demonstrated that a grape seed

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procyanidin-rich extract (GSPE) exhibits antioxidant capacity (Puiggròs et al., 2005), improves lipid metabolism (Del Bas et al., 2005), limits adipogenesis (Pinent et al., 2005), acts as an insulin-mimetic agent (Pinent et al., 2004) and reduces inflammation (Terra et al., 2011).

Procyanidin-rich foods, such as cocoa, have demonstrated antihypertensive properties (Buijsse, Feskens, Kok, & Kromhout, 2006; Taubert, Roesen, Lehmann, Jung, & Schömig, 2007). The antihypertensive properties of procyanidins are associated with different biological activities, such as nitric oxide-mediated vasodilation (Duffy et al., 2001; Fisher, Hughes, Gerhard-Herman, & Hollenberg, 2003; Mukai & Sato, 2009; Schroeter et al., 2006; Stein, Keevil, Wiebe, Aeschlimann, & Folts, 1999; Yamamoto, Suzuki, & Hase, 2008), angiotensin-converting enzyme (ACE) inhibition (Actis-Goretta, Ottaviani, & Fraga, 2006; Actis-Goretta, Ottaviani, Keen, & Fraga, 2003; Dong, Xu, Liang, Head, & Bennett, 2011; Ottaviani, Actis-Goretta, Villordo, & Fraga, 2006) and reduction of oxidative stress (Mane, Loonis, Juhel, Dufour, & Malien-Aubert, 2011; Ramiro-Puig et al., 2007). Although there are evidences in human studies that the whole grape fruit improves blood pressure and other factors related to vascular function in men with metabolic syndrome (Barona, Aristizabal, Blesso, Volek, & Fernandez, 2012) no investigation has yet been performed on the antihypertensive effects of GSPE in male hypertensive rats. As has been explained above, the phenolic composition of the grape seed differs from the composition of the whole grape. Therefore, it is necessary to extensively characterise the grape seed extract to better relate the resulting effects with the specific combination and concentration of molecules present in the extract.

The aims of the present study were to characterise and quantify both the flavonoid content present in GSPE and the total antioxidant capacity of this extract. We also evaluated the short-term effects of GSPE in an experimental model of hypertension. The underlying mechanisms involved in the antihypertensive effects of procyanidins have not been clarified in detail, but a better understanding of these mechanisms will allow a rational development of functional foods rich in polyphenols for blood pressure control. Therefore, in this study, we also investigated the possible mechanisms involved in the antihypertensive effects of GSPE.

2. Material and methods

2.1. Grape seed procyanidin-rich extract

The grape seed procyanidin-rich extract (GSPE) was obtained from white grape seeds and was kindly provided by Les Dérives Résiniques et Terpéniques (Dax, France). According to the manufacturer, the procyanidin profile of the extract was composed of monomers or flavan-3-ols (21.3%), dimers (17.4%), trimers (16.3%), tetramers (13.3%) and oligomers (5–13 units; 31.7%) of procyanidins.

2.2. Characterisation of GSPE

2.2.1. Solvents and phenolic standards

The following commercial standards were used for quantitative determination by HPLC: protocatechuic acid, eriodictyol-7-*O*-glucoside, chlorogenic acid, quercetin-3-*O*-galactoside, quercetin-4-*O*-glucoside, kaempferol-3-*O*-rutinoside, naringenin-7-*O*-glucoside, isorhamnetin-3-*O*-rutinoside, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside, eriodictyol, isorhamnetin and procyanidin B2, which were purchased from Extrasynthese (Genay, France). (+)-Catechin and (−)-epicatechin were purchased from Fluka Co. (Buchs, Switzerland), and naringenin, kaempferol, vanillic acid, *p*-coumaric acid, 3-hydroxybenzoic acid, gallic acid, rutin, and (−)-epigallocatechin gallate were purchased from Sigma Aldrich (St. Louis, MO). 2,2′-Azo-bis(2-methylpropionamidine) dihydrochloride (AAPH) was purchased from Acros Organics. Fluorescein was purchased from Fluka/Sigma-Aldrich (Madrid, Spain), and Folin–Ciocalteu's reagent and Trolox were purchased from Sigma (Barcelona, Spain). Organic solvents (high performance liquid chromatography [HPLC]-grade) were obtained from Scharlab (Barcelona, Spain) and Merck (Darmstadt, Germany).

2.2.2. Quantification of the total phenolic content of GSPE

The total phenolic content of GSPE was estimated spectrophotometrically using a Hitachi U-1900 Spectrophotometer by means of the Folin–Ciocalteu assay at 725 nm (Singleton & Esau, 1969). The assay was performed in triplicate, and the samples were dissolved in ethanol:water (1:1). The results were expressed as mg of gallic acid per g of fresh GSPE extract.

2.2.3. Analysis of individual phenolic compounds of GSPE by reverse phase chromatography coupled to mass spectrometry

To study the extract in greater detail, individual phenolic compounds of the GSPE (both flavan-3-ols and phenolic acids) were characterised by an HPLC coupled to a UV detector (Agilent 1200 Series) and a time-of-flight mass spectrometer (TOF 6210, Agilent). The HPLC–MS system consisted of an Agilent 1200 Series instrument (Agilent Technologies) with a Zorbax SB-Aq column (3.5 μm, 150 mm × 2.1 mm internal diameter [i.d.]) equipped with a Pre-Column Zorbax SB-C18 (3.5 μm, 15 mm × 2.1 mm i.d.), which was also from Agilent, and Masshunter software. During the analysis, the column was kept at 30 °C and the flow rate was 0.21 mL/min. The solvent composition of solvent A was Milli-Q water/acetic acid (99.8:0.2 v/v), and that of solvent B was acetonitrile/acetic acid (99.8:0.2 v/v). Initially, 2% solvent B was used. The proportion of solvent B was gradually increased, reaching 20% at 33 min, 22.5% at 34.2 min, 23.2% at 40 min, 25% at 63 min and 100% at 72 min. Then, solvent B was reduced to the initial proportion at 75 min and maintained at this level until 90 min to re-equilibrate the column at these initial conditions. The injection volume was 9.4 μL, and all the freeze-dried samples were re-dissolved in water:acetone: acetic acid (27.5:70:0.5 v/v/v).

The wavelength of the UV detector was set at 280 nm. Ionisation in the mass spectrometer was performed by electrospray (ESI) in the negative mode, and the source parameters were as follows: capillary voltage, 4 kV; fragmentor, 125 V, source temperature, 150 °C; desolvation gas temperature, 350 °C, with a flow rate of 9 L/min and a drying gas flow rate of 12 L/min. Nitrogen was used as the cone gas.

Individual phenols were quantified with a six-point regression curve by using standards obtained from commercial suppliers.

2.2.4. Oxygen radical absorbance capacity assay

The characterisation of the GSPE was completed with the evaluation of its antioxidant activity in terms of its hydrophilic oxygen radical absorbance capacity (ORAC assay). The ORAC assay was performed according to the methodology reported previously (Huang, Ou, Hampsch-Woodill, Flanagan, & Prior, 2002) with some modifications (Suárez, Romero, Ramo, Maciá, & Motilva, 2009). This method analyses the peroxyl radical-scavenging activity of the samples. The assay was performed in 96-well microplates with an FLx800 Fluorescence Microplate Reader (Bio-Tek-IZASA, Barcelona, Spain) with an excitation filter set at 485 nm and an emission filter set at 520 nm. The Gen5™ Data Analysis Software controlled the fluorescence plate reader. The experiment was performed at 37 °C in phosphate buffer at pH 7.4. The reaction mixture consisted of 150 μL of 68 nM fluorescein solution (substrate), 25 μL of 74 mM initiator solution (2,2′-Azobis(2-methylpropionamidine) dihydrochloride [AAPH]; prepared immediately before use in the assay buffer at 37 ºC), and 25 μL of either GSPE or Trolox at different concentrations (ranging from 0.25 to 5 μg/mL of the phenolic extract and from 12.5 to 100 μM Trolox). The assay buffer was used as a blank. The ORAC values were calculated by using the area-under-curve (AUC) results for Trolox, expressed as micromoles (μmol) of Trolox equivalents per gram of the phenolic extract, and the sample calibration curves obtained in each analysis.