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Involvement of nitric oxide and prostacyclin in the antihypertensive effect of low-molecular-weight procyanidin rich grape seed extract in male spontaneously hypertensive rats

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ABSTRACT

The aim of this study was to evaluate the involvement of endothelial-relaxing factors as possible antihypertensive mechanism of low-molecular-weight procyanidin rich grape seed extract (LM-GSPE). Thirty 17–20-week-old male spontaneously hypertensive rats (SHR) were administered water or 375 mg/kg LM-GSPE by intragastric gavage. One millilitre of saline, 30 mg/kg NG-Nitro-L-arginine methyl ester (L-NAME) or 5 mg/kg indomethacin was administrated intraperitoneally. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded before and 6 h after oral administration. Plasma concentration of 6-keto-prostaglandin F1α (PGF1α) was quantified. In addition, we evaluated the relaxation caused by LM-GSPE in different aorta preparations. The antihypertensive effect of LM-GSPE was completely and partially abolished by L-NAME and indomethacin, respectively. In addition, plasma PGF1α was increased in LM-GSPE-administered rats. Finally, LM-GSPE relaxed the intact aorta preparations but did not relax the endothelium-denuded aorta rings. L-NAME inhibited the relaxation caused by LM-GSPE in the SHR aorta rings, but indomethacin did not. Therefore, the antihypertensive effect of LM-GSPE in SHR is endothelium dependent, and it could be mediated by changes in endothelium-derived nitric oxide bioavailability. Nevertheless, prostacyclin could also contribute additionally to this effect.

1. Introduction

Endothelial tissue regulates vascular tone and exerts finely tuned control over cardiovascular homeostasis, with nitric oxide (NO) being one of the best-characterised vasodilator endothelial factors. NO is synthesised in the endothelial cells by a Ca^{2+}-dependent constitutive isoform of the enzyme NO synthase (eNOS), which can be up-regulated by an elevation in arterial blood pressure or by the presence of insulin or hormones such as adiponectin, oestrogens or thyroid hormone (Vanhoutte, Shimokawa, Tang, & Feletou, 2009). In fact, the inhibition of eNOS synthesis will increase blood pressure, as has been previously demonstrated in animal experimental models (Quiñones, Muguerza, Miguel, & Aleixandre, 2011a;

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Quiñones, Sánchez, Muguerza, Miguel, & Aleixandre, 2011b). The diet is also an important factor in the up-regulation of eNOS. It is known that flavonoid consumption potentiates NO endothelium-dependent relaxation (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).

Prostaglandin I2 (PGI_2), which is known as prostacyclin, is also an important vasodilator endothelial factor. PGI_2 is synthesised in the endothelial cells by cyclooxygenase 2, and this prostaglandin also plays an important role in limiting platelet-mediated thrombosis because it is also a potent inhibitor of platelet aggregation. Previous studies have suggested that a decrease in arterial blood pressure was caused by procyanidins and could be mediated at least in part by endothelial vascular relaxing factors (Quiñones et al., 2010; Quiñones et al., 2011a, 2011b).

We have previously demonstrated the antihypertensive effect of low-molecular-weight procyanidin rich grape seed extract (LM-GSPE) in spontaneously hypertensive rats (SHR). This grape seed extract was found to have as the most abundant phenols low-molecular-weight flavanols mainly monomers and dimers (Quiñones et al., 2013). This fact is considered important because although polyphenol bioavailability is relatively poor, flavanols with low-molecular-weight are among the most bioavailable flavanoid compounds (Tomas-Barberan et al., 2007). In addition, it is reported that the healthy properties attributed to food rich in flavanols, such as cocoa, seem to be related to the high amount of monomeric and dimeric compounds (Cooper, Donovan, Waterhouse, & Williamson, 2008). The aim of this study was to evaluate the involvement of endothelial-relaxing factors as possible antihypertensive mechanism of LM-GSPE. We used animals that had been alternatively treated with NG-Nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthesis, or with indomethacin, an inhibitor of prostacyclin synthesis. Plasma PGI_2 concentrations were evaluated in LM-GSPE-administered rats. We also studied the involvement of endothelial factors on the vasorelaxing effect of LM-GSPE in the aorta rings of untreated SHRs.

2. Materials and methods

2.1. Products and reagents

The procyanidin extract that was used in this study (LM-GSPE) was obtained from grape seeds by Les Dériveés Résiniques et Terpéniques, Dax, France. Table 1 shows the total polyphenol and phenolic compounds (adapted from Quiñones et al., 2013). Individual phenolic compounds were characterised by using an HPLC Agilent 1200 Series (Agilent, Palo Alto, CA, USA) coupled to a time-of-flight mass spectrometer Agilent TOF 6210. Commercial standards used for quantitative determination by HPLC were purchased from Extrasynthese (Genay, France), except (+)-catechin and (−)-epicatechin which were purchased from Fluka Co. (Buchs, Switzerland) and naringenin, kaempferol, vanillic acid, *p*-coumaric acid, m-hydroxybenzoic acid, gallic acid, rutin, and (−)-epigallocatechin gallate which were purchased from Sigma Aldrich (St. Louis, MO, USA). Organic solvents (high performance liquid chromatography [HPLC]-grade) were obtained from Scharlab (Barcelona, Spain) and Merck (Darmstadt, Germany). The inhibitors L-NAME and indomethacin were purchased from Sigma (Barcelona, Spain).

2.2. Experimental procedure in rats

Thirty 17–20-week-old male SHRs with an average weight of 319.07 ± 2.38 g were used for the *in vivo* experiments. All these animals were obtained from Charles River Laboratories (Barcelona, Spain). The rats were maintained at a temperature of 22 °C in 12-h light/dark cycles. They received tap water and a standard diet (A04 Panlab, Barcelona, Spain) *ad libitum* during the experiments and were divided into two groups that were administered distilled water or 375 mg/kg of LM-GSPE dissolved in distilled water by gastric intubation between 9 and 10 am. The total orally administered volume was always 1 mL, whether it was water or LM-GSPE water solution. Four hours after oral administration, five of the animals from each group were given 1 mL intraperitoneal saline, 30 mg/kg of L-NAME dissolved in 1 mL of saline or 5 mg/kg indomethacin dissolved in 1 mL of saline. Rat systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken by the tail cuff method before the initial oral administration and again at 6 h afterwards. The rats were kept at 38 °C for 10 min before the measurement to detect the pulse of the tail artery. The original method for measuring arterial blood pressure using the tail cuff provides only SBP values (Buñag, 1973), but the equipment used in this study, an LE 5001 (Letica, Spain), has a high sensitivity pulse transducer coupled with an accurate microprocessor program, which allowed us to distinguish between the SBP and the DBP. Five measurements were averaged to establish the values of the SBP and DBP. All measurements were taken by the same person in the same peaceful environment to minimise stress-induced variations in blood pressure. Moreover, to guarantee the reliability of the measurements, we established a training period of two weeks before the actual trial time, and the rats became accustomed to the procedure during this period.

Additionally, fifteen 22-week-old SHRs were sacrificed by decapitation after overnight fasting. Eight of them were treated with 375 mg/kg LM-GSPE 6 h before being sacrificed, and the remaining animals (seven) were given water 6 h before being sacrificed. The LM-GSPE and water were orally administered by gastric intubation between 9 and 10 am. Blood samples were collected from the sacrificed rats to quantify 6-keto-prostaglandin F1α (PGF1α, a stable metabolite of PGI_2. The procedures that were used to evaluate all of these parameters are described below.

2.3. Prostacyclin determination

Blood samples from the sacrificed animals were collected in tubes containing the anticoagulant lithium heparin. These samples were centrifuged at 2000 g for 15 min at 4 °C to obtain the plasma, which was divided into aliquots and stored at −80 °C until the analysis. The concentration of PGF1α, a stable metabolite of PGI_2, by using a PGF1α EIA (enzyme