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Original Contribution

Polyphenols restore endothelial function in DOCA-salt hypertension: Role of endothelin-1 and NADPH oxidase

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Abstract

Red wine polyphenols (RWPs) have been reported to exert beneficial effects in preventing cardiovascular diseases, such as hypertension. We studied the effects of chronic treatment with RWPs and apocynin, an inhibitor of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, on blood pressure, endothelial function, and oxidative status in deoxycorticosterone acetate (DOCA)-salt-induced hypertension. Rats were administered RWPs (40 mg/kg) or apocynin (33 μg/kg) daily by gavage for 5 weeks. Plasma catechin levels were detected only after RWP treatment. RWPs and apocynin prevented both the increase in systolic blood pressure and the proteinuria induced by DOCA-salt. Plasma malonyldialdehyde levels, urinary iso-prostaglandin $F_{2\alpha}$ excretion, aortic superoxide production, and aortic NADPH oxidase activity were found to be increased in animals of the DOCA group. RWP and apocynin treatments reduced these parameters in DOCA-salt rats, having no effect on control rats. However, only RWPs reduced the increase in plasma endothelin-1 (ET-1) levels and aortic $p22^{phox}$ gene overexpression found in DOCA-salt animals. RWPs and apocynin also improved the blunted endothelium-dependent relaxation response to acetylcholine in noradrenaline-precontracted aortic rings. All these results suggest that chronic treatment with RWPs prevents hypertension and vascular dysfunction. RWPs prevent vascular oxidative stress by inhibiting NADPH oxidase activity and/or by reducing ET-1 release.

Keywords: Red wine polyphenols; DOCA-salt hypertension; Endothelial dysfunction; NADPH oxidase; Endothelin-1; Free radicals

Moderate consumption of red wine is associated with a lower incidence of cardiovascular disease [1,2]. However, the source of the cardioprotective effect of wine is still uncertain. Although the alcohol component may contribute to the protective effect by increasing the concentration of high-density lipoproteins, and by decreasing the fibrinogen level and the reactivity of platelets [3–5], several recent studies suggest also a key role for the polyphenolic component (for reviews see [6,7]). Indeed, intake of red wine polyphenols (RWPs) reduced the development of atherosclerosis in several experimental models of atherosclerosis but was without effect on mature atherosclerosis [8–11].

Hypertension is a well-established risk factor for the development and acceleration of atherosclerosis. RWPs and a grape skin extract also reduced blood pressure in several models of experimental hypertension, such as N^G-nitro-L-arginine methyl ester (L-NAME)- [12,13], angiotensin II (Ang II)-

Abbreviations: Ang II, angiotensin II; DAPI, 4,6-diamidin-2-phenylindol dichlorohydrate; DHE, dihydroethidium; DOCA, deoxycorticosterone acetate; DPI, diphenylene iodonium; ET-1, endothelin-1; iso-$PGF_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$; L-NAME, N^G-nitro-L-arginine methyl ester; MDA, malonyldialdehyde; MS, mass spectrometer; NADPH, nicotinamide adenine dinucleotide phosphate; $O_2^{\bullet -}$, superoxide anion; PAD, photodiode array detector; RLU, relative luminescence units; ROS, reactive oxygen species; RT-PCR, reverse transcriptase-polymerase chain reaction; RWP, red wine polyphenol; SBP, systolic blood pressure; SEM, standard error of mean.

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[14], and deoxycorticosterone acetate (DOCA)-salt-induced hypertensive rats [15], which was related to a combination of vasodilator and antioxidant actions. Oxidative stress and the inactivation of nitric oxide (NO) by vascular superoxide anion ($O_2^{\bullet -}$) play a critical role in the pathogenesis of vascular disease, including hypertension [16,17]. Arterial $O_2^{\bullet -}$ is elevated in Ang II-induced hypertension [18], attributable to a large extent to nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation by Ang II [19,20]. However, an excess of vascular $O_2^{\bullet -}$ production has also been found in DOCA-salt hypertension [21–23], a model with a markedly depressed plasma renin activity because of sodium retention [24]. Endothelin-1 (ET-1) has been shown to contribute to the pathogenesis of salt-sensitive hypertension in animals and humans [25] secondary to a low-renin state [26,27]. ET-1 may be one of the most potent vasoconstrictors produced in the blood vessel wall known to date [28] and also augments vascular $O_2^{\bullet -}$ production, at least in part, via the ET_A/NADPH oxidase pathway [29], leading to endothelial dysfunction and hypertension. Because red wine extract [30] and polyphenol constituents [31] decreased ET-1 synthesis in cultured bovine aortic endothelial cells by suppressing transcription of the ET-1 gene, we hypothesized that in vivo RWPs would affect the development of DOCA-salt hypertension and its vascular features by interfering with ET-1 production. The involvement of the antioxidant properties of RWPs in their protective effect in this model of low-renin hypertension was also tested by comparison with the NADPH oxidase inhibitor apocynin.

Therefore, the aim of the present study was to examine whether chronic intake of RWPs prevents DOCA-salt-induced hypertension and endothelial dysfunction and, if so, to determine the underlying mechanism, focusing on the involvement of ET-1 and oxidative stress.

Material and methods

DOCA-salt-hypertensive rats and in vivo pharmacological intervention

The experimental protocol followed the European Union guidelines for animal care and protection. Twelve-week-old male Wistar rats (250–285 g) were obtained from the Laboratory Animal Service of the University of Granada (Spain). All rats were maintained at five per cage at a constant temperature (24±1°C), with a 12-h dark/light cycle and on standard rat chow. An adaptation period of 2 weeks for vehicle administration and systolic blood pressure (SBP) measurements was allowed before the initiation of experiments.

Rats were randomly divided into six groups: control, RWPs, apocynin, DOCA-salt, DOCA-salt–RWPs, and DOCA-salt–apocynin. DOCA-salt hypertension was created as previously described [32]. Briefly, DOCA-salt animals were anesthetized with 2.5 ml/kg equitensin (500 ml containing 43% w/v chloral hydrate in 81 ml ethanol, 4.86 mg Nembutal, 198 ml propylene glycol, 10.63 g $MgSO_4$, distilled water) (ip) and uninephrectomized. On the following day, they were administered DOCA subcutaneously at a dose of 12.5 mg/rat per week for 5 weeks. The remaining three groups received a sham operation. During the experimental period, DOCA-salt-treated rats were allowed free access to water containing 1% NaCl. RWPs and apocynin were given by intragastric gavage at doses of 40 mg/kg and 33 μg/kg per day, respectively. This dose of RWPs has been previously shown to prevent cardiovascular alterations in L-NAME-induced hypertension [13]. On average, the absorption of RWPs in the digestive tract was estimated to be about 5–10% [33].Thus, the dose used in the present study may lead to a concentration of polyphenols present in the plasma about 0.01 g/L, the concentration that has been found to produce the maximal endothelium-dependent relaxation in rat vascular tissues [34,35]. The dose of apocynin used has been previously used to prevent the development of DOCA-salt hypertension and to abolish the increase in $O_2^{\bullet -}$ generation [23,36]. RWP or apocynin treatment was stopped 2 days before the end of the experiments, in order to study their long-term effects without the involvement of acute administration effects. All rats of each group were then housed in metabolic cages with free access to food and their respective drinking fluids, in order to measure 24-h urine output.

In order to study the plasma levels of the main components of this mixture we prepared another four groups of rats: control, RWPs, DOCA-salt, and DOCA-salt–RWPs. After 5 weeks of treatment, the rats were anesthetized with 2.5 ml/kg equitensin (ip) 2 h after the last RWP administration and blood was collected from the abdominal aorta.

Blood pressure measurements

SBP was determined once a week, in the morning, 18–20 h after administration of the drugs in conscious, prewarmed, restrained rats by tail-cuff plethysmography (digital pressure meter, LE 5000; Letica S.A., Barcelona, Spain). At least seven determinations were made in every session and the mean of the lowest three values within 5 mm Hg was taken as the SBP level.

Cardiac and renal weight indices

At the end of the experimental period, animals were anesthetized with 2.5 ml/kg equitensin (ip) and blood was collected from the abdominal aorta. Animals were sacrificed and kidneys and hearts excised, cleaned, and weighed. The atria and the right ventricle were then removed and the remaining left ventricle was weighed. The cardiac, left ventricular, and renal weight indices were calculated by dividing the heart, left ventricle, and kidney weight by the body weight.

Plasma and urinary determinations

Plasma was obtained by blood centrifugation at 2000*g* for 15 min, aliquoted, and frozen until analysis. Plasma malonyldialdehyde (MDA) content was evaluated as described by Esterbauer and Cheeseman [37]. One hundred microliters of plasma was reacted with a chromogenic reagent, 1-methyl-2-