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Cardiovascular effects induced by northeastern Brazilian red wine: Role of nitric oxide and redox sensitive pathways

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ABSTRACT

Red wines have been shown to protect the vascular system by increasing the endothelial formation of nitric oxide (NO). This study investigated the vasoactive properties of an alcohol-free Cabernet-Sauvignon northeastern Brazilian red wine – RIOSOL (RSCS) extract. The polyphenolic-rich RSCS contained 4.2 mg GAE/L of polyphenols, and its major compounds included quercetin, myricetin and kaempferol. In normotensive conscious rats, RSCS produced hypotension and tachycardia, which were attenuated by the endothelial NO synthase inhibitor (eNOS), L-NAME. In addition, after 2 weeks of oral treatment, RSCS reduced arterial pressure in L-NAME-treated hypertensive rats. RSCS caused NO-mediated relaxations in phenylephrine contracted mesenteric artery rings, and induced the formation of NO and superoxide anions and a redox-sensitive phosphorylation of Akt and eNOS in cultured endothelial cells. In conclusion, the findings indicate that an alcohol-free lyophilized RSCS induced hypotension and endothelium-dependent vasorelaxation as a consequence of the activation of the Akt-eNOS-NO pathway in a redox-sensitive manner.

1. Introduction

Polyphenol-rich diets, food, and beverages, especially red wine, grape juice, tea, and extracts rich in polyphenols, have been shown to have a protective effect against cardiovascular diseases (Idris Khodja, Chataigneau, Auger, & Schini-Kerth, 2012; Stoclet et al., 2004). Chronic intake of red wine, a particularly rich source of phenolic compounds, caused antihypertensive effects and vasodilatation in normal and hypertensive rats (Soares de Moura et al., 2004). Red wine has also been shown to decrease lipid peroxidation, induce endothelium-derived nitric oxide (NO)-mediated relaxations, enhance NO bioavailability, and to improve the endothelial function

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(Andriantsitohaina et al., 2012; Auger et al., 2010; Diebol, Bucher, & Adriantsitohaina, 2001).

The red wine- and polyphenol-induced vasoprotective endothelial formation of NO has been shown to depend on their ability to modulate specific signalling pathways in endothelial cells (Wallerath, Polleo, Li, & Förstermann, 2003). Indeed, the stimulatory polyphenols caused the PI3-kinase/Akt-dependent activation of endothelial NO synthase (eNOS) by enhancing the phosphorylation level of Ser1177 (an activator site), and this effect is dependent on an intracellular pro-oxidant response in endothelial cells (Alhosin et al., 2013; Kim et al., 2013). However, the mechanism underlying the pro-oxidant effect of red wine polyphenols in endothelial cells still remains to be clarified.

Despite the existence of several studies with wines from different parts of the world, little information exists regarding the biological properties of northeastern Brazilian wines. The São Francisco river valley is a region generally perceived to produce good wines in northeastern Brazil. This region is situated at 8–9S (latitude) and around 40W (longitude) in the northeastern region of the country and is characterized by a semi-arid climate with high sunlight exposure almost all year. This vine growing area is associated with variations in the red wine constituents (Lucena et al., 2010). The aim of the present study was to characterize the major polyphenolic constituents of a red wine Cabernet Sauvignon RIOSOL from the São Francisco river valley (RSCS), and to determine the mechanisms underlying the cardiovascular effects using both an *in vitro* approach and an *in vivo* approach.

2. Materials and methods

2.1. Animals

Male Wistar rats (200–300 g) were housed under controlled temperature (21 ± 1 °C) and light cycle (lights on from 6 am to 6 pm). In addition, rats had free access to water and food (Labina, PURINA, São Paulo, Brazil). All experimental protocols and procedures were approved by the Institutional Animal Care and Use Committee of the Federal University of Paraiba (Protocol number 0310/08).

2.2. Drugs and solutions

All reagents were from Sigma (St. Louis, MO, USA) except diaminofluorescein diacetate (DAF-2DA) from Calbiochem (Foster City, CA, USA), and quercetin, myricetin and kaempferol from Cayman Chemicals (Ann Arbor, MI, USA). ODQ was dissolved in DMSO and the other compounds in distilled water or phosphate buffered saline solution (PBS). The composition of the Tyrode's solution was (mM): NaCl, 158.3; KCl, 4.0; $CaCl_2$, 2.0; $MgCl_2$, 1.05; NaH_2PO_4, 0.42; $NaHCO_3$, 10.0 and glucose, 5.6.

2.3. Preparation of the alcohol-free lyophilized red wine

Red wine from Cabernet Sauvignon (vintage 2006) was made from red grape varieties (*Vitis vinifera* L.) grown in the semi-arid climate with high sunlight exposure in the São Francisco river valley and provided by the Winery Santa Maria (Lagoa Grande, Pernambuco, Brazil). To obtain an alcohol-free lyophilized extract of RSCS, the wine ethanol was first evaporated under low pressure at 55 °C to obtain approximately 50% of the original volume. Then, the residual liquid was lyophilized using a LABCONCO Freezone1 lyophilizer (Kansas City, MO, USA), and kept at –20 °C until use.

2.4. Determination of the phenols content

The total polyphenol content of RSCS was determined using the Folin–Ciocalteu phenol reagent stabilized with a saturated solution of sodium carbonate and a spectrophotometer (Varian 50 Bio UV/Vis at 760 nm). The calibration curve was performed using gallic acid. Samples were analysed in triplicate and the total polyphenol content was expressed as mg of gallic acid equivalent/L of wine (mg GAE/L).

The concentration of quercetin, kaempferol and myricetin in RSCS was determined as reported previously (Vuorinen, Maatta, & Torronen, 2000), by using high performance liquid chromatography with Ultimate 3000 Dionex, Acclaim 120 Dionex C-18 column (250 mm × 4.6 mm, 5 μm). Samples were injected through a 100 μL loop. The mobile phase used was a mixture of CH_3CN: aqueous 0.1% formic acid (25:75, vol/vol) delivered at a flow rate of 2.0 mL/min. The detection of quercetin, kaempferol and myricetin was quantified at 370 nm. The samples were analysed in triplicate, and the concentration of quercetin, kaempferol and myricetin was expressed as μg/mL RSCS.

2.5. Measurement of arterial pressure and heart rate in non-anaesthetized rats

For blood pressure and heart rate determinations, protocols used were similar to those previously described (Nunes, Ribeiro, França-Silva, Medeiros, & Braga, 2010). Briefly, following ketamine and xylazine (75 and 10 mg/kg, i.p., respectively) administration, polyethylene catheters were inserted into the lower abdominal aorta and the inferior vena cava of rats through the left femoral artery and vein, respectively. Both catheters were filled with a heparinized saline solution, tunnelled subcutaneously, exteriorized and sutured at the dorsal surface of the neck. Twenty-four hours after the surgical procedure, experiments were performed in conscious rats. Changes in blood pressure and heart rate were recorded using a pressure transducer coupled to an acquisition system (PowerLab, AD Instruments, Australia) connected to a computer installed with LabChart 5.0 software (AD Instruments).

In one series of experiments, once cardiovascular parameters had stabilized, increasing doses of RSCS (10, 30, 90 mg/kg) were randomly administered i.v. Successive injections were performed at 15 min intervals in order to allow changes in arterial pressure to develop. Thereafter, 30 min after baseline recovery, L-NAME (20 mg/kg, i.v., a NO synthase inhibitor) was administered for 30 min before the administration of increasing doses of RSCS.

In a second series of experiments, after an adaptation period, 12 rats were treated orally with L-NAME (40 mg/kg/day) dissolved in the drinking water. Twelve days after the beginning of the L-NAME treatment, when rats were hypertensive, 6 rats