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Isoflavone genistein inhibits the angiotensin-converting enzyme and alters the vascular responses to angiotensin I and bradykinin

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ABSTRACT

Genistein produces antihypertensive and beneficial cardiovascular effects, although the mechanisms for these effects are not known. We examined whether genistein inhibits the *in vivo* responses to angiotensin I or enhances the responses to bradykinin in anaesthetized rats as a result of angiotensin-converting enzyme inhibition. We have also studied the *in vitro* effects produced by genistein on the angiotensin-converting enzyme activity. We measured the changes in systemic arterial pressure induced by angiotensin I in doses of 0.03 to 10 μg/kg, by angiotensin II in doses of 0.01 to 3 μg/kg, and to bradykinin in doses of 0.03 to 10 μg/kg in anaesthetized rats pretreated with vehicle (controls), or a single i.v. dose of genistein 25 mg/kg, or daily genistein 25 mg/kg i.v for two days, or a single i.v. dose of captopril 2 mg/kg. Plasma angiotensin-converting enzyme activity was determined in controls and genistein-treated rats using a fluorometric method. The effects of genistein (3–300 μmol/l) on *in vitro* angiotensin-converting enzyme activity were assessed by adding genistein to plasma samples and measuring angiotensin-converting enzyme activity. We found significant lower angiotensin-converting enzyme activity in plasma samples from rats pretreated with genistein compared with those found in the Control group (77.7 ± 8.1 his-leu nmol/min/ml and 108.7 ± 8.4 his-leu nmol/min/ml, respectively; $P = 0.01$). The incubation of genistein with plasma samples showed that genistein decreased the angiotensin-converting enzyme activity in plasma in a concentration-dependent manner ($P < 0.01$). These findings indicate that genistein inhibits the angiotensin-converting enzyme *in vivo* and *in vitro* and may explain, at least in part, the antihypertensive and beneficial vascular effects produced by genistein.

1. Introduction

Genistein is an important isoflavone that is commonly found in soya beans and their derivative foods and produces several biological effects including significant improvement in endothelial function that may lead to protective cardiovascular effects, as suggested by findings from both experimental and clinical studies (Nestel et al., 1997; Si and Liu, 2008; Vera et al., 2007). Some of the cardiovascular effects produced by this isoflavone include inhibition of platelet aggregation (Nakashima et al., 1991), arterial vasorelaxation by nitric oxide-dependent mechanisms (Mishra et al., 2000), and enhanced vasodilatation in response to acetylcholine, as previously shown in aorta from spontaneously hypertensive rats (Vera et al., 2007). In agreement with these findings, increased soya isoflavone intake has been associated with significant reductions in arterial pressure, both in animal studies (Cho et al., 2007; Si and Liu, 2008; Vera et al., 2007) and in clinical trials (Rivas et al., 2002). Although mounting evidence indicates that genistein produces antihypertensive and protective cardiovascular effects, the mechanisms underlying such beneficial effects are still poorly known (Si and Liu, 2007).

Angiotensin-converting enzyme, a zinc-containing dipeptidyl carboxypeptidase (EC 3.4.15.1), is a major player in the regulation of the renin–angiotensin system, which controls many relevant functions of the cardiovascular system. Indeed, angiotensin-converting enzyme is responsible for the conversion of relatively inactive angiotensin I to the potent vasoconstrictor angiotensin II, which is the main product of the activation of the renin–angiotensin system (Erdos and Skidgel, 1987) and a major regulator or plasma volume, blood pressure, and sympathetic nervous activity. Although angiotensin II can be generated by other enzymatic pathways, it has been widely accepted that agents with angiotensin-converting enzyme inhibiting properties produce beneficial pharmacological and clinical effects (Vane, 1999). In this regard, it has been reported that genistein decreased angiotensin-converting enzyme expression dose-dependently in rat aortic endothelial cells via estrogen receptors (Xu et al., 2006). However, no previous study has examined whether genistein inhibits the vascular responses to angiotensin I or increases the responses to bradykinin, as it is expected to result from the inhibition of angiotensin-converting enzyme activity or expression (Linz et al., 1995). Therefore the aim of the present study was to examine whether

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genistein inhibits the *in vivo* responses to angiotensin I or enhances the responses to bradykinin in anaesthetized rats as a result of angiotensin-converting enzyme inhibition. We have also studied the *in vitro* inhibitory effects produced by genistein on the angiotensin-converting enzyme activity.

2. Materials and methods

2.1. Animals and treatments

The study complied with international guidelines of the European Community for the use of experimental animal and was approved by the institutional ethics committee. Male Wistar rats (190–250 g) obtained from the colony at University of São Paulo (Ribeirao Preto Campus, Brazil) were maintained on a 12-h light/dark cycle at a room temperature (22–25 °C) with free access to standard rat chow and water.

The rats were divided into three experimental protocols. A first protocol was designed to assess the arterial pressure responses to angiotensin I, angiotensin II, and bradykinin after a single dose of genistein. The rats received vehicle (200 µl of dimethyl sulfoxide) or genistein (25 mg/kg) ($n=4$/group) via their femoral veins and then dose–response curves to angiotensin I, angiotensin II, and bradykinin were evaluated immediately as described below. A second protocol was designed to assess the arterial pressure responses to the same peptides after two doses of genistein. The rats ($n=8$/group) were treated for two days with daily intravenous injections of vehicle (200 µl of dimethyl sulfoxide) or genistein (25 mg/kg) via their penile veins and then dose–response curves to angiotensin I, angiotensin II, and bradykinin were evaluated 3 h after the second dose of genistein as described below. Finally, a third protocol was designed to assess the arterial pressure responses to the same peptides after a single dose of captopril, as a positive control of genistein. The rats ($n=3$/group) received vehicle (200 µl of saline) or captopril (2 mg/kg) via their femoral veins and then dose–response curves to angiotensin I, angiotensin II, and bradykinin were evaluated as described below.

The dose of genistein was chosen with basis on a previous study (Xu et al., 2006) showing significant inhibitory effects on plasma angiotensin-converting enzyme activity. The dose of captopril was based on previous study (Nossaman et al., 1997). The control animals received the same volumes of vehicle (200 µl) as compared with those infused in treated animals.

At the end of experiments of the second protocol, arterial blood samples were drawn from rats treated with two doses of genistein (or vehicle) to assess angiotensin converting enzyme activity.

2.2. Surgical procedures and assessment of cardiovascular responses

The rats were anesthetized with urethane (1.0 g/kg, i.p.) and the trachea was cannulated with a polyethylene tube (PE200) (Tanus-Santos et al., 2000b). A polyethylene catheter (PE50) was inserted into the left carotid and the systemic blood pressure was recorded using a data acquisition system (MP150CE; Biopac Systems Inc. CA, USA) connected to a computer (Acknowledge 3.2, for Windows). Another polyethylene catheter (PE10) was introduced into the right femoral vein for intravenous injections of drugs. The absence of somatic motor reflexes in response to tail pitching or blinking in response to a low-pressure corneal stimulation indicated deep anesthesia and analgesia. At least 15 min of stabilization was allowed before drug infusions.

2.3. Dose–response curves to angiotensin I, angiotensin II, and bradykinin

All drugs were dissolved in saline and were given in a 100 µl intravenous bolus. After baseline assessment of mean arterial pressure and heart rate for 25 min, all rats received saline followed by angiotensin I in doses of 0.03, 0.1, 0.3, 1, 3, and 10 µg/kg, followed by angiotensin II in doses of 0.01, 0.03, 0.1, 0.3, 1, and 3 µg/kg, and then bradykinin in doses of 0.03, 0.1, 0.3, 1, 3, and 10 µg/kg. These doses were chosen with basis on previous studies (Tanus-Santos et al., 2000a) and in pilot studies. The changes in mean arterial pressure were calculated as the difference between the baseline value and those recorded at the highest values of mean arterial pressure after each dose of angiotensin I or angiotensin II, and as the difference between the baseline value and those recorded at the lowest values of mean arterial pressure after each dose of bradykinin. Each dose of drug was given when the mean arterial pressure had returned to baseline after the previous injection (usually 3–6 min) and real time mean arterial pressure recordings were carried out throughout the experiments.

2.4. Effects of genistein on plasma angiotensin-converting enzyme activity

The effect of a two-day genistein treatment on plasma angiotensin-converting enzyme activity was assessed using a fluorometric method that measures the hydrolysis of the synthetic substrate hippuryl-His-Leu, as previously described (Santos et al., 1985). Briefly, 5 µl of plasma samples was incubated with 245 µl of assay solution containing 5 mM hippuryl-His-Leu in 0.4 M sodium borate buffer, pH 8.3, and 0.9 M NaCl for 15 min at 37 °C. The reaction was stopped by adding 600 µl of 0.34 M NaOH. The product of this hydrolysis (His-Leu) was measured fluorometrically (365 nm excitation and 495 nm emission) after the addition of 50 µl of o-phthalaldehyde (20 mg/ml in methanol), which was followed 10 min later by the addition of 100 µl of 3 M HCl, and centrifugation at 800 g for 5 min. A standard curve was obtained with His-Leu (0.1–30 µM), which produced a linear relationship of relative fluorescence and His-Leu concentrations (data not shown). In addition, to check for a possible interference by genistein on angiotensin converting enzyme activity assay, we constructed standard curves with His-Leu (0.1–30 µM) incubated with high concentrations of genistein (30 µM, 100 µM, and 300 µM). We found no such an interference and the linear regression coefficients and slopes obtained with this standard curve in the presence of high concentration of genistein were similar in all experiments ($r=0.999$; data not shown).

2.5. Effects of genistein on in vitro angiotensin-converting enzyme activity

To examine the effects of genistein on *in vitro* angiotensin-converting enzyme activity, we added genistein (from 3 to 300 µmol/l) to plasma samples from rats and measured plasma angiotensin-converting enzyme activity as described above. These concentrations were based on previous studies showing that such concentrations inhibit angiotensin converting enzyme activity in rat aortic endothelial cells (Xu et al., 2006). In addition, the concentrations range studied here contain the plasma genistein levels measured in clinical and experimental studies (Doerge et al., 2001; Setchell et al., 1997).

2.6. Drugs and solutions

All drugs and reagents used were purchased from Sigma Chemical Co. (St Louis, MO, USA). Genistein was dissolved in dimethyl sulfoxide. Angiotensin I, angiotensin II, and bradykinin were dissolved in saline solution immediately before use.

2.7. Statistical analysis

The results are expressed as means ± S.E.M. The between groups comparisons of hemodynamic data were assessed by two-way