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Apocynum venetum leaf extract, an antihypertensive herb, inhibits rat aortic contraction induced by angiotensin II: A nitric oxide and superoxide connection

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ABSTRACT

Ethnopharmacological relevance: The leaves extract of *Apocynum venetum* (AVLE), also known as "luobuma", have long been used in traditional Chinese medicine to treat hypertension and depression in parts of China and it has been shown to possess anti-oxidant and anti-lipid peroxidation effects. AVLE (10 μg/ml) has been reported to have a long-lasting endothelium-dependent relaxant effect and this effect has been proposed to be due to its nitric oxide(NO)-releasing and superoxide anion(SOA)-scavenging properties.

Aim of the study: The present study seeks to evaluate the differential actions of AVLE extract between Ang II- and PE-induced vasoconstriction and the involvement of superoxide anions.

Materials and methods: Single dose of Ang II (100 nM and 1 nM)- or PE (0.1 μM)-induced contraction were assessed in both endothelium-intact and -denuded aortic rings after pre-incubation of AVLE (10 μg/ml) for 15 min. The experiment was repeated in either the presence of NO synthase inhibitor, L-NAME (300 μM) or selective AT_1 receptor inhibitor, losartan (0.1 nM), or superoxide scavenger, tiron (1 mM) or a combination of L-NAME and AVLE. Superoxide production was measured by using enhanced-chemiluminescence assay.

Results: We have demonstrated that AVLE (10 μg/ml) effectively suppressed the Ang II-induced contraction (100 nM and 1 nM) of both endothelium-intact and -denuded rat aortic rings. In endothelium-intact rings, L-NAME, reversed AVLE-induced inhibition of Ang II-contraction. PE-induced contraction was significantly inhibited by AVLE in endothelium-intact rings, but not in endothelium-denuded rings. The inhibition by AVLE of PE-induced contraction was totally abolished in the presence of L-NAME. Ang II-induced SOA production concentration dependently with the optimal effect seen at 100 nM of Ang II, and AVLE (0.3, 1, 10 μg/ml) reduced this effect. SOA production in Ang II-stimulated rings was significantly higher than unstimulated control rings, while PE did not stimulate SOA production at all. SOA formation in the presence of Ang II was also inhibited in the presence of SOD (superoxide scavenger), DPI (NADPH inhibitor) and losartan (specific AT_1 receptor antagonist).

Conclusion: These results collectively suggest that the ability of AVLE in inhibiting Ang II-induced contraction via its SOA scavenging properties and nitric oxide releasing effect may account for its usage as an antihypertensive treatment in traditional folk medicine.

1. Introduction

Angiotensin II (Ang II), an active product of renin–angiotensin system causes numerous effects on the cardiovascular system such as vasoconstriction, vascular hypertrophy (Wang et al., 2002), myocardial hypertrophy and fibrosis (Rajagopalan et al., 1996) and thus it is plays a pivotal role in critical disease development. Several pathologic states have been implicated to the renin–angiotensin system, specifically Ang II, including essential hypertension, renovascular hypertension, congestive heart failure, increased systemic blood pressure, endothelial dysfunction and renal diseases associated with albuminuria (Dinh et al., 2001; Barreras and Gurk-Turner, 2003; Welch, 2008). The action of Ang II is mainly modulated by angiotensin receptors subtype 1 and 2 (AT_1 and AT_2) (Dinh et al., 2001; Siddiqui and Hussain, 2007).

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Ang II-induced hypertension has recently become an active research area in vascular biology due to the ability of Ang II to stimulate the vasoconstriction via not only the classical receptor-coupled calcium signaling pathway, but also the production of reactive oxygen species (ROS) which imposes deleterious oxidative stress subsequently leading to hypertension. This effect of ROS generation has been shown in a number of tissues through the activation of NAD(P)H oxidase, an important enzymatic source of superoxide anions (SOA), once Ang II binds to AT_1 receptor (Wang et al., 1998, 2002; Zhou et al., 2004). The resulting SOA further reacts with NO (eNO) produced from endothelial cells (Zhang et al., 1994). Herein, the lack of NO bioavailability is responsible for the impaired of vasorelaxation resulting also in endothelium-dependent vasoconstriction (Kalinowski and Malinski, 2004). Moreover, this prominent phenomenon is enhanced by contribution of ROS, which leads to vascular contraction through the activation of in mysosin light chain kinase activity (Touyz et al., 2002; Garrido and Griendling, 2009).

Throughout the years, the emerging use of Chinese medicinal plants and their extracts as effective medicinal therapies in hypertension and several cardiovascular diseases has caught increasing attention, especially those involving NO (Achike and Kwan, 2003). For example, *Eucommia ulmoides* (known as *Du-Zhong* in China) and *Eleutherococcus senticosus* (known as *Ci-mu-jia* in China or Siberian ginseng) have been described as effective antihypertensive treatment which elicit of nitric oxide endothelium-dependent vasorelaxation (Kwan et al., 2004a, 2004b, 2004c). Another potential herb that have been extensively used for hypertensive therapy over past few decades is *Apocynum venetum* Linnaeus (syn. *Trachomitum venetum* L. Woodson) which is commonly known as "Luobuma" in China (Irie et al., 2009). In addition to antihypertensive effect, *A. venetum* leaves extracts (AVLE) has been shown to possess antioxidant activity, anti-lipid peroxidation, anti-depressant, anti-anxiety, antihyperlipidemic and diuretic effect (Butterweck et al., 2003; Kao et al., 2011).

Recently, AVLE has been reported to a long-lasting endothelium-dependent vasorelaxation mediated by NO (Kwan et al., 2005) whose bioavailability may also be determined by the presence of other ROS. The fact that Ang II produces SOA as well as NO in endothelium-intact aorta as noted above, and the ROS produced may interact with each other affecting the bioavailability of NO (Wang et al., 1998), and the fact that AVLE not only enhances NO production (Kwan et al., 2005), it also inhibits the formation of other ROS (Yokozawa et al., 2002). Thus, the functional effects of AVLE on Ang II-induced vascular contraction would be intriguing. This study, therefore, is designed (1) to investigate the effect of AVLE on the vascular contractions induced by stimulation of Ang II receptor in relation to its mediation via SOA production and (2) to compared its mechanism of action to that involved in the vascular contractions induced by α-adrenoceptor stimulation, in which ROS does not seem to be directly implicated. Nevertheless, both receptors are indeed physiologically important in blood pressure regulation.

2. Materials and methods

2.1. Herbal extract and chemicals

AVLE was commercially prepared as a standard water-soluble extract as brown-colored powder form under the trade name VENETRON (Tokiwa Phytochemical Company, Tokyo, Japan). Voucher specimens of the leaves of *Apocynum venetum* Linn. were kept in the laboratory of Tokiwa Phytochemical Company, Tokyo, Japan.

Sodium-HEPES, acetylcholine chloride (ACh), PE hydrochloride, bis-N-methylacridinium nitrate (lucigenin), diethyldithiocarbamate acid (DETCA), diphenyliodonium (DPI), β-nicotinamide adenine dinucleotide phosphate (NADPH), Angiotensin II (Ang II), N^G-nitro-L-arginine methyl ester (L-NAME) and losartan were purchased from Sigma-Aldrich (USA). Sodium nitroprusside and Krebs salts (BDH, UK). AVLE extract was isolated from the dried leaves of *A. venetum* L. according to the previously published methods and provided by Tokiwa Co. Ltd. (Tokyo, Japan). DPI was dissolved in DMSO. Lucigenin, NADPH and DETCA were dissolved in Kreb–HEPES buffer and all other chemicals in distilled water.

2.2. Animals and experimental protocol

Male Sprague-Dawley rats (SD), aged about 10–11 weeks, were obtained from the University of Malaya Animal Unit and all the experimental procedures were approved by the University of Malaya Animal Care and Ethics Committee. The animals were housed in a well ventilated room (temperature: $24 \pm 1\ ^\circ C$), and had free access to standard rat chow and tap water.

2.3. Preparation of aortic rings

After one week of acclimatization period, the SD rats weighing 200–250 g were anaesthetized with a single intraperitoneal dose of pentobarbitone sodium (60 mg/kg body weight). The descending thoracic aorta was then isolated through the chest and rapidly oxygenated continuously with a mixture of 95% oxygen and 5% carbon dioxide. Following the removal of superficial fat and connective tissues, the aorta was cut into the 3–4 mm long segment and placed into the Krebs physiological salt solution (KPSS) (composition: NaCl 118.2, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.2, $MgSO_4 \cdot 7H_2O$ 1.2, glucose 11.7, and $CaCl_2 \cdot 2H_2O$ 2.5, in mM) for isometric tension measurement or in ice-cold Krebs–HEPES buffer (composition: NaCl 99.0, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.0, $MgSO_4 \cdot 7H_2O$ 1.2, glucose 11.0, $CaCl_2 \cdot 2H_2O$ 2.5 and Na-HEPES 20.0, in mM) for superoxide generation assay. Special care was taken in the procedure of cleaning the aorta except for some experiments in which the endothelium was removed by rubbing the lumen with a thin metal rod.

The aortic rings were mounted in a jacketed organ bath chambers filled with 5 ml of KPSS. The bath solution was maintained at 37 °C and continuously aerated with 95% oxygen and 5% carbon dioxide at 1 g resting tension. The tissues were allowed to equilibrate for 45 min with the bath solution changed every 15 min and rings were re-stretched as needed to maintain a final tension of 1 g. The rings were attached to isometric force-displacement transducers (Grass Instrument Co., Quincy, MA) and the output was amplified and recorded continuously using the MacLab recording system (AD Instruments, Australia). After the initial equilibration, the aortic rings were repeatedly stimulated with isotonic potassium solution (high K^+, 80 mM) for 4 min with intervals to prime the tissues and until two consecutive equal contractions were attained. Endothelial integrity was assessed with a single exposure to acetylcholine, (ACh 10^{-5} M) following a phenylephrine (PE 10^{-7} M) pre-contraction. Rings exhibiting more than 60% relaxation of the PE contraction were considered endothelium-intact and less than 20% for endothelium-denuded.

2.4. Contractile response to angiotensin II and PE

To understand the role of endothelium and NO in Ang II contraction, the concentration-contraction curve was constructed by contractions obtained over a range of single concentration of Ang II (10^{-6}–10^{-10} M), because Ang II-contraction elicits desensitization upon repeated or cumulative stimulation of Ang II. These