Biomedicine & Pharmacotherapy 102 (2018) 249–253

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Trans-anethole prevents hypertension induced by chronic exposure to both restraint stress and nicotine in rats

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ARTICLE INFO

Keywords:
Trans-anethole
Restraint
Stress
Nicotine
Hypertension

ABSTRACT

Chronic stress and smoking are major risk factors for hypertension, with stress also being a factor predisposing to smoking. Methods are needed to prevent and/or reduce hypertension induced by chronic exposure to both stress and nicotine. This study investigated whether *trans*-anethole would prevent hypertension induced by chronic exposure to both restraint stress and nicotine in rats. Rats received nicotine intraperitoneally for 21 days following restraint stress (2 h/day) and *trans*-anethole (62, 125, and 250 mg/kg) on days 4, 8, 12, 16 and 20. To confirm the preventive effects of *trans*-anethole, blood pressure and vascular tone were measured on the last day of the experiment, and compared with the results of nifedipine and aerobic exercise. The ability of *trans*-anethole, at doses of 125 mg/kg and 250 mg/kg, to prevent hypertension was comparable to that of aerobic exercise and nifedipine. Furthermore, nifedipine combined with aerobic exercise and *trans*-anethole reduced both blood pressure and vascular tone. These findings are the first to show that *trans*-anethole can prevent hypertension, suggesting that *trans*-anethole may be useful as a prophylactic antihypertensive agent.

1. Introduction

Chronic nicotine exposure through smoking elevates sympathetic nervous system activity and inflammation, leading to the development of hypertension, as shown by elevated blood pressure and impaired vascular endothelial function [1]. The vascular endothelium secretes factors regulating blood coagulation and vascular smooth muscle contraction and relaxation, and vascular endothelial dysfunction contributes to cardiovascular disease by increasing thrombus formation and vascular tone [2]. Chronic nicotine exposure can also suppress the baroreflex, causing an imbalance in the autonomic nerve system and resulting in elevated blood pressure [3]. More interestingly, 49.8% of smokers have been reported to depend on smoking to relieve stress, indicating that nicotine exposure through smoking is frequently induced by stress [4]. Chronic stress is also associated with persistent sympathetic nerve system activity and inhibits the autonomic regulation of cardiovascular function, leading to hypertension and cardiovascular disease [5].

Hypertension is an important public health issue, reducing quality of life and increasing mortality rates [6]. The global prevalence rate of hypertension in all adults is 31%, whereas the cure rate is only 55% [6]. According to the Global Burden of Disease project report in 2012, increased blood pressure causes the deaths of 9.4 million people worldwide every year, with systolic blood pressure being the factor most significantly affecting mortality due to disease [7]. Moreover, each 20 mmHg increase in systolic blood pressure was found to increase the risk of cardiovascular disease 1.2-fold [8]. Efforts to prevent an increase in blood pressure are especially needed for subjects with pre-hypertension, who have a 26% progression rate to hypertension [9]. However, pharmacologic therapy is currently recommended only for pre-hypertensive individuals with comorbidities [10]. Improvements in lifestyle through non-pharmacologic interventions such as aerobic exercise, smoking cessation, and dietary control are widely used as representative blood pressure control methods [11]. Similarly, aromatherapy using natural products has been shown to reduce blood pressure and stress, thereby enhancing blood pressure control [12].

Trans-anethole is a natural product with anti-inflammatory, antithrombotic, hypoglycemic and neuroprotective effects, primarily through the regulation of cell signaling pathways involving NF-kB and tumor necrosis factor α (TNF-α). These activities suggest that *trans*-anethole may be useful in the management of chronic diseases [13]. *Trans*-anethole was shown to have multiple beneficial effects on vascular health, with few side effects [14]. Foeniculum vulgare oil, which is composed mainly of *trans*-anethole, has antithrombotic activity without causing bleeding, a common side effect of currently prescribed antithrombotic drugs [14].

Hypertension is a direct cause of cardiovascular disease and the risk of cardiovascular disease is high in pre-hypertensive subjects, therefore

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https://doi.org/10.1016/j.biopha.2018.03.081
Received 14 December 2017; Received in revised form 14 March 2018; Accepted 14 March 2018

both the prevention and treatment of hypertension are very important [15]. Moreover, the rate of uncontrolled hypertension is increasing, despite the development of various drugs and treatments, thus further emphasizing the importance of preventing hypertension [16]. Previous studies have demonstrated the effects of *trans*-anethole on blood pressure and vascular tone regulation under normotensive conditions [17,18]. However, the preventive effects of *trans*-anethole on hypertension induced by chronic risk factors, such as stress and smoking, have not been reported. Therefore, this study assessed the preventive effects of *trans*-anethole on hypertension induced rats by chronic exposure to stress and nicotine.

2. Materials and methods

2.1. Animals and chemicals

Male Sprague–Dawley rats (Samtaco Inc., Osan City, Korea), aged 4 weeks and weighing 100–130 g, were housed under controlled conditions (22 ± 2 °C, 12-h dark/light cycle, with free access to water and food. All experimental procedures were approved by the Korea University Animal Experimental Ethics Committee (KUIACUC-2016-153). Nicotine hydrogen tartrate salt, *trans*-anethole, nifedipine, phenylephrine, and acetylcholine (ACh) were all purchased from Sigma (St. Louis, MO). Nicotine was dissolved in 0.9% normal saline and *trans*-anethole and nifedipine were dissolved in 0.1% tween 80.

2.2. Treatment with trans-anethole in a rat model of hypertension

Fifty-six rats were randomly allocated into eight groups: normotensive (n = 6), hypertensive (n = 8), *trans*-anethole 62.5 mg/kg (n = 8), *trans*-anethole 125 mg/kg (n = 8), *trans*-anethole 250 mg/kg (n = 8), aerobic exercise (n = 6), aerobic exercise + *trans*-anethole 125 mg/kg (n = 6), and nifedipine (n = 6). All rats, except normotensive rats, were placed in a properly designed acrylic cage and exposed to restraint stress for 2 h per day [19], followed by daily intraperitoneal injections of nicotine (0.8 mg/kg/day) for 21 days, followed by the injection of nicotine (3 mg/kg) on day 22 as previously described [20]. The model used in the present study, in which nicotine and immobilization stress induced hypertension in rats, has been described previously [21]. On days 4, 8, 12, 16 and 20, rats in the three *trans*-anethole groups were injected intraperitoneally with *trans*-anethole (62.5, 125, and 250 mg/kg), respectively; rats in the aerobic exercise group were allowed exercise freely for 24 h on a cage wheel, 30 cm in diameter; rats in the combined *trans*-anethole (125 mg/kg) and aerobic exercise group received both; and rats in the nifedipine group were injected intraperitoneally with 10 mg/kg of nifedipine (Fig.1).

2.3. Blood pressure measurement

Blood pressure was measured using a volume-pressure recoding tail cuff blood pressure monitoring system (CODA-6 System, Kent Scientific, Torrington, CT) on days 1, 4, 8, 12, 16, 20 and 22. To prevent changes in blood pressure due to ambient temperature and stimulation caused by measurement, rats were placed in a blood pressure measurement cage and stabilized in a quiet environment for 10–15 min at 25–30 °C on a warm plate. Blood pressure was measured 20 times and averaged [22].

2.4. Vascular tone measurement

Following sacrifice, tissue surrounding the thoracic aorta was removed gently and cut into 2.0–3.0 mm rings, ligated to a Myo-Interface Model 620 M (DMT, Aarhus, Denmark). The initial tension was set at 1.0–1.2 g for 1 h in an organ bath (37 °C) filled with Krebs' buffer (118.3 mM NaCl, 4.78 mM KCl, 25 mM $NaHCO_3$, 1.22 mM KH_2PO_4, 11.1 mM glucose, 2.5 mM $CaCl_2$, and 2.5 mM $MgCl_2$). Vascular tone was evaluated by measuring 10 μM ACh-induced maximal relaxation of aortic rings as a percentage of 10 μM phenylephrine-induced maximal contraction of aortic rings.

2.5. Statistical analysis

Results are expressed as means ± standard error of the mean (SEM). Differences between normotensive and hypertensive groups were analyzed by independent sample Student's *t*-tests, whereas differences between hypertensive and other groups were analyzed by one-way analysis of variance, with post-analysis as needed performed by determining least significant difference. All statistical analyses were performed using SPSS Statistics software (ver. 20.0), with $P < 0.05$ defined as statistically significant.

3. Results

3.1. Effects of trans-anethole on blood pressure in a rat model of hypertension

Systolic blood pressure was significantly higher in hypertensive than in normotensive rats (135.67 ± 1.88 mmHg vs. 121.62 ± 2.10 mmHg, P < 0.001) (Fig. 2A). Compared with hypertensive rats, systolic blood pressure was significantly lower in rats treated with 125 mg/kg (10.10 ± 1.66%; 121.99 ± 2.25 mmHg, P = 0.005) and 250 mg/kg (12.62 ± 2.80%; 118.57 ± 3.80 mmHg, P = 0.001) *trans*-anethole (Fig. 2B). In contrast, the mean percent reduction in rats treated with 62.5 mg/kg *trans*-anethole was 4.55 ± 4.12% (129.53 ± 5.59 mmHg), which did not differ significantly from hypertensive rats. Compared with hypertensive rats, the mean percent reductions in systolic blood pressure of rats in the aerobic exercise, aerobic exercise + *trans*-anethole 125 mg/kg and nifedipine groups were 13.18 ± 2.68% (117.82 ± 3.64 mmHg, P = 0.001), 12.97 ± 1.50% (118.11 ± 2.04 mmHg, P = 0.001) and 12.77 ± 1.57% (118.37 ± 2.14 mmHg, P = 0.001), respectively.

Diastolic blood pressure was significantly higher in hypertensive

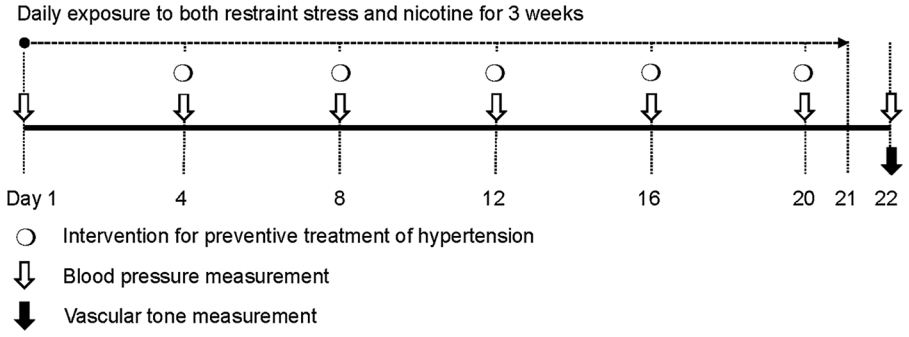

Fig. 1. Schematic diagram of the experimental design.