Anti-hypertensive Effect of the Dongchunghacho, *Isaria sinclairii*, in the Spontaneously Hypertensive Rats

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The present study examined the effect of the methanol extract of *Isaria sinclairii*, a kind of Dongchunghacho (Tochukaso), on blood pressure in spontaneously hypertensive rats (SHR). Blood pressure and heart rate were measured after treatment with the methanol extract of *I. sinclairii* by the indirect tail-cuff method and the direct in vivo model. Starting at 12 weeks of age, male SHR were treated with the extracts for 2 or 4 weeks. We found that, when compared to untreated control SHR, oral treatment with *I. sinclairii* methanol extract (30 mg/kg/day) remarkably decreased systolic blood pressure from 200 to 112 mmHg and decreased diastolic blood pressure from 114 to 88 mmHg. Furthermore, efficacy of methanol extract of *I. sinclairii* was superior to captopril (30 mg/kg/mL, positive control), an angiotensin-converting enzyme inhibitor, with a lowering effect that dropped systolic blood pressure from 201 to 130 mmHg and diastolic blood pressure from 102 to 92 mmHg. However, in normal Wistar Kyoto rats, *I. sinclairii* methanol extract did not significantly change the normal blood pressure, suggesting that this type of Dongchunghacho has a selective effect against hypertension. Therefore, methanol extract of *I. sinclairii* may be used as an anti-hypertensive food/agent. Furthermore, this extract also has multiple actions such as No production in endothelial cells, inhibiting thrombin-induced blood coagulation by thrombin and mildly decreasing in prostaglandin E2 levels in cultured macrophage cells, all of which might contribute to protection against atherogenesis and thrombus formation. HPLC and MS analysis of methanol extract of *I. sinclairii* revealed the presence of adenosine.

Key words: *Isaria sinclairii*, Hypertension, Spontaneously hypertensive rat, Adenosine

INTRODUCTION

Dongchunghacho (Tochukaso) is a traditional Oriental medicine that is composed of the fruiting bodies of *Cordyceps sinensis* and its parasitic host larva (Ikumoto et al., 1991). Nowadays, it contains various species of Cordyceps and Paecilomyces (Isaria), including *Paecilomyces tenuipes* (=*Isaria japonica*) and *Paecilomyces cicadae* (Miquel) Samson (=*Isaria sinclairii*) are also entomogenous fungi thought to be of the anamorph stage of Cordyceps (Takano et al., 2005). Cicada Dongchunghacho (*Isaria sinclairii*) has recently been introduced in a powdered form as a potential new crude drug, and contains the fruiting bodies of *Isaria sinclairii* and its parasitic host larva. FTY720 (mimic sphingosine), an isolated compound from *I. sinclairii*, was shown to be immunosuppressive because it induced apoptosis in lymphocytes (Fujino et al., 2001 and 2002) and acted on sphingosine-1-phosphate (SIP) receptors as a subfamily of G-protein-coupled receptors (Im, 2003).

Moreover, the fruiting bodies of Isaria fungi have been used to treat cancer patients in Korea (Oh et al., 2001). It was suggested that these activities are due to the effects of a combination of adenosine, 5'-adenosine monophosphate and several other nucleic acid-related compounds that are present in the extracts.

Hypertension causes hypertrophy of the heart and blood vessels, and is a major cause of stroke (apoplexy), disorders of the coronary arteries, myocardial infarction,
and cardiac insufficiency (Christopher et al., 1997; Cleland, 1999).

Since the fruiting bodies of Isaria fungi have various pharmacological effects as described above, we tested the possibility that fruit bodies of silkworms infected with *I. sinclairii* might have an anti-hypertensive activity with good safety margin, using direct and indirect hypertension assessment in the spontaneously hypertensive rats (SHR) and Wista Kyoto rats (WKY) by repeated oral treatment over one-month period.

Our study was subsequently designed to characterize a possible anti-hypertensive mechanism of action of *I. sinclairii* methanol extract which might be associated with endothelial NO dependent vasorelaxation, prostaglandin E2 level in LPS (inflammatory)-induced macrophage cell and thrombin-induced blood coagulation. We then analyzed the main components of *I. sinclairii* methanol extract to help understand one of the possible active ingredients with sensitive high performance liquid chromatography (HPLC) and tandem mass spectrometry (EI-MS).

**MATERIALS AND METHODS**

**Materials**

*I. sinclairii* was collected on Mountain Halla located in Cheju-do, South Korea. This fungus endophytically parasitizes dead or living *Cicadae* spp. This strain was isolated from conidiospores and cultured in a potato dextrose agar (PDA) medium, and then sprayed (inoculated) on silkworms for infection. By evading the defensive mechanisms of the host insect upon both the penetration of the cuticle and the reaching of the hemocoel, *I. sinclairii* proliferated inside of the insect body and was cultivated with forming fruiting bodies in the Department of Agricultural Biology, National Institute of Agricultural Science and Technology, Republic of Korea.

**Animals**

Male spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY), weighing 200±5 g at 9 weeks of age, were supplied from Japan SLC Co. (Shizuoka, Japan) and divided into four groups of ten rats including: the SHR control group, *I. sinclairii*-treated SHR group, the WKY control group and the *I. sinclairii*-treated WKY group. All procedures were performed in accordance with the NIH Guidelines for Care and Use of Laboratory Animals. The rats were kept for one week under normal physical conditions (23±2°C, 55±10%, humidity and regular day/night cycle) and fed with standard diet and water *ad libitum* before they underwent hypertension testing. The SHR were selected to have a systolic blood pressure above 160 mmHg upon the test of hypertension by the indirect (tail) method using a blood monitoring system (Japan Muromachi Co., MK-100).

**Preparation of *I. sinclairii* extract**

The dried *I. sinclairii* (50 g) was homogenized in a blender and soaked with deionized water or methanol for the extract. The samples were filtered with Whatman paper, concentrated by evaporation, and freeze-dried. The dried powder (water/methanol extract) was dissolved in saline as a test solution.

**Measurement of blood pressure, pulse, and heart rate**

**Direct model in vivo**

After an adaptation period of a week, each SHR was anesthetized using pentobarbital (35 mg/kg, i.p.), a tube of polyethylene was inserted into its carotid artery and jugular vein, and the blood pressure was measured by Grass 7p polygraph (Glass7H, Grass Instruments, Quincy, MA) connected to a Statham pressure transducer (Viggo-Spectramed, Oxnard, CA). To prevent blood coagulation, heparin (30 IU/kg) was supplied at intervals of 0.5 h. The heartbeat of each SHR was maintained with artery pressure by a tachometer under equal conditions consistent with temperature and anesthesia. Average blood pressure and pulse pressure were calculated as follows: twenty minutes after the operation, the blood pressure and heartbeat were equilibrated, the vehicle and sample were respectively injected into the jugular vein, and the change in blood pressure (systolic, diastolic, and pulse) was measured.

Average blood pressure = (systolic blood pressure + diastolic blood pressure) / 2
Pulse pressure = systolic blood pressure - diastolic blood pressure

**Measurement by tail-cuff method on a weekly basis**

The blood pressure was measured in all groups using the tail-cuff method under light general anesthesia induced by CO2. Using a blood monitoring system, we monitored SHR/WKY rats, which had received a single daily dose of 30 mg/kg of *I. sinclairii* methanol extract orally (diluted in saline) for 2 or 4 weeks. The corresponding control SHR/WKY rats received an equivalent volume of saline. All groups received normal diet and water during this period. Measurements were performed one week before and four weeks after surgery (before starting the experimental protocol).

**Statistical analysis**

For determination of systolic blood pressure (SBP), the average of three measurements was taken at the time of recording. The data presented was the mean ± SEM.