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Antihypertensive Activities of a Solid-State Culture of *Taiwanofungus camphoratus* (Chang-Chih) in Spontaneously Hypertensive Rats

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Wild and solid-state cultures (SSC) of *Taiwanofungus camphoratus* (aka *Antrodia camphorata* and Chang-chih [CC]) were sequentially extracted with cold water, methanol, and hot water to get cold-water-soluble (CWS), methanol-soluble (MS), and hot-water-soluble (HWS) extracts, respectively. Only the MS extract exhibited angiotensin-converting enzyme (ACE) inhibitory activities. The antihypertensive effects of the MS extract (10 mg/kg BW) were measured in spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats. MS extract of the SSC type was able to effectively lower the systolic blood pressure (SBP) and diastolic blood pressure (DBP) of SHR, but not of WKY rats, the results being significantly different from those for distilled water only (the blank). However, wild CC and its MS extract were not as effective as the SSC type in reducing SHR blood pressure and had no effect on WKY rats. SSC-type CC might be developed into a health food with the ability to regulate blood pressure.

Key words: angiotensin-converting enzyme; *Taiwanofungus camphoratus*; diastolic blood pressure; systolic blood pressure; spontaneously hypertensive rat

Several risk factors are associated with stroke, including age, gender, elevated cholesterol, smoking, alcohol, excessive weight, race, family history, and hypertension.[1)] Although some of these risk factors cannot be modified, one factor that can be controlled and has the greatest impact on the etiology of stroke is high blood pressure.[2)] Hypertension is considered to be the central factor in stroke with approximately 33% of deaths due to stroke attributed to untreated high blood pressure.[1)] Several classes of pharmacological agents have been used in the treatment of hypertension. One class of anti-hypertensive drugs known as angiotensin I-converting enzyme (ACE) inhibitors (*i.e.* peptidase inhibitors) has a low incidence of adverse side-effects and is the preferred class of anti-hypertensive agents for patients with concurrent secondary diseases.[3)] ACE (peptidyldipeptide hydrolyase, EC 3.4.15.1) is a dipeptide-liberating exopeptidase which has been classically associated with the renin-angiotensin system that regulates the peripheral blood pressure.[4)] ACE removes a dipeptide from the C-terminus of angiotensin I to form angiotensin II, a very hypertensive compound. Several endogenous peptides such as enkephalins, β-endorphin, and substance P have been reported to be competitive substrates and inhibitors of ACE.[4)] Several food-derived peptides that can inhibit ACE[5)] include α-lactalbumin and β-lactoglobulin,[6–8)] casein,[9–11)] zein,[12,13)] gelatin,[14)] and yam dioscorin,[15)] all of which are hydrolyzed by pepsin, trypsin, or chymotrypsin. Several antioxidative peptides (reduced glutathione and carnosine-related peptides) have also exhibited ACE inhibitory activities.[16)] Pomegranate juice,[17)] flavan-3-ols and procyanidins,[18)] and tannins[19)] have been reported to have ACE inhibitory activity.

The traditional name of the fungus Chang-chih (CC) literally means "fungus of fortune from the camphor tree." Its scientific name is *Taiwanofungus camphorates*.[20)] It belongs to the Polyporaceae, Basidiomycotina family and has a special host in the endemic

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Abbreviations: ACE, angiotensin-converting enzyme; CWS, cold-water-soluble; CC, Chang-chih; DBP, diastolic blood pressure; HWS, hot-water-soluble; MS, methanol-soluble; SBP, systolic blood pressure; SHR, spontaneously hypertensive rats

perennial tree, *Cinnamomum kanehirai* (Bull camphor tree), in Taiwan. *Antrodia camphorata* (niu-chang-chih) has been widely applied to this fungus.[20,21] CC is recognized as a folk medicine. A solid-state culture (SSC) of a CC extract has exhibited both antioxidative activity against iron-induced lipid peroxidation and hepatoprotective activity against CCl_4-induced hepatic injury.[22] The fermented filtrate of a submerged culture of CC has shown a protective effect against CCl_4-induced hepatic injury.[23] The polysaccharide from cultured mycelia and fruiting bodies of CC has exhibited an anti-hepatitis B virus effect.[24] The maleic and succinic acid derivatives of the mycelia of CC have exhibited cytotoxic activities against the LLC tumor cell line from mycelia of CC,[25] while, the methanol extract of a CC submerged culture has exhibited cytotoxic activity and induced apoptosis against the human hepatoma HepG2 cell line.[26] Due to the growth of CC on the specific tree in Taiwan, *C. kanehirai* (Bull camphor tree), it is difficult to find in the wild and very expensive to buy. SSC-CC[27] and liquid culture CC from mycelia were thus developed. In this present study, the wild (W) and SSC (batch No. WS-10) of CC were sequentially extracted with cold water, methanol, and hot water to get a cold-water-soluble (CWS) extract, methanol-soluble (MS) extract, and hot-water-soluble (HWS) extract, respectively. Each fraction was used to determine the ACE inhibitory activity. W-CC, and SSC-CC and its MS extract were orally administrated once to measure their antihypertensive effect on spontaneously hypertensive rats (SHR) during a 24-h period, and the normal blood pressure of Wistar Kyoto (WKY) rat was used for comparison.

Materials and Methods

Materials. N-(3-[2-furyl] acryloyl)-Phe-Gly-Gly (FAPGG) and ACE (1 unit/ml, rabbit lung) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). W-CC and SSC-CC (batch No. WS-10) were provided by Well Shine Biotechnology Development Co. (Taipei, Taiwan). All other chemicals and reagents were from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of the different Taiwanofungus camphoratus extracts. W-CC and SSC-CC were sequentially extracted with cold water, methanol, and hot water to get cold-water-soluble (CWS), methanol-soluble (MS), and hot-water-soluble (HWS) extracts, respectively. Briefly, 50 g each of W-CC and SSC-CC was extracted first 500 ml with cold water by stirring overnight at room temperature, and after being filtered, the residue was extracted twice with cold water under the same procedure. Each filtrate was collected and lyophilized as the CWS extract. The residue was then extracted 500 ml with methanol by stirring overnight at room temperature. After being filtered, the residue was extracted twice with methanol under the same procedure. The filtrate was collected and concentrated as the MS extract. Finally, the residue was extracted with 500 ml of boiling water for 2 h, and after being filtered, the residue was extracted again with boiling water for 2 h. All fractions were stored at $-20\,^{\circ}C$ for further investigation.

Determination of the ACE inhibitory activity of the different CC extracts by spectrophotometry. The ACE inhibitory activity was measured according to the method of Holmquist *et al.*[28] with some modifications. Twenty µl (20 mU) of commercial ACE (1 U/ml, rabbit lung) was mixed with 200 µl of the different amounts of extracted fractions from W-CC (0.1793, 0.269, and 0.3587 mg/ml) and SSC-CC (0.0896, 0.1793, and 0.3587 mg/ml), and then 1 ml of 0.5 mM FAPGG [dissolved in a 50 mM Tris–HCl buffer (pH 7.5) containing 0.3 M NaCl] was added. The decreased absorbance at 345 nm ($\Delta A_{inhibitor}$) was recorded within a 5-min period at room temperature. DMSO and distilled water were respectively used instead of the MS fraction and CWS and HWS fractions in blank experiments (ΔA_{blank}). The ACE inhibition (%) was calculated as follows: $[1 - (\Delta A_{inhibitor} \div \Delta A_{control})] \times 100\%$. The means of triplicate determinations was found in each case.

Short-term antihypertensive effects of SSC-CC, MS-SSC-CC, W-CC and MS-W-CC on SHR and WKY. The effects of orally administered SSC-CC, MS-SSC-CC, W-CC, and MS-W-CC on the reduced SBP and the reduced DBP of the animal model were measured according to the method of Lin *et al.*[29] All animal experimental procedures followed the published guidelines.[30] Male SHR or WKY (8 weeks of age, National Laboratory Animal Center, Taipei) were housed individually in steel cages kept at $24\,^{\circ}C$ and with a 12-h light-dark cycle. All had free access to a standard laboratory diet (5001 Rodent Diet, St. Louis, MO, USA) and water. After being housed for 10 weeks, the SHR weight ranged from 240 to 250 g, and SBP reached 200 mmHg. The rats were randomly divided into five groups (5 rats/treated group): control, SSC-CC, MS-SSC-CC, W-CC, and MS-W-CC for DBP and SBP determination. In a short-term antihypertensive experiment, 10 mg of W-CC or SSC-CC or its methanol extract per weight (kg) of SHR or WKY was orally administered once (each dose was suspended 0.5 ml of distilled water), and the tail blood pressure was measured four times at each desired time during 24 h with an indirect blood pressure meter (BP-98A, Softron Co., Ltd., Tokyo, Japan). A 0.5 ml amount of distilled water alone was used for a blank experiment. Before each blood pressure measurement, SHR were warmed for 10 min in a $39\,^{\circ}C$ thermostated box. The changes in blood pressure (ΔBP, involving ΔSBP and ΔDBP) were calculated as $BP_{treated\ sample} - BP_{distilled\ water}$.

Statistical analysis. Student's t-test was used for a

comparison between the blank and each treatment at the same time point. A difference is considered statistically significant when $P < 0.05$ (*) or $P < 0.01$ (**).

Results and Discussion

SSC-CC and liquid-culture CC from mycelia were developed as substitutes for W-CC, which is very expensive and not easy to find in nature. The crude extracts of CC were frequently used to test their biological activity.[22–24,26,31] We used SSC-CC as a starting material and used different solvents in sequence to obtain extracts In this study. W-CC was used for comparison. Each W-CC and SSC-CC was extracted sequentially with cold water, methanol and hot water to respectively give CWS, MS, and HWS extracts. The respective recovery from each extraction was 6.64%, 23.4%, and 2.18% for the CWS, MS, and HWS extracts from W-CC; and 11.69%, 3.19%, and 2.29% for the CWS, MS, and HWS extracts from SSC-CC. The content of CWS extract in W-CC was found to be less than that in SSC-CC, and the content of MS extract in W-CC was higher than that in SSC-CC. The HWS extract in both types of CC had similar contents. Several methanol (or ethanol)-soluble triterpenoids have been isolated and identified in W-CC.[32–34] Chen *et al.*[27] have reported that an extract by hot water or ethanol of SSC-CC contained the triterpenoids that were found in W-CC.

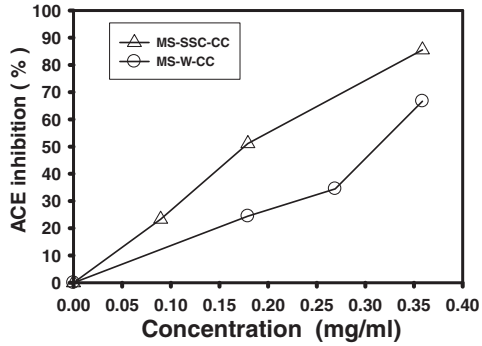

Fig. 1. Effect of the Methanol Extract of Wild Chang-Chih (MS-W-CC) and the Solid-State Culture of Chang-Chih (MS-SSC-CC) on the 20 mU ACE Activity.

The ACE inhibition (%) was calculated as follows: $[1 - (\Delta A_{inhibitor} \div \Delta A_{control})] \times 100\%$. The mean of triplicate measurement was determined. The IC_{50} value for MS-W-CC and MS-SSC-CC were 0.312 mg/ml and 0.176 mg/ml, respectively.

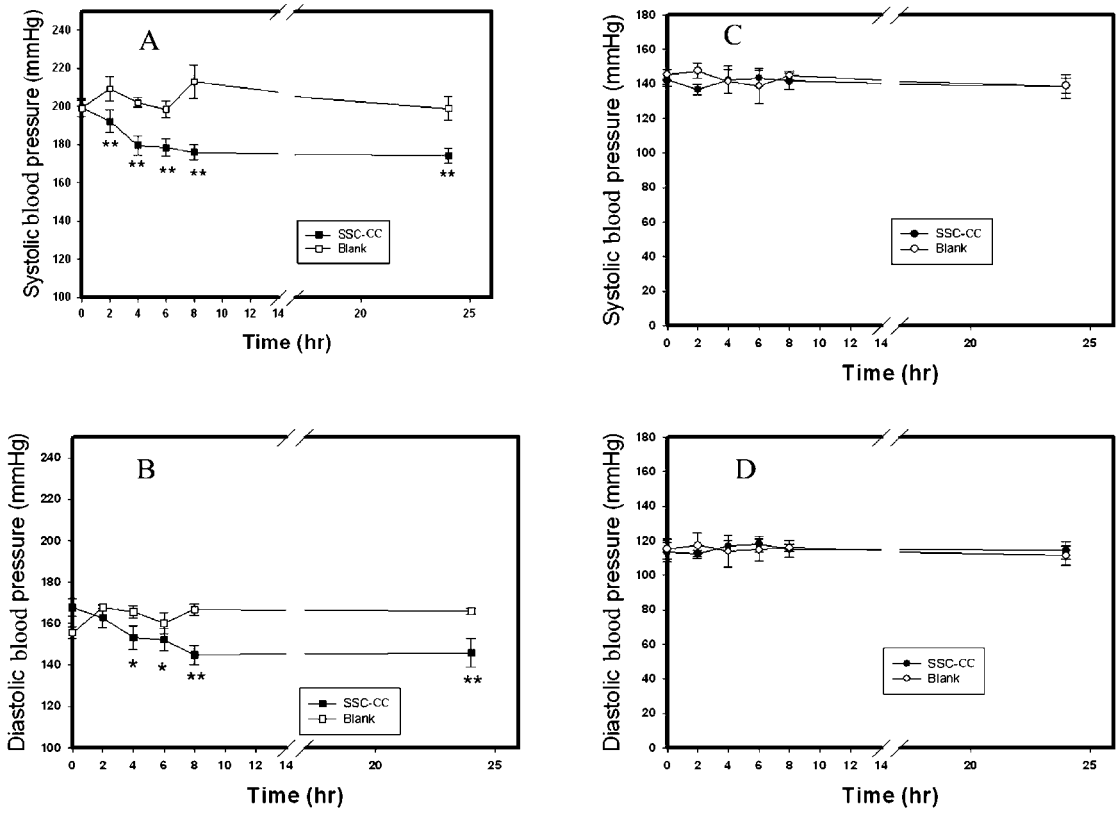

Fig. 2. Effect of the Solid-State Culture of Chang-Chih (SSC-CC) on the Changes in Systolic Blood Pressure (A) and Diastolic Blood Pressure (B) of Spontaneously Hypertensive Rats (SHR), and on the Changes in Systolic Blood Pressure (C) and Diastolic Blood Pressure (D) of Wistar Kyoto (WKY) Rats.

A 10-mg amount of SSC-CC/kg of SHR or WKY was orally administered once, and the tail blood pressure was measured four times after 2, 4, 6, 8 and 24 h by using an indirect blood pressure meter for each treatment. A 0.5-ml amount of distilled water was used for the blank experiment. A difference was considered statistically significant between the blank and treated groups when $P < 0.05$ (*) or $P < 0.01$ (**).

Table 1. Short-Term Effects of a Single Oral Administration of the Solid-State Culture of Chang-Chih (SSC-CC) and Wild Chang-Chih (W-CC) on the Changes in Systolic Blood Pressure [ΔSBP (mmHg)] and Diastolic Blood Pressure [ΔDBP (mmHg)] after 2, 4, 6, 8, and 24 h

Time (H)	SSC-CC (10 mg/kg of SHR)		W-CC (10 mg/kg of SHR)	
	ΔSBP (mmHg)[a]	ΔDBP (mmHg)	ΔSBP (mmHg)	ΔDBP (mmHg)
2	−17.1 ± 5.6	−4.9 ± 1.2	−16.5 ± 5.6	−5.8 ± 1.1
4	−22.5 ± 4.7	−12.5 ± 2.4	−14.6 ± 2.3	−3.5 ± 2.4
6	−20.0 ± 4.2	−7.8 ± 2.2	−6.8 ± 3.8	0
8	−37.2 ± 3.6	−21.8 ± 4.4	−26.3 ± 7.6	−10.6 ± 2.4
24	−24.7 ± 3.6	−20.1 ± 3.4	−5.5 ± 2.1	−0.7 ± 1.2

[a]The change in blood pressure (ΔBP, involving ΔSBP and ΔDBP) was calculated as $BP_{\text{treated sample}} - BP_{\text{distilled water}}$.

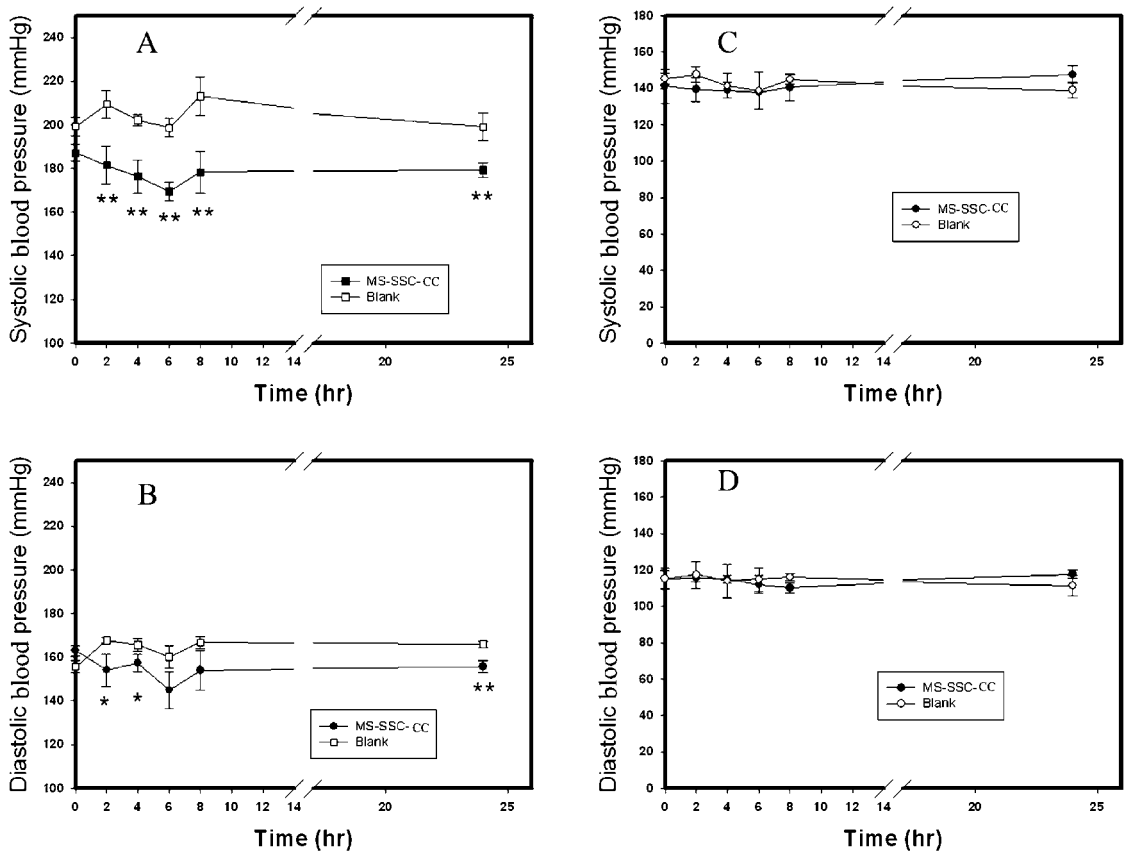

Fig. 3. Effect of the Methanol Extract of the Solid-State Culture of Chang-Chih (MS-SSC-CC) on the Changes in Systolic Blood Pressure (A) and Diastolic Blood Pressure (B) of Spontaneously Hypertensive Rats (SHR), and on the Changes in Systolic Blood Pressure (C) and Diastolic Blood Pressure (D) of Wistar Kyoto (WKY) Rats.

A 10-mg amount of MS-SSC-CC/kg of SHR or WKY was orally administered once, and the tail blood pressure was measured four times after 2, 4, 6, 8 and 24 h by using an indirect blood pressure meter for each treatment. A 0.5-ml amount of distilled water was used for the blank experiment. A difference was considered statistically significant between the blank and treated groups when $P < 0.05$ (∗) or $P < 0.01$ (∗∗).

Each fraction from SSC-CC or W-CC was analyzed for its ACE inhibitory activity. Only the MS fraction in both types of CC was found to have dose-dependent ACE inhibitory activity (Fig. 1). The triterpenes from *Ganoderma lucidum* have reportedly exhibited ACE inhibitory activity,[35] as have flavan-3-ols and procyanidins,[18] and tannins.[19] The IC_{50} values for MS-W-CC and MS-SSC-CC were 0.312 mg/ml and 0.176 mg/ml, respectively. Thus, MS-SSC-CC exhibited roughly two-fold the ACE inhibition of MS-W-CC.

A 10-mg amount of SSC-CC/kg of SHR (Fig. 2A and B) and 10 mg of SSC-CC/kg of WKY (Fig. 2C and D) were orally administered once, and SBP (Fig. 2A and C) and DBP (Fig. 2B and D) were measured after 2, 4, 6, 8, and 24 h. A 0.5-ml amount of distilled water was used for the blank experiment. SSC-CC was found able to effectively lower SBP (Fig. 2A) and DBP (Fig. 2B) of SHR, but not of WKY (Fig. 2C and D), the results differing significantly ($P < 0.05$ or $P < 0.01$) from those for the blank. Table 1 shows the changes in SBP (ΔSBP) and DBP (ΔDBP) after a single administration of SSC-CC to SHR at 2, 4, 6, 8 and 24 h. The greatest

Table 2. Short-Term Effects of a Single Oral Administration of the Methanol Extract of a Solid-State Culture of Chang-Chih (MS-SSC-CC) and the Methanol Extract of Wild Chang-Chih (MS-W-CC) in the Change of Systolic Blood Pressure [ΔSBP (mmHg)] and Diastolic Blood Pressure [ΔDBP (mmHg)] after 2, 4, 6, 8, and 24 h

Time (H)	MS-SSC-CC (10 mg/kg of SHR)		MS-W-CC (10 mg/kg of SHR)	
	ΔSBP (mmHg)[a]	ΔDBP (mmHg)	ΔSBP (mmHg)	ΔDBP (mmHg)
2	−27.9 ± 7.0	−13.6 ± 4.2	−20.1 ± 5.5	−5.4 ± 4.2
4	−25.8 ± 6.2	−8.4 ± 3.3	−5.8 ± 2.3	0
6	−29.2 ± 3.4	−12.7 ± 4.3	−2.1 ± 1.2	0
8	−34.9 ± 7.7	−15.2 ± 3.9	−15.1 ± 4.3	0
24	−19.8 ± 2.8	−10.3 ± 2.1	0	0

[a]The change in blood pressure (ΔBP, involving ΔSBP and ΔDBP) was calculated as $BP_{\text{treated sample}} - BP_{\text{distilled water}}$.

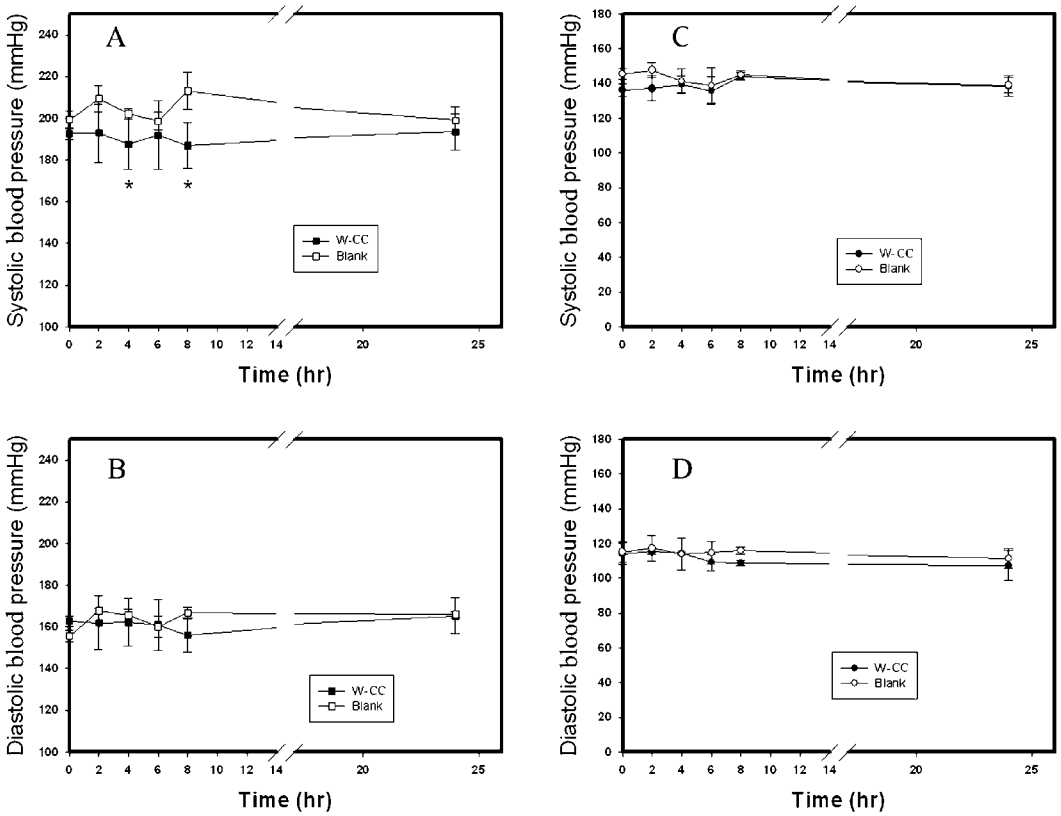

Fig. 4. Effect of Wild Chang-Chih (W-CC) on the Changes in Systolic Blood Pressure (A) and Diastolic Blood Pressure (B) of Spontaneously Hypertensive Rats (SHR), and on the Changes in Systolic Blood Pressure (C) and Diastolic Blood Pressure (D) of Wistar Kyoto (WKY) Rats. A 10-mg amount of W-CC/kg of SHR or WKY was orally administered once, and the tail blood pressure was measured four times after 2, 4, 6, 8 and 24 h by using an indirect blood pressure meter for each treatment. A 0.5-ml amount of distilled water was used for the blank experiment. A difference was considered statistically significant between the blank and treated groups when $P < 0.05$ (*) or $P < 0.01$ (**).

change in BP was reached 8 h after the single oral administration, the reduction by SSC-CC of BP being 37.2 and 21.8 mmHg, respectively, for ΔSBP and ΔDBP. It was noted that the reduction by SSC-CC of SHR BP could last for 48 h (data not shown) before returning to the original level.

Figure 3 shows the effects of 10 mg of MS-SSC-CC/kg of SHR (Fig. 3A and B) and 10 mg of MS-SSC-CC/kg of WKY (Fig. 3C and D) on SBP (Fig. 3A and C) and DBP (Fig. 3B and D) 2, 4, 6, 8, and 24 h after the single oral administration. MS-SSC-CC was found able to effectively lower SBP (Fig. 3A) and DBP (Fig. 3B) of SHR, but had no effect on WKY (Fig. 3C and D), the results differing significantly ($P < 0.05$ or $P < 0.01$) from those for the blank. Table 2 shows the changes in SBP (ΔSBP) and DBP (ΔDBP) after the single administration of MS-SSC-CC to SHR after 2, 4, 6, 8 and 24 h. The greatest change in BP was reached 8 h after the single oral administration, and the reduction by MS-SSC-CC of BP was 34.9 and 15.2 mmHg, respectively, for ΔSBP and ΔDBP. MS-SSC-CC showed dose-dependent ACE inhibitory activity (Fig. 1) and also had an antihypertensive effect on SHR (Fig. 3). This is the first report to note that SSC-CC and MS-SSC-CC

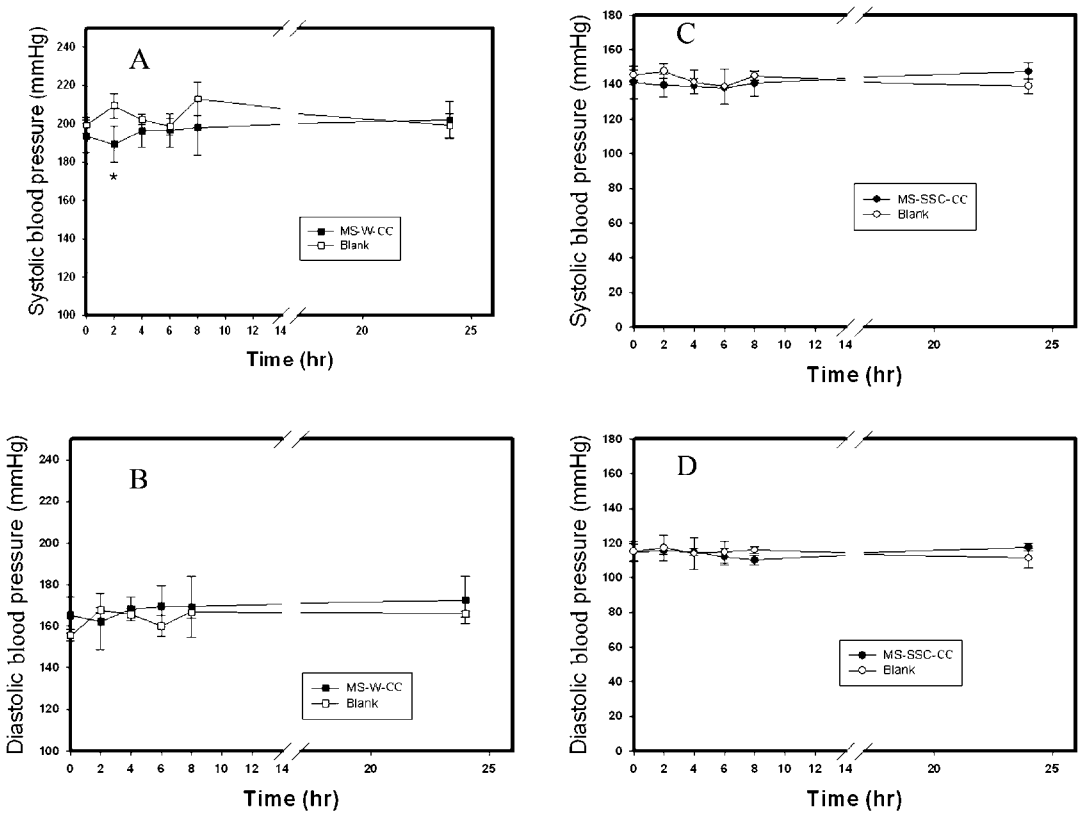

Fig. 5. Effect of the Methanol Extracts of Wild Chang-Chih (MS-W-CC) on the Changes in Systolic Blood Pressure (A) and Diastolic Blood Pressure (B) of Spontaneously Hypertensive Rats (SHR), and on the Changes in Systolic Blood Pressure (C) and Diastolic Blood Pressure (D) of Wistar Kyoto (WKY) Rats.

A 10-mg amount of MS-W-CC/kg of SHR or WKY was orally administered once, and the tail blood pressure was measured four times after 2, 4, 6, 8 and 24-h using an indirect blood pressure meter for each treatment. A 0.5-ml amount of distilled water was used for the blank experiment. A difference was considered statistically significant between the blank and treated groups when $P < 0.05$ (*) or $P < 0.01$ (**).

each exhibited an antihypertensive effect on SHR. SSC-CC (Table 1) demonstrated similar antihypertensive activity to MS-SSC-CC (Table 2). The triterpenic compounds from olive oil have exhibited vasorelaxation of the aorta in SHR.[36] The adenosine from CC mycelia have been proposed to be involved in the endothelium-dependent pathway of B85-induced vasorelaxation,[37] and it was proposed that the antihypertensive activity of SSC-CC might have been from MS fraction. Isolation and identification are currently being performed, and the pure compounds will be further investigated with an animal model.

A 10-mg amount of W-CC/kg of SHR (Fig. 4A and B) and 10 mg of SSC-CC/kg of WKY (Fig. 4C and D) were orally administered once, and SBP (Fig. 4A and C) and DBP (Fig. 4B and D) were measured after 2, 4, 6, 8, and 24 h. A 0.5-ml amount of distilled water was used for the blank experiment. On average, W-CC exhibited lower SBP than the blank group, and showed significant difference 4 and 8 h ($P < 0.05$) after the single oral administration (Fig. 4A). The reduction in DBP that followed the oral administration of W-CC was not as much as that of SSC-CC (Table 1). Table 1 shows ΔSBP and ΔDBP after the single administration of W-CC to SHR after 2, 4, 6, 8 and 24 h. The greatest change in BP was reached 8 h after the single oral administration, and the reduction by W-CC of BP was 26.3 and 10.6 mmHg, respectively, for ΔSBP and ΔDBP. In respect of the antihypertensive activity, SSC-CC had more effect on reducing SHR BP than W-CC with the same dosage. The oral administration of W-CC had no antihypertensive effect on WKY rats (Fig. 4C and D).

Figure 5 shows the effects of 10 mg of MS-W-CC/kg of SHR (Fig. 5A and B) and 10 mg of MS-W-CC/kg WKY (Fig. 5C and D) on SBP (Fig. 5A and C) and DBP (Fig. 5B and D) which were measured 2, 4, 6, 8, and 24 h after a single oral administration. MS-W-CC was also found to result in lower SBP than the blank group and showed significant difference 2 h ($P < 0.05$) after the single oral administration, while it had no effect on WKY. Table 2 shows the changes in SBP (ΔSBP) and DBP (ΔDBP) 2, 4, 6, 8 and 24 h after the single administration of MS-W-CC to SHR. The greatest change in BP was reached 2 h after the single oral administration, the reduction by MS-W-CC being 20.1 and 5.4 mmHg, respectively, for ΔSBP and ΔDBP. Although MS-W-CC showed dose-dependent ACE inhibitory activity (Fig. 1), its antihypertensive effect on SHR was not as evident as that with MS-SSC-CC (Table 2).

In conclusion, this is the first report of SSC-CC and MS-SSC-CC showing reduced BP in SHR. The isolation and identification of the pure compounds in MS-SSC-CC for investigation the mechanism for lowering BP will be performed subsequently. SSC-CC was easier to scale up than W-CC. Given its antihypertensive activity, SSC-CC might have scope for development into a healthy (or functional) food for regulating blood pressure.

Acknowledgments

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