Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 75 (2003) 635–643
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Pharmacological evidence for antidementia effect of Choto-san (Gouteng-san), a traditional Kampo medicine

Hiroshi Watanabe[a,*], Qi Zhao[a], Kinzo Matsumoto[a], Michihisa Tohda[a], Yukihisa Murakami[a], Shao-Hui Zhang[a], Tae-Hyun Kang[a], Pramote Mahakunakorn[a], Yukiko Maruyama[b], Iwao Sakakibara[c], Norio Aimi[d], Hiromitsu Takayama[d]

[a]*Department of Pharmacology, Institute of Natural Medicine, Toyama Medical and Pharmaceutical University, Toyama, 930-0194, Japan*
[b]*Traditional Medicine Group, WHO, Geneva, Switzerland*
[c]*Tsumura Central Research Laboratories, Ibaraki 300-1192, Japan*
[d]*Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 263-8522, Japan*

Received 11 November 2002; received in revised form 4 April 2003; accepted 29 April 2003

Abstract

To clarify the clinical efficacy of one of the traditional medicines in the treatment of patients with vascular dementia, we investigated the pharmacological activities of Choto-san in animal models. Pretreatment with Choto-san (0.75–6.0 g/kg po), a component herb, Chotoko (75–600 mg/kg po), and indole alkaloids and phenolic fractions of Chotoko prevented ischemia-induced impairment of spatial learning behaviour in water maze performance of mice. A single administration of Choto-san (0.5 to 6.0 g/kg po) or Chotoko (*Uncaria* genus) produced a dose-dependent antihypertensive effect in spontaneously hypertensive rats (SHR) and partly inhibited the induction of the apoplexy in stroke-prone SHR (SHR-SP). Choto-san, Chotoko, and its phenolic constituents, (−)epicatechin and caffeic acid, significantly protected NG108-15 cells from injury induced by H_2O_2 exposure in vitro and also inhibited lipid peroxidation in the brain homogenate. Indole alkaloids, rhynchophylline and isorhynchophylline (1–100 μM), reversibly reduced *N*-methyl-D-aspartate (NMDA)-induced current concentration dependently in NMDA receptor-expressed *Xenopus* oocytes.

These results suggest that antidementia effects of Choto-san are due to antihypertensive, free radical scavenging and antiexcitotoxic effects, which are attributed at least partly to phenolic compounds and indole alkaloids contained in Chotoko.

Keywords: Choto-san; *Uncaria sinensis*; Indole alkaloids; Rhynchophylline; Geissoschizine methyl ether; Phenolic compounds; (−)Epicatechin

1. Introduction

Herbal medicines were first introduced to Japan by a Chinese monk Ganjin Wajo in 5 AC. Since then, various Chinese herbal medicines together with the medical diagnosis/treatment system, which had been developed in Han and the following dynasties of China, were transmitted to Japan for over 1000 years. Over the years, the traditional Chinese diagnosis/treatment system that used herbal medicines was modified by the Japanese and Kampo medicine was developed.

Choto-san (Gouteng-san) is a Kampo medicine that consists of 10 medicinal herbs and gypsum fibrosum (Table 1). The indication of Choto-san is for chronic headache and hypertension. Target group of patients based on the Kampo diagnosis is as follows: considerably built patients with weak physical constitution and of middle age, chronic headache, painful tension of the shoulder and cervical muscle, vertigo, morning headache, heavy feeling of the head, feeling of uprising heat, tinnitus, and insomnia (Terasawa, 1993). The clinical efficacy of Choto-san in patients with vascular dementia has been demonstrated by a double blind and placebo controlled study (Terasawa et al., 1997). Choto-san (4.5 g/day po) and a placebo were each given three times a day to a group of patients with vascular dementia for 12 weeks. At the end of the 12-week administration, Choto-san was statistically superior to the placebo in global improvement rating (GIR), utility rating, GIR of subjective symptoms (heaviness of head, headache, dizziness of vertigo, etc.), GIR of psychiatric symptoms (spon-

* Corresponding author. Tel.: +81-76-434-7610; fax: +81-76-434-5056.
E-mail address: hwatanab@ms.toyama-mpu.ac.jp (H. Watanabe).

0091-3057/03/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0091-3057(03)00109-6

Table 1
Composition, Kampo diagnosis, indication and usage of Choto-san

Item	Component herb	Chemical constituents
Choto-san (Gouteng-san)	Dried hook of *U. sinensis* (Oliv.) Havil. (3)	Indole alkaloids
	Dried peel of *Citrus unshiu* Mare. (3)	D-limonene, auraptene, hesperidin
	Dried tuber of *Pinellia ternata* Breit. (3)	Homogentisic acid
	Dried root of *Ophiopogon japonicus* Ker-Gawler (3)	Ophiopogonin A, B
	Dried *Poria cocos* (Fr.) Wolff (3)	Pachyman
	Dried root of *Panax ginseng* C.A. Meyer (2)	Ginsenosides
	Dried flower of *Chrysanthemum morifolium* Hemsl. (2)	
	Dried root of *Ledebouriella seseloides* Woll. (2)	Psolaren, deltoin, calcium
	Gypsum, $CaSO_4 \cdot 2H_2O$ (5)	Glycyrrhizin
	Dried root of *Glycyrrhiza uralensis* (1)	Zingiberene
	Dried tuber of *Zingiber officinale* Roscoe (1)	
Kampo diagnosis	Yang disease stage 2, Yang deficiency, Oketsu type, KI deficiency	
Indication	Chronic headache and hypertension	
Usage	4.5 g/day po (divided into two to three times)	

Numbers in parentheses indicate the weight of herbs (grams) that is used to make the decoction everyday.

taneity, emotion, intellectual ability, etc.) and GIR of disturbance in daily living activities (sitting, standing, walking, washing face and hands, etc.), whereas GIR of neurological symptoms (aphagia, dysarthria, motor disturbance, etc.) was not significantly different from the placebo at the evaluation points. There was a tendency of the improvement of the revised version of Hasegawa's dementia scale between Choto-san and the placebo. In patients with asymptomatic cerebral infarction, it was shown that subchronic treatment with Choto-san (28 g dried herbs/day po) for 4 weeks improved microcirculatory flow of bulbar conjunctiva, erythrocyte aggregability, and blood cell deformability, and suggested that these effects of Choto-san contribute partly to inhibit the deterioration of vascular dementia following the cerebral infarction. (Yang et al., 1999).

To confirm the clinical efficacy of Choto-san pharmacologically, the effects of Choto-san and the major component herb, Chotoko (*Uncaria* genus), on hypertension in spontaneously hypertensive rats (SHR) and hypertension and apoplexy in the stroke-prone SHR (SHR-SP), on ischemia-induced deficit of spatial learning ability in mice and on oxidative stress-induced cell damage and lipid peroxidation in vitro, and effects of the constituents of Chotoko on *N*-methyl-D-aspartate (NMDA) receptor function using a receptor expression model employing *Xenopus* oocytes were studied.

2. Method

2.1. Subjects

The subjects were experimentally naïve male ICR mice at 8 weeks old and SHR, SHR-SP at 4 weeks old, and age-matched Wistar–Kyoto rats. They ware obtained from the colony of specific pathogen-free mice and rats maintained by Japan SLC (Shizuoka, Japan). The mice and rats were housed in groups of eight per cage ($31 \times 20 \times 13$ cm) and four per cage ($36 \times 30 \times 17$ cm), respectively, on a 12-h light/dark cycle (light on 0730–1930 h) at 24 ± 1 °C with constant humidity for at least 1 week before the experiments. Food and water were given ad libitum. All experiments were performed at the same time of day to avoid the effect of physiological cycling. All experimental procedures were performed in accordance with the standards established by the Guide for the Care and Use of Laboratory Animals at Toyama Medical and Pharmaceutical University.

2.2. Drugs

The following drugs and reagents were purchased from the indicated sources: aminopterin, sodium azide, trichloroacetic acid, 2-thiobarbituric acid, bismalondialdehyde tetraethyl acetal, (−)-epicatechin, and caffeic acid from Sigma (St. Louis, MO, USA); curcumin, urethane, tacrine, and KCL from Nacalai Tesque (Kyoto, Japan); H_2O_2 from Santoku Chemical Industries (Tokyo, Japan); thymidine and hypoxanthine from Kohjin (Tokyo, Japan); minomycin from Lederle (Saitama, Japan); Dulbecco's modified Eagle's medium (DMEM) from Gibco BRL, Life Technologies (New York, USA); fetal bovine serum from Canasera International (Canada), $AcONH_4$ and CH_3CN from Wako Chemical (Tokyo, Japan); nifedipine from Bayer Japan (Osaka), nicardipine hydrochloride from Nihon Iyakuhin Kogyo (Toyama).

2.3. Choto-san preparation

Choto-san was prepared by mixing the 11 herbs (purchased from Tochimoto, Osaka, Japan), all in dried, crude form and preparing an extract from them. Twenty five grams of the mixed materials were mixed with 300 ml of distilled water and boiled for 45 min at 100 °C, then 3 g of *Uncaria sinensis* was added and boiled for 15 min more. The extract was filtered, and the residue was extracted once more according to the same procedure. Finally, all extract frac-

tions were pooled. The supernatant was lyophilized to form a dried powder. The freeze-dried powder was stored at room temperature in the desiccator.

2.4. Analysis of 3-D HPLC fingerprint of Choto-san

Choto-san (2.5 g, Tsumura, Tokyo, Japan) was filtered and then submitted for high performance liquid chromatography (HPLC) analysis. HPLC equipment was controlled with an SLC-10A (Shimazu, Kyoto, Japan) using a TSK-GELODS-80TS column (4.6 × 250 mm), eluting with solvents (A) 0.05 M $AcONH_4$ (pH 3.6) and (B) CH_3CN. A linear gradient of 100% A and 0% B changing over 60 min to 0% A to 100% B was used. The flow rate was controlled with an LC-10AD pump at 1.0 ml/min. The eluate from the column was monitored and the 3-D date was processed by an SPD-M10A diode array detector. The profile is shown in Fig. 1. To clarify the chemical constituents of Choto-san in this study, analysis of 3-D HPLC was carried out.

2.5. Transient cerebral ischemia

Mice were subjected to transient cerebral ischemia induced by bilateral common carotid occlusion 1 h after the administration of the drug. In brief, the mice were anesthetized with urethane (1.5 g/kg ip). The bilateral common carotid arteries were exposed, carefully separated from the adjacent veins and sympathetic nerves, and then occluded by artery clips (Roboz Surgical Instrument, MD, USA) for 20 min. While the arteries were clamped, 0.3 ml of blood was withdrawn from the tail vein. Then the artery clips were removed and cerebral blood flow was restored. The skin incision was closed and the mice were kept in an air-conditioned room at 25 °C. Sham-operated mice were subjected to the same procedure without carotid clamping and withdrawal of the blood.

2.6. Morris water maze learning performance

One day before the start of learning, mice were given a pretraining session in which they were allowed to swim freely in a pool (70 cm diameter, with a depth of 13 cm of water) for 60 s without an escape platform. The pool was placed in a dimly lit and large test room and surrounded by visual cues. In the learning block, the pool was filled to a depth of 13 cm with water maintained at 25 ± 1 °C. A platform (5 cm diameter) was situated 1 cm below the surface of the water. The pool was divided into four quadrants with the platform in a fixed position in one quadrant. Daily learning consisted of four trials in which the mouse was placed in the water from four different starting points and the latency of escaping onto the platform was recorded. This was conducted for 5 consecutive days. A maximum of 60 s was allowed during which the mouse had to find the platform and climb onto it. On the sixth day, each mouse was subjected to a probe trial in which there was no platform present. The time of crossing the former platform quadrant and the total time of crossing all quadrants were recorded for 1 min.

2.7. Measurement of the blood pressure and heart rate in awake rats

Awake rats were lightly supported in a mesh holder made of cloth and the arterial blood pressure from the tail artery was indirectly measured using a tail-cuff apparatus (BP-98, Softron, Japan) equipped with a pair of photodiode and light-emitting diode, an air pump, and a pressure transducer. The apparatus was controlled with a personal computer. Signals from the photocell amplifier and cuff pressure transducer were recorded continuously on the computer monitor. Regression curve was calculated from peak values of each pulse volume oscillation by least squares method. Systolic and mean blood pressure were determined at the pressure where this curve started and became maximum, respectively. Diastolic blood pressure was calculated from the values of systolic and mean blood pressure. All of these results were displayed and saved in a floppy disk.

2.8. Cell cultures and H_2O_2-induced oxidative cell damage in NG108-15 cells

NG108-15 cells were continuously cultured in DMEM supplemented with 4% fetal bovine serum, 100 μM hypoxanthine, 16 μM thymidine, 1 μM aminopterin and 1 μg/ml minomycin. The culture medium was changed every 2–3 days. All cultures were maintained at 37 °C under 10% CO_2 with 95% relative humidity. For experiments, cells were planted onto 3.5-cm polyornithine-coated plates and used after 3–4 days of incubation.

A test compound was first dissolved in dimethyl sulfoxide (DMSO) and later mixed with the culture medium to give a final concentration of 12.5, 25, 50, or 100 μM with a DMSO concentration of 0.5% vol/vol. DMSO was also present at this level in control cells not treated with test compounds. For co-incubation with a test compound and H_2O_2 in order to induce cell damage, the cells were incubated in culture medium containing 500 μM H_2O_2 and 0.05% trypan blue with or without various concentrations of the test compound (12.5–100 μM) for 3 h. Then cell viability was measured by the trypan blue exclusion method and expressed as the percentage of unstained cells among the total cells.

2.9. Malondialdehyde measurements and antilipid peroxidation activity

Malondialdehyde (MDA) was measured using a modified method (Esterbauer et al., 1991; Gluck et al., 2001). Brain tissue was homogenized in 1.15% KCl/0.4 mM of sodium azide and incubated at 37 °C for 15 min. Proteins were precipitated by the addition of 20% (wt/vol) trichloroacetic

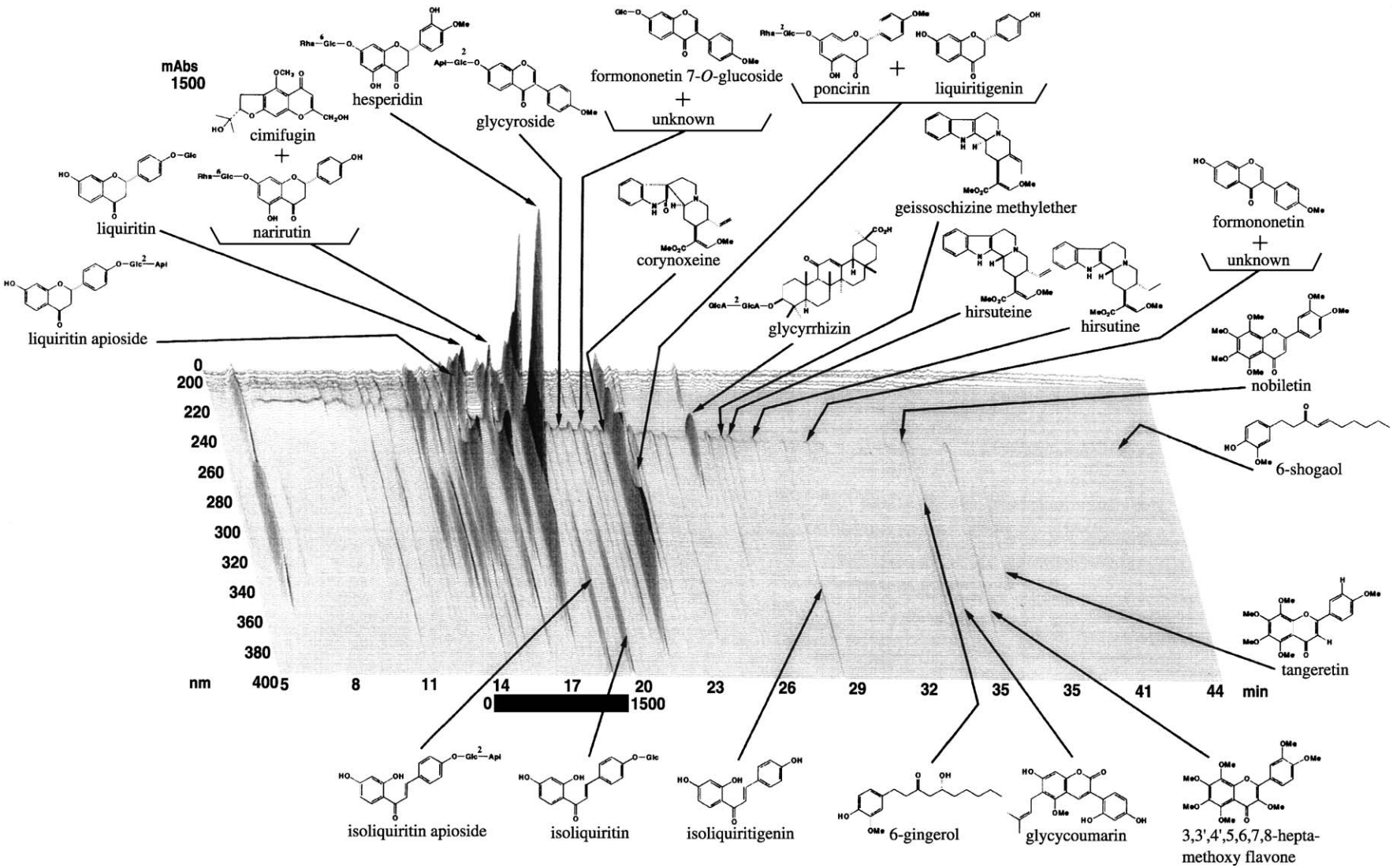

Fig. 1. Three-dimensional HPLC profile of Choto-san extract. Major constituents are detected in the profile.

acid. The tissue samples were centrifuged at 12,000 × g for 15 min and the supernatant was added to an equal volume of 0.8% (wt/vol) 2-thiobarbituric acid. These samples were incubated at 100 °C for 15 min. After the cooling period, the thiobarbituric acid reactive substance (TBA-RS) generated was spectrophotometrically determined at 532 nm. Bismalondialdehyde tetraethyl acetal was used as a standard. Protein concentrations were assayed by using the biuret method.

2.10. Isolation of total RNA, preparation of oocytes, and electrophysiological recordings

The cerebral cortex was dissected after the decapitation of male Wistar rats (12–14 weeks old, Japan SLC) and frozen in liquid nitrogen. The frozen tissues were homogenized in Sepasol-RNA I Super (Nacalai Tesque) and the total RNA was extracted according to the protocol provided by the manufacturer. *Xenopus* oocytes at stage V or VI were prepared by the method described in the previous report (Leewanich et al., 1998), injected with 47 nl of 5 mg/ml total RNA prepared from the cerebral cortex and incubated at 18 °C for 2 days in modified Barth's solution supplemented with antibiotics. Oocytes expressing total RNA from the cerebral cortex were used to examine NMDA-, kainic-acid-, and AMPA-induced current responses. The membrane currents were recorded from oocytes at a holding potential of −60 mV using a two-electrode voltage clamp method (Gene Clamp 500, Axon Instruments, Foster City, CA).

2.11. Data analysis

All results are expressed as the mean ± S.E.M. Statistical significance between different groups for the Morris water maze test was analyzed by two-way analysis of variance (ANOVA) among the groups. A Student's *t* test was used for the analysis of significant differences between the two groups. One-way ANOVA followed by the Dunnett test was used for multiple comparisons. Differences of $P < .05$ were considered significant.

3. Results

3.1. Prevention of ischemia-induced impairment of spatial memory in mice

Mice subjected to transient cerebral ischemia required a longer time to locate the hidden platform than the sham-operated control mice during the learning trials, although the ischemia did not affect the swimming ability of the mice in the pretraining trial of the water maze.

Pretreatment of mice with Choto-san (750–6000 mg/kg po) significantly shortened the latency of escaping onto the platform during the learning period for 5 days and significantly increased the time of crossing the former platform quadrant in the probe trial of the sixth day after the transient ischemia as compared with the ischemic control (Fig. 1). Pretreatment with Chotoko (75–600 mg/kg po), a component herb of Choto-san, also prevented the prolongation of the latency of escaping onto the platform in the water maze performance (Fig. 2). Furthermore, both the alkaloid fraction (188 mg/kg po) and the phenolic fraction (188 mg/kg) of Chotoko prevented the ischemia-induced impairment of escape latency. The indole alkaloids rhynchophylline (10 mg/kg po) and geissoschizine methyl ether (10 mg/kg po) significantly prevented the prolongation of the escape latency during the learning period for 5 days. The percentage of crossing the former platform quadrant in the rhynchophylline group was higher than that in the geissoschizine

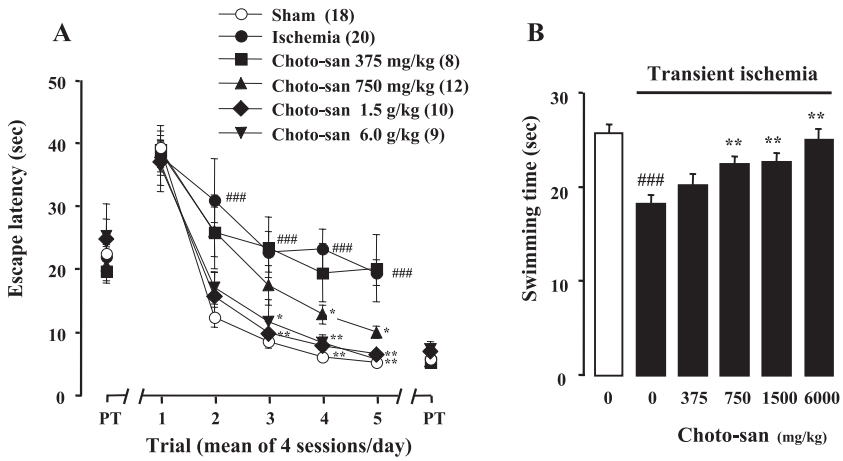

Fig. 2. Effect of Choto-san on transient ischemia (2VO)-induced impairment of Morris water maze performance in mice. Choto-san was orally given to animals 1 h before the ischemia. Two days after the ischemia, the trial test was performed, four trials/block/day for 5 days. (A) Time course of the change in the latency of escaping to the platform in the pool. Each point represents the mean of the latency with the S.E.M. (B) The swimming time in the platform quadrant was recorded at the probe trial for 1 min after the platform was removed on the sixth day of the test. Each column represents the mean swimming time in the quadrant with S.E.M. PT: platform is visible during the trial. ###$P < .001$ vs. sham group. *$P < .05$, **$P < .01$ vs. ischemic control.

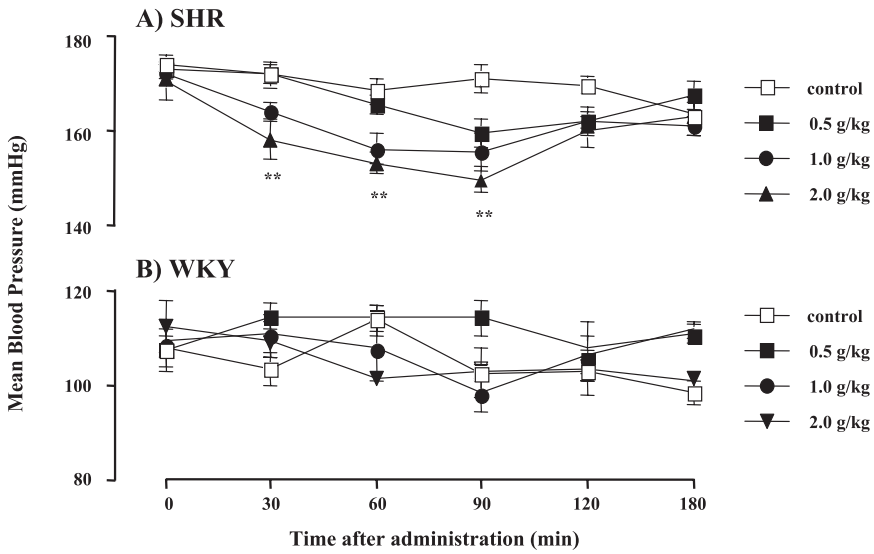

Fig. 3. Antihypertensive effect of a single administration of Choto-san extract in SHR (upper) and WKY rats (lower). The vertical axis represents the mean blood pressure (mmHg) and doses represent oral administration of the extract. Vertical bars show the standard error of the mean ($n=6$). $**P<.01$ vs. each control group.

methyl ether group in the probe test. A reference agent, tacrine (1 and 2.5 mg/kg ip), significantly shortened the latency of escaping onto the platform in the water maze performance.

3.2. Antihypertenstive effects in SHR and SHR-SP

A single administration of Choto-san (0.5, 1.0, and 2.0 g/kg po) produced a dose-dependent hypotensive effect in SHR, whereas it did not affect the heart rate in SHR. It affected neither blood pressure nor heart rate in normotensive Wistar–Kyoto rats (Fig. 3). A single administration of the extract of Chotoko showed a hypotensive action at a dose of 0.2 g/kg po. A single administration of nifedipine (5 and 10 mg/kg po), a reference agent, produced a dose-dependent hypotensive effect in SHR, but did not affect the normal blood pressure at the same doses in Wistar–Kyoto rats.

In SHR-SP, the subchronic administration of Choto-san at a dose of 0.5 or 5 g/kg/day po caused a significant hypotensive effect (Fig. 4) and a tendency to inhibit the induction rate of the apoplexy. The subchronic administration of Chotoko at a dose of 0.05 g/kg/day po also produced a significant hypotension and showed a tendency to inhibit the rate of apoplexy in SHR-SP, whereas another Kampo medicine, Saiko-keishi-to (0.5 and 5.0 g/kg/day po) affected neither the blood pressure nor the apoplexy (Fig. 4). The subchronic administration of a reference agent, nicardipine, produced prominent hypotensive effect at doses of 1 and 10 mg/kg/day po and inhibition of apoplexy at 10 mg/kg/day po.

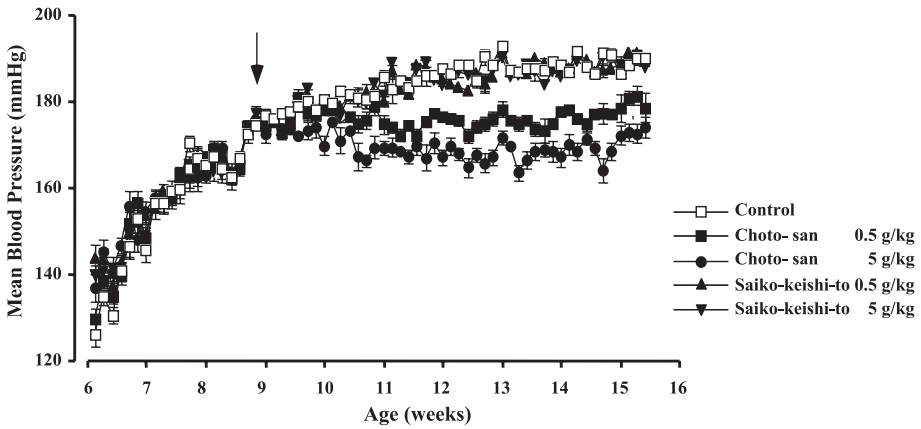

Fig. 4. Antihypertensive effect of subchronic administration of Choto-san extract in SHR-SP. Choto-san extract (0.5 and 5 g/kg/day), Saiko-keishi-to (0.5 and 5 g/kg/day), a negative control, or tap water (Control) was given to animals as drinking water for 7 weeks after the blood pressure reached a level over 170 mmHg ($n=9$). The mean blood pressure significantly decreased at 10 (a low-dose group) to 14 days (a high-dose group) after the start of drinking of Choto-san.

3.3. Antioxidant and antilipid peroxidation activities in vitro

Application of H_2O_2 significantly reduced the viability of NG108-15 cells in a concentration-dependent manner with an IC_{50} of 500 μM. A reference immunosuppressive ligand FK506 (100 to 1000 nM) inhibited the H_2O_2-induced reduction in the cell viability in a concentration-dependent manner. Choto-san (250–1000 μg/ml) significantly increased the viability of the cells compared to that of the control treated with H_2O_2 alone. Chotoko (250–1000 μg/ml) also significantly increased the cell viability. Constituents of Chotoko, (−) epicatechin and caffeic acid, inhibited H_2O_2-induced decrease in the cell viability at the concentration of 200 μM.

The antilipid peroxidation activity was determined by quantification of TBA-RS using a conventional method in vitro. Vitamin E, a reference agent, at a concentration range of 15–375 μM, exhibited a suppressive effect on the lipid peroxidation caused by radical-generating system in the brain homogenate. Choto-san (50–250 μg/ml) and methanol extract of Chotoko significantly inhibited the formation of TBA-RS in a concentration-dependent manner when compared with the vehicle-treated control. (−)Epicatechin at the concentration of 3–75 μM also showed a significant inhibition on TBA-RS formation. IC_{50} of Choto-san, methanol extract of Chotoko, (−) epicatechin, and vitamin E in the brain homogenate were 124.7μg/ml, 4.6 μg/ml, 39.3 μM, and 153.7 μM, respectively.

3.4. Inhibition of NMDA receptor functions by indole alkaloids

To clarify possible molecular underlying mechanism(s) of the actions of an indole alkaloid, rhynchophylline, which has been purified from *Uncaria rhynchophylla*, the effect of the alkaloid on NMDA receptor function using a receptor expression model employing *Xenopus* oocytes was investigated. Rhynchophylline and isorhynchophylline (1–100 μM) per se did not induce membrane current, but reversibly reduced NMDA-induced current in a concentration-dependent but not voltage-dependent manner (Fig. 5). The IC_{50} values of rhynchophylline and isorhynchophylline were 43.2 and 48.3 μM, respectively. The alkaloids had no effect on the currents mediated either by ionotropic kainic acid-type and (+)-alpha-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptors or by the metabotropic glutamate receptors 1 and 5. The alkaloids (30 μM) significantly reduced the maximal current response evoked by NMDA and glycine (a co-agonist of NMDA receptor), but had no effect on the EC_{50} values and Hill coefficients of NMDA and glycine for inducing currents.

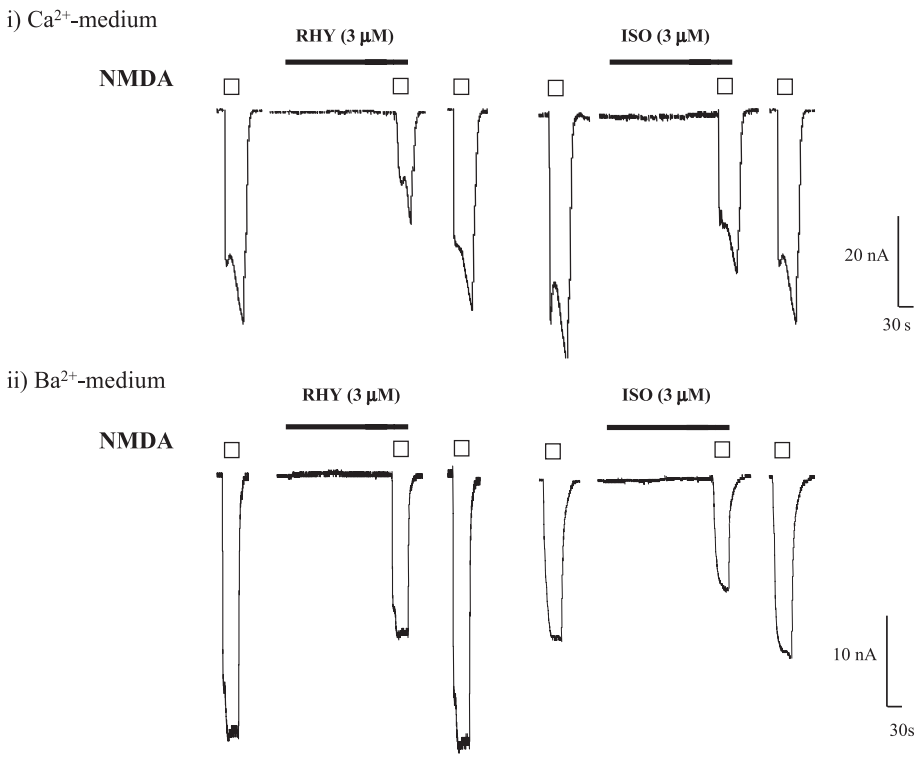

Fig. 5. Rhynchophylline and isorhynchophylline inhibit NMDA current in oocytes injected with rat cortex total RNA. Traces represent NMDA currents recorded in (i) Ca^{2+}-containing, Mg^{2+}-free MBS or in (ii) Ba^{2+}-containing, Mg^{2+}-free MBS supplemented with EGTA. NMDA (white square: 100 μM) was applied for 30 s. Horizontal bars above the middle of the traces represent application of 3 μM rhynchophylline (RHY) or isorhynchophylline (ISO).

4. Discussion

The present results showed that Choto-san, a traditional Kampo medicine, exerted a protective effect on spatial learning impairment induced by transient cerebral ischemia in mice. The neuroprotective effect of Choto-san may be due at least partly to the antihypertensive, antioxidative, and receptor-blocking effects.

The decrease in cardiac norepinephrine content and the reductions in plasma angiotensin II, plasma aldosterone, and urine levels of adrenaline and noradrenaline have been shown to be involved in the antihypertensive effect of Choto-san (Watanabe et al., 1987; Yokose et al., 2000). It has been reported that Choto-san also inhibits norepinephrine- and high K^+-induced contractions in isolated mesenteric arteries in a concentration-dependent manner, suggesting that the antihypertensive effect of Choto-san is mediated by the calcium channel antagonistic action (Ishii et al., 1987). The calcium channel blocking activity of Choto-san has been demonstrated more clearly in the pharmacological analysis of hirsutine, an indole alkaloid isolated from *U. rhynchophylla* Miq. (Horie et al., 1992; Watanabe et al., 1999; Yamahara et al., 1987; Yano et al., 1991). Furthermore, several alkaloids such as hirsutine, dihydrocorynantheine, geissoschizine methyl ether, rhynchophylline, and 3-alpha-dihydrocadambine extracted from *U. rhynchophylla*, have been reported to induce hypotensive and vasodilative effects in anesthetized rats and dogs (Aisawa et al., 1985; Ozaki, 1989, 1990; Sakakibara et al., 1999). There is another possibility that the antihypertensive effect of Choto-san resulted from radical scavenging activity of Chotoko, a component herb of Choto-san in the isolated aorta with the endothelium (Goto et al., 1998).

In the present study, Choto-san significantly protected NG108-15 cells against H_2O_2-induced damage in a concentration-dependent manner. Thus, it is possible that the protective effect of Choto-san on the H_2O_2-induced decrease in cell viability may be due to its antioxidant and free radical scavenging properties. This possibility was supported by the present results that Chotoko and its constituents, (−)epicatechin and caffeic acid, produced the protective effect against H_2O_2-induced damage. Although its precise mode of action has not been clearly elucidated, some studies have shown that cellular glutathione (GSH) plays an important role in protection against oxidative stress-induced injury (Tanaka et al., 2001).

Hydrogen peroxide is believed to cause cell damage by reacting with the cell membrane, resulting in lipid peroxidation of the membrane. Lipid peroxidation has been implicated as one of the main processes responsible for ischemic cell damage. Several studies have shown that pretreatment with either a free radical scavenger or antioxidants reduced ischemia reperfusion-induced lipid peroxidation. The inhibitory effect of Choto-san on lipid peroxidation may be contributed by its constituents. Choto-san was reported to contain many phenolic antioxidants such as epicatechin, caffeic acid, procyanidin, and quercetin, which are known to prevent lipid-peroxidation-induced cell damage by interrupting lipid peroxidation chain reactions initiated by free radicals at the cell membrane (Ishige et al., 2001). Chotoko has been reported to have antioxidant and free radical scavenging activities, detected by electron spin resonance method, and to inhibit lipid peroxidation in the brain of iron-induced epileptic rats (Liu and Mori, 1992)). Taken together with these results, the present study suggests that Choto-san protects the brain due to the attenuation of free radical formation following ischemia.

NMDA subtype of glutamate receptors in the cerebral cortex and hippocampus plays an important role in learning and memory (Bliss and Collingridge, 1993; Cotman et al., 1989). Excessive activation of NMDA receptors induces the neuronal cell death mediated by intracellular Ca^{2+} overload. Such excitotoxic neuronal death appears to contribute to various neurological disorders such as cerebrovascular dementia and Alzheimer's disease (Choi, 1992; Cotman et al., 1989; Muller et al., 1995; Parsons et al., 1998). Various indole alkaloids isolated from *U. sinensis* such as isorhynchophylline, isocorynoxeine, and rhychopylline at relatively high concentrations prevented glutamate-induced cell death in cultured cerebellar granule cells and global cerebral ischemia in rats (Shimada et al., 1999; Suk et al., 2002). Furthermore, NMDA receptor antagonists provided protection against neuronal damage attributed to cerebral ischemia (Block, 1999; del Zoppo et al., 1997). The present findings suggest a possibility that rhynchophylline exerts noncompetitive antagonism by allosterically inhibiting NMDA binding to the NMDA recognition site and/or glycine binding to the glycine recognition site on the NMDA receptor channel protein. However, further investigation is required to identify the site of action of rhynchophylline on the NMDA receptors.

In conclusion, the present results suggest that antidementia effects of Choto-san are due to antihypertensive, free radical scavenging, and antiexcitotoxicity effects, which are attributed at least partly to phenolic compounds and indole alkaloids contained in Chotoko.

Acknowledgements

FK506 was donated by Fujisawa Pharmaceutical Co. (Osaka, Japan).This work was supported by a grant-in-aid for Leading Research Utilizing Potential of Regional Science and Technology from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

Aisawa K, Hattori Y, Kihara T, Ishihara T, Endo K, Hikino H. Hypotensive action of 3-*alpha*-dihydrocadambine, an indole alkaloid glycoside of *Uncaria* hooks. Planta Med 1985;51:424–7.

Bliss TVP, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993;361:31–9.

Block F. Global ischemia and behavioral deficits. Prog Neurobiol 1999;58: 279–95.

Choi DW. Excitotoxic cell death. J Neurobiol 1992;123:1261–76.

Cotman CW, Bridges RT, Taube JS, Clark AS, Geddes JW, Monaghan DT. The role of NMDA receptor in the central nervous system plasticity and pathology. J NIH Res 1989;11:65–74.

del Zoppo GJ, Wagner S, Tagaya M. Trends and future developments in the pharmacological treatment of acute ischemic stroke. Drugs 1997; 54:9–38.

Esterbauer H, Schau RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med 1991;11:81–128.

Gluck MR, Moy LY, Jayatilleke E, Hogan KA, Manzino L, Sonsalla PK. Parallel increases in lipid and protein oxidative markers in several mouse brain regions after methamphetamine treatment. J Neurochem 2001;79:152–60.

Goto H, Tanigawa K, Sekiya N, Kusano Y, Shimada Y, Terasawa K. Effect of *Uncariae ramulus* on the endothelium in spontaneously hypertensive rats. J Trad Med 1998;15:410–1 [in Japanese].

Horie S, Yano S, Aimi N, Sakai S, Watanabe K. Effects of hirsutine, an antihypertensive indole alkaloid from *Uncaria rhynchophylla*, on intracellular calcium in rat thoracic aorta. Life Sci 1992;50:491–8.

Ishige K, Schubert D, Sagara Y. Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. Free Radic Biol Med 2001;30:433–46.

Ishii K, Kano T, Ando J. Pharmacological effect of Diao-Teng-San, a blended traditional Chinese herb medicine, in spontaneously hypertensive (SHR) and normotensive Wistar–Kyoto (WKY) rats (Abstract in English). J Med Pharm Soc Wakan-Yaku 1987;4:107–15.

Leewanich P, Tohda M, Matsumoto K, Subhadhirasakul S, Takayama H, Kitajima M, et al. A possible mechanism underlying corymine inhibition of glycine-induced chloride current in *Xenopus* oocytes. Eur J Pharmacol 1998;348:271–7.

Liu J, Mori A. Antioxidant and free radical scavenging activities of *Gastrodia elata* Bl. and *Uncaria rhynchophylla* (Miq.) Jacks. Neuropharmacology 1992;31:1287–98.

Muller WE, Mutschler E, Riederer P. Noncompetitive NMDA receptor antagonists with fast open-channel blocking kinetics and strong voltage-dependency as potential therapeutic agents for Alzheimer's dementia. Pharmacopsychiatry 1995;28:113–24.

Ozaki Y. Pharmacological studies of indole alkaloids obtained from domestic plants, *Uncaria rhynchophylla* MIQ. and *Amsonia elliptica* ROEM (Abstract in English). Folia Pharmacol Jpn 1989;94:17–26.

Ozaki Y. Vasodilative effects of indole alkaloids obtained from domestic plants, *Uncaria rhynchophylla* MIQ. and *Amsonia elliptica* ROEM (Abstract in English). Folia Pharmacol Jpn 1990;95:47–54.

Parsons CG, Danysz W, Quack G. Glutamate in CNS disorders as a target for drug development—an update. Drug News Perspect 1998;11:523–69.

Sakakibara I, Terabayashi S, Kubo M, Higuchi M, Sasako H, Okada M. Evaluation of Gou-teng (hooks and stems of Uncariae plants): III. Hypotensive principle from *Uncaria rhychophylla* and alteration of its content during extraction process (in Japanese). Nat Med 1999;53: 308–12.

Shimada Y, Goto H, Itoh T, Sakakibara I, Kubo M, Sasaki H, Terasawa K. Evaluation of the protective effects of alkaloids isolated from the hooks and stems of Uncaria sinensis on glutamate-induced neuronal death in cultured cerebellar granule cells from rats. J Pharm Pharmacol 1999; 51:715–22.

Suk K, Kim SY, Leem K, Kim YO, Park SY, Hur J, et al. Neuroprotection by methanol extract of *Uncaria rhynchophylla* against global cerebral ischemia in rats. Life Sci 2002;70:2467–80.

Tanaka K, Fujita N, Yoshioka M, Ogawa N. Immunosuppressive and non-immunosuppressive immunophilin ligands improve H_2O_2-induced dell damage by increasing glutathione levels in NG108-15 cell. Brain Res 2001;889:225–8.

Terasawa K. Kampo; insights from clinical cases (in Japanese). Tokyo: K.K. Standard McIntyre; 1993.

Terasawa K, Shimada Y, Kita T, Yamamoto T, Tosa H, Tanaka N, et al. Choto-san in the treatment of vascular dementia: a double-blind, placebo-controlled study. Phytomedicine 1997;4:15–22.

Watanabe K, Hirokawa Y, Shibata A, Wakabayashi H, Shimada K. Effects of Daisaiko-to, Choto-san and Sairei-to in spontaneously hypertensive rats (in Japanese). J Med Pharm Soc Wakan-Yaku 1987;4:356–7.

Watanabe K, Yano S, Horie S, Yamamoto LT, Takayama H, Aimi N, et al. Pharmacological properties of some structurally related indole alkaloids contained in the Asian herbal medicines, hirsutine and mitragynine, with special reference to their Ca^{2+} antagonistic and opioid-like effects. In: Watanabe H, Shibuya T, editors. Pharmacological research on traditional herbal medicines. Amsterdam: Harwood Academic; 1999. p. 163–77.

Yamahara J, Miki S, Matsuda H, Kobayashi G, Fujimura H. Screening test for calcium antagonist in natural products. The active principles of *Uncaria ramulus* et *Uncus* (Abstract in English). Folia Pharmacol Jpn 1987;90:133–9.

Yang Q, Kita T, Hikiami H, Shimada Y, Itoh T, Terasawa K. Effects of Choto-san on microcirculation of bulbar conjunctiva and hemorheological factors in patients with asymptomatic cerebral infarction (Abstract in English). J Trad Med 1999;16:135–40.

Yano S, Horiuchi H, Horie S, Aimi N, Sakai S, Watanabe K. Ca^{2+} channel blocking effects of hirsutine, an indole alkaloid from *Uncaria* genus, in the isolated rat aorta. Planta Med 1991;57:399–510.

Yokose T, Okubo H, Sakurai Y, Yoshizawa T, Oka T, Akamine Y, et al. Effects of Choto-san on hemodynamics, the sympathetic nervous system and the renin–angiotensin system in spontaneously hypertensive rats (Abstract in English). Jpn J Clin Physiol 2000;30:125–31.