Effects of Choto-san and Chotoko on thiopental-induced sleeping time

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Abstract

Choto-san has been used for treatment of centrally regulated disorders such as dementia, hypertension, headache and vertigo. Our laboratory showed that Choto-san improved learning memory in ischemic mice. It is noticeable that Choto-san treated animals and animals that underwent conducting occlusion of common carotid arteries (2VO) operation slept longer than the normal animals. Therefore, this study aimed to clarify the effects of Choto-san and its related component; Chotoko and Choto-san without Chotoko on thiopental-induced sleeping time. The results show that Choto-san (0.3, 1 and 3 g/kg, p.o.) dose dependently prolonged the sleeping time induced by thiopental (50 mg/kg, i.p.). Chotoko (71.4 mg/kg, p.o.) and diazepam (1 mg/kg, i.p.) also increased the sleeping time while Choto-san without Chotoko (1 g/kg, p.o.) had no effect. This indicates that the effects of Choto-san and Chotoko, the main active constituent of Choto-san, may be mediated in part via their suppressive effect on the central nervous system of which the precise mechanisms remain unknown.

Key words Choto-san, *Uncaria* sp., *Uncaria rhynchophylla*, *Uncaria sinensis*, thiopental-induced sleep.

Introduction

Choto-san, a Japanese Kampo medicine composed of 11 medicinal plants, has been prescribed for treatment of dementia, hypertension, headache, vertigo and dizziness.[1] The uses of this Kampo medicine have been supported by several studies.[2-6] These diseases or symptoms are well known for their centrally regulated homeostasis. Our laboratory showed that Choto-san improves learning memory in transient global ischemic mice.[2] Conducting occlusion of common carotid arteries (2VO) of anesthetized rats, we noticed that Choto-san treated animals slept longer than the water treated control group. Therefore, this study aimed to further clarify the potentiating effect of Choto-san on the sleeping time induced by thiopental barbiturate. In addition, the effects of Chotoko, an active component of Choto-san, and of Choto-san without Chotoko were also examined.

Materials and Methods

Animals : Male ICR mice (7-8 weeks) were obtained from Sankyo Labo Service, Hamamatsu, Japan. Animals were housed in a 12-hour light/dark cycle (light on during 7.30am - 7.30pm) at a room temperature of 24 ± 1℃ with a relative humidity of 55 ± 5 %. Food and water were supplied ad libitum.

Drugs : Choto-san water extract was prepared from the mixture of 11 medicinal plants as the following recipe: Aurantii Nobilis pericarpium (Peel of *Citrus unshiu* MARKOVICH) 3 g, Ophiopogonis tuber (root of *Ophiopogon japonicus* KER-GAWLER) 3 g, Pinelliae tuber (tuber of *Pinellia ternata* BREITENBACH) 3 g, Hoelen (fungus of *Poria cocos* WOLF) 3 g, Uncariae Ramulus et Uncus (hooks and branch of *Uncaria rhynchophylla* MIQUEL, and of *Uncaria sinensis* OLIVER) 3 g, Ginseng radix (root of *Panax ginseng* C. A. MEYER) 2 g, Saposhnikoviae radix (root and rhizome of *Saposhnikovia divaricata* SCHISCHKIN) 2 g, Chrysanthemis flos (flower of *Chrysanthemum morifolium* RAMATULLE, *Chrysanthemum indicum*

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LINNE) 2 g, Glycyrrhizae radix (root of *Glycyrrhiza uralensis* FISHER, *Glycyrrhiza glabra* LINNE) 1 g, Zingiberis rhizoma (rhizome of *Zingiber officinale* ROSCOE) 1 g, and Gypsum Fibrosum ($CaSO_4 \cdot 2H_2O$) 1 g. All the components except Ramulus et Uncus were put together and boiled with 230 ml of water for 60 min. Romulus et Uncus was added at the 45-min time and boiled for 15 min. The water extract was filtered and then freeze dried. Thiopental (sodium salt containing sodium carbonate) was obtained from Wako Pure Chemical Industries, Ltd., Japan, and was dissolved by saline. Diazepam (Cercine® injection 10 mg/2ml) was purchased from Takeda Chemical Industry, Ltd., Japan.

Sleeping-time investigation : The extracts of Choto-san (0.3, 1 and 3 g/kg), Chotoko (71.4 mg/kg) and Choto-san without Chotoko were dissolved in water and orally administered one hour before thiopental injection (50 mg/kg, i.p.). Diazepam (1 mg/kg), used as a positive control, was intraperitoneally injected 30 min before the injection of thiopental (50 mg/kg, i.p.). Sleeping time was defined as the time interval between disappearance and reappearance of the righting reflex. The maximum sleeping times were 281 min in diazepam treated mice and 182 min in 3 g/kg Choto-san treated mice.

Results

Thiopental injection induced sleep in mice for 19.2 ± 3.8 min (mean ± S.E.) (Fig. 1). Pretreatment with Choto-san (0.3, 1 and 3 g/kg) dose dependently prolonged the thiopental-induced sleeping time to 42.8 ± 7 ($p<0.005$), 72.2 ± 24.6 ($p<0.01$), and 102.5 ± 27.2 min ($p<0.001$), respectively. Diazepam and Chotoko also prolonged the sleeping time to 166.3 ± 25.6 ($p<0.0001$) and 44.1 ± 12.6 min ($p<0.05$), respectively. Whereas Choto-san without Chotoko had no effect on thiopental-induced sleep.

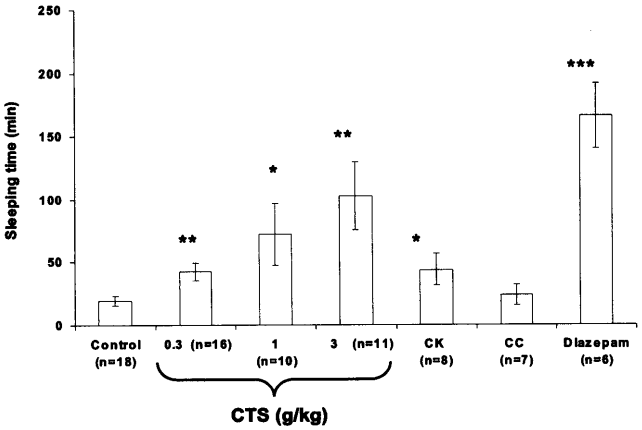

Fig. 1 Effects of Choto-san (CTS, 0.3, 1 and 3 g/kg), Chotoko (CK, 71.4 mg/kg), Choto-san without Chotoko (CC, 1 g/kg) and diazepam (1 mg/kg) on thiopental-induced sleeping time. Data are in mean ± S.E. *** $p<0.0001$, ** $p<0.005$ and * $p<0.05$.

Discussion

This study provides another evidence to support a central effect of Choto-san. This finding corresponds with previous studies that Choto-san and its active component act mainly in the central although different study models were employed. They showed that the water extract of *Uncaria sinensis* possesses a protective effect against glutamate-induced neuronal cell death.[1] In addition, water extracts of *Uncaria rhynchophylla* revealed anti-convulsive activity in kainic acid-induced epileptic seizure.[7] Therefore, the prolongation effect of Chotoko on thiopental-induced sleeping time while Choto-san without Chotoko is devoid of this effect further confirms that Chotoko is the active component of Choto-san. The results from this study corresponds with a previous study reporting that the tetracyclic oxindole alkaloids; isorhynchophylline, corynoxine and corynoxine B, isolated from *Uncaria macrophylla* WALL. prolonged thiopental-induced sleep.[8]

In one of our studies on the effects on Choto-san in 2VO-induced dementia rat, the preliminary results showed that an administration of Choto-san to the pentobarbital anesthetized 2VO rats one hour after the operation resulted in animal death, while the water-administered animals were still alive (unpublished data). Both thiopental and pentobarbital induced unconsciousness by binding to a receptor residing within the GABA-benzodiazepine -Cl^- ionophore receptor complex, leading to an increase in the opening time of Cl^- channel.[9] Pentobarbital provides long periods of anesthesia but thiopental is a very short type. Choto-san and Chotoko may directly act as an enhancer of the GABA/ Cl^- channel system as well as diazepam, and induced death in the case of pentobarbital because of the strong effect, or prolonged the sleeping time which was induced by the weak barbiturate, thiopental. It is well known that other kinds of receptor systems are also involved in the anesthesia and prolongs the sleeping time such as N-methyl-D-

asparatate (NMDA), nicotinic acetylcholine, serotonin, α_2-adrenergic and so on. Choto-san is used as a treatment for hypertension, headache, and dizziness. Many reports have shown the decrease in the blood pressure by Choto-san.[10] Then it can not be ruled out that the α_2-adrenergic system is involved in the decrease of blood pressure and prolongs the sleeping time induced by Choto-san and Chotoko. Alkaloid components of the uncaria family, pteropodine and isopteropodine, positively modulate the serotonin 2 receptor response.[11] Furthermore, rhyncophylline and isorhyncophylline, which are components of Chotoko, inhibit the NMDA response.[12] These results show the possibility that serotonin and NMDA receptor systems may also be concerned with the prolongation of the sleeping time. However, further studies are required before any conclusion on precise mechanisms can be drawn.

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和文抄録

釣藤散の脳虚血マウスの学習行動改善作用を検討する過程において，釣藤散を投与した動物の睡眠時間が延長することを観察した。そこで本研究では，釣藤散およびその構成生薬・釣藤鉤ならびに，釣藤鉤をのぞいた釣藤散の，チオペンタール誘発睡眠に及ぼす効果を検討した。釣藤散は，0.3，1，3 g/kg 経口投与により用量依存的に 50 mg/kg チオペンタール腹腔内投与による正向反射消失時間を有意に延長した。釣藤鉤（71.4 mg/kg p.o.：釣藤散 1g/kg 中と等量）も有意な効果を示したが，釣藤鉤を除いた釣藤散（1 g/kg）は無効であった。本結果より，釣藤散およびその構成生薬・釣藤鉤が中枢抑制作用を有することが推定された。

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