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EXPERIMENTAL STUDY

Effect of Sini San Freeze-dried powder on sleep-waking cycle in insomnia rats

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Abstract

OBJECTIVE: To investigate the effects of the Sini San at different doses on each sleeping state [slow-wave sleep 1 (SWS1), slow-wave sleep 2 (SWS2), rapid-eye-movement (REM), wakefulness (W)] in insomnia rats and to identify its mode of action for improving sleep.

METHODS: The insomnia rats were randomly divided into a high-, medium- or low-dose group of Sini San (equal to crude drug 8.8, 4.4, or 2.2 g/kg, respectively) for seven consecutive days.

RESULTS: Compared with pre-administration, SWS2 was significantly increased after administration of the low dose. Compared with pre-administration, W was significantly decreased and SWS1, SWS2, and the total sleeping time (TST) were markedly increased after administration of the medium dose. Compared with pre-administration, W was significantly decreased and SWS1, SWS2, rapid-eye-movement sleep, and TST were significantly longer after administration of the high dose. The effects of Sini San on sleep-wake cycle are dose-dependent.

CONCLUSION: The results suggest that Sini San extends SWS1 and SWS2, which increases the total sleeping time.

Key words: Sleep initiation and maintenance disorders; Electroencephalography; Sleep stages; Medicine, Chinese Traditional; Sini San

INTRODUCTION

Sedative and hypnotic drugs mainly reduce slow-wave sleep 2 (SWS2) and rapid-eye-movement (REM) sleep and increase slow-wave sleep 1 (SWS1) to prolong the total sleeping time (TST). Long-term use of these drugs leads to drug addiction and dependence,[1] and re-

bound phenomena after abstinence follows drug withdrawal.[2] Traditional Chinese Medicine (such as Sini San) is effective for treating insomnia and has fewer side effects than synthetic drugs. Recent studies of the pharmacodynamics of Sini San have shown that this compound improves sleep quality.[1,3-5] In the present study, we investigated the effects of different doses of Sini San on each of the sleeping states in rats affected by insomnia *via* cortical electroencephalography (EEG) recordings and calculations of the duration of each sleeping state.

MATERIALS AND METHODS

Animals

Thirty male Wistar rats [3 months old; (220±10) g] of SPF grade were purchased from the Experimental Animal Centre of Gansu University of Traditional Chinese Medicine [Certificate of quality: SCXK (gan) 2010-0008-0000204]. All experiments followed a protocol approved by the local animal ethics committee and the local government, and all experiments followed a protocol approved by the local Animal Ethics Committee and the local government. The experiments were performed in the Department of Pharmacology of Chinese Materia Medica, Gansu University of Traditional Chinese Medicine between January 2010and July 2011.

Materials

Pentobarbital sodium was purchased from Shanghai General Reagent Factory (Shanghai, China), and prepared with distilled water to a 1% solution before use. Dental acrylic water and dental acrylic cement were purchased from Gansu Dental Equipment Factory (Lanzhou, China), and benzylpenicillin sodium from the General Pharmaceutical Factory of Gansu Pharmaceutical Group (Lanzhou, China). The ingredients of Sini San included: Chaihu (*Radix Bupleuri Chinensis*), Baishao (*Radix Paeoniae Alba*), Zhishi (*Fructus Aurantii Immaturus*), and Gancao (*Radix Glycyrrhizae*), which were kindly provided by Dr. Chengyi Li (Department of Pharmacy, Gansu University of Traditional Chinese Medicine, Lanzhou 730020, China). Other purchases included: ND-97 Digital Polysomnography (Shanghai Medical Instrument High Technology Company, Shanghai, China), the electromagnetically shielded recording chamber (Stoelting Company, Chicago, IL, USA), the stereotaxic apparatus (Stoelting Company, Chicago, IL, USA), plexiglass boxes (Lanzhou Research Institute of Electrical Instruments, Lanzhou, China), and the footplate electrical stimulator (Lanzhou Research Institute of Electrical Instruments, Lanzhou, China).

Preparation of the freeze-dried Sini San

The mixture (total of 580 g) of Chaihu (*Radix Bupleuri Chinensis*), Baishao (*Radix Paeoniae Alba*), Zhishi (*Fructus Aurantii Immaturus*), and Gancao (*Radix Glycyrrhizae*) was decocted for 30 min with boiling distilled water (equal to 10-fold weight of the mixture), and then filtered. The drug residue was decocted for 20 min with boiling distilled water (equal to 6-fold weight of the mixture), and then filtered. Filtrates from the two decoctions were combined, concentrated to the required volume, and lyophilized to produce the high-dose freeze-dried Sini San (0.88 g/mL). Dilutions of this dose gave a medium and low dose of Sini San (0.44 or 0.22 g/mL, respectively).

Implantation for EEG

Animals were anesthetized with pentobarbital sodium (45 mg/kg), fixed in the stereotaxic apparatus, and the skull exposed. Two screw electrodes (1 mm diameter) were implanted into the skull (AP-2, R2; AP + 2, R2, AP-2, R2 referring to the former anterior fontanelle 2mm, next to the right side to open 2 mm.AP + 2, R2 referring to the anterior fontanelle 2 mm, next to the right side to open 2 mm; AP + 5, R0 refers 5 mm after anterior fontanelle every on sagittal.) asthe cortical electrodes, and the remaining electrode was placed in the center of the frontal bone (AP + 5, R0) as the ground electrode. The cortical electrodes touched, but did not cut, the dura. The electrodes were connected to a socket by leads, which were fixed to the skull with dental acrylic cement.[6] Postoperative rats were put into separate plexiglass boxes and housed in an electromagnetically shielded recording chamber under standard conditions (21℃±2℃, 40%-45% humidity, illumination between 7∶00 and 21∶00 h, and ventilation). Each rat was administered 45 000 U penicillin (intraperitoneally) for three days and allowed to recover for seven days. Prior to testing, the EEG recording cable was connected to a socket for 5.5 h for habituation to the experimental conditions. During the EEG recording, the behavior of the rats was observed using a video-monitoring system.

Insomnia measurement

EEG signals of the rats in a non-stressed state were recorded 8 days after the operation (recording time of 10 h from 08∶00 to 18∶00). Animals were placed in separate plexiglass boxes (14 mm × 25 mm × 28 cm) on an electrified grid on which electric shocks (0.5 mA, 1 Hz for 18 ms) were delivered.[1] The electric shocks lasted 30 s with a 30-min interval between sessions, and the EEG signals were recorded. The EEG was then analyzed with the outcome measures of wakefulness (W), SWS1, SWS2, and REM sleep.

Digital polysomnography

EEG signals were passed through a low- and high-pass filter at 0.5 and 35 Hz, respectively. In preliminary experiments, 10 rats were used to obtain the optimal gain and speed.[7] Electrical signals in each channel were clear

with no overlap when the gain of the equipment was set at 200 and the speed was 2 (20 s per screen).

Differentiation of sleeping states

According to the polygraphic recordings and other related studies,[8-13] the sleep-wake cycle of rats is divided into four states based on the waveform: (a) W; (b) SWS1; (c) SWS2; and (d) REM sleep. During W, the EEG signals are different because the rats are in different behavioral conditions. Two kinds of EEG signals exist.(a) waking (W): when rats are moving, climbing, exploring, or scanning, the cortical EEG shows predominantly theta rhythm (6-9 Hz) waves, and when rats are grooming or standing still, the EEG shows predominantly low voltage waves with high frequency. (b) SWS1: when rats are lying with their eyes closed or sleeping, the cortical EEG shows predominantly high amplitude waves (0.5-5 Hz) with sleep spindles (10-15 Hz). During SWS1, high amplitude waves occupy less than 50% of the period. (c) SWS2: SWS2 is characterized by high amplitude waves with low frequency and also with sleep spindles. High amplitude and low frequency waves occupy more than 50% of the period. (d) REM sleep: REM sleep is characterized by theta waves, which are not markedly different from W. Thus, REM sleep is determined according to the EEG signal together with the behavior of the rats. Because wakefulness does not directly transform into REM sleep, SWS may appear before REM sleep, and REM sleep can directly revert to SWS or W. Generally, the duration of REM sleep is less than 3 min. Any separate state lasting at least 20 s and within a period of 20 s is considered as an analytic unit.

Drug administration

Insomnia rats were randomly divided into three groups 10 rats/group): low, medium, or high doses of Sini San (equal to crude drug 8.8, 4.4, or 2.2 g/kg, respectively administered intragastrically). Drug treatments occurred at 8∶00 for seven consecutive days. EEG signals of each rat in an electrically stimulated state were recorded for 10 h, 30 min after the last administration.

Statistical analysis

All the data were analyzed by the paired Student's *t*-test. Significance was reached at values of $P<0.05$ or $P<0.01$. Using the paired Student's *t*-test to verify the successful establishment of insomnia models, Statistical analysis was performed using SPSS 17.0 (SPSS Institute, Chicago, IL, USA).

RESULTS

Analyses of W, SWS1 SWS2, REMS and TST

Analytical results of the effect of Sini San freeze-dry powder on sleep phase in insomnia rats (Table 1).

The insomnia rats were randomly divided into a high-, medium- or low-dose group of Sini San (equal to crude drug 8.8, 4.4, or 2.2 g/kg, respectively) for seven consecutive days.

Significant differences were found in all states of the sleep-wake cycle when the pre- and post-shock recordings were compared. These results indicated that the rat model of insomnia was successfully established. In the low-dose group, SWS2 was significantly ($P<0.01$) longer after administration. Compared with pre-administration, W was significantly ($P<0.05$) decreased and SWS1, SWS2, and TST were markedly ($P<0.01$) increased after administration of the medium dose. Compared with pre-administration, the duration of W was significantly ($P<0.05$) shorter and SWS1, SWS2, REM sleep, and TST were significantly ($P<0.01$) longer after administration of the high dose.

DISCUSSION

Previous studies on the efficacy of Sini San on sleeping time have been limited to the clinical observation

Table 1 Efects of Sini San at different doses on the sleep-wake cycle in insomnia rats

Group		W	SWS1	SWS2	REM sleep	TST
High-dose	PrS	152.5±16.5	279.3±16.6	15.4±3.5	17.7±6.1	319.4±18.4
	PS	201.3±35.5[a]	271.2±29.7[d]	4.3±2.4[c]	7.1±3.2[c]	272.6±34.6[a]
	PA	150.8±29.2[b]	306.6±26.3[b]	10.4±3.4[e]	15.1±7.0[f]	344.3±29.0[b]
Medium-dose	PrS	163.2±16.9	286.3±14.9	12.9±3.3	14.9±5.9	322.8±16.3
	PS	215.1±31.2[c]	242.1±37.3[a]	4.1±2.0[c]	8.0±5.4[d]	269.4±31.1[c]
	PA	158.4±30.2[b]	285.3±35.7[e]	11.5±6.8[b]	11.0±6.2	316.4±31.1[b]
Low-dose	PrS	179.1±15.1	262.4±13.7	8.3±2.4	21.4±5.9	287.0±13.2
	PS	219.9±24.1[a]	248.4±20.9[d]	1.8±1.4[c]	10.8±5.3[d]	268.1±22.5[a]
	PA	235.9±12.5	239.1±16.2	5.9±2.8[b]	9.2±4.5	249.1±13.0

Notes: PrS: pre-shock; PS: post-shock; PA: post-administration; W: wakefulness; SWS1: slow-wave sleep 1; SWS2: slow-wave sleep 2; REM: rapid-eye-movement; TST: total sleeping time. Low, medium, and high doses groups were given Sini San (equal to crude drug 8.8, 4.4, or 2.2 g/kg, respectively administered intragastrically) for seven consecutive days. [a]$P<0.01$, [c]$P<0.005$, [d]$P<0.05$, *vs* PrS; [b]$P<0.01$, [f]$P<0.05$, [e]$P<0.005$, *vs* PS.

phase. The present study investigated the effects of Sini San on sleeping states in insomnia rats with pre- and post-shock. Our findings revealed that Sini San markedly increased the sleeping time. Furthermore, we showed that Sini San extended the SWS2 and SWS1, which increased the total sleeping time. Therefore, Sini San may be more efficacious for ameliorating insomnia compared with synthetic drugs.

Overall, the present study provides evidence of a possible underlying mechanism of action of Sini San for its sedative-hypnotic effects, thus further strengthening its potential for clinical use.

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