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EXPERIMENTAL STUDY

Sedative and hypnotic effect of freeze-dried paeoniflorin and Sini San freeze-dried powder in pentobarbital sodium-induced mice

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Abstract

OBJECTIVE: To investigate the sedative and hypnotic activity of paeoniflorin and freeze-dried Sini San powder on mice and provide a reliable method for determining the pharmacodynamic material basis of Sini San.

METHODS: Male adult mice weighing 20-22 g were used in this study. Three experiments were carried out. Synergism with pentobarbital was used as an index for hypnotic effect. Loss of the righting reflex was used to determine the start of sleep. Sleep latency and sleeping time were recorded in each experiment.

RESULTS: The coefficient of variation of the suprathreshold dose (55 mg/kg) was significantly lower than that of the threshold dose. The sleep latency of mice was significantly decreased, and the sleeping time of mice was significantly prolonged. The effects of paeoniflorin and Sini San on prolonging the sleeping time of mice induced by pentobarbital sodium were significantly stronger than those in the control group.

CONCLUSION: Paeoniflorin produces significant sedative and hypnotic effects, and there is an obvious dose-effect relationship.

Key words: Paeoniflorin; Pentobarbital; Hypnotics and sedatives; Sleep; Sini San

INTRODUCTION

At present, drug therapy is one of the most important methods for the treatment of insomnia. In China, treatment mainly includes sedative hypnotic drugs, Traditional Chinese Medicine (TCM) (single herbs and compounds), and natural hypnotic substances. TCM has been used for treating insomnia for thousands of years and traditionally shows few toxic side effects.

Sini San was first recorded in *Shang Han Lun*[1] and is composed of four herbs, including Chaihu (*Radix Bupleuri Chinensis*), Baishao (*Radix Paeoniae Alba*), Zhishi (*Fructus Aurantii Immaturus*), and Gancao (*Radix Glycyrrhizae*). Sini San is a basic prescription of TCM for alleviating ShaoYang and coordinating the liver and spleen. This effectively disperses pathogens and alleviates mental depression, soothes the liver, regulates the spleen, and exhausts the stagnation of *Qi* and blood. According to TCM clinical observations, the stagnation of liver-*Qi* induces insomnia and accounts for

more than 80% of the total insomnia cases. Compared with other drugs, Sini San is effective in treating insomnia and has fewer side effects. Therefore, we selected a representative prescription of Sini San to study and discuss for improving sleep pharmacology.

Baishao (*Radix Paeoniae Alba*) can stimulate the nervous system, decrease depression, enhance work performance, resist anoxia, eliminate fatigue, act as a sedative, act as an analgesic, and improve sleep.[2,4] Furthermore, it has been found to have a potential effect on extending human life. [4] The major active constituent of this herb is paeoniflorin (5beta-[(Benzoyloxy)methyl] tetrahydro-5-hydroxy-2-methyl-2,5-methano-1H-3,4-dioxacyclobuta[cd]pentalen-1alpha[2H]-yl-beta-D-glucopyranoside, $C_{23}H_{28}O_{11}$: 480.45). The purpose of this study, therefore, was to confirm that paeoniflorin can improve sleep and exert sedative and hypnotic effects.

MATERIALS AND METHODS

Agents

Diazepam (DZP, 2.0 mg/kg, Sigma) was used as the standard hypnotic drug. Pentobarbital sodium (Batch No. 080605) was purchased from Shanghai General Reagent Factory (Shanghai, China). Pentobarbital was prepared with distilled water to a 1% solution before use. Chaihu (*Radix Bupleuri Chinensis*), Baishao (*Radix Paeoniae Alba*), Zhishi (*Fructus Aurantii Immaturus*), and Gancao (*Radix Glycyrrhizae*) were purchased from Harbin Shiyitang medicine plant (Harbin, China) and were kindly authenticated by Dr. Chengyi Li, professor of Pharmacognosy. Peoniflorin (purity 99%) was purchased from Tianjin jianfeng Natural Product R&D Co., Ltd. (Tianjin, China). Drugs were dissolved in water for injection before use.

Ethical approval of the study protocol

All animal experiments used in this study were conducted in accordance with the European Community guidelines for the use of experimental animals and approved by the Animal Care Committee of Heilongjiang University of Chinese Medicine.

Animals

A total of 310 SPF male adult mice weighing 20-22 g (Experimental Animal Center of Heilongjiang University of Chinese Medicine, Harbin, China) were used in this study. Each mouse was used only for one experiment. All animals were housed in acrylfiber cages (450 mm × 290 mm × 189 mm, 15-18 per cage) in an air-conditioned, sound-attenuated room, with controlled temperature 26℃ ± 2℃ and humidity (53% ± 16%). This room was kept on a 12∶12-h light-dark cycle (lights on at 7∶00 am, 50-120 lux). The animals were allowed free access to food (standard laboratory animal food) and water except the experiments and acclimated 7 days before they were used.[3]

Preparation of Sini San and other herbs freeze-dried powder

The mixture (580 g) of Chaihu (*Radix Bupleuri Chinensis*), Baishao (*Radix Paeoniae Alba*), Zhishi (*Fructus Aurantii Immaturus*), and Gancao (*Radix Glycyrrhizae*) each 145 g were decocted for 1 h with boiling distilled water (equal to 10-fold weight of the mixture) and then filtered. The drug residue was decocted for 1 h with boiling distilled water (equal to 8-fold weight of the mixture) and then filtered. Filtrates from the two decoctions were put together and concentrated to the required volume (0.8 g/mL) (crude drugs) under the water bath at 70℃. We decocted Sini powder first, and then used freeze-drying to prepare the final Sini powder for storage until use. The constituents of Sini powder were detected and the four major constituents (paeoniflorin, naringin, hesperidin, and licorice acid) were quantified by thin-layer chromatography and high-performance liquid chromatography. Preparation of Chaihu (*Radix Bupleuri Chinensis*), Baishao (*Radix Paeoniae Alba*), Zhishi (*Fructus Aurantii Immaturus*), and Gancao (*Radix Glycyrrhizae*) (freeze-dried powder) were the same as Sini powder (freeze-dried powder).

Evaluation of sleep latency and sleeping time

It is suggested that the righting reflex is a useful method for assessing whether or not animals are asleep.[4,5] Observers were blinded to the drug treatment. Following the pentobarbital injection the index of hypnotic effect was recorded. The indexes were recorded as follows: time elapsed between the administrations of pentobarbital until loss of righting reflex was recorded as the sleep latency, while the time from the loss to its recovery was considered the sleeping time.[6]

Effects of different doses of pentobarbital sodium on sleeping time of mice

The method of pentobarbital induced sleeping in mice is a classic pharmacological experiment for screening sedative and hypnotic drugs. The differential dose of pentobarbital can affect experimental results significantly. Through this experiment, we can determine a best dose of pentobarbital sodium on sleeping time of mice. The experiments were carried out from 10∶00 to 14∶00 h in a quiet room in which the temperature was maintained at 20℃±23℃. Mice were grouped of 10 in each. Mice were treated as follows: groups 1, 2, 3, 4, 5, 6, and 7 received the pentobarbital sodium (at dose of 35, 40, 45, 50, 55, 60, and 65 mg/kg, intraperitoneal injection, respectively). Sleeping time of mice was recorded.

Effects of drug on the sleep latency and duration of sleep in pentobarbital sodium treated mice

Experiments were carried out from 10∶00 to 14∶00 h in a quiet room in which the temperature was maintained at 20℃±23℃. Mice were randomly divided into ten groups (ten mice in each). Group 1 received water for injection as the control, group 2 received 2.0 mg/kg