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The ameliorating effects of LiuWei Dihuang Wang on cycloheximide-induced impairment of passive avoidance performance in rats

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Abstract

The ameliorating effects of aqueous and ethanolic extracts of LiuWei Dihuang Wang (LDW_W and LDW_E) after single, 1-week or 2-week consecutive treatment on the cycloheximide-induced amnesia by using the passive avoidance task in rats were studied. After single treatment, LDW_W and LDW_E (1 and 2 g/kg) significantly prolonged the shortened step-through latency induced by CXM and their potency was equal. LDW_W at 1 g/kg after 1-week consecutive treatment or at 0.1 g/kg after 2-week consecutive treatment almost completely reversed CXM-induced amnesia. LDW_W at any dose alone after single, 1-week or 2-week consecutive treatment did not influence the step-through latency in the training trial in rats. Furthermore, muscarinic antagonist scopolamine, peripheral cholinergic antagonist scopolamine methylbromide, serotonin precursor 5-hydroxytryptamine and serotonin releaser *p*-chloroamphetamine could block the ameliorating effects of LDW_W. $GABA_A$ receptor antagonist bicuculline and $GABA_B$ receptor agonist baclofen also blocked the ameliorating effects of LDW_W. These results suggest that the ameliorating effects of LDW whose potency were parallel to treatment duration might be related to activating peripheral cholinergic neuronal system and modulating the central nervous system.

Keywords: LiuWei Dihuang Wang; Passive avoidance task; Cycloheximide; Memory

1. Introduction

LiuWei Dihuang Wang (abbreviated as LDW), a traditional Chinese medicinal prescription, consists of dihuang (Rhizoma of *Rehmannia glutinosa* LIBOSCH., Polygonaceae), shanzhuyu (Fructus of *Cornus officinalis* SIEB. et ZUCC., Cornaceae), shanyao (Radix of *Dioscorea opposita* THUNB., Dioscoreaceae), zexie (Rhizoma of *Alisma orientalis* (SAM.) JUZEP., Alismataceae), mudanpi (Cortex of *Paeonia suffruticosa* ANDR., Ranunculaceae) and hoelen (*Poria cocos* (SCHW.) WOLF, Polyporaceae). It has long been employed in the clinical treatment of diabetic mellitus and neurosis, and described as an anti-aging prescription in ancient Chinese herbal books. Recent study revealed that LDW ameliorated the deterioration of memory ability in senescent accelerated mice and hydrocortisone-treated mice (Zhou et al., 1999b; Wei, 2000; Chen et al., 2001). Our previous study indicated that acute and chronic treatment of LDW could alleviate the drug-induced learning acquisition deficit and facilitate the two-way active avoidance performance in rats (Hsieh et al., 2003).

In general, memory processes are divided into three stages: learning acquisition, memory consolidation and retrieval. According to biochemical studies, memory consolidation needs the participation of protein, especially new protein transcription and synthesis (Hatakeyama et al., 2006). Protein synthesis inhibitors as cycloheximide (CXM) impair memory consolidation in rodents (Barraco and Stettner, 1976; Davis and Squire, 1984). Therefore, this study was desired to investi-

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gate the ameliorating effects of LDW extracted by distilled water or 95% ethanol on the CXM-induced impairment of passive avoidance performance in rats. Some studies pointed out that memory consolidation involves the activation, by neurotransmitters such as acetylcholine, dopamine and serotonin, of receptor-linked enzymes responsible for synthesis of intra- and inter-cellular messages (Wang et al., 1997). Hence, Nabeshima et al. pointed out that cycloheximide-induced memory consolidation deficits mainly via disturbances in the cholinergic, serotonergic and GABAergic neuronal system (Nabeshima et al., 1988, 1989, 1991). Then, we further clarified the mechanism of the ameliorating effects of LDW on the CXM-induced impairment of passive avoidance performance by combining with some transmitter agonists or antagonists in rats.

2. Materials and methods

2.1. Materials

All composed raw materials of LDW were bought from the Taichung Traditional Material Pharmacy and identified by Professor Dr. Chung-Chuan Chen of Department of Chinese Medicinal Resources, China Medical University. All plant samples, whose voucher numbers are ICPS-LDW-RR-050920, ICPS-LDW-FC-050920, ICPS-LDW-RD-050920, ICPS-LDW-RA-050920, ICPS-LDW-CP-050920 and ICPS-LDW-P-050920, were deposited in the Institute of Chinese Pharmaceutical Sciences, China Medical University. The ratio of all composed raw material of LDW (2.5 kg) was 8:4:4:3:3:3 followed as our previous report (Hsieh et al., 2003) and the description of Yang et al. (2006). LDW (2.5 kg) was extracted with 5 L distilled water or 95% ethanol at 50 °C four times, and the aqueous or ethanolic extract (LDW_W or LDW_E) reduced to dryness with a vacuum rotary evaporator. The yield of LDW_W and LDW_E are 13.52 and 10.56%, respectively. LDW_W or LDW_E were applied in distilled water solution (0.2 mL/100 g). Scopolamine (SCOP), scopolamine methylbromide (M-SCOP), 5-hydroxytryptamine (5-HTP), *p*-chloroamphetamine (PCA), bicuculline (BIC), baclofen (BAC) and cycloheximide (CXM) were purchased from Sigma–Aldrich Chemical Co., and dissolved in 0.9% saline (0.1 mL/100 g). The vehicle-treated rats received the same volume of saline.

2.2. Animals

Male Sprague–Dawley rats, weighing 200–250 g, were obtained from Animal Center of China Medical University and used in the below experiments according to the guidelines of the Committee on Care and Use of Laboratory Animals of the China Medical University. All rats were housed for at least 1-week before starting experiment in a temperature (23 ± 1 °C) and humidity (60%) regulated environment with free access to standard food in pellets (supplied and designed by Fwusow Industry Co. Ltd., Taiwan) and tap water, on a 12–12 h light/dark cycle (light phase: 08:00–20:00 h) was maintained. Then, the rats were randomly assigned into each group supported the passive avoidance performance.

2.3. Passive avoidance performance

The passive avoidance test was carried out in two compartments with a steel-rod grid floor (consisted of 36 parallel steel rods, 0.3 cm in diameter with 1.5 cm apart from the neighboring rod). The light compartment (48 cm × 20 cm × 30 cm) with a 20 W lamp centrally 30 cm was seated above the grid floor, whereas the dark compartment of the same size was connected to the light compartment through a guillotine door (5 cm × 5 cm). The room was kept dark during the experiments, which were conducted between 09:00 and 12:00 h. In the training trials, the guillotine door between the light and dark compartments was closed. When the rat was placed in the light compartment with its back to the guillotine door, the door was opened and the step-through latency (STL) was measured simultaneously until the rat entered the dark compartment. After the rat entered the dark compartment, the door was closed. An inescapable scrambled footshock (1.0 mA for 2 s) was delivered through the grid floor. Then the rat was replaced back into the home cage until the retention trial. Twenty-four hours later, the retention trial was performed. The rat was again placed in the light compartment, as in the training trial, the guillotine door was opened and the STL was recorded and would be used as a measure of retention in the analysis. The upper cut-off time was set as 300 s (Hsieh et al., 1998).

In the first experiment, rats were orally given with LDW_W or LDW_E (0.01, 0.1, 1 and 2 g in term of aqueous or ethanolic extract/kg) for single, 1-week or 2-week consecutive treatment and the training trial was carried out 1 h after the last dosage of LDW_W or LDW_E. CXM (1.5 mg/kg, s.c.) used to induce amnesia was administered only once immediately after the training trial (Hsieh et al., 2001b).

In the second experiment, rats were orally given with LDW_W (0.1 g/kg) for 2-week consecutive treatment and the training trial was carried out 1 h after the last dosage. CXM (1.5 mg/kg, s.c.) was administered immediately after the training trial. SCOP (0.3 mg/kg, i.p.), M-SCOP (0.5 mg/kg, i.p.), 5-HTP (50 mg/kg, i.p.), PCA (1.0 mg/kg, i.p.), BIC (0.025 mg/kg, i.p.) or BAC (0.01 mg/kg, i.p.) were also administered immediately after the training trial (Hsieh et al., 1998, 2001a).

2.4. Data analysis

All data of the passive avoidance performance were expressed as mean ± standard errors of means (S.E.M.) and analyzed using a Kruskal–Wallis non-parametric one-way analysis of variance, followed by Mann–Whitney's *U*-test. The criterion for statistical significance was $p < 0.05$ in all-statistical evaluations. All data collected during motor activity and shock sensitivity was expressed with means and standard errors and analyzed using a one-way analysis of variance, followed by Scheff's test. The criterion for statistical significance was $p < 0.05$ in all-statistical evaluations.