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The memory enhancement effect of Kai Xin San on cognitive deficit induced by simulated weightlessness in rats

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ABSTRACT

Ethnopharmacological relevance: It is vital for astronauts to develop effective countermeasures to prevent their decline of cognitive performance in microgravity to make space-flight missions successful. The traditional Chinese herbal formula Kai Xin San (KXS) has been used to treat amnesia for thousands years. It is a traditional complex prescription comprising of ginseng (Panax ginseng C. A. Meyer), hoelen (Poria cocos (Schw.) Wolf), polygala (Polygala tenaifolia Willd), and acorus (Acorus tatarinowii Schott). Previous study showed KXS could improve CMS-induced memory impairment in rats.

Material and methods: In this paper, a unique environmental factor—microgravity (weightlessness) was simulated as hindlimb suspension (HLS) by tail in rats for two weeks as the HLS animal model. The KXS at the doses of 0.3 or 0.6 g/kg p.o. daily was administrated to HLS rats for two weeks at the same time of HLS, the memory behavior tests were investigated with Morris water maze (MWM) and Shuttle Box (SB) test. The levels of ROS, 8-OHdG and 3-nitrotyrosine (3-NT) in the serum, and AChE and ChAT activity in the brain of rats were determined by ELISA or biochemical analysis.

Results: After HLS for two weeks, the escape latency and the swimming distance were significantly increased in the MWM test in rats in the HLS group, compared with control group. The percent of swimming distance in target quadrant and the number of target crossing was significantly decreased in rats in the HLS group compared with the control group. Performance in the SB test showed, the numbers and the distance of active avoidance was decreased from day 4 to day 7, the time spent in electric area was increased in rats in the HLS group compared with the control group.

Administration of KXS 0.3 or 0.6 g/kg to the HLS rats for two weeks significantly reduced the escape latency and the swimming distance, increased the percentage of swimming distance in target quadrant and the number of target crossings ($P < 0.01$, compared with the HLS group) in the MWM test. Similar treatment with KXS increased the numbers and the distance of active avoidance ($P < 0.01$, compared with the HLS group) and reduced the time spent in electric area after training 3 days in the SB test ($P < 0.01$, compared with the HLS group).

The HLS induced the increase of the ROS, 8-OHdG and 3-NT in the serum of rats, but has little influence on the AChE, ChAT activity in the brain. Only the AChE activity in the cortex and the ChAT activity in the hippocampus had some changes in rats in the HLS model group.

After administration of KXS 0.6 g/kg for two weeks, the abnormal levels of ROS, 8-OHdG, 3-NT were found reversed in the serum of rats ($P < 0.05$, compared with HLS model group). And KXS 0.3 g/kg was found reversed the increased AChE activity in the cortex.

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[4] Chen Yi-Xi and Jiang Ning performed biochemical analysis.

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Conclusions: Experimental results from this study show that KXS may improve memory deficiency induced by HLS, its mechanisms are major related to antioxidant activities, rather than the central cholinergic system.

1. Introduction

In space, astronauts experience unique environmental factors such as microgravity, which could negatively affect their physiological and psychological state, and performance, especially learning and memory, thus endangering the safety of the crew and the mission's success (Koppelmans et al., 2013). It is vital to develop effective countermeasures to offset or minimize these deleterious consequences. The U.S. National Aeronautics and Space Administration (NASA) together with partner countries have developed various recommendations for these countermeasures, including procedures, training, exercise, and counteracting therapies (Smith et al., 2014). In China, in addition to conventional treatments used in Western countries, it was recommended that astronauts be treated with traditional Chinese medicine (TCM), such as herbal medicine, acupuncture, and massage therapy to enhance cognitive and emotional function (Fan et al., 2007; Liu et al., 1995).

Kai Xin San (KXS) was originally recorded in 'Bei Ji Qian Jin Yao Fang' in the Tang Dynasty in China, which is a category of traditional complex prescription comprising of ginseng (Panax ginseng C. A. Meyer), hoelen (Poria cocos (Schw.) Wolf), polygala (Polygala tenaifolia Willd), and acorus (Acorus tatarinowii Schott). It was reported that KXS could cure symptoms including desolation, moodiness, forgetfulness etc., which are similar to the neuroses such as depression, anxiety, and impairment in learning and memory (Wang et al., 2007). Experimental studies demonstrated that KXS ameliorates the learning and memory deficit in amygdala-lesioned mice, senescence accelerated mice, mice with learning and memory deficits induced by ethanol and scopolamine (Nishiyama et al., 1994a), as well as mice with thymectomy-induced impairment of learning behaviors (Nishiyama et al., 1994b) in the step-down or spatial memory test. However, the effects and mechanisms of KXS on cognitive functions are still not well-understood.

The HLS rat model is a popular model which is used to make simulated microgravity on earth and widely used to study microgravity-induced alterations related to fluid redistribution in skeletal muscle (Frigeri et al., 2001), and also used to study simulated weightlessness induced impairment of learning and memory (Sun et al., 2009). This paper will focus on the study of the memory enhancement effects of KXS on the HLS rat model and the possible mechanisms.

2. Materials and methods

2.1. KXS preparation and administration procedure

The herbs (ginseng, hoelen, polygala, and acorus) for the preparation of KXS aqueous extract were purchased from Beijing Tongrentang Drug store (Beijing, China) and authenticated by professor Bengang Zhang (Institute of Medicinal Plant Development, Beijing, China) based on their macroscopic characteristics. The quality of these crude drugs is controlled by the Chinese Pharmacopoeia (2010), and a voucher specimen of each plant (No. 2013013, No. 2013014, No. 2013015, No. 2013016, respectively) was prepared and deposited in the herbarium of the Institute of Medicinal Plant Development.

The crude drugs were cut into small pieces and then mixed together in a ratio of 3:3:2:2 as the formula in 'Tai Ping Hui Min He Ji Ju Fang' in Song dynasty in China (Liu et al., 2005; Dang et al., 2009). Dried samples weighing about 3000 g were boiled in 30 L of distilled water for 1 h. After extraction, the decoction was collected, and the residue was subjected to further extraction in 30 L of boiling water for 1 h. The process lasts for 3 times uninterruptedly. The decoctions collected from the three successive extractions were mixed and passed through filter paper. The filtrate was collected and concentrated in a water bath kept below 70 °C under reduced pressure using a rotary evaporator. The percentage (w/w) yield of the water extract of KXS was 18%.

2.2. Drugs and regents

Huperzine A was purchased from Shanghai Tauto Biotech Co., Ltd. The 3-nitrotyrosine (3-NT), 8-OHdG, ROS commercial ELISA kits were bought from R&D, USA. The AChE and ChAT commercial kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing City, PR China).

2.3. Animal preparation

Fifty male SD rats (weighing about 180–200 g) were purchased from the Laboratory Animal Center (Beijing, China). The rats were housed individually in the Specific Pathogen Free animal house, and were allowed to acclimate to the environment of the animal room for 3 days before the onset of each experiment. Food and water were provided ad libitum throughout the course of the experiment. The temperature in the animal room was maintained at 20 ± 2 °C and on a 12:12 h light–dark cycle (lights on at 08:00 AM). All animal handling procedures were performed in compliance with the 'Principles of Laboratory Animal Care' (NIH publication No. 85–23, revised 1985) and PR China legislation for the use and care of laboratory animals.

2.4. Experimental design

Rats were randomly divided into five groups, including the control group, the simulated weightlessness (HLS) group (untreated control), the HLS+huperzine A 0.1 mg/kg (HLS+huperzine A 0.1 mg/kg) group, the HLS+KXS 0.3 g/kg group, and the HLS+KXS 0.6 g/kg group (10 rats for each group). The HLS experimental animals were housed individually and exposed to HLS for two weeks. The rats in HLS+huperzine A or HLS+KXS groups were administered huperzine A or KXS intragastrically for two weeks at the same time as HLS procedure. The control animals were housed individually in a different room but did not undergo HLS procedure. On the 14th day, the MWM test or the SB test was used to study the behavior of the rats. After the behavior test, the rats were suspended by the tails as usual till the last day of the behavior tests. At the end of the behavior test, the rats were sacrificed, the brains were removed immediately, the blood was collected and the serum was separated. The samples were placed in liquid nitrogen for later determination.