Antidepressant Effects of Gammakdaejo-Tang on Repeated Immobilization Stress in the Ovariectomized Female Rats

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Gammakdaejo-Tang (GMT) is a traditional oriental medicinal formula, a mixture of 3 crude drugs, and it has been clinically used for treating mild depressive disorders. The purpose of the study was to examine the effect of Gammakdaejo-Tang (GMT) on repeated stress-induced alterations of learning and memory on a passive avoidance test (PAT) test and also the anxiety-related behavior on the elevated pulse maze (EPM) in ovariectomized female rats. We assessed the changes in the reactivity of the cholinergic system by measuring the immunoreactive neurons of choline acetyltransferase (ChAT) in the hippocampus after behavioral testing. The rats were exposed to the immobilization (IMO) stress for 14 days (2hours/day), and Gammakdaejo-Tang (400 mg/kg, p.o.) was administered 30 min before IMO stress. Treatments with GMT caused significant reversals of the stress-induced deficits in learning and memory on a working memory test, and it also produced an anxiolytic-like effect on the EPM, and increased the ChAT reactivities ($p<0.001$, respectively). These results suggest that Gammakdaejo-Tang might prove to be an effective antidepressant agent.

Key words : Gammakdaejo-Tang (GMT), passive avoidance test (PAT), choline acetyltransferase (ChAT)

Introduction

Postmenopausal women often suffer from symptoms called postmenopausal syndrome. The decrease of E_2 has an influence on regulating the production of corticosterone and changing the behavioral response under the stress condition. So, postmenopausal woman was weak on the stress condition. Also, stress and more stress elicit the depression. Depression is a common, debilitating, life-threatening illness with an increasing morbidity and mortality. Furthermore, the World Health Organization revealed that depression is the fourth leading cause of disability worldwide, exceeded by lower respiratory infections, perinatal conditions and HIV/AIDS[1]. Recent antidepressant drugs, including various monoamine reuptake inhibitors and monoamine oxidase inhibitors, have proven to be effective and are available in clinic, but they are burdened with such disadvantage as slow onset of action, relatively low response and side effects, which make the research and development of new type antidepressants urgent[2-5]. Plant products are frequently considered to be less toxic and have fewer side effects than synthetic ones. Many traditional oriental medicine has been used in clinical practice for several centuries[6,7].

Gammakdaejo-Tang consists of 3 kinds of medicinal herbs: Glycyrrhizae Radix, Date, Wheat. Gammakdaejo-Tang is a traditional oriental herbal medicine, and it has been used in the treatment of mental stress, anxiety and manic-depressive illness. In the previous study, repeated Gammakdaejo-Tang treatment induced release of serotonin and melatonin[8]. Also, GMT and their constituent herbs helped regulation of blood pressure and heart rate[9]. In the previous study demonstrated the treatment of Glycyrrhizae Radix, component of Gammakdaejo-Tang, reduced the anxiety and improved the learning and memory in the repeated stress induced rats[10]. Date was used for sleep disorder and hot water extract of common wheat (HY6228) is effective in preventing ischemia reperfusion acute renal failure[11].

Taking into account that only few studies have been conducted with Gammakdaejo-Tang in animal models to clarify its mechanism of antidepressant action, the present

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work aimed to extend literature data by further investigating the involvement of the cholinergic system in the antidepressant-like effect of Gammakdaejo-Tang in the elevated plus maze and passive avoidance test in the rats.

Materials and Methods

1. Subjects and stress procedure

Sprague Dawley female rats at the age of 8 weeks (Samtaco, Inc. Korea) were used for the study. The rats were housed under a controlled temperature (22～24℃) with a 12 h light/dark cycle. The lights were on from 8:00 to 20:00. Food and water were available ad libitum. They were allowed at least 1 week to adapt to their environment before the experiments. The animal experiments were carried out in accordance with the Prevention of Cruelty to Animals Act 1986 and NIH guidance for the care and use of laboratory animals for experimental procedures, and were approved by local committee review.

The female rats were randomly divided into four groups (n=6 per group): the nonoperated and nonstressed group (Normal), the ovariectomized and stressed group (Control) and the ovariectomized, stressed and GMT treated group (GMT). Using aseptic conditions, bilateral ovariectomy was performed under general anesthesia with pentobarbital sodium (50 mg/kg, i.p.). After postoperative recovery for 7 days, the ovariectomized rats were stressed daily. Stress was produced by forcing the animals into an immobilizer device (a disposable rodent restraint cone, Havard Instrument, U.S.A.) for 2 h (10:00～12:00 a.m.) for 14 days. From the 8th day after the first immobilization, GMT group was treated daily with the GMT extract (400 mg/kg, p.o.) for 2 weeks, and other groups were given sterile saline. Immobilization began 30 min after the treatments.

2. Preparation of herbal extracts.

The dried Gammakdaejo-Tang (GMT) samples (200 g) were immersed in a 10-fold volume of dH_2O, boiled at 80℃ for 1h, and then the water extract was collected. The process was repeated once, and the extracts were combined and concentrated with a rotary evaporator and vacuum-dried to yield 8.1% (w/w) of the extract.

3. Elevated plus maze

The plus-maze apparatus was constructed with black wood. It consisted of two open-arms (the arms extended from a central 50×10 cm space) and two enclosed arms (50×10×40 cm). The arms extended from a central platform (10×10 cm). The apparatus was elevated 50 cm above the floor. The animals were transported to the testing room at least 1 hr prior to starting the experiment. After all of the stress sessions, the rats were individually placed in the central platform facing a closed arm and they were allowed to explore the maze for a 5-min test period. The duration of time spent in the open arms and closed arms were the behavioral measures that were recorded for each rat.The apparatus was wiped clean with a damp sponge and dried with paper towels between tests.

4. Passive avoidance task

A passive avoidance task was performed after stress procedure. The rats were trained in a step-through inhibitory avoidance task. On the training trial, each rat was placed on a lighted platform outside a hole leading to a dark compartment. When the rat stepped into the dark compartment, a constant current foot-shock (5 V, 0.5 mA, 10seconds) was delivered twice. For the retention test, at 24 hours (day 1), and at 2, 3 and 4 days later, each rat was again placed on the platform and the latency to step-through was recorded.

5. ChAT immunohistochemistry

At the end of the behavioral observation, the animals were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and then perfused transcardially with 100 ml of saline, followed by 500 ml of a 4% solution of formaldehyde prepared in phosphate buffer. The brains were then removed, postfixed in the same fixative for two to three hours at 4℃ and then placed overnight at 4℃ in PBS containing 20% sucrose. On the following day, the brain was cut into coronal sections that were sliced to 30 μm- thicknesses. Sections were processed for choline acetyltransferase (ChAT) immunoreactivity by using sheep anti-ChAT polyclonal antibody (Chemicon international, Temecula, CA). The primary antibody was prepared at a dilution of 2000X in 0.3% PBST, 2% normal rabbit serum and 0.001% kehole limpit hemocyanin (Sigma, USA). The sections were incubated in the primary antibody for 72 h at 4℃. Following rinsing in PBST, the sections were incubated for 2 h at room temperature in biotinylated rabbit anti-sheep secondary antibody (Vector Laboratories, Burlingame, CA) that was diluted 200X in PBST containing 2% normal rabbit serum. After three more rinses in PBST, the sections were placed in Vectastain Elite ABC reagent (Vector laboratories, Burlingame, CA) for 2 h at room temperature. Following a further rinsing in PBS, the tissue was developed using diaminobenzadine (sigma, USA) as the chromogen. Images were captured using an Axio Vision 3.0

imaging systems (Zeiss, Oberkochen, Germany) and processed in Adobe Photoshop. For measuring cells of ChAT, the grid was placed on CA1 and CA3 in the hippocampus areas according to the method of Paxinos G. et al[12]. The number of cells was counted at 100x magnification using a microscope rectangle grid measuring 200×200 μm.

6. Statistical analysis

Statistical comparisons were done for the behavioral and histochemical studies using the one-way ANOVA, respectively, and LSD post hoc was done. All of the results were presented as means ± S.E.M., and we used SPSS 18.0 for Windows for analysis of the statistics. The significance level was set at $p<0.05$.

Results

1. Effect of GMT on elevated plus maze

Fig. 1 shows mean group spent time in the arms for all groups. The Control group showed that the time spent in the open arm was decreased compared to the Normal group. Howevr, treatment of GMT was significantly increased time spent in the open arm compared to the Control group.

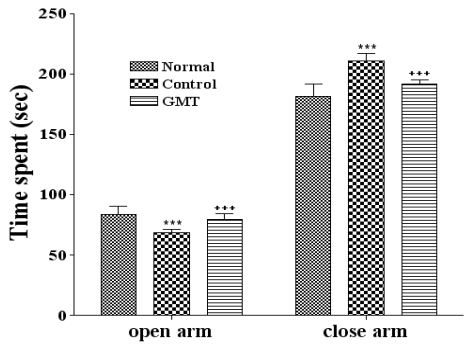

Fig. 1. The effects of GMT extract on the elevated plus maze. Data represent means±SEM. ***p<0.001 compared to Normal, +++p<0.001 compared to the Control.

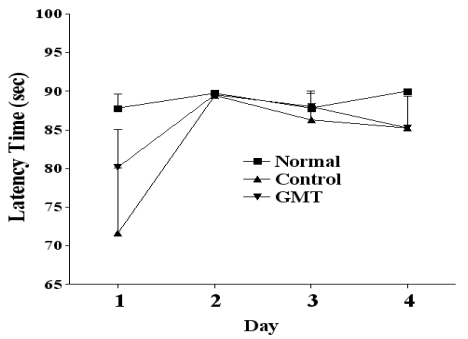

Fig. 2. The effects of GMT extract on the passive avoidance test. Data represent means±SEM.

2. Effect of GMT on passive avoidance task

Fig. 2 shows mean group escape latencies in the PAT for all groups for 4 days. The escape latency was not differed from among the groups when the results were averaged over all the session. However, the Control group showed a worse performance than did the Normal group.

3. ChAT immunoreactive neurons of the hippocampus

The results of determining the ChAT immunoreative cells per section from different hippocampal formations are shown in Fig. 3 (A) and (B). The number of ChAT neurons in the CA1 area was 10.0±0.5 in the Normal group, 3.2±0.5 in the Control group, 5.4±0.5 in the GMT group [$F_{2,44}=68.9$, $p<0.001$]. The ChAT immunoreactive cells in the CA3 area were 18.4±0.6 in the Normal group, 11.0±0.7 in the Control group, 12.4±1.1 in the GMT [$F_{2,44}=32.9$, $p<0.001$].

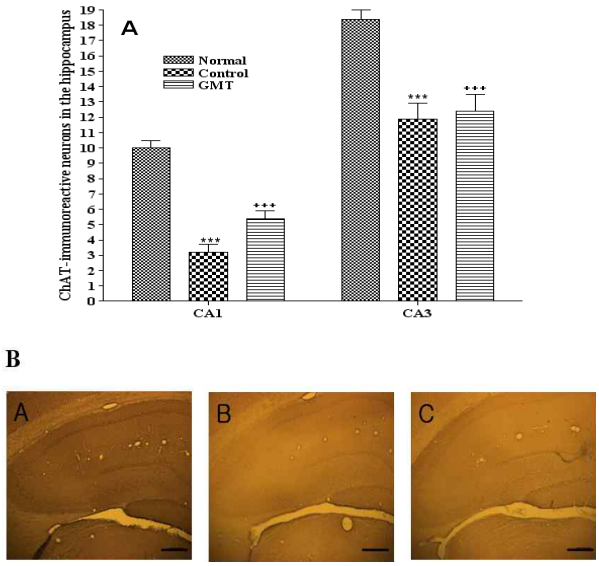

Fig. 3. A) The mean (±S.E.M.) values of quantities of choline acetyltransferase (ChAT) immunostained nuclei in the hippocampus. ***p<0.001 compared to Normal, +++p<0.001 compared to the Control. B) Representative photographs of ChAT-immunostained brain sections. A: Nomral group, B: Control group and C: GMT group. Sections were cut coronally at 30 ㎛ and the scale bar represents 100 ㎛ (100x100)

Discussion

The present study investigated a dose-dependent antidepressant effect following Gammakdaejo-Tang in the elevated plus maze, passive avoidance test and ChAT immunostaining. Gammakdaejo-Tang has been used frequently in traditional Oriental medicine for treating depressant. There may be a lot of chemical constituents in the formulation and the interaction of these constituents are not well known. However, Glycyrrhiza uralensis, compounds of

GMT, it is widely used as a flavoring adjuvant in drug preparations and an ingredient of cigarettes for its taste and properties that reduce irritation[13,14]. It has also been reported that an aqueous extract of G. Glara L. Showed significant antidepressant-like activity in mouse tail suspention test[15]. Zhao et al., repoted that the antidepressant-like effect of liquiritin from *Glycyrrhiza uralensis* in chronic variable stress induced depression model rats[16].

In the EPM experiment, the Control group had more anxiety than the Normal group did. The treatment of Gammakdaejo-Tang significantly decreased the anxiety. Although the mechanisms associated with these findings are unknown, synaptic plastic changes occur in the central nervous system[12], perhaps through the immediate induction of early gene expression[17,18]. Severe changes shown interference of previous stressful situations in animal models of anxiety. For example, restraint stress or exposure to predator was decreased time spent in open arm and increased time spent enclosed arm exploration of the EPM[19,20]. Thus the spent time in the open arms was increased after treatment of Gammakdaejo-Tang and the increased latency may reflect the anxiolytic-like effects of acupuncture.

Also, chronic stress situations also resulted in deficits in new learning and memory for escape behavior. In the passive avoidance task experiment, the Control group showed the memory impairment compared to the Normal group (at 2nd day). However, the GMT group exhibited improved memory function compared to the Control group. During the training trial in a step-through inhibitory avoidance task after shock, there were no differences among the groups. GMT seems to be more effective inreversing the memory impairment of late phase, rather than early phase, suggesting that it may facilitate process of memory consolidation. In ChAT histochemistry, the GMT group showed higher ChAT reactivity in both hippocampal CA1 and CA3. This study demonstrated that the cholinergic system might be affected by exposure to chronic stress[10]. These results are consistent with previous reports showing that the cholinergic neurons in the brain are involved in learning and memory in humans and animals[21,22]. In particular, the hippocampus cholinergic neurons are involved in the formation and maintenance of short-term working memory or retention and retrieval processes in long-term reference memory[23-27]. Based on a previous study, this result suggests that the GMT administration can promote the memory function.

In conclusion, our results suggest that GMT, an oriental herbal medicine, exerts antidepressant-like effects in rats. However further studies are necessary to confirm and extend these result. Somehow, the findings presented here are relevant because they validate the uses of GMT, an important medicinal formula used in many Asia counties.

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