

Brief Communication

Effect of Hochu-ekki-to (TJ-41), a Japanese Herbal Medicine, on Daily Activity in a Murine Model of Chronic Fatigue Syndrome

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We aimed to evaluate the effect of a Japanese herbal medicine, Hochu-ekki-to (TJ-41), on daily activity in a murine model of chronic fatigue syndrome (CFS). CFS was induced by repeated injection of *Brucella abortus* (BA) antigen every 2 weeks. TJ-41 was orally administered to mice in a dose of 500 mg/kg/day for 1 week before injecting BA and for 4 weeks thereafter. We evaluated daily running activity in mice receiving TJ-41 as compared with that in untreated mice. Survival of both mouse groups was also monitored during the observation period. Body weight (BW), spleen weight (SW), SW/BW ratio and expression levels of interleukin-10 (IL-10) mRNA in spleen were determined in both groups at the time of sacrifice. The daily activity was significantly higher in the treated group than in the control. Two mice in the untreated group died 2 days after the second injection of BA, whereas no mice in the group treated with TJ-41 died. The SW and SW/BW ratio were significantly lower in the treated mice than in the control. Suppressed IL-10 mRNA levels were observed in the spleens of the mice treated with TJ-41. Our data suggest that Hochu-ekki-to might possess an inhibitory effect on the marked decrease in running activity following BA injection.

Keywords: Hochu-ekki-to – herbal medicine – daily activity – chronic fatigue syndrome

Introduction

Fatigue is a common symptom observed in general population, with up to 50% of the respondents reporting fatigue in a survey (1). It is also presented as a symptom by at least 20% of individuals seeking medical care (2). This fatigue is usually transient, self-limiting and explained by prevailing circumstances (3). When the fatigue cannot be explained by a medical condition, it may represent chronic fatigue syndrome (CFS).

Typical presentation of CFS is characterized by sudden onset of a flu-like illness in which the predominant symptom is severe and lasting fatigue that greatly reduces activity. The hypotheses regarding the etiology of CFS propose the involvement of a specific viral infection and immune dysfunction associated with

the infection, although it remains an important area for research. Studies in the past have reported an immunologic model of fatigue following intraperitoneal injection of bacterial antigen; however, the duration of fatigue evaluated by wheel running was found to be short (4). Ottenweller *et al.* (5) have described the establishment of a potential murine model to study CFS which could be induced by *Brucella abortus* (BA) treatment. In this model, the mice were placed in running wheels and intravenously injected with the killed BA antigen, which produces an acute immune response (5). The mice were found to decrease their voluntary activity in the running wheel after the injection of the BA antigen (5). The administration of this antigen has previously been reported to produce modification in cytokine gene expression in the lymphocytes of mice (6).

Survey data from the United Kingdom show that herbal medicine has been tried by about 30% of the British population (7). Hochu-ekki-to (TJ-41) is one of the herbal medicines prescribed for viral infections, bacterial infections or post-surgical shock. It has been reported to promote certain biological

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activities including enhancement of NK cells (8) and macrophage (9) activity. It has also been indicated that Hochu-ekki-to has an inhibitory effect on influenza virus infection via enhancement of host immune responses in virus-infected mice (10).

In this paper, we describe the effect of a Japanese herbal medicine, Hochu-ekki-to (TJ-41), on daily activity in a mouse model of CFS.

Subjects and Methods

Mice and Experimental Conditions

Female BALB/c mice, 8 weeks of age, were obtained from Charles River (Kanagawa, Japan), and housed singly in cages (230 × 100 × 100 mm) including running wheels (230 mm in diameter), counters showing running wheel activity and water taps, which were obtained from Natsume Seisakusho Co., Ltd. (Tokyo, Japan). These cages were maintained under a light-dark photoperiod (10 h vs 14 h) provided by fluorescent bulbs fitted in the cage floor. We fed all the mice ($n = 20$) every day during the course of the experiment. Environmental air temperature was maintained at 24–25°C. The daily running activity of mice was defined as the number of wheel turns per 24 h. The running activity was measured at 9 o'clock when the environmental lighting was turned on. Approval for this experiment was obtained from the animal experiment committee in Kanazawa Medical University.

Induction of CFS by BA

Fixed killed whole BA ring test antigen was obtained from the National Veterinary Services Laboratories in the United States Department of Agriculture. CFS was induced by repeated twice injection of original BA antigen solution (0.2 ml per mouse) via the tail vein every 2 weeks. In the pilot experiment, we found that the BA-injected mice showed less running activity for 2–3 weeks after injection.

Treatment of Mice with Hochu-ekki-to

We obtained Hochu-ekki-to (TJ-41) that contains a mixture of spray-dried hot water extracts of 10 medicinal plants from the Ibaraki Plant of Tsumura Co., Ltd. (Tokyo, Japan). The 10 medicinal plants are *Astragali radix* (16.7%), *Atractyloclis lanceae rhizoma* (16.7%), *Ginseng radix* (16.7%), *Angelicae radix* (12.5%), *Bupleuri radix* (8.3%), *Zizyphi fructus* (8.3%), *Aurantii nobilis pericarpium* (8.3%), *Glycyrrhizae radix* (6.3%), *Cimicifugae rhizoma* (4.2%) and *Zingiberis rhizoma* (2.0%). This agent was dissolved in distilled water and diluted with water to the appropriate concentration. TJ-41 solution was administered orally in a dose of 500 mg/kg once daily through a feeding needle inserted down the throat of the mice ($n = 10$) for 1 week before the induction of CFS and for 4 weeks thereafter. The dose of the herbal medicine was determined on the basis of findings of previous reports (11). Untreated mice ($n = 10$) were given saline during the same period. The mice enrolled into the experiment were randomly assigned to the treated or the control group.

Parameters Determined for Evaluation of Hochu-ekki-to

We started to examine the running activity (2 weeks) at baseline 2 weeks after the mice were housed, since the activity was stabilized after 2–3 weeks of housing (5). Daily activity during 2 weeks after each injection of BA was evaluated in the mice receiving TJ-41 as compared with that in the untreated mice. Survival in both groups was also monitored during the observation period. The mice in both groups were sacrificed by cervical dislocation 4 weeks after the first BA injection. Ratios of spleen weight (SW) (mg) to body weight (BW) (g) (SW/BW), thymus weight (TW) (mg) to BW (TW/BW), heart weight (HW) (mg) to BW (HW/BW) and lungs weight (LW) (mg) to BW (LW/BW) as well as the weights of the organs and the body were assessed between both groups at the time of sacrifice. Expression levels of interleukin-10 (IL-10) mRNA in spleens from both groups were determined by using real-time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) as described previously (5). Optimum number of cycles within the RT-PCR was examined for the IL-10 mRNA levels. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an endogenous internal standard, and was amplified with specific primers for the number of cycles. The IL-10 mRNA levels were calculated as comparative values, which were normalized to the cytokine mRNA in the spleen from normal female BALB/c mouse (value = 1).

Statistical Analysis

Data are expressed as mean values \pm SD. Data differences between the mice treated with Hochu-ekki-to and the control were analyzed by the unpaired Student's *t*-test. A *P* value of <0.05 was considered to be statistically significant.

Results

Daily Running Activity in Mice

The daily running activity of both mouse groups before and after the induction of CFS is presented in Fig. 1. Baseline levels of daily activity were not significantly different between both the groups. However, the daily activity was significantly higher in the group treated with herbal medicine than in the control during the 2 weeks after each BA injection (8157.6 ± 3287.6 vs 5884.3 ± 2977.5 and 5584.9 ± 2897.4 vs 2758.5 ± 2612.1 during the 2 weeks after the 1st and 2nd injection, respectively, $P < 0.05$). Two mice in the untreated group died 2 days after the 2nd injection of BA, whereas no mice in the group treated with TJ-41 died.

Organ Weights and Expression Levels of IL-10 mRNA in Spleen

BW, SW, TW, HW and LW in both groups are shown in Table 1. Significant decrease in SW was observed in the mice treated with TJ-41 as compared with that in the control (546 ± 37 mg vs 693 ± 107 mg, respectively, $P < 0.05$), while there

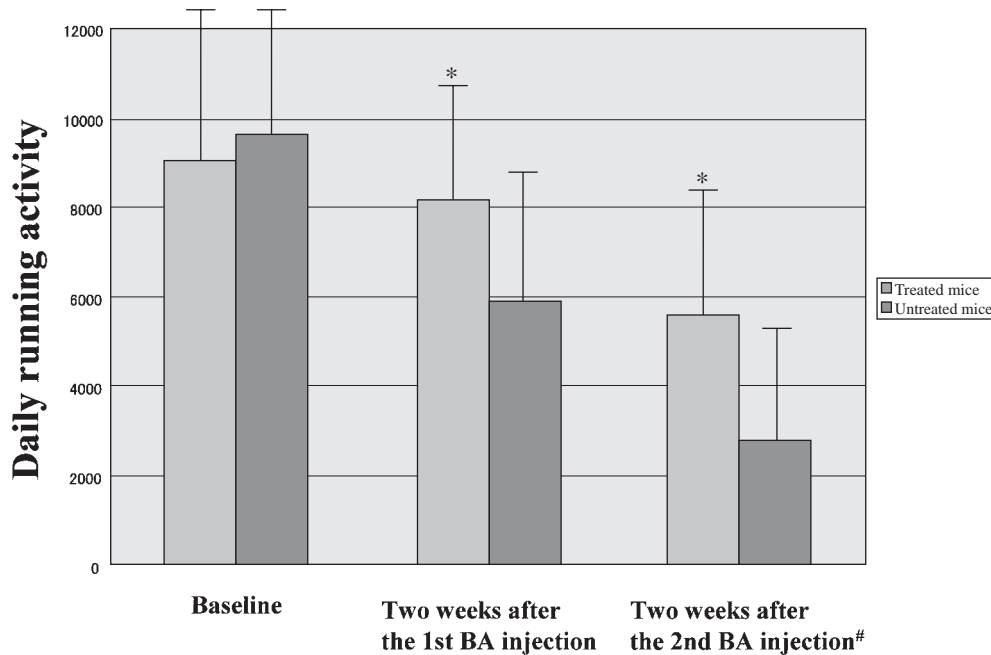


Figure 1. Effect of Hochu-ekki-to on the daily running activity in a mouse model of chronic fatigue syndrome. Data are expressed as means \pm SD. BA, *Brucella abortus*. Daily running activity was defined as the number of wheel turns per 24 h. * $P < 0.05$ compared with the daily activity in the untreated mice. † Two mice in the untreated group died 2 days after the second injection of BA, while no mice in the treated group died.

Table 1. Effect of Hochu-ekki-to on body weight and weight of organs including spleen, thymus, heart and lungs at the time of sacrifice

	BW (g)	SW (mg)	TW (mg)	HW (mg)	LW (mg)
Treated mice ($n = 10$)	22.6 \pm 2.3	546 \pm 37*	43 \pm 12	118 \pm 19	149 \pm 11
Untreated mice ($n = 8$)	21.8 \pm 1.1	693 \pm 107	47 \pm 25	110 \pm 22	154 \pm 21

Data are expressed as means \pm SD. BW, body weight; SW, spleen weight; TW, thymus weight; HW, heart weight; LW, lungs weight. * $P < 0.05$ compared with spleen weight in the untreated mice.

were no differences in BW and the weights of other organs between both the groups (Table 1). SW/BW ratio was significantly lower in the group treated with TJ-41 than in the untreated group at the time of sacrifice (24.2 ± 1.4 vs 31.8 ± 6.3 , respectively, $P < 0.05$), whereas TW/BW, HW/BW and LW/BW ratios were not significantly different between both the groups. IL-10 mRNA levels in spleens of the treated mice ($n = 6$) were significantly lower than those in the control ($n = 4$) (5.47 ± 1.81 vs 11.39 ± 3.99 , respectively, $P < 0.05$).

Discussion

Running wheel activity is a comparatively sensitive indicator to measure the severity of CFS. It has been reported that blinded observers could not distinguish BA-injected mice from saline-injected mice 11 days after the injection on the basis of poor grooming (5). In practice, treatment of CFS, whether pharmacological or non-pharmacological, has generally been directed toward relieving symptoms and improving the impaired functions (3). Therefore, the therapeutic effect of this herbal medicine on the running behavior is considered to be a more robust evidence than the effect on grooming.

Chao *et al.* (4) have previously reported that splenic enlargement (mg/g body weight) was found on day 12 post-inoculation in a murine model of immunologically mediated fatigue

(24.1 ± 1.8 in the *Corynebacterium parvum* group and 20.2 ± 1.6 in the *Toxoplasma gondii* group vs 5.3 ± 0.2 in the saline group). Marked elevation of SW/BW ratio after the repeated BA injection was also observed in the present experiment, and a significant reduction in SW/BW ratio was found in the mice treated with the herbal medicine, suggesting that the measurement of splenic enlargement might be useful to monitor the improvement in immune stimulation during treatment.

We found a significant decrease in IL-10 mRNA levels in the spleens of the mice treated with TJ-41 as compared with those from the untreated mice. Abnormal expression of various cytokine (IL-10, IL-4, IL-2 and interferon- γ) genes in the spleen has been demonstrated in previous reports (5,6). Circulating concentrations of these cytokines also need to be measured for the evaluation of therapeutic effects in this mouse model, since the measurement of circulating cytokine levels will potentially be applied to future clinical studies.

CFS has been described to possess significant symptom overlap and comorbidity with psychiatric disorders (3). Effects of herbal medicines including Hochu-ekki-to on behavioral despair and acetic acid-induced writhing in mice has been reported earlier, suggesting that Kampo medicines may have anti-depressive and anti-nociceptive properties (12). Results of our study may also be associated with the psychiatric profile of Hochu-ekki-to.

There are few studies demonstrating the effectiveness of alternative and complementary approaches including herbal therapies for patients with CFS (3). Daily running activity in a mouse model of CFS was significantly higher in the group treated with Hochu-ekki-to (500 mg/kg/day) than in the control. SW/BW ratio was significantly lower in the treated mice than in the control at the time of sacrifice. Our results suggest that Hochu-ekki-to treatment might have an inhibitory effect on the marked decrease in running activity following the BA injection via modulation of host immune responses, although the optimum dose and the appropriate time of initiating the treatment of this herbal medicine have not been established in this study. The difference between the effectiveness of this agent and a positive control drug such as a western medicine should also be assessed. Intraperitoneal treatment with potent antioxidants such as carvedilol (5 mg/kg) and melatonin (5 mg/kg) has been shown to produce a significant decrease in the immobility period in a mouse model of CFS (13). Further studies are required to elucidate these issues.

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