Effects of Kamikihi-To, a Traditional Chinese Medicine, on Passive and Conditioned Avoidance Performance Impairment in Senescence Accelerated Mouse (SAM)

Koji NISHIZAWA*, Hiroshi SAITO and Nobuyoshi NISHIYAMA
Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo Bunkyo-ku, Tokyo 113, Japan

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Abstract—Effects of Kamikihi-To (KMK), a traditional Chinese medicine (Chinese name: Jia-Wei-Gui-Pi-Tang), on learning performance impairment caused by aging were evaluated in senescence accelerated mice (SAM). Normal diet containing 8% KMK extract was given to SAM-P/8, a senile-prone strain, and to SAM-R/1, a resistant strain, from 2 months old. Effects of KMK on learning performance were evaluated in 5 and 10 month old SAM using step through and step down type passive avoidance tests and shuttle box and lever press type conditioned avoidance tests. At 5 months old, KMK increased the retention rate in the step through test and decreased the number of errors in the step down test in SAM-P/8, though KMK had no effects in conditioned avoidance tests. KMK had no effects in any tests in SAM-R/1. At 10 months old, the decrease of the number of errors in the step down test and increase of the rate of the conditioned avoidance response in the shuttle box test were observed in SAM-P/8 treated with KMK. These results suggest that chronic administration of KMK can improve learning performance in the senescence model.

Kamikihi-To (KMK, Chinese name: Jia-Wei-Gui-Pi-Tang) is a traditional Chinese medicine which was first described in "Nei-Ke-Zhai-Yao" (1529 A.D.). It has been used to treat insomnia, anemia, amnesia, palpitation, and neurosis brought on by physical and mental overwork. KMK consists of Astragalus, Ginseng, Atractylodes, Hoelen, Polygala, Jujube, Longan, Zizyphus, Angelica, Licorice, Ginger, Saussurea, Bupleurum and Gardenia. Among these herbs, Hoelen, Astragalus, Ginseng, Longan and Polygala have been described as having anti-amnesia properties as their chief action in the Chinese herbal dictionary "Zhong-Yao-Da-Ci-Dian". In the previous studies, we reported that KMK ameliorated ethanol-induced or electroconvulsive shock-induced memory impairment in young adult mice (K. Nishizawa et al., unpublished observation).

Senescence-accelerated mouse (SAM) consists of the senescence-accelerated prone mouse (SAM-P) and senescence-accelerated resistant mouse (SAM-R), the latter of which shows normal aging. SAM-P/8 separated from several SAM-P substrains is characterized by age related deficits in learning performances (1).

In this investigation, the effects of chronic KMK administration on learning performances of SAM-P/8 were evaluated in passive and conditioned avoidance tests in comparison with those of control mice, SAM-R/1.

Materials and Methods

Animals

The substrains of SAM, SAM-P/8 and R/1 were originally obtained from Prof. T. Takeda (Chest Disease Research Institute, Kyoto University) and bred in our laboratory. Mice were housed individually in aluminum cages
under temperature- and humidity-controlled conditions (22±1°C, 55±2%), and they were given food and water ad libitum.

**Extraction of KMK**
KMK was provided by Kanebo Co., Ltd. (Osaka, Japan). The drug is a spray-dried powder of a hot-water extract prepared (with 10 parts of water at 95–100°C for 1 hr, yield: about 19%) from a mixture of the following fourteen constituent herbs: Astragalus (*Astragali radix*), Ginseng (*Ginseng radix*), Atractylodes (*Atractylodes rhizoma*), Hoelen (*Hoelen*), Polygala (*Polygalae radix*), Jujube (*Zizyphi fructus*), Longan (*Longanae arillus*), Zizyphus (*Zizyphi spinosi Semen*), Angelica (*Angelicae radix*), Licorice (*Glycyrrhizae radix*), Ginger (*Zingiberis rhizoma*), Saussurea (*Saussureae radix*), Bupleurum (*Bupleuri radix*) and Gradenia (*Gardeniae fructus*).

**Administration of KMK**
Male SAM-P/8 and R/1 were given the normal diet (CE-2, Japan Clea Co. Ltd., Tokyo, Japan) until 2 months of age. Thereafter, SAM-P/8 and R/1 were given continuously the normal diet (P/8-C and R/1-C, respectively) or that containing 8% KMK extract for 3 or 7 months (P/8-KMK and R/1-KMK, respectively: 5 months old; P/8-C N=12, P/8-KMK N=12, R/1-C N=12, R/1-KMK N=12: 10 months old; P/8-C N=10, P/8-KMK N=9, R/1-C N=12, R/1-KMK N=11). The average of the KMK dosage was about 10 g/kg/day. There were no significant differences in food intake and in body weight between the control and KMK treated group in both SAM-P/8 and R/1.

**Behavioral experiments**
Behavioral experiments were conducted in SAM when they were 5 and 10 months old, respectively. KMK diet or normal diet was given until the final day of the behavioral experiments.

1. **Passive avoidance tests**
The step through test was performed before the step down test every day using the same animals.

1-1. **Step through test:** The apparatus consists of a bright compartment and a dark one which were partitioned by a wall with a round opening in the middle (2). A mouse was placed in the bright compartment. When a mouse stepped into the dark compartment through the opening, it was given a punishing shock of 36 V AC and turned back to the bright compartment as a learning trial. From the following day on, the mouse was placed in the bright compartment again for a maximum of 5 min (when the mouse stepped through into the dark compartment within 5 min, it was taken out immediately and returned to its home cage) every day at the same time of day for 10 days as a testing trial. The numbers of mice which had not stepped through into the dark compartment in the testing trials were recorded every day and the percentage of animals which passed the 5 min cut-off period successively until the designated day was computed (retention rate). At the end of the experiment, the number of days on which the mice stepped through during the 10 day-testing trials was also recorded.

1-2. **Step down test:** A mouse was put on the rubber platform (35 mm in diameter at the top, 40 mm in height) in the apparatus with a grid floor for 10 min (2). When the mouse stepped down and its forepaws touched the grid floor, it was given a punishing shock of 50 V AC and jumped back onto the platform as a learning trial. From the following day on, the mouse was put on the rubber platform for 3 min every day at the same time of day for 10 days as a testing trial. The numbers of mice that had not stepped down onto the floor in the testing trials were recorded every day and the percentage of animals that cleared the 5 min cut-off period successively until the designated day was computed (retention rate). At the end of the experiment, the number of step-down events during the 10 day-testing trials was also recorded.

2. **Motor activity**
The experimental apparatus for the measurement of motor activity in mice was a tilting-type round activity cage (18 cm in diameter and 18 cm in height) MA001; O'Harra Co., Ltd., Tokyo, Japan). Measurement of motor activity was conducted for 30 min at 5 and 10 months old.

3. **Conditioned avoidance tests**
The apparatuses and conditions were described previously (3). The temporal parameters were as follows: intertrial interval of 40 sec; warning sound with durations of 20 sec, sound only for the first 10 sec and sound with
shock (an intensity of 36 V AC) for the remaining 10 sec. Each session consisted of 60 trials per 1 hr a day. From the next day of the last passive avoidance tests, the shuttle box test was conducted for 7 days, and subsequently, the lever press test for 8 days. The indices of the avoidance behavior were conditioned avoidance response (CAR: the number of avoidance responses during warning term/the number of trials), escape response (the number of escape responses during shocking term/the number of trials), escape failure (the number of non-avoidance or non-escape responses/the number of trials) and intertrial response (ITR: spontaneous movement or lever press not induced by conditioned and unconditioned stimuli/session) during each session.

**Statistics**

The number of mice that did not fail in the passive avoidance tests was analyzed with the χ²-test. Step through latency was analyzed with the Mann-Whithey U-test. For CAR, escape response, escape failure, ITR, motor activity and the number of step through and step down events, ANOVA followed by Student’s t-test was used.

**Results**

1. Passive avoidance tests

1-1. Effects of KMK on step through type passive avoidance tests in SAM-P/8 and R/1

![Graph A](image1)

![Graph B](image2)

Fig. 1. Effects of KMK on retention rate in the step through test in SAM-P/8 and R/1. Retention rates, incidence of mice which had not stepped through into the dark compartment until the designated day of the 10 day testing period in the step through test in SAM-P/8 and R/1 at 5 months old (A) and 10 months old (B) are shown. P/8-C: normal diet group in SAM-P/8, P/8-KMK: KMK extract diet group in SAM-P/8, R/1-C: normal diet group in SAM-R/1, R/1-KMK: KMK extract diet group in SAM-R/1 (N=9-12). *P<0.05 between P/8-C and P/8-KMK, **P<0.01 between P/8-C and R/1-C.
at 5 and 10 months old: Effects of KMK on the retention rate in the step through test in SAM-P/8 and R/1 at 5 months old are shown in Fig. 1A. On the first day of the testing trials, all groups retained high retention rates (83–100%). From day to day, the number of mice which had not failed in the testing trials decreased in the normal diet group of P/8 (P/8-C). In contrast, the KMK diet group of P/8 (P/8-KMK), normal diet group of R/1 (R/1-C) and KMK diet group of R/1 (R/1-KMK) continuously retained high retention rates than P/8-C. On the last day, retention rates of P/8-C, P/8-KMK, R/1-C and R/1-KMK were 25%, 58%, 75% and 67%, respectively. While P/8-C showed a lower retention rate than R/1-C in every testing trial except for the first day, P/8-KMK showed significantly higher retention rate than P/8-C on the 4th day of the testing trials. However, KMK did not affect the retention rate in SAM-R/1.

More severe decrement of retention rate, which may be caused by aging, was observed in SAM-P/8 and R/1 at 10 months old (Fig. 1B). There was no difference between P/8-C and R/1-C on retention rate. KMK showed no influence on retention rate in SAM-P/8 and R/1.

The total number of errors when the 5- and 10-month old mice stepped through into the dark compartment during the 10 day-testing trials was as follows: Five months old, P/8-C: 1.0±0.2 (P<0.05 vs. R/1-C), P/8-KMK: 0.6±0.2, R/1-C: 0.3±0.1, R/1-KMK: 0.4±

![Fig. 2. Effects of KMK on retention rate in the step down test in SAM-P/8 and R/1. Retention rates, incidence of mice which had not stepped down onto the foot shock floor until the designated day of 10 day testing period, in the step down test in SAM-P/8 and R/1 at 5 months old (A) and 10 months old (B) are shown (N=9–12). *P<0.05, **P<0.01, ***P<0.001 between P/8-C and R/1-C. See the legend to Fig. 1 for details.](image-url)
0.2; Ten months old, P/8-C: 2.7±0.7, P/8-KMK: 2.1±0.5, R/1-C: 1.3±0.3, R/1-KMK: 1.3±0.3. Thus KMK had no effect on the number of errors in the step through test in SAM-P/8 and R/1 at 5 and 10 months of age.

1-2. Effects of KMK on step down type passive avoidance tests in SAM-P/8 and R/1 at 5 and 10 months old: Effects of KMK on the retention rate in the step down test in SAM-P/8 and R/1 at 5 months old are shown in Fig. 2A. The retention rate decreased in P/8-C earlier than in R/1-C, whereas KMK did not affect the retention rate in SAM-P/8 and R/1.

More severe decrement of retention rate was observed in SAM-P/8 and R/1 at 10 months old which was considered to be caused by aging. There was no difference between P/8-C and R/1-C on the retention rate in the step down test as well as in the step through test at 10 months old (Fig. 2B). Furthermore, KMK had no effect on the retention rate in 10 months old SAM.

Table 1 showed the effects of KMK on the number of step down events during 10 day-testing trials. The number of step down events during 10 day-testing trials was significantly higher in P/8-C than R/1-C at both 5 and 10 months old. At 5 months old, KMK tended to decrease the number of step down events in SAM-P/8. At 10 months old KMK significantly decreased the number of step down events in SAM-P/8. KMK had no influence on the number of step down events in SAM-R/1.

2. Motor activity

Effects of KMK on motor activity in SAM-P/8 and R/1 at 5 and 10 months old are shown in Table 2. Motor activity of each group at 10 months old was lower than that at 5 months old. At 10 months old, motor activity of P/8-C was lower than that of R/1-C, although there was no difference between P/8-C and R/1-C in motor activity at 5 months old. KMK ameliorated the decrease of motor activity in SAM-P/8, without affecting that of SAM-R/1.

3. Conditioned avoidance tests

3-1. Effects of KMK on acquisition in the shuttle box test in SAM-P/8 and R/1 at 5 and 10 months old: Effects of KMK on acquisition in the shuttle box test in SAM-P/8 and R/1 at 5 and 10 months old are shown in Fig. 3. The conditioned avoidance response (CAR) of P/8-C was continuously lower than that of R/1-C at both 5 and 10 months old. KMK significantly increased CAR and decreased the escape response of the first session in 10 month-old SAM-P/8. KMK, however, had no effects on CAR and escape response of any other session in SAM-P/8 and R/1 at both 5 and 10 months old. There were no significant differences in intertrial response (ITR) between P/8-C and R/1-C at 5 months old. ITR of P/8-C was lower than that of R/1-C at 10 months old.

Table 1. Effects of KMK on the number of step down events during 10 days in SAM-P/8 and R/1

<table>
<thead>
<tr>
<th>Group</th>
<th>5 Months old</th>
<th>10 Months old</th>
</tr>
</thead>
<tbody>
<tr>
<td>P/8-C</td>
<td>3.6±0.8**</td>
<td>4.3±0.6**</td>
</tr>
<tr>
<td>P/8-KMK</td>
<td>1.8±0.4</td>
<td>2.6±0.5*</td>
</tr>
<tr>
<td>R/1-C</td>
<td>0.3±0.1</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td>R/1-KMK</td>
<td>0.3±0.1</td>
<td>0.8±0.3</td>
</tr>
</tbody>
</table>

Number of step down events during 10 days is shown. Each value represents the mean and S.E.M. (N=9-12). *P<0.05, between P/8-C and P/8-KMK; **P<0.01, between P/8-C and R/1-C.

Table 2. Effects of KMK on motor activity in SAM-P/8 and R/1

<table>
<thead>
<tr>
<th>Group</th>
<th>5 Months old</th>
<th>10 Months old</th>
</tr>
</thead>
<tbody>
<tr>
<td>P/8-C</td>
<td>293±35</td>
<td>102±17#</td>
</tr>
<tr>
<td>P/8-KMK</td>
<td>297±36</td>
<td>228±34**</td>
</tr>
<tr>
<td>R/1-C</td>
<td>426±67</td>
<td>244±67</td>
</tr>
<tr>
<td>R/1-KMK</td>
<td>460±72</td>
<td>289±66</td>
</tr>
</tbody>
</table>

Motor activity during 30 min is shown. Each value represents the mean and S.E.M. **P<0.01, between P/8-C and P/8-KMK; *P<0.05, between P/8-C and R/1-C.
KMK did not alter the ITR at both ages, suggesting that its effect on CAR was not due to the increase of motor activity. There was no difference of escape failure in two substrains at both ages.

3-2. Effects of KMK on acquisition in the lever press test in SAM-P/8 and R/1 at 5 and 10 months old: Effects of KMK on acquisition in the lever press test in SAM-P/8 and R/1 at 5 and 10 months old are shown in Fig. 4. CAR of each group in the lever press test was lower than that in the shuttle box test in both SAM-P/8 and R/1. Especially, CAR of P/8-C and P/8-KMK were hardly increased after 8 sessions at both ages. KMK did not influence CAR in both SAM-P/8 and R/1. KMK increased ITR of SAM-R/1 at 10 months old without affecting that of SAM-P/8, the reason for which is not yet elucidated. KMK had no influences on escape response and escape failure in any tests (data not shown).

Discussion
At 5 months old, KMK ameliorated memory retention in the step through test and the number of errors in the step down test in SAM-P/8. However, KMK had no effects on any tests concerning learning and memory in SAM R/1. At 10 months old, the ameliorating effects of KMK on the number of errors were observed in the step down test and those on memory acquisition in the shuttle box test in SAM-P/8. These suggested that KMK improved memory dysfunction in senile animals.

The several age-related pathomorphological changes in the SAM-P/8 brain are summarized as follows: cell loss in the locus coeruleus and dorsolateral tegmental nucleus, lipopigmentation, thalamic neuronal inclusion, astrocytosis (4), PAS-positive intracellular granular structures (5), spongiform degeneration (6), and reduction of dendritic spine of the hippocampal pyramidal neurons (7). Tanaka et al. (8) have indicated that norepinephrine contents in the hippocampus and thalamus in SAM P/8 were lower than those in SAM-R/1. The memory impairment of SAM-P/8 may be caused by
these pathomorphological and biochemical changes.

Yagi et al. (9) reported that SAM-P/8 showed a remarkable age-related deterioration in ability of memory acquisition without impairing any other memory stages in the step through test. In this investigation, impairment of memory acquisition was observed in conditioned avoidance tests in SAM-P/8. However, the normal diet group in SAM-P/8 retained good memory, comparable to that of SAM-R/1, at the first day of the testing trials in the step through test at 5 months old, suggesting that SAM-P/8 had the ability of memory acquisition. Furthermore, the retention rate in the step through and step down tests and the number of errors in the step down test were also impaired in SAM-P/8 at 5 months old. These indicated that memory deficits of SAM-P/8 at 5 months old were not caused only by memory acquisition impairment.

In the previous investigation, we indicated that KMK ameliorated the impairments of memory registration, consolidation and retrieval in an acute memory impaired model (K. Nishizawa et al., unpublished observation). In the present study, KMK ameliorated some parameters of memory acquisition, retention and total number of step down events in SAM-P/8, emphasizing that KMK can promote several memory stages in memory impaired animals.

There were no differences in food intake and body weight between normal and KMK treated groups in SAM-P/8 and R/1. This result suggests that the ameliorating effects of KMK in SAM-P/8 were not due to general metabolic alteration but due to the memory promotion per se.

Peroxidized lipids occurring in animal tissues have been generally recognized to affect several cardiovascular, pulmonary, or hepatic diseases (10) and to be a principal cause of aging (11). Wang et al. (12) suggested that the contents of peroxidized lipid in the liver was higher in SAM-P/8 than in SAM-R/1, although those in brain were similar between them. Furthermore, they demonstrated that deer antler extract, the Japanese name is

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Fig. 4. Effects of KMK in the lever press test in SAM-P/8 and R/1. Data of the lever press test in SAM-P/8 and R/1 at 5 months old (A) and 10 months old (B) are shown (N=9–12). See the legend to Fig. 3 for details.
“Rokujo”, decreased peroxidized lipid contents in the brain and liver of SAM-P/8 and R/1. Therefore, we examined the effects of KMK on peroxidized lipid levels in the brain and liver by the thiobarbiturate method (13). KMK had no effects on peroxidized lipid contents in the brain and liver (K. Nishizawa et al., unpublished observation). This indicates that memory improvement effects of KMK in SAM are not caused by protection from lipid peroxidation.

There is not difference in motor activity between SAM-P/8 and R/1 at 5 months old. At 10 months old, the motor activity of SAM-P/8 is lower than that of SAM-R/1. KMK improved the decrease of motor activity in SAM-P/8 without affecting SAM-R/1 at 10 months old. This demonstrates that KMK ameliorates the decrease of volition induced by aging, which is one of the symptoms of Alzheimer’s and other senile diseases.

KMK ameliorated the transient memory dysfunction induced by ethanol or electroconvulsive shock (K. Nishizawa et al., unpublished observation). Furthermore, in this investigation, chronic administration of KMK showed ameliorating effects on learning and memory impairment in SAM, a senile model animal. From these results, we speculate that KMK might be useful for the treatment of memory deficits caused by aging such as senile dementia or decrease of volition induced by aging.

References
3 Segawa, M., Saito, H. and Nishiyama, N.: Alterations in choline acetyltransferase and tyrosine hydroxylase activities of various brain areas after the acquisition of active avoidance tasks in mice. Biogenic Amines 7, 171–180 (1990)