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HERBAL MEDICINE AND THE STUDY OF AGING IN SENESCENCE-ACCELERATED MICE (SAMP1TA/Ngs)

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Abstract—Two types of herbal medicine, Toki-Shakuyaku-San (TSS) and Boui-Jiou-Tou (BJT), were considered from the view point of their possible preventive effect on senility using the Senescence-Accelerated Mouse (SAMP1TA/Ngs), which is thought to be a useful model of human aging. The investigation of the effects of TSS and BJT on the behavior, learning ability, longevity, and histological changes in animals may be important for basic studies on aging in humans. Pellet feed containing each herbal medicine was given to the treated group *ad libitum* for 24 weeks starting at the age of six weeks. Learning ability and the longevity of mice were assessed and spontaneous motor activity was also measured. Morphological examinations were performed. On the passive avoidance task, the period until acquisition of standard achievement rate was shorter in the treated groups than in the control groups. The mean number and the density of dendritic spines were higher in the BJT treated group than in the control group. With regard to life span, median survival time tended to be longer in the treated groups than in the control groups. The results of this study suggested that TSS and BJT have some valuable effect for the prevention of senility. *Copyright © 1997 Elsevier Science Inc.*

Key Words: herbal medicine, aging, Senescence-Accelerated Mice, SAM, SAMP1TA/Ngs, aging of brain, brain dysfunction

INTRODUCTION

HERBAL MEDICINES are being used more and more frequently by physicians because drugs produced by chemical synthesis have not had the expected effect on age-related brain dysfunction and, in fact, sometimes cause side effects. Japanese herbal medicines basically reflect the traditions of Chinese medicine. But in the course of their use in Japan over hundreds of years, Japanese herbal medicines have shown a unique development as so-called "Kampo." In recent years it has become necessary to take their use one step further on the basis of past experience and to conduct a more scientific investigation of their effects.

Two types of herbal medicine were considered from the viewpoint of studies on aging. One

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is called "Toki-Shakuyaku-San (TSS)," an extract of six types of herbs, that has been used traditionally for a wide range of gynecological diseases such as ovarian insufficiency and endometriosis. Recently, TSS was also reported to have a useful pharmacological effect on neural transmitters in the brain (Koyama, 1989). The other is called "Boui-Jiou-Tou (BJT)," an extract of five types of herbs, that has been described in certain traditional Chinese books as a remedy for poriomania of several diseases such as senile dementia. The investigation of the effects of TSS and BJT on the behavior, learning ability, longevity, and histological changes in animals may be important for basic studies on aging and the quality of life in humans.

MATERIALS AND METHODS

Experimental animals

Male SAMP1TA/Ngs, an inbred strain of Senescence-Accelerated Mice (SAM) (Takeda *et al.*, 1981) raised in more than 20 successive generations under clean conventional conditions at the Laboratory Animal Center of Nagasaki University (Kishikawa *et al.*, 1994) was employed for the present study. The TSS study group consisted of 21 mice receiving TSS as a treated group and 22 untreated mice as a control group. The BJT study group consisted of 25 mice receiving BJT and 24 untreated mice as a control group.

Method of administration

In the TSS group, solid pellet feed (CE-2: Nihon CLEA, Tokyo, Japan) containing 0.044% TSS (Table 1), or a total dose of 80 mg/kg/day, was given to the treated group *ad libitum* for 24 weeks from the age of six weeks. In the BJT group, solid pellet feed containing 0.052% BJT (Table 2), or a total dose of 95 mg/kg/day, which is the recommended dosage for humans, was given to the treated group *ad libitum* for 24 weeks from just after weaning, that is, from the age of six weeks.

Learning ability and behavior

The measurement of learning ability and behavior was conducted at the age of seven months, just before sacrifice. Learning ability was measured by the Morris water maze method (Morris, 1981) and the step-down passive avoidance task (Nishimura *et al.*, 1990; Kishikawa *et al.*,

TABLE 1. COMPOSITION OF TSS

Ingredients [Herbs]	*Components*
Angelicae radix (3 g)	ligustilide, *n*-butylidenphthalide, sedanonic acid, safrol, palmitic acid, linolic acid, bergaptene, scopoletin, falcarinol, falcarindiol, vitamin B12, nicotic acid
Paeoniae radix (4 g)	paeoniflorin, oxypaeoniflorin, benzoylpaeoniflorin, albiflorin, paeonol
Cnidii rhizoma (3 g)	ligustilide, cnidilide, neocnidilide, butylphthalide, butylidenephthalide
Atractylodis lanceae rhizoma (4 g)	hinesol, β-eudesmol, elemol, atractylodin
Alismatis rhizoma (4 g)	alisol A, B, C; alisol A, B, C monoacetate; D-glucose; D-fructose; sucrose; β-sitosterol; lecithin; choline
Hoelen (4 g)	pachyman, eburicoic acid, pachymic acid, dehydroeburicoic acid, ergosterol, 3β-*o*-acetyltumulosic acid, 3β-*o*-acetyldehydrotumulosic acid

The above quantities are used to produce 4.0 g TSS extract, which is the daily dose for humans (80 mg/kg/day).

TABLE 2. COMPOSITION OF BJT

Ingredients [Herbs]	*Components*
Sinomeni caulis et rhizoma (2 g)	sinomenine, disinomenine, sinactine, tuduranine, acutmine, acutumidine, magnoflorine, tetrandrine, isotetrandrine, β-sitosterol, stigmasterol
Glycyrrhizae radix (2 g)	glycyrrhizin, glabric acid, liquiritin, licoricone, licoflavone, licoricidin, formononetin, putrescin, glycyrol, isoglycyrol, glycyrin, deoxoglycyrrhetol
Cinnamomi cortex (6 g)	cinnamic aldehyde, methoxycinnamic aldehyde, cinnamyl acetate, phenylpropyl acetate, cinnamic acid, cinncassiol A ~ E, cinnzeylanol, cinnezeylanine, cinnamoside, D-glucose, D-fructose, sucrose, (−)-epicatechin, procyanidin B-2, B-5, procyanidin C-1, cinnamtannin 1, melilotic acid, melilotic acid-*o*-glucoside
Saposhnikoviae radix (6 g)	deltoin, bergapten, psoralen, hamaudol, cimifugin, 5-O-methylvisamminol, falcarindiol, saposhikovan A, B, C
Rehmanniae radix (6 g)	catalpol, aucubin, β-sitosterol, mannitol, D-glucose, D-galactose, D-fructose, sucrose, raffinose, stachyose, arginine

The above quantities are used to produce 4.75 g BJT extract, which is the daily dose for humans (95 mg/kg/day).

1993). In the latter, the mice were subjected not only to a single trial, but also to electric shock when they stepped down from the second day onwards. This experiment was conducted to determine on what day the mice reached the standard achievement of 180 s. Spontaneous motor activity was measured using a tilting-type ambulometer (Kishikawa *et al.*, 1993). The tests were carried out on each mouse in the above order and at the same afternoon time, but an interval of at least one day was left between each test.

Age-related grading score

The grading score system (except the ocular findings) established by Hosokawa *et al.* (1984) was employed for this study.

Longevity

The survival time of the treated mice and control mice was also assessed.

Morphological examination

Soon after the measurement of behavior at seven months of age, the mice, other than those used for the longevity study, were sacrificed under pentobarbital sodium anesthesia. After fixation, 8 μm light microscope specimens were made from sections of the hippocampus and other regions of the brain. After H&E, PAS, and myelin staining, the sections were histologically examined. A morphometrical study on vacuolar change in the pontine reticular formation was carried out using an image analysis processor (nexusQube: Nexus Inc., Tokyo, Japan). Basal dendrites and dendritic spines of hippocampal pyramidal neurons from the BJT group were also studied by the rapid Golgi method (Ferrer and Gullotta, 1990).

Statistical analysis

The following methods were employed in the statistical analysis. The *t*-test was used to analyze brain weight and the Wilcoxon's rank-sum test to analyze spontaneous motor activity, number of vacuolar changes, and number and/or density of dendritic spines because the results

were all compared as mean values. Analysis of covariance was employed for body weight, grading score, and the assessment of latent periods in the water maze method because the serial changes had to be considered. The log-rank test, which is a common analysis method for survival, was suitable for assessments of longevity and latent periods in the step-down task because the latent periods in the passive avoidance task (step-down type) should be considered the censoring points of a maximum 180 s as standard achievement. All statistical analyses were performed by means of the statistical software SAS (SAS Institute, North Carolina, USA).

RESULTS

Body weight

No significant statistical difference was observed between the treated group and the control group in either the TSS or BJT study group (Figs. 1 and 2).

Age-related grading score

The patterns are almost identical in the groups fed TSS and BJT. There was no significant statistical difference, therefore, between the two groups using either herbal medicine, even at different ages (Figs. 3 and 4).

Learning ability

With regard to the Morris water maze method in the 24-week feeding groups, the TSS-treated group and control group scored an average of 46.5 and 61.0 s, respectively, on the first trial on the first day. On the last trial on the third day, they scored 16.9 and 23.0 s, respectively. In this study group, there seems to be a difference between the treated group and the control group (Fig. 5). But in fact the variance is large and no statistically significant difference exists. The groups

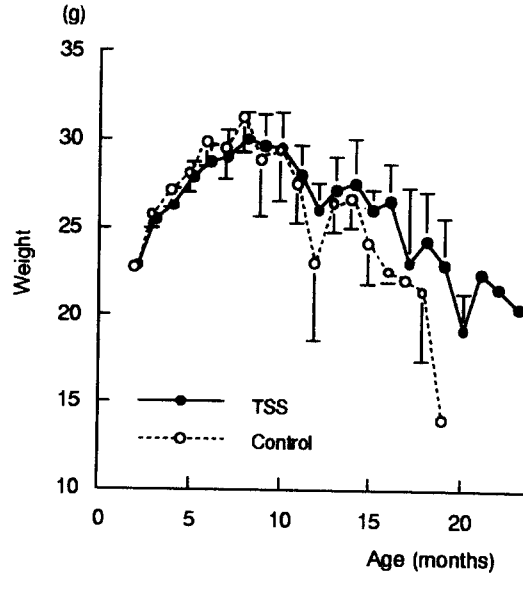

FIG. 1. Comparison of body weight of TSS group.

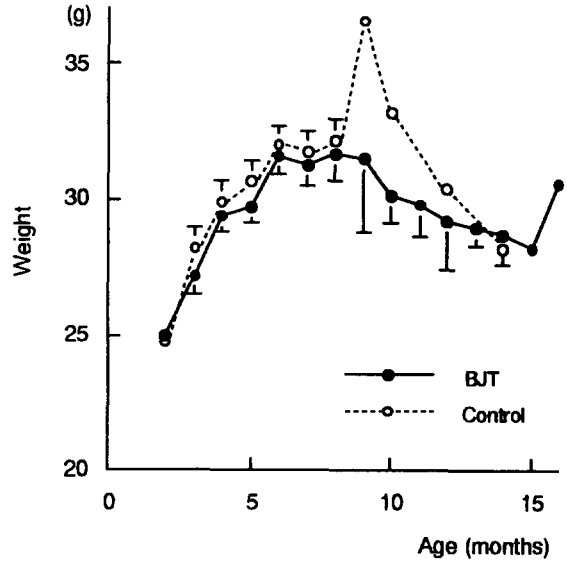

FIG. 2. Comparison of body weight of BJT group.

fed BJT for 24 weeks scored 51.2 and 49.1 s, respectively, on the first trial on the first day. On the last trial on the third day, the BJT treated group and control group scored an average of 14.8 and 19.0 s, respectively, or an almost identical pattern (Fig. 6). There was no significant statistical difference, therefore, between the two groups.

In the training trial on the first day in the passive avoidance task, the latent period was short

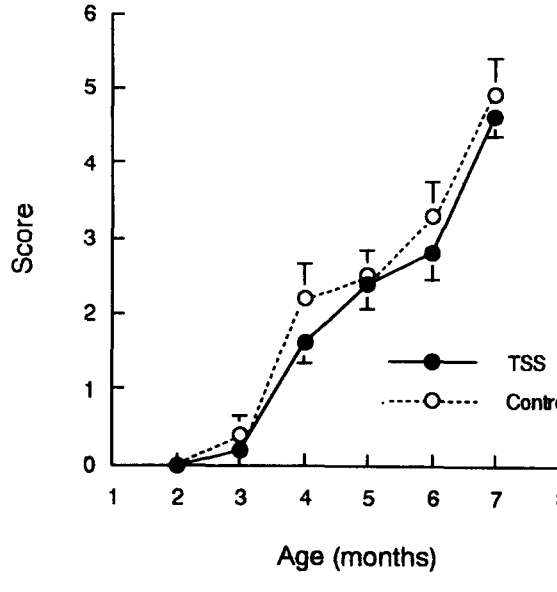

FIG. 3. Age-related grading score of TSS group.

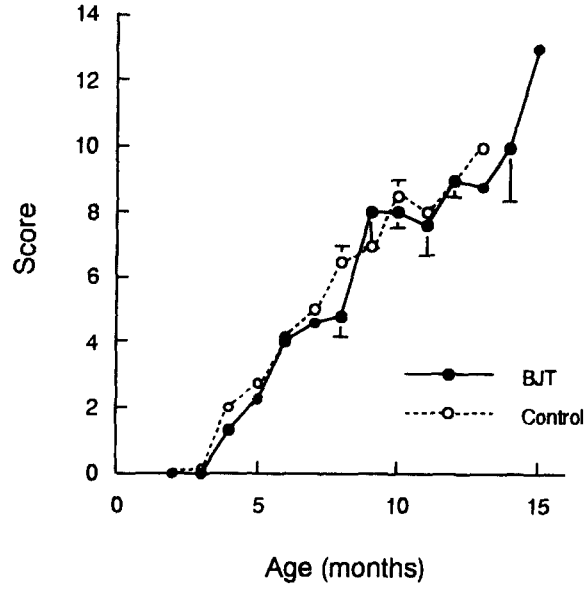

FIG. 4. Age-related grading score of BJT group.

in each study group in treated and control groups in each study group and there was no significant statistical difference between the treated groups and the control groups. In the first test trial (second day in Fig. 7), the median latent period was 60.4 s in the TSS treated group and 11.1 s in the control group, but the difference was not statistically significant. With regard to the day at which the mice reached the standard achievement of 180 s, the control groups

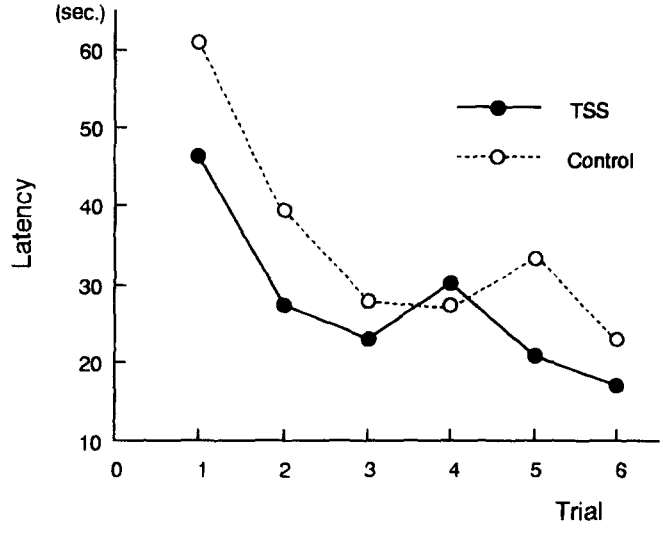

FIG. 5. Water maze task response of TSS group.

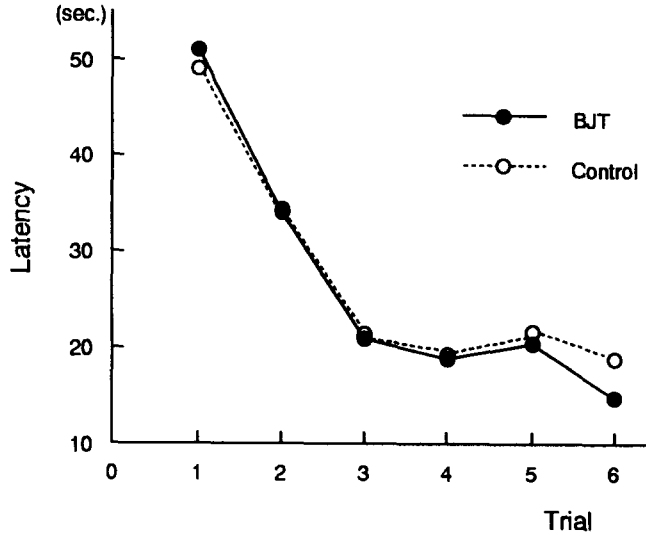

FIG. 6. Water maze task response of BJT group.

required seven days for acquisition but the groups treated with TSS seemed to require only four days for the establishment of learning ability (Fig. 7).

In the groups fed BJT, the median latent periods at the first test trial (second day in Fig. 8) were 39.1 s in the control group and 180 s in the treated group. Although there seemed to be a significant difference between the control group and treated group, no statistically significant difference was found due to the large variance. With regard to the day at which the mice reached

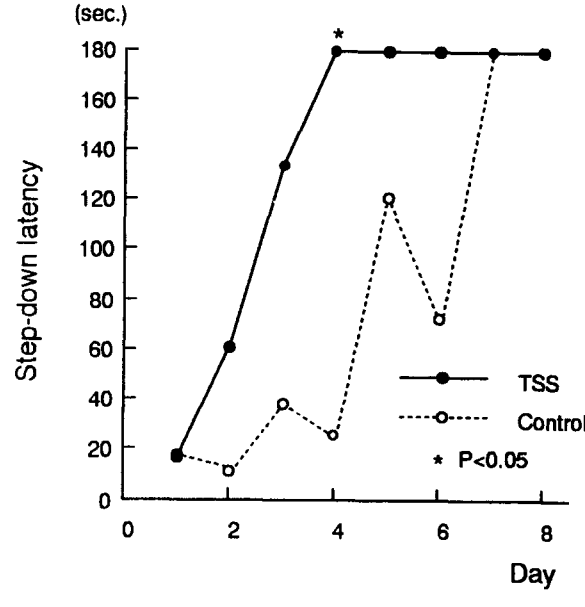

FIG. 7. Step-down passive avoidance task of TSS group.

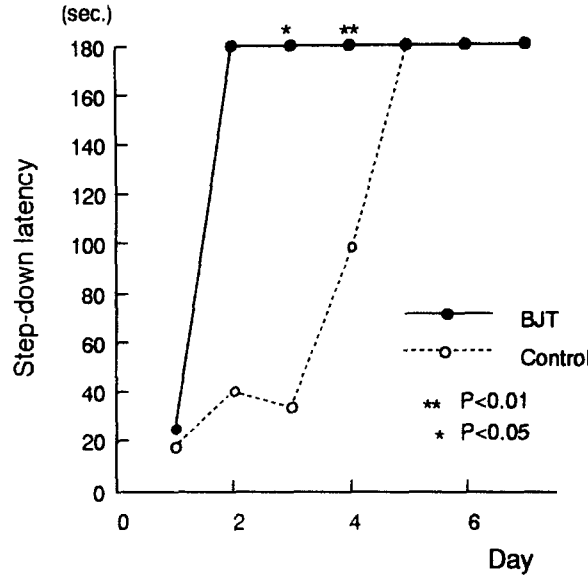

FIG. 8. Step-down passive avoidance task of BJT group.

the standard achievement of 180 s, the treated group seemed to require only two or three days for the establishment of learning acquisition while the control group required five days (Fig. 8).

Spontaneous motor activity

Throughout the 150-min experiment, the amount of activity every 10 min seemed to be almost the same, and there was no statistically significant difference between the treated group and the control group at each duration (Figs. 9 and 10).

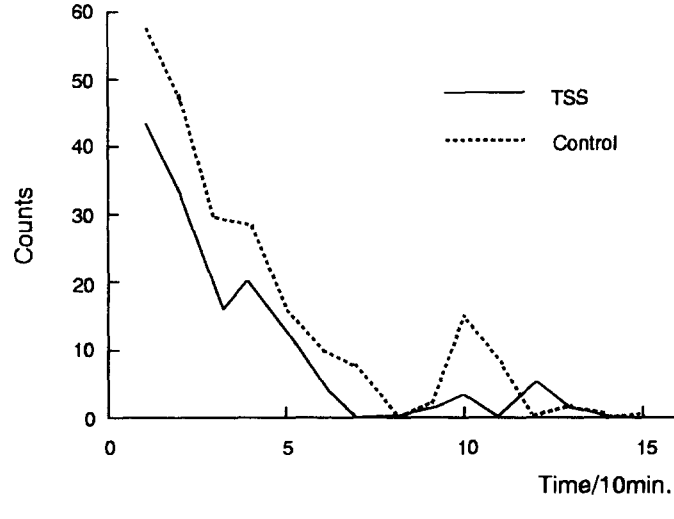

FIG. 9. Spontaneous motor activity of TSS group.

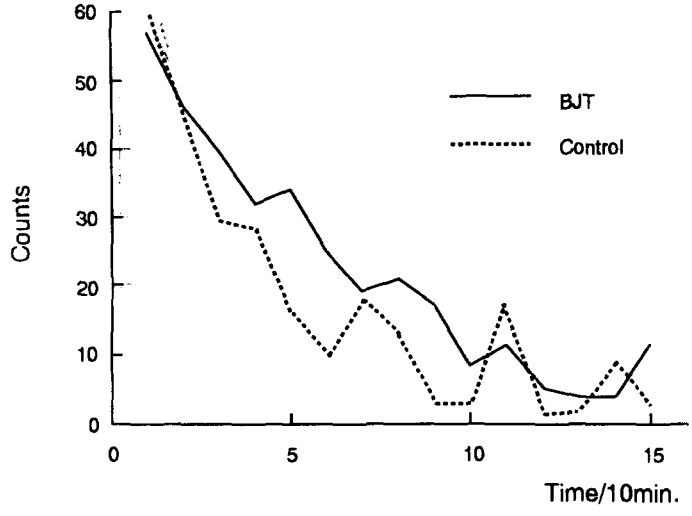

FIG. 10. Spontaneous motor activity of BJT group.

Longevity

The 50% mortality rate was prolonged by 26.4% in the TSS treated group as compared with the control mice and 31.7% in the BJT treated group as compared with the control group (Figs. 11 and 12). The longest survival in the groups fed TSS and BJT was 26.8% and 25.0% longer than that in each control group, respectively (Figs. 11 and 12).

Morphological study

No significant difference was observed in brain weight between the treated group and the control group in either the TSS or BJT study group. Macroscopically, no particular difference was detected in the brains of the TSS and BJT study groups. Although not actually statistically

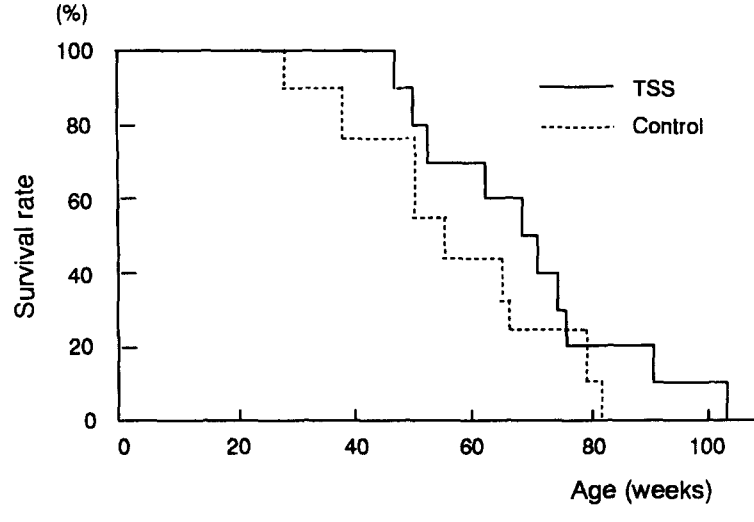

FIG. 11. Comparison of survival time of TSS group.

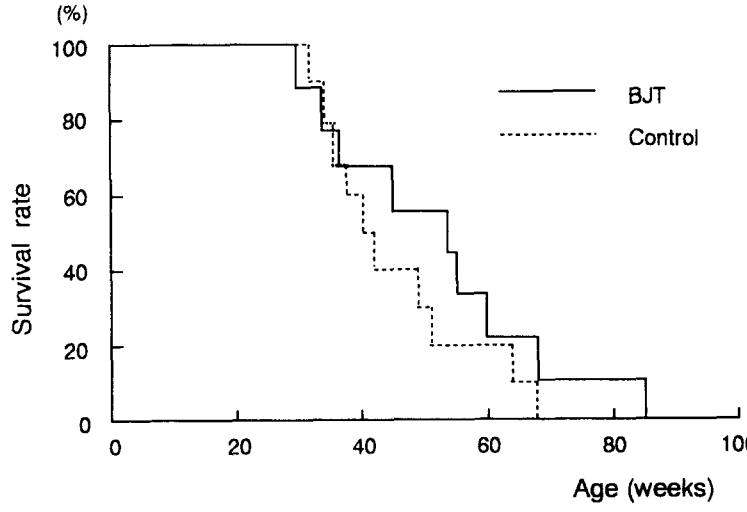

FIG. 12. Comparison of survival time of BJT group.

significant, the extent of histological changes such as PAS-positive granular structures (PPGS) in the hippocampal region was mild in the treated groups as compared to that in the control groups. Small to minute vacuolar changes were observed from the midbrain to the medulla oblongata with particular prominence in the pontine reticular formation. By image analysis procession, the changes seemed more distinct in the control groups than in the treated groups. The mean number of vacuoles in the pontine reticular formation was 44.8% higher in the control group than in the TSS treated group, but, again, there was no statistically significant difference between the two groups because of the large variance. On the other hand, the mean number of vacuoles was 87.1 ± 6.7 in the BJT treated group but 116.4 ± 13.0 in the control group. The p-value of 0.063 by Student's t-test and 0.085 by Wilcoxon's rank-sum test between the BJT study group and the control group indicate a difference close to statistical significance (Fig. 13).

The mean number and density of basal dendritic spines were higher in the BJT treated group than in the control group (Figs. 14 and 15). There was no actual significant difference between the two groups, however, except for the density of spines at the portion more than 150 μm (p = 0.08, Fig. 15).

DISCUSSION

As the dawn of an "aging society" rapidly approaches, various issues concerning human aging have arisen in both social and scientific fields. The aging and age-related diseases of the brain, which is the center of life maintenance, as well as remedies for brain dysfunctions, are attracting special attention. No effective drug to alleviate age-related brain dysfunction has been established despite remarkable development in the field of neuroscience. One of the reasons for the delay in establishing procedures is, of course, that the actual pathogenesis of age-related brain dysfunction remains obscure. But another important reason is that a useful animal model has not yet been found (Kishikawa *et al.*, 1993; Games *et al.*, 1995). In this respect the present study has great potential importance because it employs the senescence accelerated mouse (SAM), which has been proposed as a model for the elucidation of some of the phenomena of

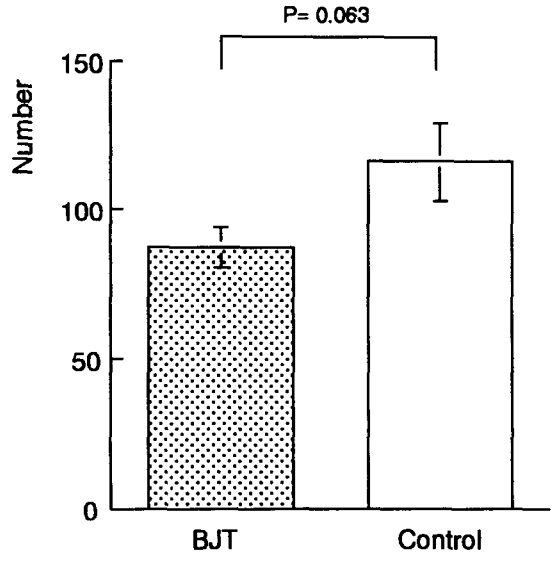

FIG. 13. Vacuolar change of pons.

aging (Takeda *et al.*, 1981). Although spontaneously occurring learning and memory disturbances are well known in SAMP8 and SAMP10 (Miyamoto *et al.*, 1986; Shimada *et al.*, 1992), it is also reported that the strain SAMP1TA/Ngs exhibits a learning disturbance without amyloid deposition (Kishikawa *et al.*, 1994). The learning disturbance in SAMP8 is characterized by onset at two months of age and subsequent progression with age. In contrast, SAMP1TA/Ngs displays a high level of learning behavior at around five months of age and subsequently suffers

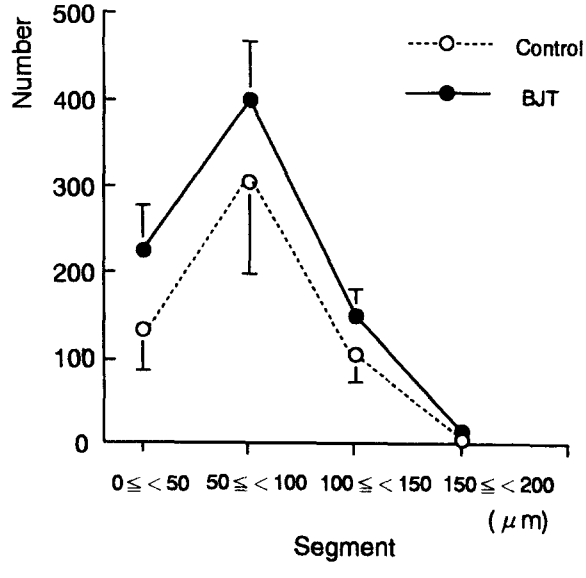

FIG. 14. Number of dendritic spine.

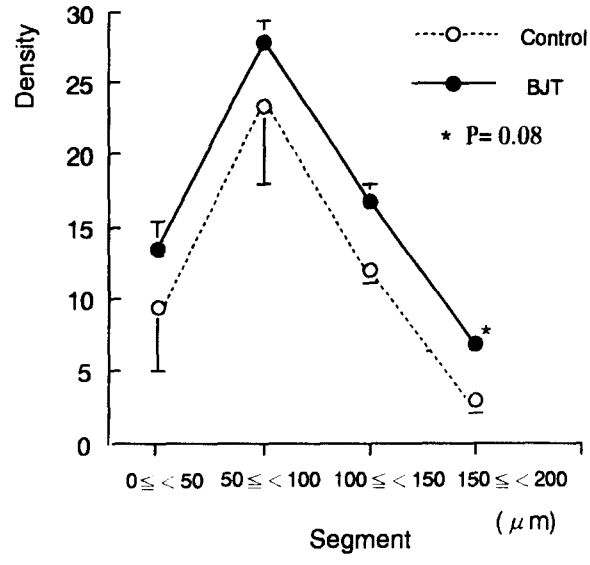

FIG. 15. Density of dendritic spine.

a loss of learning ability due to accelerated senescence. The increase up to the age of five months and subsequent decrease in dendritic spines in the rapid Golgi study may be evidence of the behavioral pattern of learning in SAMP1TA/Ngs, reflecting the process by which learning ability once acquired is impaired by accelerated senescence (Kawaguchi *et al.*, 1994, 1995). This pattern resembles the process of brain dysfunction appearing in human beings as a result of senescence. This is the reason we chose the strain SAMP1TA/Ngs from among the various strains of SAM for this study.

It is extremely interesting that, in the study of a passive avoidance task, a marked acquisition disturbance appeared in the control groups. From the fact that there was no difference in spontaneous motor activity between the treated and control groups, it is unlikely that a difference in spontaneous activity on the platform affected the latent period. In other words, it is unlikely that a dulling of the activity of mice due to TSS or BJT prolonged the latent period in the test trial and/or latent step-down period. The reasons for the acquisition disturbance observed in SAMP1TA/Ngs can be categorized into two patterns: (1) at around three months of age, it is due to the immaturity of the neuronal network, especially dendritic spines (Kawaguchi *et al.*, 1995); and (2) at seven months of age, it is a result of the loss of dendritic spines due to accelerated senescence. The favorable results of the passive avoidance task in the group treated for 24 weeks suggest that either the ongoing development of dendritic spines is promoted by TSS and BJT or that the loss of dendritic spines due to aging is suppressed by TSS and BJT.

Although not actually significant, the extent of morphological changes such as PPGS in hippocampal region and vacuolar change in the brain stem was relatively indistinct in the treated group. It is reported that PPGS observed at the light microscopic level corresponded to abnormal synaptic terminals (Irino *et al.*, 1994). We also have findings suggesting that the PPGS corresponds to the synaptic terminal of neuronal dendrites (Kishikawa *et al.*, 1995). Although TSS has been used traditionally for a wide range of gynecological diseases, it is recently reported to have a useful pharmacological effect on neural transmitters in the brain (Koyama, 1989). It is

also known that the nuclei in the reticular formation of the brain stem have long and ascending projections to the limbic system and cerebral cortex, with a significant relation to learning and memory (Kornblith and Olds, 1973; Gabriel *et al.*, 1986; Takeda *et al.*, 1991). Septal nuclei also receive information from the brain stem, particularly its reticular formation (Vertes, 1985). These facts strongly suggest that vacuolar changes, PPGS, and learning ability are interrelated. TSS and BJT seem to play some important role in preventing the impairment of the synapse and in repairing the synaptic transmitting system.

It is important to evaluate the morphometry of the dendritic spines in the hippocampal pyramidal neurons in both the TSS and BJT study groups, because the SAMP1TA/Ngs used in our experiments demonstrate an intimate correlation between the results of the step-down passive avoidance task and the number of basal dendritic spines in the hippocampal CA1 pyramidal neurons (Kawaguchi *et al.*, 1995). Thus, an investigation concerning the basal dendritic spines of hippocampal pyramidal neurons was also conducted in the BJT study group. The mean number and density are higher in the BJT group than in the control group; but there was no actual significant difference between the two groups, except for the density of dendritic spines at the portion more than 150 μm. The study on dendritic spines of hippocampal neurons, not only basal dendrites but also apical dendrites should be continued because the present study employed only a few materials for the Golgi study (eight specimens from BJT-treated mice and three specimens from control mice). That might be one of the reasons why no statistically significant difference was obtained.

To our knowledge, there is no scientific description with special reference to the relationship between longevity and TSS or BJT. The results of the present study suggested that TSS and BJT have a tendency to prolong the survival time of SAMP1TA/Ngs. Because there were no predominancies of neoplastic lesions or pneumonia in the control mice of either the TSS or BJT study groups, it is very difficult to explain the 26.4% and 31.7% prolongation of longevity due to TSS and BJT, respectively. Studies on immune activity should be considered as a way to shed light on this question.

It is very important to determine which component of these Kampo medicines exerts an effect on behavior, prolongation of median survival time, and longest survival. At present, no one can answer this very difficult question, even though pharmaceutical scientists have suggested two or three specific ingredients of TSS and BJT.

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REFERENCES

FERRER, I. and GULLOTTA, F. Down's syndrome and Alzheimer's disease: Dendritic spine counts in the hippocampus. *Acta Neuropathol.* **79,** 680–685, 1990.

GABRIEL, M., GREGG, B., CLANCY, A., KITTRELL, M., and DAILEY, W. Brain stem reticular formation neuronal correlates of stimulus significance and behavior during discriminative avoidance conditioning in rabbits. *Behav. Neurosci.* **100,** 171–184, 1986.

GAMES, D., ADAMS, D., ALESSANDRINI, R., BARBOUR, R., BERTHELETTE, P., BLACKWELL, C., CARR, T., CLEMENS, J., DONALDSON, T., GILLESPIE, F., GUIDO, T., HAGOPIAN, S., JOHNSON-WOOD, K., KHAN, K., LEE, M., LEIBOWITZ, P., LIEBERBURG, I., LITTLE, S., MASLIAH, E., MCONOLOGUE, L., MONTOYA-ZAVALA, M., MUCKE, L., PAGANINI, L., PENNIMAN, E., POWER, M., SCHENK, D., SEUBERT, P.,

SNYDER, B., SORIANO, P., TAN, H., VITALE, J., WADSWORTH, S., WOLOZIN, B., and ZHAO, J. Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein. *Nature* **373,** 523–527, 1995.

HOSOKAWA, M., KASAI, R., HIGUCHI, K., TAKESHITA, S., SHIMIZU, K., HAMAMOTO, H., HONMA, A., IRINO, M., TODA, K., MATSUMURA, A., MATSUSHITA, M., and TAKEDA, T. Grading score system: A method for evaluation of the degree of senescence in senescence accelerated mouse (SAM). *Mech. Ageing Dev.* **26,** 91–102, 1984.

IRINO, M., AKIGUCHI, I., and TAKEDA, T. Ultrastructural study of PAS-positive granular structures (PGS) in brains of SAMP8. In: *The SAM Model of Senescence,* Takeda, T. (Editor), pp. 371–374, Excerpta Medica, Elsevier Science B.V., Amsterdam, 1994.

KAWAGUCHI, S., KISHIKAWA, M., IKEMATSU, K., SAKAE, M., ISEKI, M., KONDO, H., and NAKANE, Y. Age-related changes in hippocampal pyramidal cells (CA1) among SAMP1TA/Ngs. A quantitative rapid Golgi study of basal dendrites and dendritic spines. In: *The SAM Model of Senescence,* Takeda, T. (Editor), pp. 331–333, Excerpta Medica, Elsevier Science B.V., Amsterdam, 1994.

KAWAGUCHI, S., KISHIKAWA, M., SAKAE, M., and NAKANE, Y. Age-related changes in basal dendrite and dendritic spine of hippocampal pyramidal neurons (CA1) among SAMP1TA/Ngs. A quantitative analysis by the rapid Golgi method. *Mech. Ageing Dev.* **83,** 11–20, 1995.

KISHIKAWA, M., NISHIMURA, M., SAKAE, M., and ISEKI, M. The learning ability and motility of Senescence-Accelerated Mice (SAM-P/1) treated with Toki-Shakuyaku-San. *Phytother. Res.* **7,** S63–S66, 1993.

KISHIKAWA, M., SAKAE, M., ISEKI, M., KAWAGUCHI, S., IKEMATSU, K., KONDO, H., SASANO, S., SATO, H., and HIGUCHI, K. Ecology and learning ability of SAMP1TA/Ngs raised at Nagasaki. In: *The SAM Model of Senescence,* Takeda, T. (Editor), pp. 381–384, Excerpta Medica, Elsevier Science B.V., Amsterdam, 1994.

KISHIKAWA, M., ISEKI, M., IKEMATSU, K., SAKAE, M., INOUE, Y., SASANO, S., and SATO, H. Fine structures of PAS-positive granular structure observed in SAMP1TA/Ngs and SAMP8/TaNgs. Proceedings of the 11th Annual Meeting of Council for SAM Research; 1995:95–96, 1995 (in Japanese with English abstract).

KORNBLITH, C. and OLDS, J. Unit activity in brain stem reticular formation of the rat during learning. *J. Neurophysiol.* **36,** 489–501, 1973.

KOYAMA, T. Effect of Toki-shakuyaku-san on neurotransmitter in the brain. *Gendai Iryogaku* **14,** 89–95, 1989 (in Japanese).

MIYAMOTO, M., KIYOTA, Y., YAMAZAKI, N., NAGAOKA, A., MATSUO, T., NAGAWA, Y., and TAKEDA, T. Age-related changes in learning and memory in the senescence-accelerated mouse (SAM). *Physiol. Behav.* **38,** 399–406, 1986.

MORRIS, R.G.M. Spatial localization does not require the presence of local cues. *Learn. Motiv.* **12,** 239–260, 1981.

NISHIMURA, M., SHIIGI, Y., and KANETO, H. State dependent and/or direct memory retrieval by morphine in mice. *Psychopharmacology (Berlin)* **100,** 27–30, 1990.

SHIMADA, A., OHTA, A., AKIGUCHI, I., and TAKEDA, T. Inbred SAM-P/10 as a mouse model of spontaneous, inherited brain atrophy. *J. Neuropathol. Exp. Neurol.* **51,** 440–450, 1992.

TAKEDA, T., HOSOKAWA, M., TAKESHITA, S., IRINO, M., HIGUCHI, K., MATSUSHITA, T., TOMITA, Y., YASUHIRA, K., HAMAMOTO, H., SHIMIZU, K., ISHII, M., and YAMAMURO, T. A new murine model of accelerated senescence. *Mech. Ageing Dev.* **17,** 183–194, 1981.

TAKEDA, T., HOSOKAWA, M., and HIGUCHI, K. Senescence-accelerated mouse (SAM): A novel murine model of accelerated senescence. *J. Am. Geriatr. Soc.* **39,** 911–919, 1991.

VERTES, R.P. Brainstem-septohippocampal circuits controlling the hippocampal EEG. In: *Electrical Activity of the Archicortex,* Buzsaki, G. and Vanderwolf, C.H. (Editors), pp. 33–45, Akademiai Kiado, Budapest, 1985.