Anti-aging Effect of DX-9386 in Senescence Accelerated Mouse

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The effects of DX-9386, a traditional Chinese prescription (ginseng, acorus, polygala and hoelen) were studied on life span, the degree of senescence, motor activity and the antibody production response in senescence accelerated mouse (SAM). DX-9386-containing food was given to SAM for 13 consecutive months from 2 months of age. DX-9386 significantly prolonged the life span of SAM, prevented body weight decrease with aging and tended to improve the senile syndrome. The motor activity of SAMP8 was higher than that of SAMR1, and DX-9386 tended to increase the activity in SAMP8. The *in vivo* antibody production was markedly decreased in SAMP8 and DX-9386 showed no ameliorating effect on that. These results suggest that DX-9386 has anti-aging impact.

Keywords DX-9386; SAM; life span; senile syndrome; motor activity; PFC

DX-9386 is a traditional Chinese prescription consisting of ginseng, acorus, polygala and hoelen in the ratio of 1 : 25 : 1 : 50 (dry weight). It has long been employed in the treatment of mental disorders, brain hypoxia, senile amnesia and other aging-related diseases among Chinese people.[1] While there has been a great number of clinical application of DX-9386 in China, basic research on the anti-aging effect of this traditional prescription from a modern pharmacological viewpoint is much less developed. In this report, we observed the effect of DX-9386 chronically administered for 13 months on life span and senile syndrome using the short-lived animal model, senescence accelerated mouse (SAM). We also evaluated the effect of DX-9386 on body weight, motor activity and immune response, since these parameters are known to be altered during the aging process.

MATERIALS AND METHODS

Animals and Drug Treatment Male SAMR1 and SAMP8, originally provided by Prof. Takeda (Kyoto University, Kyoto, Japan) and bred in our laboratory for 4 years, were called SAMR1/HS and SAMP8/HS, respectively, according to the general rule of nomenclature for distributed SAM. They were kept in a temperature and humidity-controlled (22±1 °C, 55±2%) room under clean conventional conditions with food and water *ad libitum*. The test groups were divided as follows: SAMR1 and SAMP8 control groups (SAMR1-CON and SAMP8-CON); SAMR1 and SAMP8 DX-9386-treated groups (SAMR1-DX and SAMP8-DX). Each group consisted of 10 animals at the beginning of the experiments. Control groups were fed normal CE-2 food (Clea Co. Ltd. Japan) and drug-treated groups were fed DX-9386-containing food (1%, w/w) from the age of 2 months until all experiments had been completed.

Life Span and Senescence Degree Evaluation The life span of mice was recorded as the natural survival rate at the age of 15 months. The degree of senescence was evaluated according to the method of Hosokawa *et al.*[2] In this evaluation system, the parameters of activities, conditions of the hair and skin, and signs of the eyes were recorded and scored, with a lower score indicative of the better condition.

Measurements of Motor Activity (MA) The apparatus (Animate, AT-342; Toyo Sangyo Co. Ltd., Toyama, Japan) was a doughnut-shaped cage controlled by computer. Thirty-six units of three-dimensionally arranged photodetectors in it recorded the three dimensional activities of mice. MA was measured for 1 h for each mouse in this apparatus.

Plaque-Forming Cell (PFC) Assay PFC assay was performed according to the methods used elsewhere.[3,4] Briefly, mice were immunized by peritoneal injection of 10% (v/v in saline) sheep red blood cells (SRBC), 0.2 ml/mouse, 4 d before PFC assay. On the testing day, spleens were taken out and single splenocyte suspension was prepared individually in Eagle's minimum essential medium. Cell suspension (5×10^6 cells/ml, 0.2 ml) was mixed with 0.025 ml complement (1 : 1 v/v fresh guinea pig serum) and 0.025 ml (1 : 10) SRBC. An aliquot of 0.1 ml mixed cell suspension was added to Cunningham's chamber and incubated at 37 °C for 1 h. The hemolytic plaques formed in the chamber were counted under a light background and the result was expressed as the number of PFC/10^6 splenocytes.

RESULTS

Effect of DX-9386 on Life Span SAMP8-CON began to die at the age of about 10 months and the number of surviving mice had decreased markedly to 40% by the time of the evaluation (15-months, Fig. 1). Nevertheless, all mice in the SAMP8-DX group survived (100%). The survival rates of SAMR1-CON and SAMR1-DX were 90% and 100%, respectively.

Effect of DX-9386 on Body Weight and Degree of Senescence As shown in Table I, the body weight of SAMP8-CON was significantly decreased compared with SAMR1-CON. DX-9386 treatment did not affect the body weight of SAMR1 but significantly increased that of SAMP8. The conditions of hair glossiness and hair loss were significantly better in SAMR1-CON and the other indices showed obviously better tendencies than those of SAMP8. The mean score of SAMR1-CON was significantly less than that of SAMP8. DX-9386 treatment did

not alter senile indices in SAMP8 or SAMR1. The mean score was less in the DX-9386-treated group than that of SAMP8 control, but was not statistically significant.

Effect of DX-9386 on MA The numerical evaluation of horizontal activity and average distance were significantly increased in SAMP8 compared to those of SAMR1, and all the other parameters showed a more increasing tendency in SAMP8 than in SAMR1. In SAMP8, MA tended to increase in the DX-9386-treated group compared with control group (Table II).

Effect of DX-9386 on *in Vivo* Antibody Production Response The number of PFC by the splenocytes declined more markedly in SAMP8 than in SAMR1. DX-9386 had no effect on the number of splenic PFC in SAMR1 or SAMP8 (Fig. 2).

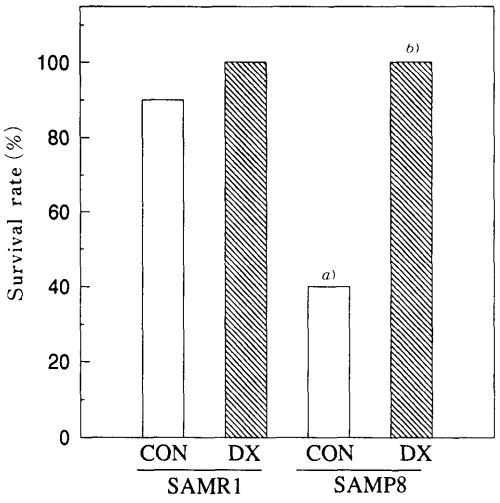

Fig. 1. Effect of DX-9386 on Life-Span

The natural life span was recorded as survival rate and evaluated when mice were 15 months old. The number of mice in each group was 10 at the beginning of drug administration. *a*) $p<0.05$ *vs.* SAMR1-CON; *b*) $p<0.05$ *vs.* SAMP8-CON in x^2-test.

DISCUSSION

In our previous work, DX-9386 ameliorated the learning and memory deficiencies not only in chemically impaired or forebrain-lesioned model animals, but also in SAM, suggesting that the preparation improved aging-related deterioration of learning and memory (unpublished). In this study, it was demonstrated that chronic oral ingestion of DX-9386 resulted in a survival rate of 100% of SAMP8 at 15 months of age and prevented the decrease of body weight in these animals. It has been reported that the natural median survival time is 12.1 months in SAMP8 and 18.9 months in SAMR1[5] and our result showed that DX-9386 significantly prolonged the natural life span of

TABLE I. Effect of DX-9386 on Degree of Senescence

Parameter		SAMR1		SAMP8	
		CON ($n=9$)	DX ($n=10$)	CON ($n=4$)	DX ($n=10$)
	Body weight (g)	33.61±0.67	31.11±0.36	30.25±0.63[a]	32.34±0.43[b]
General	Reactivity	0.67±0.29	0.60±0.22	1.05±0.29	0.83±0.13
	Passivity	1.56±0.24	1.00±0.21	2.00±0.14	1.75±0.15
Hair	Glossines	0.44±0.24	0.20±0.13	2.00±0.41[a]	2.00±0.33
	Coarseness	0.78±0.28	0.40±0.16	2.25±0.48	2.60±0.34
	Hair loss	0.33±0.17	0.10±0.10	1.50±0.29[a]	1.20±0.36
Skin ulcer		0.11±0.11	0.10±0.10	1.50±0.65	1.00±0.39
Eye	Periophthalmic lesion	0.00±0.00	0.00±0.00	0.75±0.25	0.80±0.20
	Cataracta	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Corneal ulcer	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Skeleton kyphosis		0.00±0.00	0.00±0.00	0.25±0.25	0.20±0.13
Mean score		0.39±0.16	0.24±0.11	1.18±0.27[a]	1.04±0.28

DX-9386-containing special diet was given to mice for 13 months. Senescence degree was evaluated at their age of 15 months by the method of Hosokawa *et al.*[2] In each parameter, the maximal score is 4.00 and the scores are gradually decreased to 0.00 in case of no signs. *a*) $p<0.05$ *vs.* SAMR1-CON; *b*) $p<0.05$ *vs.* SAMP8-CON in Student's *t*-test for body weight and total score, in Mann Whitney's *U* test for the others: mean±S.E.M.

TABLE II. Effect of DX-9386 on MA

Parameter	SAMR1		SAMP8	
	CON ($n=9$)	DX ($n=10$)	CON ($n=4$)	DX ($n=10$)
No. of movements	8310±680	7495±770	10243±700	11288±1350
Movement time (min)	34.9±2.2	32.4±3.0	40.8± 2.8	43.1± 3.2
No. of horizontal activity	337± 15	365± 22	258± 22[a]	276± 24
Total distance (cm)	8750±810	7780±1010	13150±1770	14880±3040
Max. continuous time (min)	41.4±7.3	26.7±1.4[a]	72.5±18.0	74.2±18.6
No. of vertical activity	595± 60	539± 63	499± 73	558± 69
Average distance (cm)	26.0±2.1	20.6±1.8	53.2±10.5[b]	65.5±23.9
Average speed (cm/s)	4.1±0.2	3.8±0.2	5.4± 0.8	5.5± 0.9

MA was measured for 1 h with Animate apparatus. *a*) $p<0.05$; *b*) $p<0.01$ *vs.* SAMR1-CON in Mann-Whitney's *U* test, mean±S.E.M.

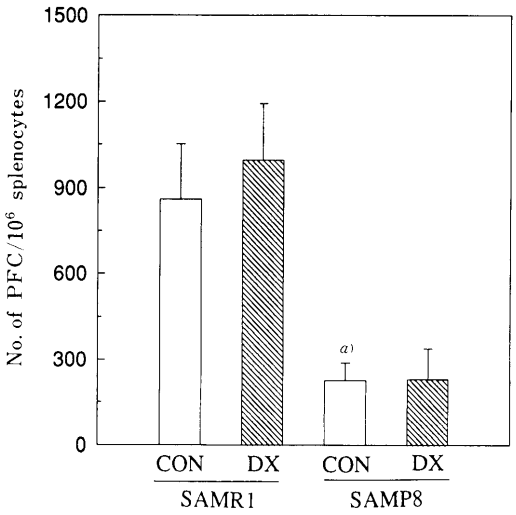

Fig. 2. Effect of DX-9386 on *in Vivo* Antibody Production Response

Mice were immunized with SRBC (10% v/v, 0.2 ml, i.p.) and PFC formed by the splenocytes was tested 4 d thereafter. *a*) $p<0.05$ *vs.* SAMR1-CON in Student's *t*-test, mean ± S.E.M., $n=4-10$.

SAMP8, suggesting an anti-aging effect of the prescription. The body weight of SAMP8 showed an age-related decline compared with SAMR1. DX-9386 prevented the decrease of body weight and tended to improve the senile syndrome, suggesting that the general physiological condition of SAMP8 was improved.

MA in SAMP8 was higher than in SAMR1, in accordance with the report that MA of SAMP8 during the light period (9:00 a.m.—11:30 a.m.) in a novel environment was higher at any age than that of the SAMR1 control.[6] Thus, we considered that hyperactivity or restlessness may be one of the characteristics of SAMP8. The tendency of DX-9386 to increase MA in SAMP8 may be the result of an improvement in general physiological condition or physical strength of the body.

Senescence is believed to be a complicated changing process in physiological and biochemical functions of the multi-systems of the body.[7] The neuroendocrine-immune modulation network (NIM)[8] has been considered to play an important role in the aging process.[9,10] It has been reported that the immune function was significantly declined in SAMP1 substrain[4,11,12] compared with SAMR1, and there was an age-related decrease of splenocyte proliferation induced by phytohemagglutinin parenthesis in SAMP8,[13] suggesting a close relationship between the accelerated senescence and the low immune function in SAM. It was found in our study that the *in vivo* antibody production to SRBC challenge was significantly decreased in SAMP8 compared with SAMR1, which suggests that the declination of immune response is another characteristic of SAMP8, in addition to the well known characteristics of learning and memory deficiency.[5,14,15] The fact that DX-9386 did not improve the immune response suggests that its anti-aging effect is not related to amelioration of the immune status, and that it is less probable that the drug affects the immune system or NIM network. The mechanism of DX-9386 is not yet understood and further experiments are being conducted in our laboratory.

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