Improving Effects of DX-9386, a Traditional Chinese Medicinal Prescription, on Thymectomy-Induced Impairment of Learning Behaviors in Mice

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The subject mice were thymectomized 4 weeks after birth. Ten months after the thymectomy, learning behaviors in passive and active avoidance performances and a spatial memory task, the contents of brain monoamines and brain choline acetyltransferase (ChAT) activity, as well as the immune response were evaluated. DX-9386, a traditional Chinese medicinal prescription consisting of ginseng, polygala, acorus and hoelen, was prepared in CE-2 mouse food (1%, w/w) and given to the thymectomized mice after the operation until all the experiments were finished. DX-9386 treatment significantly ameliorated the learning and memory ability impaired by thymectomy in passive avoidance performances and in a spatial memory task, and the mice tended to improve in the active avoidance performance of a lever press test. However, DX-9386 treatment did not improve the thymectomy-reduced immune response. The contents of hypothalamic norepinephrine, 3,4-dihydroxyphenylacetic acid and homovanillic acid, and hypothalamic ChAT activity were significantly increased in thymectomized mice, and DX-9386 restored them to the control levels. These results suggested that DX-9386 mainly affected the cognitive process of the central nervous system to ameliorate the learning and memory deficit induced by thymectomy.

Keywords DX-9386; thymectomized mouse; learning behavior; monoamine; choline acetyltransferase activity; plaque-forming cell (PFC)

DX-9386, a traditional Chinese medicinal prescription, consists of ginseng, polygala, acorus and hoelen in the ratio of 1:1:25:50 (dry weight). It has long been employed in the clinical treatment of some mental disorders such as brain hypoxia and senile amnesia in China,¹⁾ but studies of its pharmacological effects are still under-developed. In our previous studies, DX-9386 ameliorated the learning and memory ability of both chemically and physically lesioned model mice, but had no effect on the learning behaviors of normal mice. In addition, the chronic ingestion of DX-9386-containing food improved the learning behaviors in senescence accelerated mice (unpublished data). These findings suggested that DX-9386 affected the central nervous system (CNS) by ameliorating deficiencies of learning and memory ability, although its precise mechanism remains to be elucidated.

The neuropharmacological effects of ginseng, the primary component of DX-9386, have been well studied during recent years. It has been found that ginseng or its main active ingredients, ginsenosides, ameliorated the learning behaviors which had been impaired either chemically or electroconvulsively,^{2–4)} or had deteriorated with aging.³⁾ Ginsenosides were shown to potentiate nerve growth factor (NGF)-mediated nerve fiber outgrowth in organ cultures of chick dorsal root and sympathetic ganglia, and supported the neuronal survival of both chick and rat cerebral cortex⁵⁾; they also promoted the metabolism of central cholinergic neurons.⁶⁾ These findings indicate that ginseng affects central cognitive processes *via* multiple mechanisms which ameliorate learning and memory ability. In addition, ginseng also potentiates immune function,^{7–9)} which could be beneficial in improving cognitive deficits due to systematic aging accelerated by immunodeficiency. However, whether DX-9386 possesses an immunopotentiating effect, or whether

it is effective simply on learning and memory deficits induced by immunological aging, is presently unknown.

Recent studies demonstrated that the neuroendocrine immunomodulation (NIM) network is closely related to systematic aging, in which the thymus plays an important role.^{10–19)} Thymectomy of mice not only reduced the immune function, but also caused disorders of the peripheral endocrine hormones,²⁰⁾ which are supposed to accelerate the aging process.¹⁷⁾ Our recent studies revealed that thymectomy in young adult mice also impaired learning and memory ability (unpublished data), in addition to reducing their immune response. In this study, therefore, the effect of DX-9386 on learning behaviors was studied from the angle of the NIM network using thymectomized (ATx) mice.

MATERIALS AND METHODS

Mice and Drug Administration Male ddY mice, 4 weeks old, were thymectomized by sterilized surgery under anesthesia. Sham-operated (SHAM) mice, which were subjected to all the same operation procedures as the ATx mice except for thymus removal, and age-matched normal (CON) mice served as controls. DX-9386 was provided by Daiichi Pharm. Co., Ltd. (Japan), and DX-9386-containing CE-2 food was prepared by Clea Co., Ltd. (Japan). In this study, DX-9386-treated thymectomized (ATx + DX) mice were begun to be fed DX-9386-containing food (1%, w/w) after the operation until all the experiments were finished. Mice in the control groups (including CON, SHAM and ATx) were fed CE-2 control food. All mice were kept in a temperature and humidity controlled ($22 \pm 1^\circ\text{C}$, $55 \pm 2\%$) room under clean conventional conditions with food and water *ad libitum*, and were used in the experiments 10 months after the operation.

Passive Avoidance Performances Step Through (ST) Test: The ST test was performed according to the method employed in our laboratory.^{21,22} The apparatus (PA M1, O'Hara & Co., Ltd., Tokyo, Japan) consisted of two compartments separated by a black wall with a hole in the lower middle part. Of the two chambers, one is illuminated and the other dark. The test was conducted for 10 consecutive days, including one learning trial and nine testing trials, at the same time of the day. In the learning trial (day 1), a mouse was put into the bright chamber and it would suffer an electric foot shock (36 V, AC) through the stainless steel grid floor when it entered the dark compartment. The time when the mouse entered the dark chamber was recorded automatically and described as latency. The number of mice which did not enter the dark compartment (successful mice) was also recorded. In the testing trials (days 2–9), the same test procedures were performed. The number of mice which stayed in the bright compartment without entering the dark one by the time of each testing trial was recorded as memory retention, and the average number of entering events per testing trial was recorded as the total number of errors. Mice were allowed to stay in the bright compartment for up to 60 s in the learning trial and 300 s in the testing trials, respectively.

Step Down (SD) Test^{21,22}: The chamber of the SD test was equipped with a stainless steel grid floor through which an electric shock (50 V, AC) was given. On the grid floor, there was a platform on which a mouse stayed to avoid the electric shock. In the learning trial (day 1), mice were exposed to a 10-min learning course in which they were put on the platform, and punished by a foot shock when they stepped down from the platform (error). The numbers of errors and of mice which did not step down (successful mice) during the latter half (5 min) of the learning trial were recorded. In the testing trials (days 2–9), the same test procedures were performed at the same time of the day for 3 min. The memory retention and the total number of errors were defined as in the ST test, and recorded.

Measurement of Motor Activity (MA): A mouse was put into a round testing cage (18 cm in diameter and 18 cm in height) with a tilting-type ambulator (MA001, O'Hara & Co., Ltd.), and the amount of MA was measured for 30 min before the ST and SD tests during the 10-d trials of passive avoidance performance.

Active Avoidance Performance Shuttle Box (SB) Test^{23,24}: The SB test was performed for a consecutive 7-d course with 60 trials per day. The chamber of the apparatus (Model GT-8332S, O'Hara Co., Ltd.) was equipped with a stainless grid floor connected to an electric stimulator. Two sets of infra-red beams were arranged apart at the lower part of the long sides, and a buzzer and lamp were set on the ceiling. The SB was computer-controlled and the programmed schedule of one trial was 60 s, of which 40 s was for the interval, 10 s for conditioned stimulation (CS) with a warning buzzer and the light on, and 10 s for unconditioned stimulation (US) with a real electric shock (36 V, AC) concurrently with the warning buzzer and light. When a mouse moved from one side of the apparatus to the other to cut both of the

two infra-red beams during the period of CS or US, the electric shock was either avoided or canceled immediately. The perturbation of the photo beam which occurred in the period of CS or interval were recorded as either a conditioned avoidance response (CAR), or spontaneous response (SR), respectively.

Lever Press (LP) Test^{23,24}: Mice were subjected to a consecutive 10-d testing course, with 60 trials per day of an LP test. A steel lever was vertically set at one side of the testing chamber of the LP apparatus (Model GT-8332, O'Hara Co., Ltd.). The chamber was also equipped with a warning buzzer and a lamp on the ceiling, and a stainless grid floor for giving an electric shock. The program and schedule of the trials, and the recorded parameters were the same as those in the SB test, except that the mouse had to press the lever to cancel or avoid the electric shock in this test.

Spatial Memory Test A Morris water maze was used to evaluate the spatial memory performance in mice. The water maze pool was a circular plastic tank, 74 cm in diameter, 32 cm deep and divided into 4 equal fan-shaped regions. A translucent plastic platform (10 cm in diameter, 26 cm in height), was located in a constant position in the center of one quadrant (region 1) at 0.5 cm below the surface of the water. Thus, a mouse inside the pool was unable to locate it visually. The temperature of the water was kept at $18.5 \pm 0.5^\circ\text{C}$. Some cues were arranged around the pool, which were visible from the inside of the pool and kept unchanged throughout the period of the trials.

The procedure of the trials was as follows: a mouse was put into the pool, from one of the 4 starting points which divided the pool into 4 quadrants. The swimming route of the mouse was traced by a video camera and imaged on a television monitor connected to the computer. The total time it took for a mouse to find and get onto the platform (escaping latency), the duration of time a mouse was actually swimming, but not floating, to find the goal (swimming time), and the swimming speed were recorded automatically. A mouse was allowed to stay on the platform for 30 s after reaching it. When the mouse failed to find the platform within 90 s, it was forced to be put on the platform for 30 s. Each mouse accepted 4 trials which were started randomly from the 4 different starting points per day, and the interval between each trial was set at 5 min. The trials were performed for 7 consecutive days. In the last testing day (day 7), the platform was taken out and a mouse was put into the pool from one randomly arranged starting point. The time a mouse spent in region 1 where the platform used to be (staying time in region 1), the number of times that a mouse came across the platform position (crossing the goal), and the ratios of the swimming distance in region 1 (swimming distance ratio) and the swimming time in region 1 (swimming time ratio) over the total values in the 4 regions, were recorded.

Monoamine Assay The monoamine contents, including norepinephrine (NE), 3,4-dihydroxyphenylacetic acid (DOPAC), dopamine (DA), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) and serotonin (5-HT), of the cortex, hippocampus and hypothalamus were measured with high pressure liquid chromatogra-

phy (HPLC) combined with an electrochemical detector (EC). The HPLC-EC system (BAS Co., Ltd.) consisted of a column (Bio-phase ODS IV, 4.0×110 mm), pump (PM-60), sample injector (CAM/240), degassor (CD-22), EC (Amperometric detector, LC-4C) and recorder (Chromatocorder 12, SIC Co., Ltd.). The mobile phase was 0.1 M tartaric acid/0.1 M sodium acetate (5/4), 0.5 mM EDTA-2Na, 5% acetonitrile and 550 μ M sodium 1-octane sulfonate. Its pH value was adjusted to 3.2 and the flow rate was 700 μ l/min.

The desired brain regions were dissected individually on ice and frozen in liquid nitrogen. The samples were homogenized in 0.1 M perchloric acid solution after being weighed and centrifuged (15000 rpm $\times$ 20 min). The supernatant was used for monoamine analysis. The monoamine content was determined using an external standard method, and the results were expressed as nanograms of monoamines per milligram tissue weight (ng/mg).

Brain Choline Acetyltransferase (ChAT) Activity Assay Acetylcholine synthesizing enzyme, ChAT, activity was measured. Brain samples were taken as described above and homogenized in 0.32 M sucrose solution. An aliquot of 0.02 ml homogenate from each sample was mixed in the test tube with 0.04 ml 1% triton-X 100 and 0.04 ml cocktail of [14 C]acetyl CoA which was composed of 12.5 mM potassium phosphate buffer, 20 mM choline chloride (Wako Pure Chem. Ind. Co., Ltd., Japan), 0.25 mM physostigmine sulfate (Wako Pure Chem. Ind. Co., Ltd. Japan), 250 mM NaCl, 2.5 mg/ml bovine serum albumin (BSA) and 0.125 mM [14 C]acetyl CoA (3.15 TBq/mmol, Amersham, Co., Ltd.). The mixtures were incubated at 37°C for 15 min with constant shaking. The reaction was stopped by adding 0.5 ml of a 500 mM potassium phosphate buffer, and then 1 ml acetonitrile containing kalibor (Wako Pure Chem. Ind. Co., Ltd.) and 4 ml toluene were added in turn. After being well mixed and extracted with a vortex, the tubes were centrifuged to separate the organic phase from the water phase. Two ml of the organic phase was transferred to the vial containing 2 ml scintillation liquid, and the dpm was counted for 1 min with a liquid scintillation counter (LSC-700, Aloka Co., Ltd.). The ChAT activity was expressed as the quantity of acetylcholine produced per milligram tissue protein per minute (nmol/mg/min). Each brain sample was tested in duplicate and the mean dpm was used to calculate the ChAT activity.

Plaque-Forming Cell (PFC) Assay A PFC assay was performed according to Cunningham's method.²⁵ Mice were immunized by a peritoneal injection of 10% (v/v in saline) sheep red blood cells (SRBC), 0.2 ml/mouse, 4 d before the PFC assay. On the testing day, the spleens were removed and single splenocyte suspensions were prepared individually in Eagle's minimum essential medium. The cell suspension (0.2 ml) was mixed with 0.025 ml complement (1:1 v/v fresh guinea pig serum) and 0.025 ml SRBC (1:10). Then, the mixture (0.1 ml) was added into 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 results were expressed as the number of PFC/ 10^6 splenocytes.

Statistics The latency in the ST test and the number of errors in the SD test, MA, CAR and SR in SB and LP tests were analyzed using Mann-Whitney's *U* test; the number of successful mice in both the ST and SD test, with χ^2 -test; the body weight and data of the water maze test, with the Bartlett test and ANOVA followed by Tukey's or Steel-Dwass's test; the number of PFC, monoamine content and ChAT activity, with ANOVA followed by Duncan's test.

RESULTS

Effect of DX-9386 on the Body Weight of ATx Mice

The mice were weighed regularly after the thymectomy. The body weights of the ATx mice significantly declined from 8 months after the operation. However, DX-9386 treatment showed no effect on the decrease of body weight induced by thymectomy (data not shown).

Effect of DX-9386 on Memory Registration and Memory Retention Impaired by Thymectomy in the ST Test The latency or the number of successful mice of the ATx mice in the first testing trial (day 2), which is supposed to reflect the capacity for memory registration in mice, tended to decrease. The memory retention and the total number of errors also displayed an impaired tendency regarding learning and memory ability in ATx mice, but the differences were not statistically significant compared with the SHAM. DX-9386 treatment tended to prolong the latency and to increase the number of successful mice, which, however, was not statistically significant compared to that of ATx mice. Memory retention was significantly extended in the trials on days 6, 7 and 10, and the total number of errors was also significantly decreased in

Fig. 1. Effect of DX-9386 on Memory Registration and Memory Retention Impaired by Thymectomy in SD Test

A: Number of errors indicates the number of the times that mice stepped down to the floor in the learning trial (open column) and in the first testing trial (dashed column). B: Total number of errors indicates the average number of errors, i.e. stepping down from the platform, in each testing trial. a) $p < 0.05$, b) $p < 0.01$ vs. SHAM; c) $p < 0.05$, d) $p < 0.01$ vs. ATx; bar indicated S.E.M, $n = 10$.

Fig. 2. Effect of DX-9386 on Active Avoidance Performances Impaired by Thymectomy

CAR: the responses made by mice during the period of conditioned stimulation in SB (A) or LP (B) tests. ○, control; ●, SHAM; ▽, ATx; ▼, ATx+DX. *a*) $p < 0.05$, *b*) $p < 0.01$ vs. SHAM, $n = 10$.

Fig. 3. Effect of DX-9386 on Spatial Memory Performances Impaired by Thymectomy

In the trials of day 1 to 6, each mouse was subjected to 4 trials per day starting from 4 randomly arranged positions. A: escaping latency is the sum of time for a mouse to get onto the platform in the 4 trials. B: total swimming time of the 4 trials for a mouse to reach the platform. C: the average swimming speed of the mice in the 4 trials. D: the number of crossings over the platform position in the last trial without the platform. ○, CON; ●, SHAM; ▽, ATx; ▼, ATx+DX. *a*) $p < 0.05$, *b*) $p < 0.01$ vs. SHAM; *c*) $p < 0.05$, *d*) $p < 0.01$ vs. ATx; $n = 10$.

ATx+DX group (data not shown).

Effect of DX-9386 on Memory Registration and Memory Retention Impaired by Thymectomy in the SD Test Thymectomy significantly impaired memory registration, in which the number of errors was significantly increased and the number of successful mice tended to decrease. The memory retention also tended to decrease and the total number of errors was significantly increased in ATx mice. DX-9386 treatment significantly decreased the number of errors (Fig. 1A) and tended to increase the number of successful mice in the first testing trial. Similar to the results of the ST test, DX-9386 tended to extend the memory retention, but it was not statistically significant compared to that with ATx mice. The total number of errors was significantly decreased in ATx+DX group (Fig. 1B).

Effect of DX-9386 on SB Test Performance Impaired by Thymectomy The memory registration, in terms of

CAR, was significantly delayed by thymectomy, and the significantly low CAR was recorded from the trials of day 2 in ATx group. DX-9386 showed no effect on CAR in the SB test (Fig. 2A).

Effect of DX-9386 on LP Test Performance Impaired by Thymectomy Similar to the results of the SB test, a decreased response in CAR was found with ATx mice compared to the SHAM mice, but the difference did not reach statistical significance. DX-9386 treatment showed a tendency toward an ameliorating effect on this performance in the first seven trials (Fig. 2B).

Effect of DX-9386 on the Spatial Memory Test Performance of ATx Mice In a water maze test, escaping latency and swimming time were significantly prolonged in ATx mice compared to those in the SHAM mice in the testing trials of days 2, 3 and 5. The swimming speed of ATx mice was not altered by thymectomy (Fig. 3A–C). In the group of ATx +DX mice, the escaping latency

was significantly shortened and the swimming distance and swimming time was significantly decreased, correspondingly, but the swimming speed was not affected by DX-9386 (Fig. 3A—C). In the last trial (day 7), the staying time in region 1 and the ratios of swimming time and distance in region 1 tended to decrease in ATx mice (data not shown). The number of crossing the platform position was significantly decreased in ATx mice compared with the SHAM mice (Fig. 3D). DX-9386 significantly increased the number of crossings of the goal; however, only ameliorating tendencies were observed on the other three parameters (data not shown).

Effect of DX-9386 on the MA or SR of ATx Mice MA measured with a tilting-type ambulometer in ATx mice tended to decrease compared with SHAM. DX-9386 showed no effect. The SR in the SB test was significantly decreased in the trials on days 2, 5, 6 and 7 in ATx mice, while DX-9386 treatment tended to raise it to the normal level. In the LP test, ATx mice showed a decreased tendency of SR after the testing trial of day 3. DX-9386 treatment also restored the SR to around the control levels (data not shown).

Effect of DX-9386 on Brain Monoamine Content in ATx Mice The hypothalamic content of NE, DOPAC and HVA in ATx mice were significantly elevated. DA also tended to increase when compared to that of the SHAM mice. DX-9386 treatment restored them to the control levels (Fig. 4). Neither thymectomy nor DX-9386 altered

Fig. 4. Effect of DX-9386 on Hypothalamic Monoamine Contents in ATx Mice

The monoamine contents were determined by HPLC analysis 10 months after thymectomy. a) $p < 0.05$, b) $p < 0.01$ vs. SHAM; c) $p < 0.01$ vs. ATx; bar indicated S.E.M., $n = 5-8$. □, NE; ■, DA; ▨, DOPAC; ▩, HVA; ▤, 5-HT; ▦, 5-HIAA.

Fig. 5. Effect of DX-9386 on Hypothalamic ChAT Activity in ATx Mice

The ChAT activity was tested 10 months after thymectomy. a) $p < 0.01$ vs. SHAM; b) $p < 0.05$ vs. ATx; bar indicated S.E.M., $n = 8$.

the monoamine levels of the cortex and hippocampus, except that the 5-HIAA of the cortex was significantly decreased in ATx+DX group (data not shown).

Effect of DX-9386 on Brain ChAT Activity in ATx Mice Similar to the results of monoamines, thymectomy increased the hypothalamic ChAT activity. DX-9386 administration inhibited the elevation of the ChAT activity in the hypothalamus (Fig. 5). The ChAT activities of the cortex and hippocampus were not influenced by thymectomy or DX-9386 treatment (data not shown).

Effect of DX-9386 on the Antibody Production Response in ATx Mice The number of PFC formed by the splenocytes of ATx mice was markedly decreased. DX-9386 showed no effect on the reduced immune response induced by thymectomy (data not shown).

DISCUSSION

Our previous studies indicated that DX-9386 treatment did not affect the learning performances of normal mice (unpublished observation). Chronic ingestion of DX-9386, however, significantly ameliorated the impairment of memory registration and retention in passive avoidance performances and a spatial memory in a water maze test in ATx mice, suggesting that DX-9386 improved the thymectomy-induced deterioration of learning and memory. Thymectomy impaired the SB-type but not LP-type active avoidance performance, which DX-9386 treatment alleviated. These results indicated that different mechanisms were responsible for the performance of SB and LP tests. MA, or SR in SB and LP tests, was found to decrease or tended to decrease in ATx mice, while DX-9386 treatment did not affect any of them. These indicated that the thymectomy-induced impairment of learning behaviors was not due to the non-specific factor of hyperactivity, and also that the ameliorating effects of DX-9386 on learning and memory performance were not achieved by altering motor activity. These results are in accordance with our previous findings that DX-9386 ameliorated the learning behaviors in memory-impaired model mice or in senescence accelerated mice, and suggested that DX-9386 is also effective in amelioration of the learning and memory deficit induced by thymectomy in mice.

Senescence is a common cause for the development of cognitive deficit. Manifestation of learning and memory deficit and immune deficiency are two predominant features of the aging process. Thymectomy in mice can cause profound immunodeficiency²⁶⁻³²) and neuroendocrine unbalances following a latency of more than 5 months after thymectomy.^{20,26}) An imbalance in the NIM network caused by thymectomy is supposed to accelerate the aging process.¹⁷) Thus, it has been suggested that ATx mice can be used as an animal model for studying the immunopharmacology of aging.²⁶) With these findings and dissertations, we consider that thymectomy-induced learning and memory deficit may be one of the features of systematic aging induced by an imbalance in the NIM network, although the influence of the thymus or immune function on the central cognitive process remains unclear. Therefore, chronic application of a drug

which possesses an immunopotentiating or immunomodulating effect, or modulates the neuroendocrine balance, is expected to restore the balance of the NIM network, which may lead to the amelioration of thymectomy-induced learning and memory deficit.

Ginseng, one of the primary components of DX-9386, has been reported to augment the immune function. Administration of ginseng or its extracts increased the number of PFC in the spleen and the number of T-helper cells,⁷⁾ stimulated basal natural killer cell activity in mice⁸⁾ and enhanced the cell-mediated immune function in clinical studies.⁹⁾ In addition, ginseng affects the pituitary-adrenocortical system in rats,³³⁾ and modulates the serum level of growth hormone and prolactin in old rats.³⁾ These findings suggest that ginseng modulates the function of the NIM network. The extract of hoelen, another component of DX-9386, was reported to inhibit the secretion of cytokines, such as interleukin 1 β and interleukin 6, from human monocytes *in vitro*,³⁴⁾ suggesting its immunosuppressive effect. Hoelen has also been reported to have a luteotropic effect on stimulating the secretion of progesterone,^{35,36)} which could lead to a change in the balance of sex hormones to influence the NIM network. Therefore, it is reasonable to postulate that DX-9386 should have another way of ameliorating learning and memory ability, especially in thymectomy-induced impairment. Unlike the effects of ginseng, however, DX-9386 did not improve the immune response reduced by thymectomy. The differences in pharmacological effects between the individual drug and the prescription may be partly due to interactions among the components or to a difference in doses of the components. This may also be one of the characteristics of the prescription. These results suggested that the ameliorating effect of DX-9386 on learning and memory ability impaired by thymectomy is not directly related to the improvement of the immune function, and that this prescription mainly affects the CNS to improve cognitive function. Our recent study discovered that a single oral administration of DX-9386 promoted the generation of long-term potentiation of an evoked potential in the dentate gyrus of anesthetized rats (unpublished observation), suggesting its direct effect on the central learning and memory processes. However, the possibility cannot be ruled out that other mechanisms are involved in the central effect of DX-9386. It was also quite reasonable to consider that the other components of the prescription played different roles in thymectomized animals.

The monoamine content and ChAT activity in the hippocampus and cortex were not significantly affected by thymectomy or DX-9386 treatment, except that the 5-HIAA of the cortex was decreased in the DX-9386-treated group. These results suggested that the thymectomy-induced impairment of learning and memory ability and the ameliorating effects of DX-9386 were not significantly related to the functions of the adrenergic or cholinergic neurons in these brain regions. The hypothalamus has been known to be the center of neuroendocrine immunomodulation.³⁷⁻⁴¹⁾ Hypothalamic NE and DA modulate the release of hypophysiotropic peptide hormones, such as gonadotropin releasing hormone,

thyrotropin-releasing hormone and growth hormone-releasing hormone,⁴²⁾ which could lead to changes in the peripheral levels of their corresponding hormones. And these hormones were known to modulate immune function.²⁰⁾ Our results showed that the hypothalamic NE, DOPAC and HVA, and ChAT activity were significantly elevated in ATx mice. Although these were restored to normal levels by DX-9386 treatment, the immune response remained unchanged. These results suggest that DX-9386 modulates both adrenergic and cholinergic transmission in the hypothalamus, which might be related to alterations of the NIM network.

The present study indicated that DX-9386 improved the learning and memory ability impaired by thymectomy, without improving the immune response, suggesting that the ameliorating effect of this prescription on thymectomy-induced learning and memory deficit is not likely achieved by way of improving the immune function to restore the balance of the NIM network. Although the mechanisms of the effects of DX-9386 remain unclear, the present work supports the suggestion that this prescription mainly affects the cognitive processes of the CNS in ameliorating learning and memory ability. Studies to elucidate the effective mechanisms of DX-9386 are now being conducted in our laboratory.

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