原著

Dementia and memory improvement due to histological changes in the brain hippocampus and hormone secretion of brain by lecithin administration

Yeun-Hwa GU[1)], Takenori YAMASHITA[2)], Ki-Mun KANG[3)]

Department of Radiological Science, Faculty of Health Science, Junshin Gakuen University[1)]
Department of Radiological Science, Faculty of Health Science, Suzuka University of Medical Science[2)]
Department of Therapeutic Radiology, Gyeongsang National University Hospital, Gyeongsang Institute of Health Sciences, Jinju Korea[3)]

Abstract: In this study, senescence accelerated mice (SAMP8 male, 8w), were used for the study of spatial recognition ability. We studied the effects on the brain hippocampus by administering lecithin (500 mg/kg, po). As compared to sham control groups, the peroxy radical was inhibited significant in the lecithin administration group. The brain peroxidized fat level had a tendency to decrease was found in the lecithin group. Also, in the intracerebral serotonin concentration, was increased in the sham control more than the lecithin administration group (referred to as the lecithin group thereafter). It was realized that the delivery time was shortened in the water maze experiment of the lecithin group as compared to the sham control. Furthermore, activation of pyramidal cells in the CA1 region of the hippocampus were observed in the lecithin group. Therefore, a peroxy radical was extinguished in this study by lecithin intake, and lipid hydroperoxides in brain of the SAM (Senescence-Accelerated Mouse) mouse were decreased by the lecithin administration. Also, it was found that lipid peroxidation of the brain was inhibited. Furthermore, hippocampal nerve cells were activated as suggested thus cognitive function was improved. Also, intracerebral serotonin concentration was normalized so we can see some effects on affective disorders that showed demented peripheral symptom by homeostatic effect. Because of lipid peroxidation inhibition, learning and effect for the improvement effect of the memory impairment were found by lecithin administration, and the lecithin was effective in the memory improvement in dementias as well.

Keyword： Hippocampus, histological change, *Lecithin*, Intracerebral hormone, Dementia, Memory improvement

Introduction

In the recent past, due to the number of people aging rapidly, the number of senile dementia patients is increasing and becoming a serious problem which cannot be avoided and poses a serious dilemma. As for the dementia, a complex higher level of cerebral cortex sexual function is affected under a clear consciousness. A mneme disorder, even a simple thought, the reduction of the cognitive function including the reduction of the judgment are core symptoms and, for a disease to have both peripheral symptom such as the abnormality of the neurologic functions, affective disorders, a volition reduction, or delirium.

The dysautonomia are classified roughly for multiinfarct dementia (Vascular dementia; VD) and Alzheimer disease (Alzheimer's disease; AD), and these patients account for approximately 80% of all dementia patients[1)- 2)]. Brain-specific denaturation by blood circulation disorder and a medionecrosis due to the hardening of the small and medium size artery in the parenchyma of the brain, fibrous thickening, the deposition of the protein ingredient, cerebral infarction and a cerebral hemorrhage occur, and VD develops as a result that a nerve cell received an obstacle secondarily. On the other hand, in AD, an old man squad by the amyloid deposition, the formation of the neurofibrillary tangle by the hyperphosphorylation of the Tau protein are done with a cause for reported progressive neurodegenerative disease pathologically in 1907 by Alzheimer[2)- 4)]. Also, a reduction of the acetylcholine synthase colin acetyl transferase (CAT)

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Department of Radiological Science,
Faculty of Health Sciences, JUNSHIN GAKUEN University
Professor

activity in the autopsy brain of AD was found[5)-7)]. It is reported that points of the cognitive functions correlate with an acetyl cholinergic system afferent fiber disorder degree in the cerebral cortex well[6), 8)-10)]. Furthermore, it is reported that oxidative stress (OS) and the accumulation of the free radical to cause the contribution of the inflammation process, lipid peroxide and neurodegeneration of the brain from microglial accumulation activated increase and a senile plaque of the acute phase reaction protein are associated with AD[11), 12)].

It is phospholipid including the choline which is a precursor of the acetylcholine (ACh) which is a neurotransmitter, and lecithin is the main material constituting a cell membrane.

We can expect effect for prevention from getting older, the activation of the brain cell, neuronal activation, and there is a report that a memory, teachability was improved in these patients by a cholinesterase inhibitor (AChE inhibitor) and combination oral administration of the lecithin in the AD patients by the clinic[13)].

Furthermore, the lecithin activates various kinds of phosphorus oxidase that unsaturated fatty acid of the second place of the lecithin was removed by a phospholipase A2 in vitro, and it is revealed that it functions as a messenger in the intracellular[14), 15)]. As a remedy, since the reduced activity of acetylcholine system that is associated with learning and memory in AD is found, acetylcholine system activation drug is the most often used, others such as anti-inflammatory drugs, steroids, and antioxidants are used as well[16)- 21)]. Currently, there are AChE inhibitors used as a drug that is effective for treating and preventing AD, but only donepezil hydrochloride is approved in Japan. In drug therapy for VD, preventing the progression by controlling risk factors such as diabetes or high blood pressure is the subject of treatment. When it comes to the elderly in particular, the interaction of the drug will become a problem when administering this multi-drug combination and an increase in frequency of side effects. In addition, because it takes a long-term administration for the treatment of VD and AD, it is necessary to select a material with fewer side effects that can withstand long-term dosing.

Therefore, we focused on lecithin which is a natural substance with almost no side effects when used in this study. Using senescence accelerated mice; conduct *in vivo* experiments using (Senescence Accelerated Mouse; SAM), into the brain hippocampus and the presence or absence of memory improvement of lecithin, we have investigated the effects[24)-29)] .

Material and Methods

Five weeks old male ICR mice (20-24g / mouse) were purchased from CLEA Japan, Inc, Japan. and 4-week-old (10-15g / mouse) male SAMR1 mice and male SAMP8 mice were purchased from SLC Inc[15),23)], Japan. To prepare for testing, the mice were kept in room with a temperature of 22±3℃, 60% humidity, 14 hours/day of lighting (10 hours/day of no lighting). The mice were allowed to drink tap water and eat animal feed (provided by Japan CLEA Inc.) freely. HEMATOCRIT KH-120 centrifuge from KUBOTA was used for blood drawn from the fundus of the eye, and Hitachi Koki Co. CHIBITAN-II MILLIPORE was used for blood drawn from the heart. EIA Kit: LDN Serotonin EIA RUO 10-0900 was used for Serotonin measurements, EIA Kit: LDN Dopamine EIA RUO 10-0300 was used for Dopamine measurements, and ELISA Kit：MP Biomedicals Noradrenalin ELISA 196594 was used for Noradrenalin measurements. Other devices also used included MONOSIN Ⅱ a stirrer from TAIYO, Star LC1011C for 450 nm absorbance measurements, and Micro plate reader MPR A4 from TOYOSODA for 490 nm absorbance measurements.

Hormone Measurements in the Brain of ICR Mice

Forty mice were used as a control group and another 40 was used as a *lecithin* medicated group. The *lecithin* medicated group was forcibly given 500mg/kg of *lecithin* in 0.2mL dosage daily for 4 weeks using stomach sonde and the control group was given water in the same dosage. Blood was drawn from the fundus of the eye and measured from each group before

medication and 8 weeks and 12 weeks after medication. Centrifuge was used on the blood for 10 minutes at 10,000 rpm and then preserved frozen. Blood was drawn again on the 16th week after the last medication, serum was removed after the blood was processed in the centrifuge and then preserved frozen. Each brain hormone (Serotonin, Dopamine, Noradrenalin) was measured from the serums using the EIA kit and ELISA kit. Also, the body weight of the ICR mice was measured every 3 days.

Morris Water Maze Experiment and Brain Hormones Measurements for SAM Mice

Morris water maze test

The water maze experiment followed the Morris method[24-29].

The device consists of a circular tank (150cm in diameter, 50cm in depth) and platforms (12.5cm in diameter, 15cm in height) to be able to evade the mouse when we filled with water.

The platform was made using crystalline acrylic and was put 1 cm below the water level of 23 ± 2℃ (mouse mixes 2% of skim milks so that a platform is not caught visually).

Also, we divided the wall surface of the circular tank into four segments of the north, south, east and west and established division into four central (the northeast) platforms (Fig. 1A).

The experimenter performed by two people used a melanotic curtain and covered it around a circular tank and we did background constantly and performed experiment to Step1-3 under quiet environment (Fig. 1B).

[Step1: pre-training session]

After having let the mouse swim apart from the platform no more than 15 seconds, we guided it to return to the platform. We left the intervals ten seconds or more because one of them and repeated this operation three times.

[Step2: training session]

We measured the time before arriving at the platform from death by drowning and left unattended after

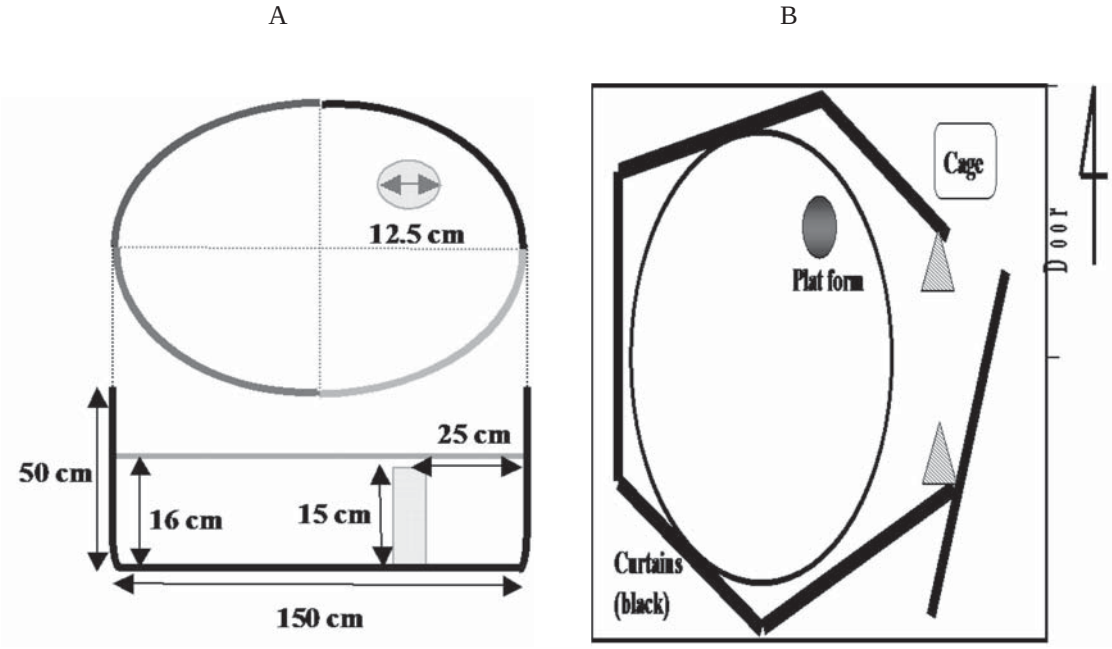


Fig.1. Cross-sectional diagram of pool (A), and its position in the laboratory (B). The water maze was a cylindrical pool (diameter 50 cm) filled with water (depth 16 cm, 23 ± 2℃) that was made opaque by the addition of a quantity of skim milk. Approximately 1 cm below the surface was a 12.5 × 12.5 cm^2 hidden platform made of transparent acrylic. Four points around the circumference of the pool are arbitrarily designated North, South, East, or West and, on this basis, the pool area was divided into 4 quadrants. There were numerous distal cues in the laboratory (doors, students (△), fluorescent lights on the ceiling, black curtains).

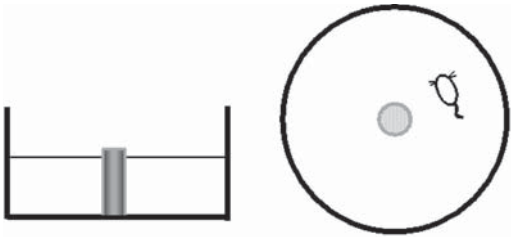


Fig.1-1.The day before we conducted a training session, and several centimeters immobilized a platform in a high locus from the surface of the water in the center of the circle tank, and a platform was able to knock mouse flat.

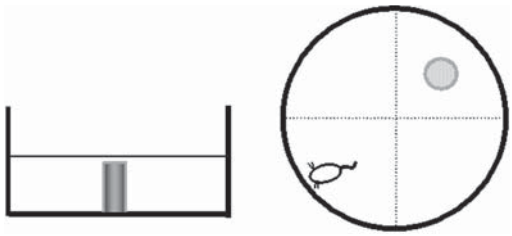


Fig.1-2. We made mouse randomly die by drowning from three points in the equidistance with the state that turned a head to the wall surface of the circular tank from a platform.

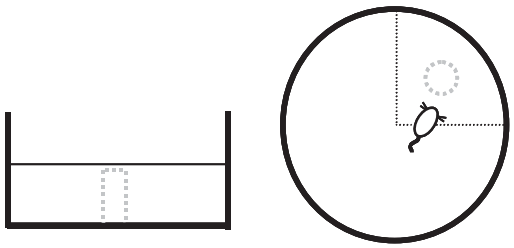


Fig.1-3. We conducted one time of probe test on the last day of the training session.

delivery for ten seconds and made the locus memorized. In addition, when the mouse did not arrive at the platform even if 120 seconds passed, we moved it forcibly to a platform and left it unattended for ten seconds and made the locus memorized. In this case, delivery time for the mouse to be able to put was 120 seconds[30]. Thus the trial was carried out twice a day and we tried the first 30-60 minutes after oral administration, and then the second after the first trial ended around 60-90 minutes later. We repeated this daily from 10:00 to 16:00 and was carried out for nine days and measured the delivery time to the platform. We calculated a conative delivery time mean and screened space recognition ability over time twice.

[Step3: probe test]

After having made a record, we removed the locus of the platform and gave off mice similar to a training session on the surface of the water and recorded the 120-second swimming behavior. We aimed at the frequency that sailed across the sojourn time with divisions into four and the virtual platform area that established a platform for the data analysis and screened space recognition ability[31].

Test groups included 10 mice for each of the SAMR1 Mice control groups, SAMR1 mice *lecithin* medicated group, SAMP8 mice control group, and SAMP8 mice *lecithin* medicated group. Following the completion of the preparation period, daily for 4 weeks, 500 mg/kg of *lecithin* was given at 0.2mL dosage to the *lecithin* medicated group and the same amount of water was given to the control group. 8 weeks after the last dosage was given, the Water Maze experiment was conducted for 1 more week using the Morris method.

Blood was drawn from each of the groups, 9 weeks after the last dosage. The blood was then processed in a centrifuge, and then the serum was removed and preserved frozen. Each of the brain hormones (Serotonin, Dopamine, and Noradrenalin) were measured from the serum using the EIA kit and ELISA kit. Also, the body weights of the SAM mice were measured every 3 days.

The water maze device is composed of a cylindrical tank (150 cm in diameter and 20 cm in depth.) filled with 23℃ of water and has invisible acrylic platform (12cm in diameter and 25cm in height) placed 25 cm away from the wall and 1cm below the water surface. The walls of the tank were separated into zones by 4 different colors (red, yellow, green, and white) in order for the mouse to be able to learn its relative location in the tank. The tests were conducted in a quiet, static environment.

SAM mice were entered randomly into one of three-fixed locations in the tank equal distance away from the platform with their head facing toward the wall. The goal time was measured from when the mice first

entered the water to when the mice reached the safety of the platform. Once reaching the platform, the mice were left on the platform for 10 seconds to give them time to memorize their relative location. For cases in which the mice were not able to find the platform within 120 seconds, they were forcibly moved to the platform and left for 10 seconds. The test was conducted every day for one week. The test was conducted to rate one of mice's higher brain functions - space recognition ability[32].

Preparation of frozen Slides

The cervical dislocation mice in each group 18 weeks after oral administration were bled left ventricular saline perfusion by (1mL/min), and fixed by (15 min, 1mL/min) perfusion with 10% formalin solution . The Brain was removed, immersion for 24 hours in 10% formalin solution dip to (4 ℃), further 4 hours (4 ℃) 10% sucrose/0.1 M PBS, 20% sucrose/0.1 M PBS 4 hours (4 ℃) , at the end immersion overnight/0.1 M PBS 30% sucrose dip was (4 ℃). Next, they were freeze-embedded using the OCT compound (embedding agent for frozen packages), or sliced to 9 μm the brain in cryostat, and was attached to a glass slide. Thereafter, it was dried for 1 hour or more in cold air, immersed 30-60 min EtOH 50%, then washed 2-4 minutes with running water, and subjected to a histological exam by staining. In this study, we referenced to the structure of the hippocampus in the rat brain of David from upon section manufacturing and was sliced in the part of fig.2.

Manufacture of the freeze slide specimen

We performed a cervical dislocation of mice of each group 18 weeks after oral administration and bled each mouse by left ventricular saline perfusion (1mL/min) and immobilized it by the perfusion (1mL/min, 15min) with the 10% Formalin solution.

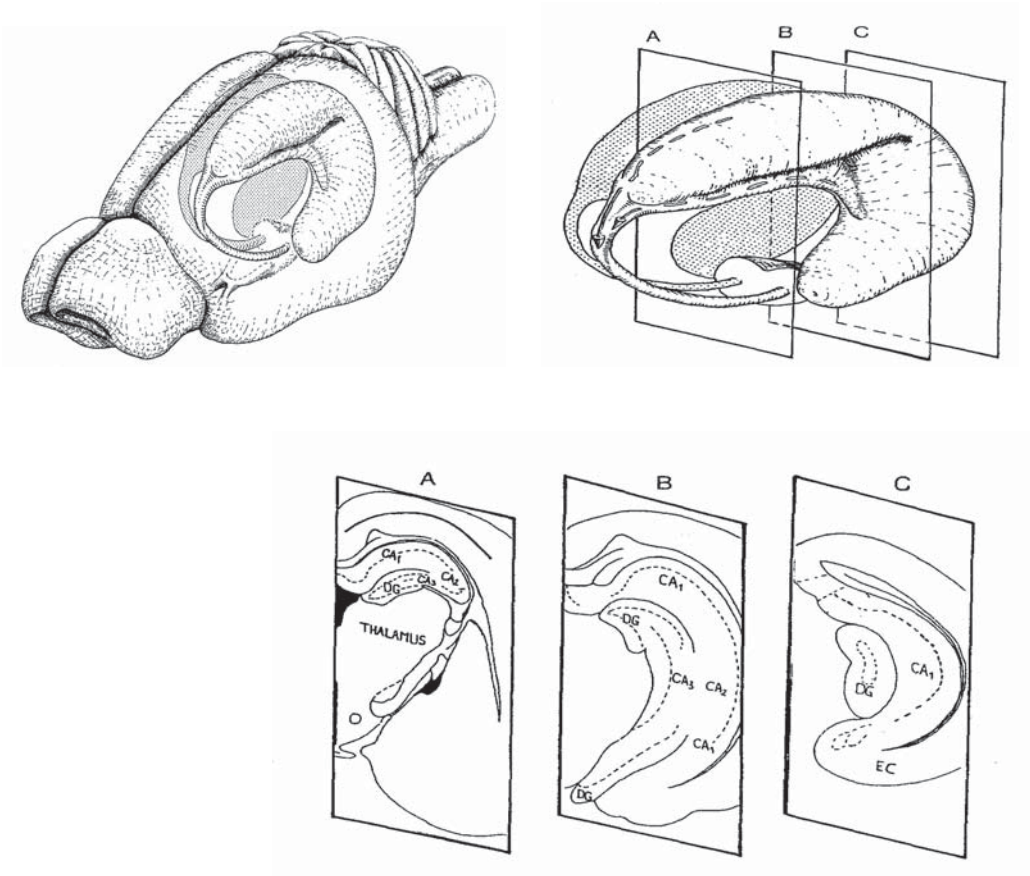


Fig.2. Line drawings showing the three-dimensional organization of the hippocampal formation in the rat brain. Three coronal sections (A, B and C) are shown at different rostrocaudal levels through the hippocampal formation. CA1 and CA3, fields of the hippocampus; EC, entorhinal cortex; DG, dentate gyrus. (David G. A, Menno P. W., The rat nervous system. Second edition, Academic Press, Inc., (1995))

We resected the brain and did an insuccation (4℃) with a 10% Formalin solution for 24 hours, and (4℃) did an insuccation (4℃) in 30% sucrose/0.1 M PBS in 20% sucrose/0.1 M PBS in 10% sucrose/0.1 M PBS (4℃) overnight no more than four hours.

Then, we frozed it using an OCT compound (embedding agents for freezes) and embedded it and thinly sliced the brain to 9 μm in cryostat and were able to stick them to a slide. We conducted a histologic examination by each staining subsequently after being dried in a cold wind more than one hour, and immersing it in 50% EtOH for 30-60 minutes, then douched it under running water for 2-4 minutes.

We performed it in reference to the hippocampal structure in the rat brain of David et al. on the occasion of graft manufacture and, in this study, did a section in a site of fig. 2 [6].

Staining of the brain cell slice

Hematoxylin and eosin methods

We stained each groups' mice brain slice specimen with Mayer of hematoxylin solution after making for five minutes and washed itwith water under running water for 20 minutes. We stained it with a 1% eosin solution next for 2-4 minutes and it was light and washed with water for around ten seconds and flushed an extra eosin solution and repeated this operation twice. We immersed it in 70%, 80%, 90%, 95%, 99% (I, II) of ethanol for about ten seconds each and dehydrated it with differentiation and occluded it after penetration using Oikit in xylene I, II.

The Kluver-Barrera's stain; KB stain methods

We immersed each groups' mice brain slice specimen in 95% ethanol after making for 2-5 minutes and stained it with 0.1% Luxor fast blue liquid overnight (56-60 ℃). After staining, we immersed it in 95% ethanol for 1-3 minutes and rinsed it out with distilled water three times, each for around 30 seconds. After that we immersed it in a 0.05% lithium carbonate water solution next for five minutes, and soaked it in 70% of ethanol three times appropriately, and having adjusted it to objective thinness, we interspersed distilled water three times and completely removed rinsing ethanol for each for around 30 seconds. Furthermore, we stained it in 0.1% cresylecht violet chrome for 5-8 minutes and we soaked it in 95% ethanol three times appropriately and adjusted it to objective thinness. We immersed it in 70%, 80%, 90%, 95%, 99% (I, II) of ethanol for by for each ten seconds and, after dyeing, dehydrated it with differentiation and occluded it after penetration using Oikit in xylene I, II.

Chemical structure of lecithin

We show below the chemical structure of lecithin.

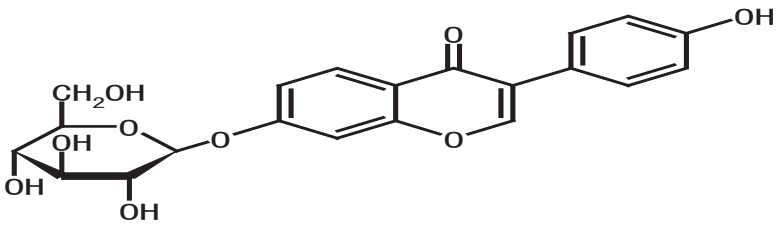
Statistical analysis

The mean significant difference official approval calculated it in $p<0.05$ and $p<0.01$ using Dunnett-test. About surviving neurons in the hippocampal CA1 region in each SAM used by Student's t-test.

Results

Antioxidant action (SOD-like activity)

We showed SOD-like activity in the mice serum 30 days after the lecithin administration in fig.3. With the SOD-like activity, we couldsee a high value in the lecithin group (35.9 ± 7.2%) as compared with the sham control group (25.2 ± 6.2%) ($p < 0.01$).



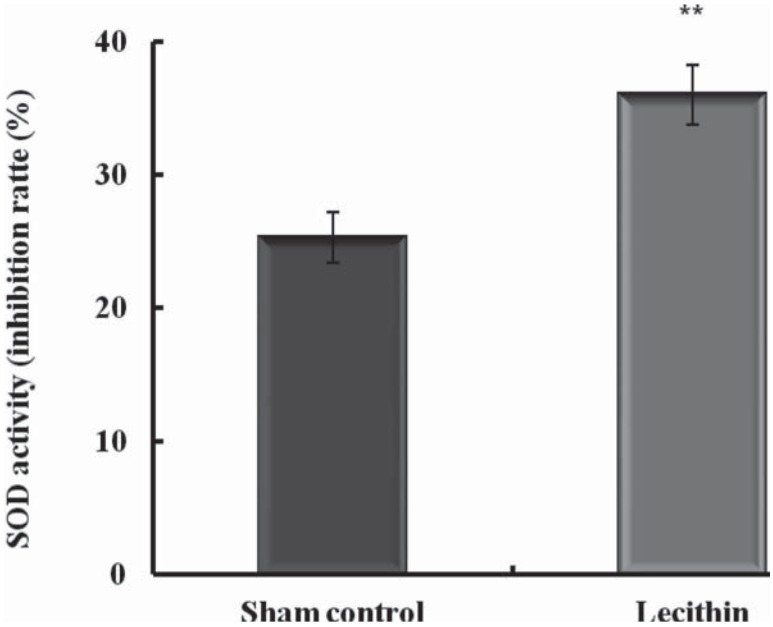


Fig.3. Effect of lecithin on SOD activity (inhibition rate, %) in SAMP8 serum. DW, lecithin was administered (p.o., each dose was 10 mL/kg body weight) for 30 days. The results represent the mean ± S.E. (n=10). Asterisks indicate groups significantly different from sham control at $p < 0.01$ (**).

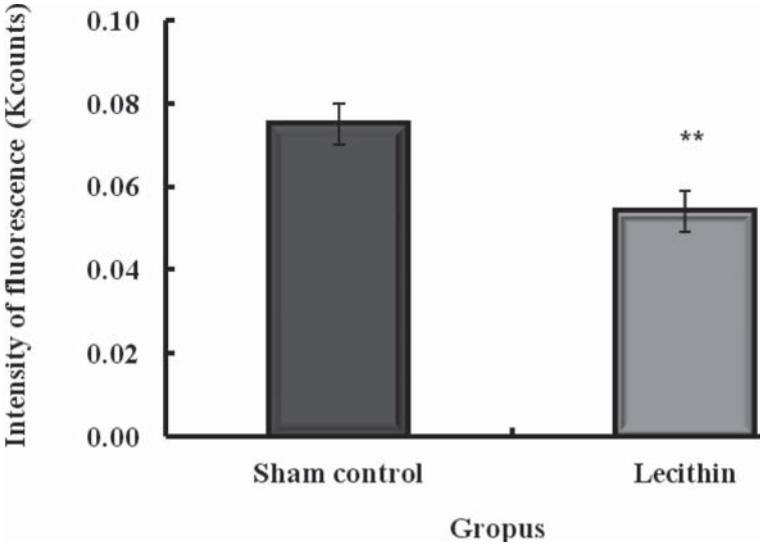


Fig.4. Effect of lecithin on blood levels of anti oxidation activity in SAMP8 serum. DW, lecithin was administered (p.o., each dose was 10 mL/kg body weight) for 30 days. The results represent the mean ± S.E. (n=10). Asterisks indicate groups significantly different from sham control at $p < 0.01$ (**).

Antioxidant action (Luminol measurement)

We took the emission intensity measured on the vertical axis, are shown in fig.4. Results scavenging action on the peroxy radical in mice serum lecithin and sham control group 30 days after administration. Compared with the luminous intensity of the sham control group(0.075 ± 0.004 Kcounts), a significant suppression emission was observed in the lecithin group(0.054 ± 0.005 Kcounts)($p<0.01$). Therefore, the peroxy radical scavenging effects can be expected in the lecithin, further, a synergistic effect was suggested.

Peroxidized fat level (Peroxidized fat level in the brain)

Sham control after the water-T-maze test end and the peroxidized fat density in the mouse brain of lecithin group was screened by tissue TBARS value

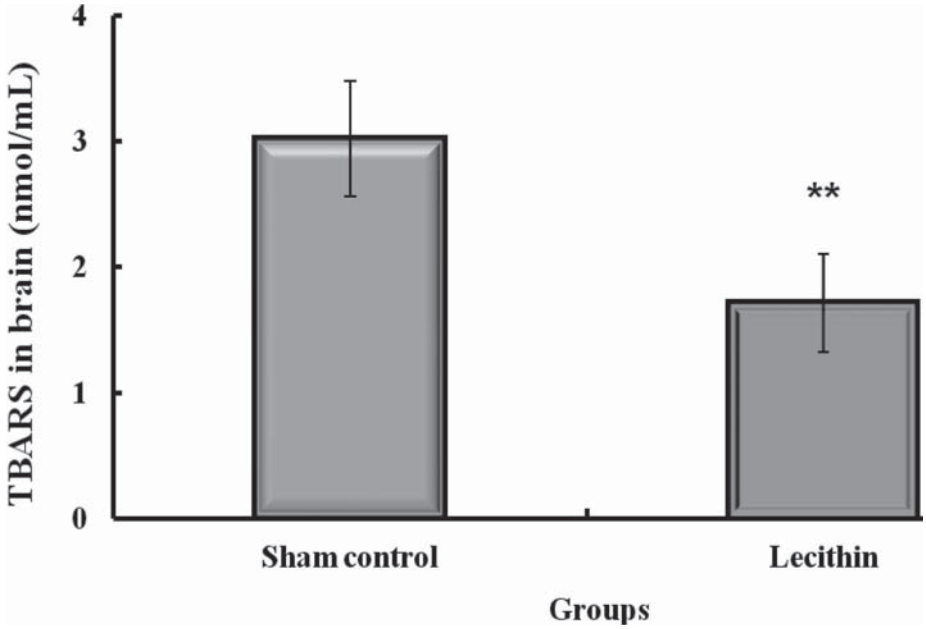


Fig.5. Effect of lecithin on TBARS levels in SAMP8 brain homogenate. The results represent the mean ± S.E. (n=10). Asterisks indicate groups significantly different from sham control at $p < 0.01$ (**).

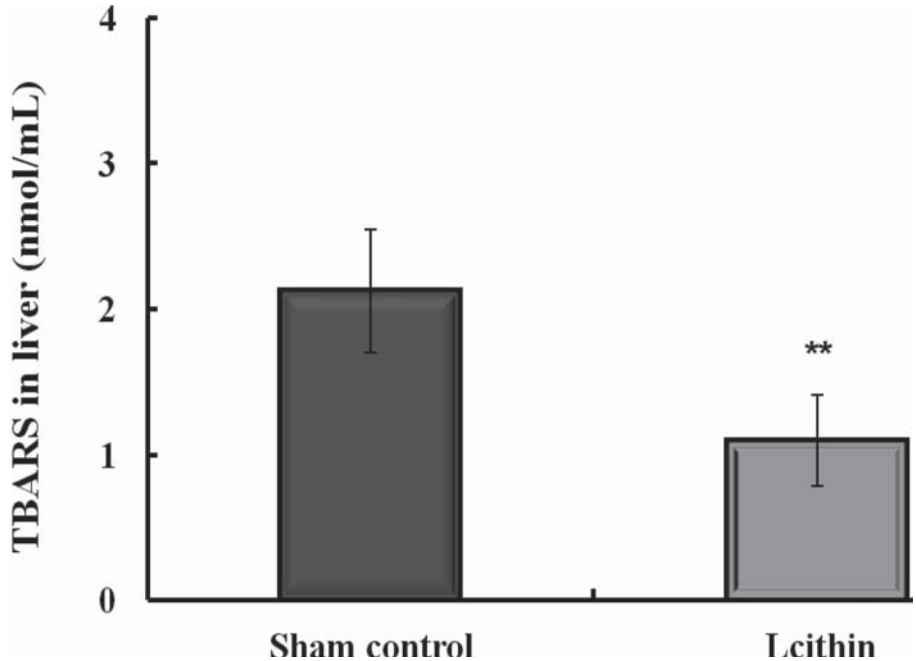


Fig.6. Effect of lecithin on TBARS levels in SAMP8 liver homogenate. The results represent the mean ± S.E. (n=10). Asterisks indicate groups significantly different from sham control at $p < 0.01$ (**).

(Fig.5).

The decrement of the TBARS value was found in the lecithin group(1.7 ± 0.3nmol/mL) as compared with sham control group(3.0 ± 0.04 nmol/mL)($p < 0.01$).

Thus, by lecithin administration, the suppression of the brain lipid hyperoxidation was found.

Peroxidized fat level in the liver

Sham control after the water-T-maze test end and the peroxidized fat density in the mouse liver of lecithin group screened by tissue using TBARS value (Fig.6). The lecithin group (1.0 ± 0.3 nmol/mL) had a decrease of the TBARS value as compared with the sham control group (2.1 ± 0.4 nmol/mL) ($p<0.01$).

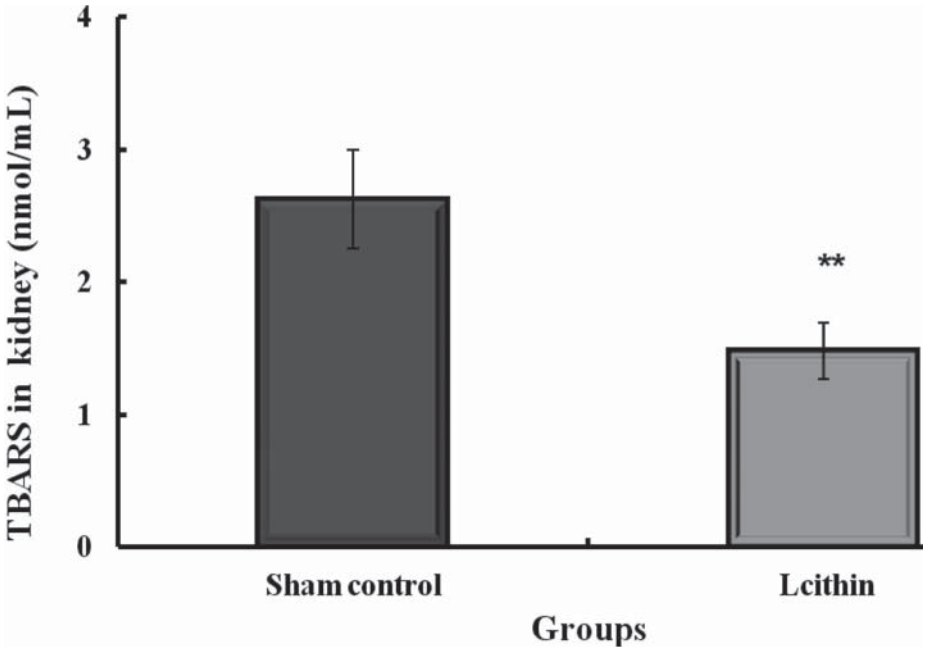


Fig.7. Effect of lecithin on TBARS levels in SAMP8 kidney homogenate. The results represent the mean ± S.E. (n=5-10). Asterisks indicate groups significantly different from sham control at $p < 0.01$ (**).

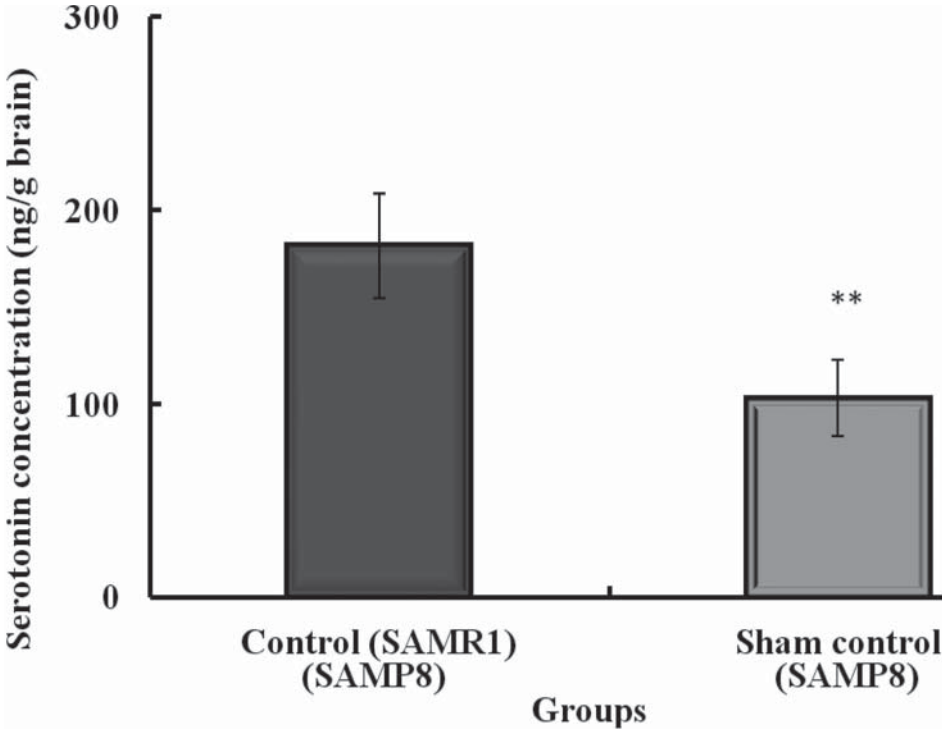


Fig.8. Serotonin concentration (ng/g brain) of SAM brain after water maze test. DW was administered (p.o., each dose was 10 mL/kg body weight) 30-60 min before the acquisition trial. The results represent the mean ± S.E. (n=10). Asterisks indicate groups significantly different from sham control at $p < 0.01$ (**).

Thus, inhibition of the lipid peroxidation in the liver was suggested by lecithin administration.

Peroxidized fat level in the kidney

Sham control after the water-T-maze test end and the peroxidized fat density in the mouse brain of lecithin group screened by tissue using TBARS value

(Fig.7). The lecithin group (1.4 ± 0.2 nmol/mL) had a decrease of the TBARS value as compared with the sham control group (2.6 ± 0.3 nmol/mL) ($p<0.01$). Therefore, we can conclude that lipid peroxidation, etc.

Serotonin concentration

We showed results of the serotonin concentration in the mouse brain just after the water-T-maze test. The training trial in control group (SAMR1(Senescence-Accelerated Mouse Resistant) mice:) which was a negative senility model and the sham control group which was a senescence accelerating model (SAMP8 (Senescence-Accelerated Mouse Prone) mice in fig. 8. A decrease in serotonin concentration was found in the sham control group(102 ± 19 ng/g brain) for control group(181 ± 25ng/g brain)($p<0.01$). Thus, it was found that the SAMP8 mice had decreased serotonin concentration as compared with SAMR1 mice.

We showed results of the serotonin concentration in the mice brain just after the training trial of the water-T-maze test by the lecithin administration in fig.9. Increase of the serotonin concentration was found in lecithin group(201 ± 36 ng/g brain) as compared with the sham control group(102 ± 65 ng/g brain)($p<0.01$). Also, it was similar among a range of the brain serotonin concentration of the control group (SAMR1) and the lecithin treated group than fig.8. Therefore, it was suggested that a decrease in the serotonin concentration by the senescence accelerating was inhibited by lecithin administration.

Morris water maze test

We performed a Morris water maze test as evaluation of the space perception and showed test results in control group (SAMR1 mice) which was a negative senility model and the sham control group which was a senescence accelerating model (SAMP8 mice) in fig.10. A delay of significant platform delivery time was found in sham control group and the

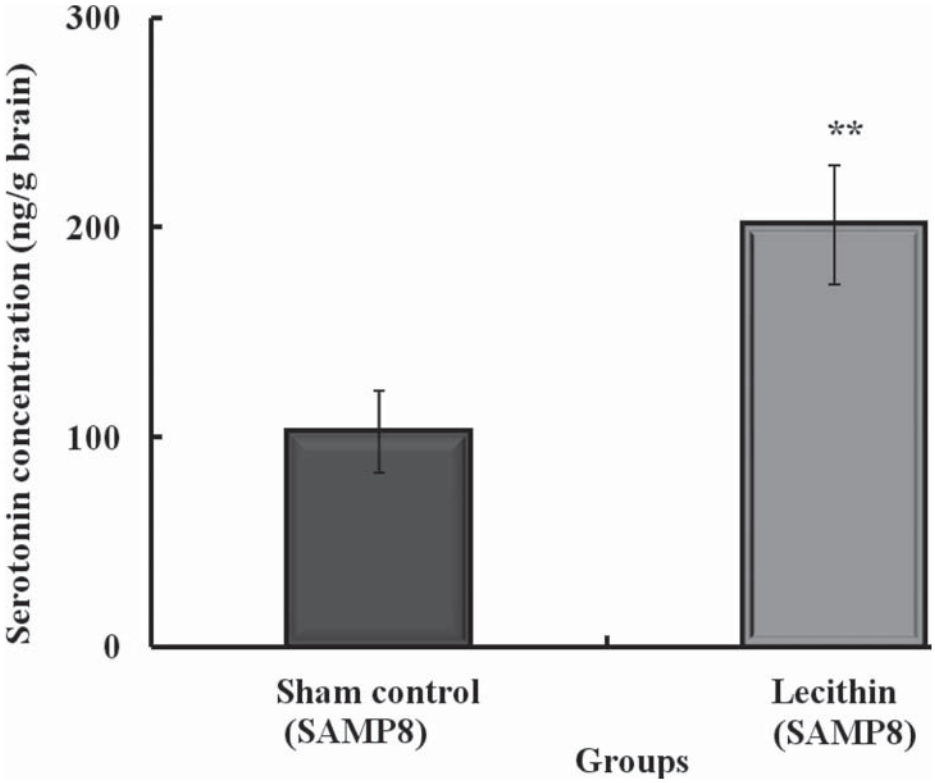


Fig.9. Serotonin concentration (ng/g brain) of SAMP8 brain after water maze test. DW (Sham control) and lecithin were administered (p.o., each dose was 500 mg/kg body weight) 30-60 min before the acquisition trial. The results represent the mean ± S.E. (n=10). Asterisks indicate groups significantly different from sham control at $p < 0.01$ (**).

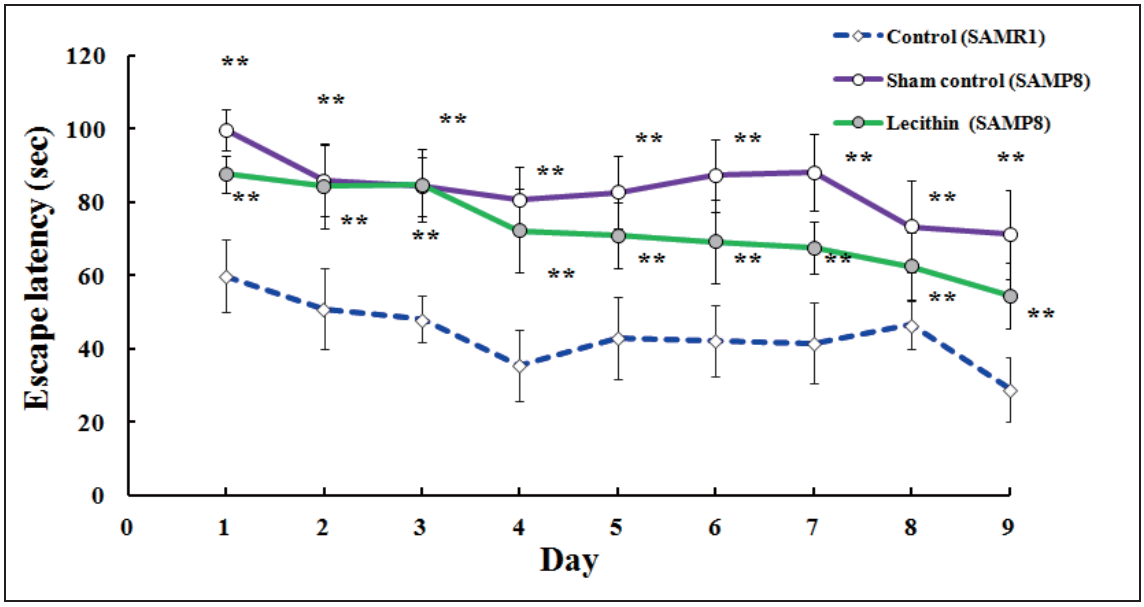


Fig.10. Latency to escape from the water. DW was administered (p.o., each dose was 10 mL/kg body weight), and EGb were administered (p.o., each dose was 500 mg/kg body weight) 30-60 min before the acquisition trial. The results represent the mean ± S.E. (control, n=17; sham control, n=25; lecithin, n=15). Asterisks indicate groups significantly different from control at $p < 0.01$ (**).

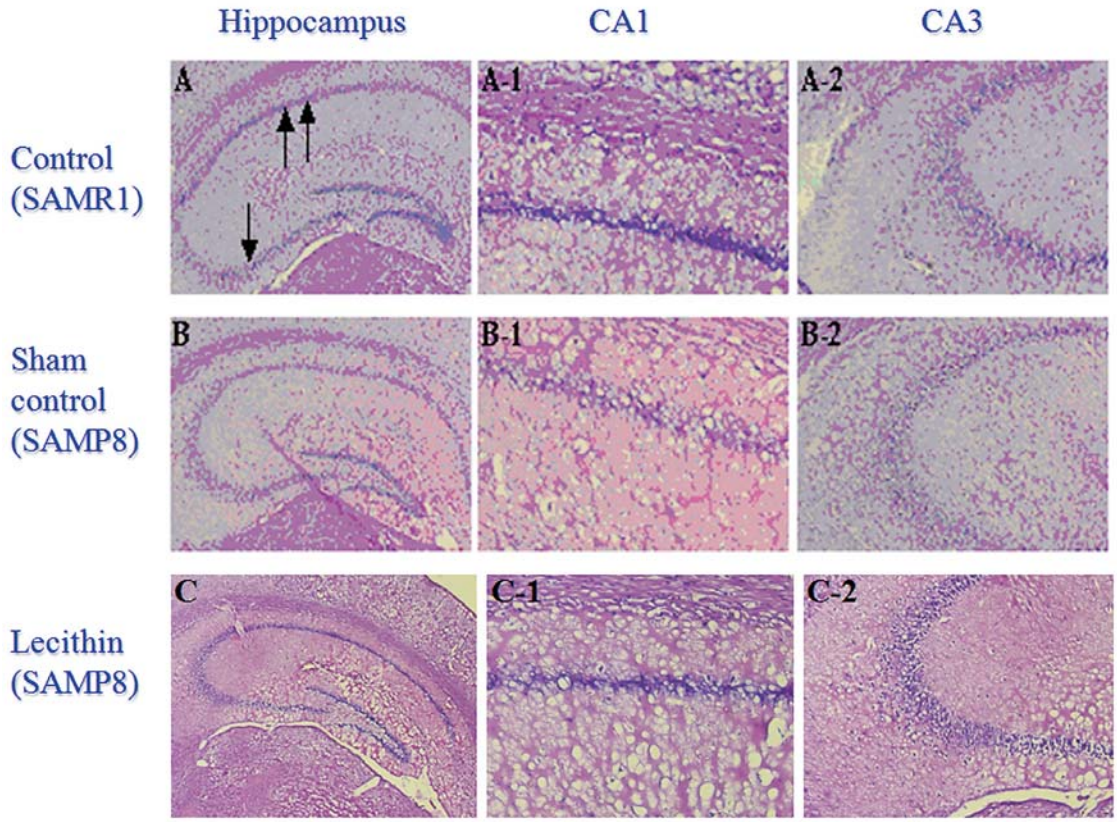


Fig.11. Histopathological alterations of the hippocampal CA1 (double arrow ↑ ↑) and CA3 (arrow ↓) regions in 26-27 week-old SAM. Microphotographs of coronal sections of the hippocampus in control (A), sham control (B) and lecithin (C) were stained with hematoxylin and eosin. Scale bar, 40 ?mx1 (A-D), 200 ?mx5 (A1-C1), 100 ?m x 2.5(A2-C2)

group of lecithin as compared with control group ($p<0.01$). The significant difference of the platform delivery time was not found from sham control group as compared with the lecithin group.

Brain tissue dyeing

The hematoxylin and eosin method

We showed results of the histologic change by the HE staining in the sea lion after the chronic administration of lecithin in fig.11 and screened histologic change by observation of CA1 domain and the CA3 domain. The density of the cone cell in the hippocampal CA3 region in sham control group (SAMP8 mice) decreased as compared with the control group (SAMR1 mice) (A2, B2). Furthermore, this density reduction was remarkable in CA1 domain (A1, B1). However, cell density showed cell density similar to a hippocampal domain of the control group in CA1 and CA3 region highly (C1) in the lecithin group. Also, we showed the density of the cone cell in the hippocampal CA1 region in the sham control group in Table 1 as compared with the control group. It was more remarkable in the sea lion whole area including CA1 in the lecithin group and CA3 region than sham control group, and, in the CA1 domain, the density of the cone cell was high. In the CA1 domain, the density of the cone cell of the CA1 domain in the lecithin group was in particular significantly higher than the sham control group ($p<0.01$) .

Klüver-Barrera method

We showed results of the histologic change by the Kl?ver-Barrera dyeing in the sea lion after the chronic administration of lecithin in fig.12 and screened histologic change by the observation of CA1 domain and the CA3 domain. Also, we transcribed control group, sham control group, a tissue slice of the lecithin group into each A, B, C and transcribed the sea lion domain similar to fig.12. The density of the cone cell in the whole sea lion which included CA1 and CA3

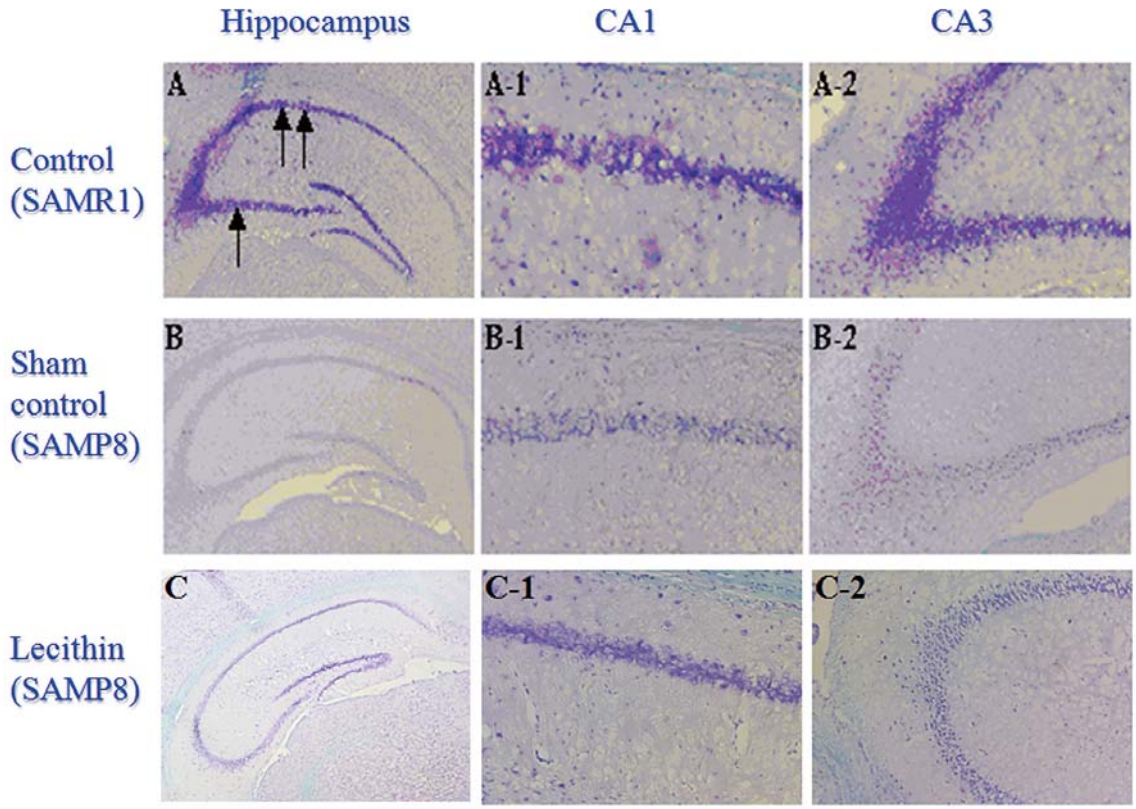


Fig.12. Histopathological alterations of the hippocampal CA1 (double arrow ↑ ↑) and CA3 (arrow ↑) regions in 26-27 week-old SAM. Microphotographs of coronal sections of the hippocampus in control (A), sham control (B) and lecithin (C) were stained with Kluver-Barrera. Scale bar, 40 ?mx1 (A-C), 200 ?mx5 (A1-C1), 100 ?m x 2.5(A2-C2)

Table 1. Surviving neurons in the hippocampal CA1 region in each SAM strain

	Number of surviving neurons in the CA1 region
	26-27 week-old
Control (SAMR1)	243.5 ± 4.6
Sham control (SAMP8)	198.3 ± 7.5**
Lecithin (SAMP8)	256.2 ± 5.8##

The surviving pyramidal neurons per unit length (1 mm) in the entire hippocampal CA1 region were counted five times in independent fields under a light microscope. Results are the mean ± S.E.M. of three to four independent experiments. **P<0.01, (Control vs. Sham control); ## P<0.01, (Sham control vs. EGb), compared with 26-27 week-old SAM by Student's t-test.

region was remarkable, and it was found in sham control group (SAMP8 mice) as compared with control group (SAMR1 mice) to decrease (A, B). In contrast, it was found in CA1 and CA3 region in the lecithin group that cell density greatly (C) showed cell density similar to a hippocampal domain of the control group was conspicuous.

Discussion

SAM model

The SAM (Senescence Accelerated Mouse) model is based on the mouse and other systems mouse of AKR/J origin and a cross not to anticipate and is the inbred strain mouse which has been developed by repeating the selection mating which was based on pathological finding for a senility degree score, a life[33]. Close to q cardinal sign and 14 SAMP (Senescence Accelerated-prone Mouse) systems to do and 4 SAMR (Senescence Accelerated-resistant Mouse) systems by senescence accelerating and negative senility, furthermore, the SAMP quodque system is a system relatively-specific with aging for promotion senility, and naturally occur in various senility pathosis[33),34)]. Inevitably, involving a primary thing of pathosis thought about are included in these pathosis with physiologic senility in human elderly people.

Because the SAMP8 mouse which they used this time shows pathosis such as learning, a mneme obstacle, affective disorders, immunity dysfunction, the daily rhythm abnormality, and they are senescence accelerating symptoms than SAMR1 mouse, it is placed in the demented model animal in the wide sense[35)-38)]. In contrast, the SAMR1 mouse follows negative senility, and it is more significant as compared with the SAMP mouse and which inhibited a slowdown, and the age-related increase of a senility degree score screened by a senility degree score decision system is often used as a contrast of the SAMP mouse in a study of a life of the conventional breeding SAMR1 mouse because (50% survival) and has a longer life-span which is 40% longer when compared with the SAMP mouse[34)].

Antioxidant effects

It is these prevalence rates that increase with aging, and it is wide that OS(Oxygen Species;OS) is associated with one of the age-related regressive change closely, and the epidemiological characteristic of AD is known[39), 40)]. Reactive oxygen species (Reactive Oxygen Species;ROS) are always generated in human bodies with energy using oxygen. ROS causes the disorder of the cell function by modifying function molecules such as protein, lipids, the DNA from the high reactivity. The cells comprise defense mechanisms for ROS, but OS generates it inside and outside the cells when we cannot handle generated ROS enough excessively. Contribution of ROS is pointed to for an episode of the neurodegenerative disease including AD[41)]. Also, the material about organic OS protection is divided into oxidation injury repair enzymes such as SOD, free radical development prevention type antioxidants such as the glutathione peroxidase, vitamin C, vitamin E, free radical supplement type antioxidants such as the flavonoid and lipase, the protease 31). As a result of having

evaluated the elimination ability of the peroxy radical derived from AAPH as an index of the antioxidant action in this study, significant emission of light inhibition was found in an emission of light strength from the control group (SAMR1) as compared with an emission of light strength of the sham control group (SAMP8). In this study, we did the elimination ability of the peroxy radical derived from SOD-like activity that was an O_2 · - degrading enzyme and AAPH with the index of the antioxidant action and examined the influence for the antioxidant action of lecithin.

The SOD-like activity was not found in the lecithin group, but it was done through scavenging the peroxy radical which we generated by using lecithin, and it is speculated what was attenuated, as a result, *in vivo* OS by an antioxidant of the mice serum by AAPH because a luminol emission of light strength derived from AAPH was inhibited conspicuously. Therefore, lecithin is thought to prevent the progression of demented prophylaxis and these diseases.

Peroxidized fat level

In the affected part of AD, accumulation of TBARS becoming the index of the lipid peroxidation response is found[43]. Also, the Hirai et al. measure Wistar origin rat intracerebral TBARS and reported increase of the peroxidized fat with the senility first[43]. Takeuchi et al. and Uchiyama et al. report increase of TBARS with the senility in rat serum, liver microsome in rat brain, liver, kidney subsequently[40), 44]. In the longitudinal study using the SAM mice, we found that increase of the peroxidized fat preceded the manifestation of the senility symptom, and it was found that the lipid peroxidation response with free radicals was associated with senescence accelerating directly [45]. Also, serum TBARS of the SAMP mice shows an upward trend 2-8 months after birth as compared with the SAMR mouse later, and it is reported to show the change that is similar in the liver and the skin tissue[46), 47]. In this study, we measured SAMP8 mice brain, liver, TBARS in the kidney and examined the effect on lipid peroxidation of lecithin in each organ and LPC (lyso phosphatidyl choline). Because we thought to correlate with a quantity of peroxidized fat to some extent, as for the quantity of peroxy radical which resulted from AAPH, it was speculated that the reduction of the peroxy radical quantity by lecithin caused a peroxidized fat quantity reduction of the brain, liver, the kidney, but, actually showed a significant difference in the sham control group and the lecithin group.

Serotonin concentration level

The memory consists of the highly complicated intentions including many nervous structures and the neurotransmitter system. Colin and a glutamine-based system are tied so far with the cognitive process such as attention, learning and the memory function, and it is suggested that serotonin (5-HT) plays an important role more in highly advanced cognitive processes[48), 49]. The 5-HT1A receptor shows high distribution in 5-HT receptor subtype in particular in the sea lion domain that it is apparent to have a limbic system, the role that are above all important to learning and memory[50), 51].

Also, it is thought that the serotonin is associated with the affective disorders that are demented peripheral symptom because the serotonin is associated with an effect, and it is shown that we reduce a rage and aggressiveness measured by a psychological test in 5-HT agonist[52]. Whereas, in the AD disease brain, it is known that abnormality occurs in neurotransmitters, and it is reported that serotonin and the deep raphe nuclei of the relation, a median raphe nucleus, the nerve cell number of the posterior tegmental nucleus decrease[52), 53]. Also, the Gu et al. found that serotonin concentration increased in SAMP8 mouse serum by a lysolecithine administration when apparent[54]. Therefore, in this study, we studied effect on intracerebral serotonin concentration of the SAM mouse in lecithin. The statistical significance was found in intracerebral serotonin concentration in 2 groups. Because lecithin indicates the antioxidant action resulted in the increase of the serum serotonin level, the action for the serum serotonin level of lecithin and action for the intracerebral serotonin of lecithin, action of lecithin for neurotransmitter such as

the acetylcholine is thought about.

Morris water maze test

We learned what we progress to a specific landmark seen over a labyrinthine wall that allied learning is necessary for the Morris type water maze which we tried by this experiment and moves in maze[55]. It is one of three methods of experiments which is regarded as the golden standard for experiment procedures using the mouse solid, and this Morris water maze test can screen a spatial memory with a thing using the evacuation action that assumed space information a clue. We conducted a water-T-maze test of the SAM mouse in lecithin in this study and screened the space recognition ability. From training session start day one to the last day, an extension at the delivery time when the sham control group was significant was found when we compared the sham control group of the SAMP8 mouse with the control group of the SAMR1 mice. Furthermore, in the probe test, remarkable increase of the number of cutting across of the sojourn time with division into four and the virtual platform area was found. Also, the shortening at delivery time was not found in the group of sham control until study day 7, and the change over time was not found. We confirmed learning in the space perception and memory impairment with the SAMP8 mice which was senescence accelerating system than the SAMR1 mice showed senility more negative than this. A significant difference was found in sham control groups and lecithin groups as compared with the control group. Also, a significant difference had even sham control group and a group of lecithin. As for the cell death inhibition of the hippocampal cone cell by the lecithin administration, improvement of space perception learning and the memory impairment was suggested.

Histologic change of the brain

It is said that it is apparent that dementia develops most due to a neuronal remarkable decrease, and it is checked for an experiment case using the animal that a sea lion is associated with a memory deeply. The sea lion is comprised of gyrus dentatus, CA1, CA3, subiculum (the low rank region). The most important input site to a sea lion is an entorhinal cortex, and a neuron having axon to finish is present in gyrus dentatus, CA1, CA3 [56]. The important space information is input into a sea lion by an entorhinal cortex and a direct combination of CA1[57]. Morris reported that the results of the water-T-maze test decreased in the rat of the NMDA receptor antagonist administration in the cone cell domain of CA1 in the specimen of the hippocampal slice [29]. Also, the Riedel et al. find that we inhibit the accomplishment of the problem that hippocampal inhibition already learned and show that a sea lion is necessary for the recall of not only the fixation but also the spatial memory[58]. Furthermore, it is reported in the hippocampal CA1 region of the SAMP8 mouse as compared with SAMR1 mice of ten years of age of the month that a nerve cell decreases[58]. About the observation of the histologic change in this study, we performed it in reference to a hippocampal structure in the rat brain of David et al. and judged CA1 in the SAM mouse sea lion and the CA3 domain[59]. The rodent hippocampal CA3 cone cell creates an arch type, and a cone cell is clogged up a little, and pigment extremely thickens. In contrast, the ganglionic layer of optic nerve of the CA1 domain was clogged up thinly and judged CA1 domain, ↑ site with CA3 domain in Fig. 9, 10 of ↑ ↑ sites because CA1 cells were parvus more clearly than CA3 cone cell[59]. In whole sea lion which included CA1 in sham control group (SAMP8 mouse) and CA3 domain as compared with control group (SAMR1 mouse), the findings were remarkable, and, consequently as a result of HE staining and KB dyeing, the density of the cone cell decreased. Therefore, it was speculated that the space recognition ability evaluation in the water-T-maze test lowered by senescence accelerating as a result that the nerve cell in the sea lion decreased, and input ability of the space information decreased. Whereas the cell density of the CA3 domain in the lecithin group increased more than the sham control group. Also, it was more remarkable in whole sea lion including CA1 in the lecithin group and CA3 region than sham control groups, and in the

CA1 domain, the density of the cone cell was high. In the CA1 domain, the density of the cone cell of the CA1 domain in the lecithin group was in particular significantly higher than the sham control group (Table 1). Therefore, the nerve cell (CA1 domain) in the sea lion becomes dense, and memory improvement is expected by a direct combination of CA1.

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