Differential effects of melatonin on hippocampal neurodegeneration in different aged accelerated senescence prone mouse-8

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Abstract

OBJECTIVES: A purpose of this study is to compare the differential effects of melatonin on hippocampal neurodegeneration in accelerated senescence prone mouse-8 (SAMP8) which is initiated treatment at different age.

METHODS: The 4-months old SAMP8 mice were injected subcutaneously with melatonin (1 mg/kg/day) for 4 months. Similar treatments were performed in the 7-months old mice. When the animals were complete 11-months old, a series of tests were performed. Y maze test and Eight-arm radial maze task were used to assess cognitive performance. Hippocampal pyramidal cells were estimated by Nissl's staining. By using Gomori's methenamine silver methods, the methenamine silver staining granules (MSSG) were observed in area CA1 of hippocampus. A computer-assisted morphometric study was carried out on the ultrastructure of perikaryal CA1 pyramidal cell mitochondria. The volume density (Vv), surface density (Sv), numerical density (Nv) and mean volume (V) of the mitochondria were calculated.

RESULTS: Melatonin treatment obviously reduced the deposition of MSSG and elevated hippocampal pyramidal cell number while improving the learning and memory deficits of SAMP8. The mice initiated treatment from 4-months old exhibited a greater response to melatonin supplementation than 7-months old mice. It also decreased mean volume (V) and significantly elevated the Sv and Nv of the mitochondria in hippocampal CA1 region. However, 7-months old mice showed little effects on it.

CONCLUSIONS: Our results indicate that the protective effects of melatonin on hippocampal neurodegeneration of SAMP8 are age dependent.

ORIGINAL ARTICLE

Abbreviations:
MT – melatonin
CSF – cerebrospinal fluid
SAM – senescence accelerated mouse
APP – amyloid precursor protein
Aβ – β-amyloid protein
ATP – adenosine 5′-[tetrahydrogen] triphosphate
MSSG – methenamine silver staining granules
Vv – volume density
Sv – surface density
Nv – numerical density
V – mean volume
mtDNA – mitochondrial deoxyribonucleic acid

INTRODUCTION

Aging, accompanied by cognitive deficits, involves a very complex series of changes both at the genetic and the phenotypic level. Increased brain oxidative stress and mitochondrial dysfunction may be involved in hippocampal neuron degeneration and seem to have an important role in cognitive impairment caused by normal aging and neurodegenerative diseases (Ames *et al.* 1993; Lin & Beal, 2006).

The senescence-accelerated mouse (SAM), established from its progenitor strain AKR/J mice, was developed by Takeda *et al.* (1981) as a murine model of accelerated aging. Nine senescence-prone (SAMP) strains and four senescence-resistant (SAMR) strains have been developed. SAMP8, a substrain of SAMP, shows rapid advancement of senescence and exhibits some age-related neuropathological features after 4–6 months of age, such as age-related deficits in learning and memory, periodic acid Schiff-positive granular structures in the hippocampus (Akiyama *et al.* 1986), dysfunction of the glutamatergic, cholinergic and noradrenergic neurotransmission (Nomura & Okuma, 1999), mitochondrial dysfunction in hippocampus (Xu *et al.* 2007), and neuronal loss of cerebral cortex (Miyamoto *et al.* 1986). The impairment of spatial learning and memory in SAMP8 mice becomes apparent after 4 months of age (Ikegami *et al.* 1992). Among these mechanisms of the cognitive impairment in SAMP8 mice, the oxidative stress damage and mitochondrial dysfunction in hippocampus and the cerebral cortex neuron remain to be the most primary causes (Farr *et al.* 2003; Passos *et al.* 2006).

Melatonin has potential utility both in slowing normal brain aging and in treatment of neurodegenerative conditions. Melatonin levels both in cerebrospinal fluid (CSF) and in postmortem human pineal gland are decreased in aging (Liu *et al.* 1999). Pinealectomy, which removes the major source of melatonin synthesis within the brain, appears to accelerate the aging process (Payão *et al.* 2001; Reiter *et al.* 1999). Melatonin shows a remarkable function versatility exhibiting antioxidant (Okatani *et al.* 2002), anti-amyloid (Matsubara *et al.* 2003), neurogenesis promotion (De Butte & Pappas, 2007), synaptic plasticity modulation (El-Sherif *et al.* 2003), mitochondrial protection (Jou *et al.* 2004; Jou *et al.* 2007) and immunomodulatory properties (Giannoulia-Karantana *et al.* 2006). Melatonin's antioxidative and neuroprotective properties have repeatedly been demonstrated in the hippocampus (Baydas *et al.* 2005; Letechipía-Vallejo *et al.* 2007; Rosales-Corral *et al.* 2003). Melatonin also preserves mitochondrial homeostasis, reduces free radical generation, e.g., by enhancing mitochondrial glutathione levels, and safeguards proton potential and ATP synthesis by stimulating complex I and IV activities (Feng & Zhang, 2005).

Recently, Lardone *et al.* (2006) demonstrated that endogenous melatonin levels and oxidative damage in some tissues of SAMP8 mice exists an inverse correlation, which indicated a low level of melatonin in these animals. It has also been demonstrated that chronic melatonin administration was able to reduce the age-dependent oxidative damage of neural lipids and proteins in SAMP8 mice through the ability of melatonin to scavenge oxygen free radicals and to stimulate antioxidant enzyme activity (Okatani *et al.* 2002). Melatonin also can restore the hepatic mitochondrial physiology in old SAMP8 mice by stimulating mitochondrial respiratory chain activity (Okatani *et al.* 2003). Not only is melatonin an excellent antioxidant, but so are its metabolites, e.g., AFMK, AMK, etc (Manda *et al.* 2007; Tan *et al.* 2007). However, some researcher recently showed that the different age at initiation of melatonin treatment in the transgenic Tg2576 mouse yield opposite result concerning the antioxidant and the anti-amyloid effect (Quinn *et al.* 2005). Whether the discrepancy would also exist in other animal model of aging and neurodegenerative diseases remain to be determined. In the current experiment, we compared the effect of chronic melatonin treatment on hippocampal neuron degeneration between the middle-aged and senile SAMP8 mice. Through these experiments, we hope to explore the possible neuroprotectant mechanism of melatonin in different stages of aging and neurodegenerative diseases. The protective effect of melatonin was measured on the basis of learning and memory impairing, neuron loss, A-beta loading and morphological structure of the mitochondria in hippocampal CA1 region.

MATERIAL AND METHODS

Chemicals

Melatonin (Sigma, St. Louis, MO) was dissolved in absolute ethanol and then diluted with 0.9% saline; the final ethanol concentration was less than 0.5%. All other chemicals were analytical grade.

Animals

The progenitors were purchased from Vital River Laboratory Animal Company (Beijing, China). They were all housed and maintained in a room at 20 ± 2 °C with automatic light cycles (12-hr light/dark) and a relative humidity of 40–50%. Food and water were offered ad libitum throughout the study. All experiments were car-

ried out with the approval of the local animal use committee. Efforts were made to minimize animal suffering and to reduce the number of animals used.

Melatonin treatment regime

30 male SAMP8 were randomly divided into three groups (n = 10 for each group). The 4 months old SAMP8 mice (MT-4 group) were injected subcutaneously (S.C.) (AM 10:00) with melatonin (1 mg/kg/day) for 4 months. Similar treatments were performed in the 7 months old mice (MT-7 group). The mice in control group were injected S.C. (AM 10:00) from 4 months old with 0.9% saline and 0.5% ethanol for 4 months. A series of praxiology tests, such as Y-maze test and eight-armed radial maze test, were done on the next day when the animals were complete 11 months old before they were killed.

Behavioral testing

Y maze test

The apparatus consisted of three opaque-white arms (30 × 5 × 20 cm) connected into a Y shape. Each arm had a signal light at its distal end and the floor of each arm was installed with electric wires through which an electric shock (40 ± 5 V for 10 s) can be applied. Only one arm lacking electric current remained illuminated throughout the test (because rats are typically neophobic, they prefer to avoid an illuminated area). Mice were adapted to the apparatus for 3 min before the start of testing. During the testing, mice were placed in the stem of the Y maze. When animals received an electric shock, they entered into one or the other of the two branch arms. Entries into the nonshock (illuminated) arm no more than 10 seconds were recorded as correct choices. The test was stopped when the animal entered into the nonshock (illuminated) arm in 9 out of 10 consecutive trials. Twenty-four hours latter, the correct choices in the 10 consecutive trials were measured. The maze was cleaned with ethanol between each animal to minimize odor cues.

Eight-arm radial maze learning performance

The apparatus consisted of an octagonal central platform (30 cm across) and eight radial arms (10 × 66 cm) projecting from the center, all elevated 70 cm above the floor. Each arm had a signal light at its distal end and the floor of each arm was installed with electric wires through which an electric shock (40 ± 5 V for 10 s) can be applied. Only one arm lacking electric current remained illuminated throughout the test. Mice were adapted to the apparatus for 3 min before the start of testing. The mice were placed at the end of one arm and received an electric shock. Entries into the nonshock (illuminated) arm were recorded as correct choices. Mice were given three trials on the first day. Twenty-four hours latter, the time of correct choice was measured as memory result (escape latency). The maze was cleaned with ethanol between each animal to minimize odor cues.

Tissue samples

After the praxiology tests, the mice were deeply anesthetized intraperitoneally with 10% chloral hydrate (30 mg/kg) and perfused transcardially with 50 ml of ice-cold physiological saline, followed by a fixative of 80 ml of 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains of dorsal hippocampus were postfixed overnight in 4% paraformaldehyde at 4 °C, transferred to 30% sucrose in PBS for 24 h at 4 °C, and frozen. 20 μm-thick serial coronal sections containing both the ipsilateral and contralateral hippocampus were cut every 5th section through the dorsal hippocampus [from bregma –1.34 mm to bregma –2.54 mm according to the atlas of Paxinos & Franklin (2001)] with a Leica SM200R microtome. The free-floating sections were prepared for double. One was treated with Nissl staining, and the other was performed with Gomori's methenamine silver methods. The ventrohippocampus were prepared for the electron microscopy analysis.

Cresyl Violet (Nissl) staining and neuron counting

The sections were mounted and air-dried on gelatin-coated microscopic slides and stained briefly (30 s) with 0.25% cresyl violet. Sections of each hippocampus sampled at regular intervals of 120 μm were taken for analysis. Quantitative analysis of pyramidal cells was performed as described previously (Onozuka *et al.* 1999). Round, clear, medium, and large cells were counted in the dorsal hippocampus using a 40 × microscope objective. For each section, all cells meeting the criteria described above were counted in the hippocampal pyramidal layer in the CA1 and CA3 region.

Gomori's methenamine silver method

A-beta load was measured in the hippocampus on 5 coronal plane sections [bregma −1.76, −1.90, −2.04, −2.18, and −2.32 mm; see Ref. (Paxinos & Franklin, 2001)] per animal using a 40 × microscope objective by Gomori's methenamine silver methods (Takemura *et al.* 1993). Tissue sections were mounted on to gelatin-coated slides and air-dried. The sections were washed thrice in distilled water and immersed in 0.5% periodic acid solution for 15 min. After rinsed in water for 5 min, the sections were immersed in 8% chromic acid for 30 min and then washed slightly in water. After treatment with 1% Sodium Pyrosulfite for 1 min, sections were replaced in the methenamine silver solution at 60 °C about 35 min until they develop a golden brown color. The reaction was stopped using hypo (3% w/v sodium thiosulphate) for 2 min. Sections were rinsed, dehydrated, cleared and mounted. For each section, clusters number, granules number and the area of granules were counted by image analytical system (Leica Q500IW,

Germany) in the two distinct zones of the hippocampal CA1 region.

Electron microscopy

Mice were perfused through the heart with 2% paraformaldehyde and 2.5% glutaraldehyde in PBS. Brains were removed and the ventrohippocampus was cropped several 1×1×3 mm tissue blocks and then fixed in the same solution for 48 h at 4 °C. The tissue were washed in PBS, postfixed for 90 min with 1% osmium tetroxide in PBS, rinsed in PBS, dehydrated with graded ethanol and then infiltrated overnight in half acetone and half epon resin. The tissue was fixed in Epon 812 at 60 °C for 48 h. Semithin sections (1 μm) were cut on an AO microtome. After dyeing by toluidine blue, selected areas of CA1 hippocampus were identified, trimmed, and mounted on blank plastic blocks. Serial ultrathin sections were cut on an ultramicrotome and collected on Formvar- and carbon-coated slot grids. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Hitachi H-600 electron microscope (Hitachi, Tokyo, Japan).

Stereological analysis

Electron micrographs were taken at a final magnification of 15000 × to determine the volume density (Vv), surface density (Sv), numerical density (Nv), and mean volume (V) of the mitochondria in hippocampal CA1 region. Six sections per group and three distinct microscope fields every micrograph was chosen randomly. The microscope images were stored and further analyzed using a computer imaging analysis system (KONTRON IBAS 2.0, Germany). Unbiased quantitations were determined in final prints of 10192 mm^2 at magnification × 15000 according to the method of Weibel *et al.* (1979) as follows. Vv, the relative volume fraction of the mitochondria, was determined as the relative surface fraction of the unit area comprised by all mitochondrion slices. Nv depends on mitochondria sections (N_A) counted per standard measuring surface, on the size distribution (K, for mitochondria = 1) and, finally on the shape of the mitochondria (β, in our case = 1.57), writing $Nv = K \cdot N_A^{3/2}/\beta \cdot Vv^{1/2}$. V, the mean volume per mitochondria, was obtained by simple division of Vv by Nv. The surface density (Sv), the surface area of mitochondria in a certain volume, was calculated according to the equation: $Sv = 4 \cdot \sum Bx/\pi \cdot \sum Ar$ where $\sum Bx$ is the total mitochondrial circumference per standard measuring surface and $\sum Ar$ is the total area of the standard measuring surface.

Statistical analysis

Data are shown as means ± SEM. Statistical analyses were performed with the SPSS program. One-way ANOVA followed by the Student-Newman-Keuls test was used for statistical comparison of the mean number. All group comparisons having $P < 0.05$ were deemed significant.

RESULTS

In the Y-maze test, the SAMP8 mice treated with melatonin showed a significant increase in the number of the correct choices in the 10 consecutive trials after 24 h (vs. control, $P < 0.01$). However, no differences were observed between the MT-4 (treatment with melatonin from 4-months old) and MT-7 (treatment with melatonin from 7-months old) group (Fig. 1 A). In radial eight arm-maze task, treatment with melatonin from 4-months old obviously improved short-time memory in a comparison with control group and the MT-7 group, indicated by a decrease of escape latency ($P < 0.05$). The MT-7 group showed no significant improvement (Fig. 1 B).

To quantitatively estimate the levels of A-beta load in the hippocampal CA1 regions, we used Gomori's methenamine silver methods (Fig. 2 A-C). In MT-4 group, a significant decrease in the area of granules was noted (vs. control and MT-7 group, $P < 0.01$, Fig. 2 D). The number of granules of MT-7 group was significantly less than that of the control groups ($P < 0.05$, Fig. 2 E). There were no differences of the number of clusters among three groups ($P > 0.05$) (data not show).

Fig. 3 summarizes quantitative histopathological analysis of hippocampal CA1 and CA3 regions of experiment animals. In hippocampal CA1 regions, treat-

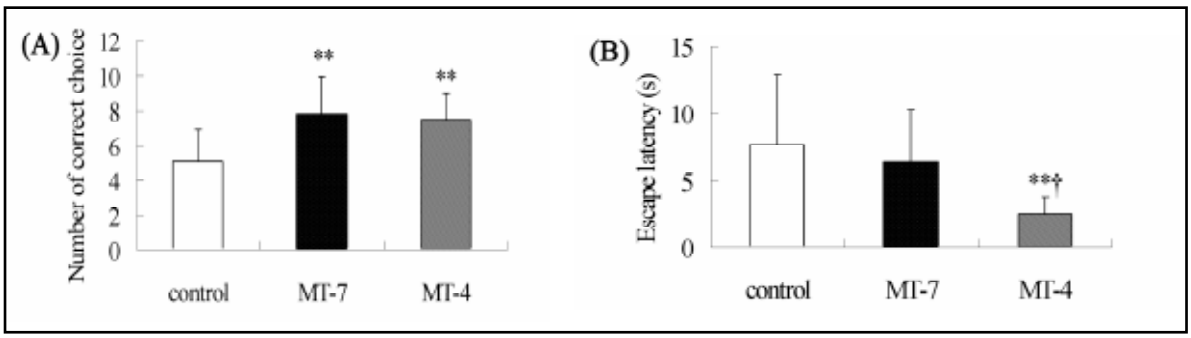

Figure 1. Effects of melatonin treatment on the number of correct choice in Y maze test (A) and the escape latency in radial 8 arm-maze task (B). MT-4: treatment with melatonin from 4-months old; MT-7: Treatment with melatonin from 7-months old. Data represent mean ± S.E.M. ($n = 10$). **$P < 0.01$ versus control, †$P < 0.05$ versus MT-7 group.

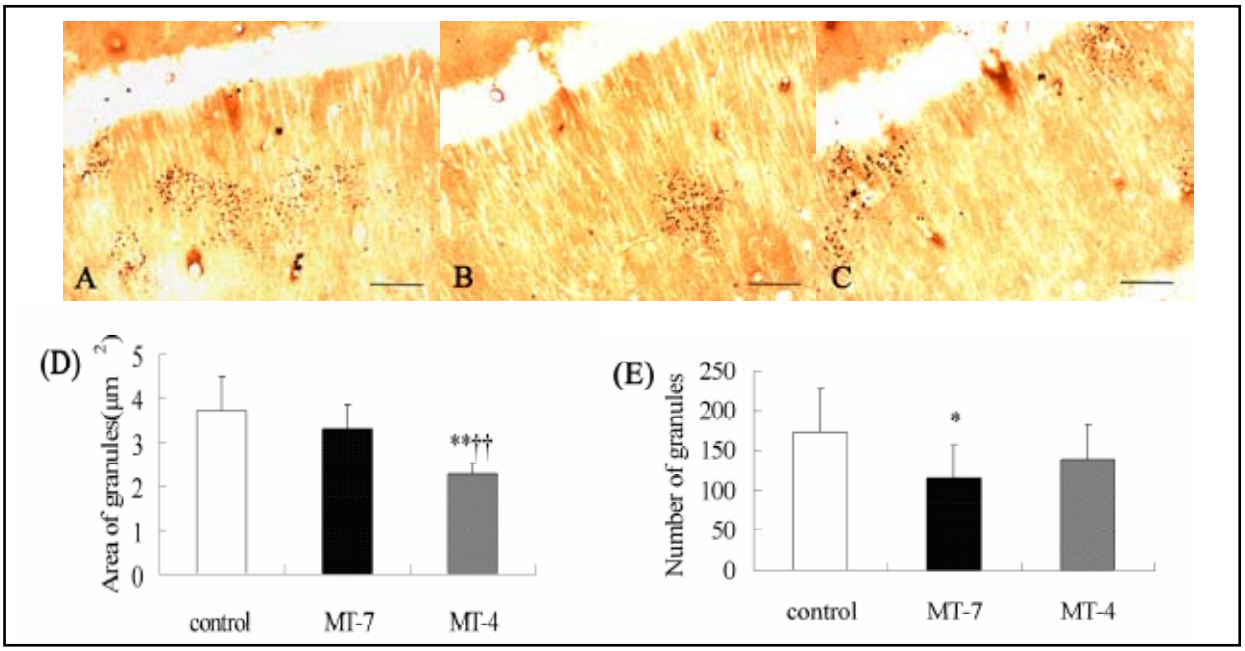

Figure 2. The methenamine silver staining granules (black dots) in the hippocampal CA1 of SAMP8 and the granules' area and number analysis. (**A-C**) Representative photomicrographs of methenamine silver stained sections of the hippocampal CA1 regions control (**A**), MT-4 (B) and MT-7 (**C**) groups (Scale bar, 50 μm). Mean value (±S.E.M.) of the area (**D**) and the number (**E**) of the granules in hippocampal CA1 regions. MT-4: treatment with melatonin from 4-months old; MT-7: Treatment with melatonin from 7-months old. Data represent mean ± S.E.M. (n = 10). *P < 0.05, **P < 0.01 versus control; ††P < 0.01 versus MT-7 group.

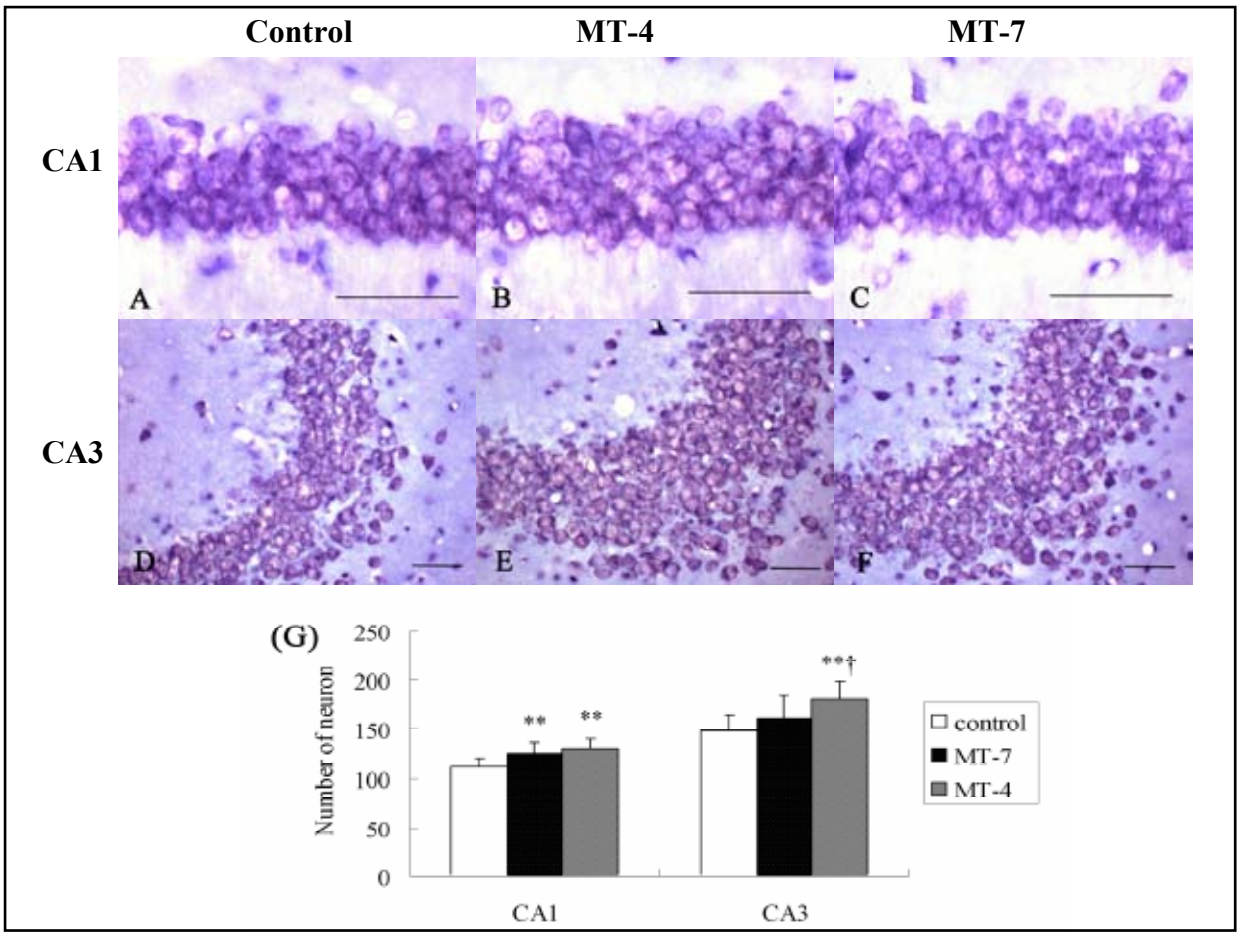

Figure 3. Cresyl Violet staining in the hippocampus of SAMP8 and neuronal number analysis. (**A-F**) Representative photomicrographs of cresyl–violet stained sections of the hippocampal CA1 and CA3 regions (Scale bar, 50 μm). (**G**) Mean value (±S.E.M.) of the neuronal number in the stratum pyramidal of CA1 and CA3 regions. MT-4: treatment with melatonin from 4-months old; MT-7: Treatment with melatonin from 7-months old. Data represent mean ± S.E.M. (n = 10). **P < 0.01 versus control, †P < 0.05 versus MT-7 group.

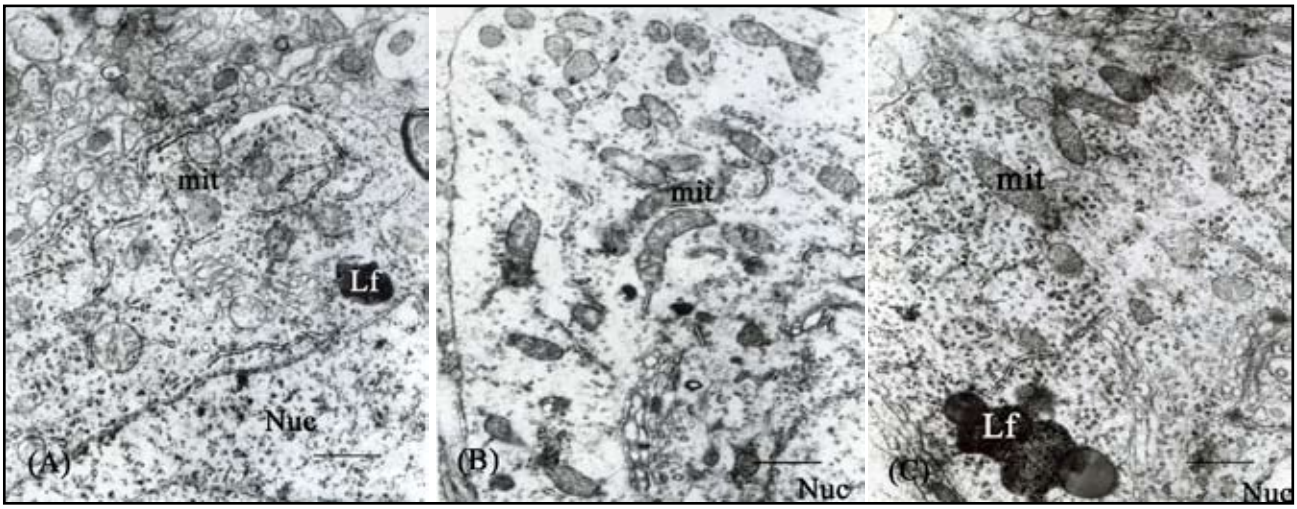

Figure 4. Representative electron microscopic picture of the hippocampal CA1 regions control (**A**), MT-4 (**B**) and MT-7 (**C**) groups (Scale bar, 1 μm). MT-4: treatment with melatonin from 4-months old; MT-7: Treatment with melatonin from 7-months old; Lf: lipofuscin granules; mit: mitochondria; Nuc: nucleus.

Table 1. Morphometric parameters of mitochondria in the hippocampal CA1 regions of different initiated melatonin treatment aged SAMP8.

groups	Vv	Sv	Nv	V
Control	5.62±2.22	4.33±1.56	0.11±0.02	0.32±0.10
MT-7	7.04±2.81	4.22±1.26	0.12±0.03	0.20±0.05*
MT-4	7.99±2.15	7.06±1.58*†	0.37±0.13**††	0.12±0.05**

MT-4: treatment with melatonin from 4-months old; MT-7: Treatment with melatonin from 7-months old; Vv ($\mu m^3/\mu m^3$, $\times 10^{-2}$): Volume density; Sv ($\mu m^2/\mu m^3$, $\times 10^{-2}$): Surface density; Nv (No. mito/μm^3): Numerical density; V (μm^3): mean volume. Data represent mean ± S.E.M. (n = 10). *P < 0.05, **P < 0.01 versus control; †P < 0.05, ††P < 0.01versus MT-7 group.

ment with melatonin increased the pyramidal neurons as compared to the nontreated animals (P < 0.01). No differences were observed between the MT-4 and MT-7 group. However, in hippocampal CA3 regions, an age-dependent difference was observed: treatment with melatonin from 4-months old significantly increased the pyramidal neurons (vs. control and MT-7 group, P < 0.01 and P < 0.05 respectively). No differences were seen between the MT-7 and control group.

In both different initiated treatment ages, melatonin did not influence the Volume Density (Vv) of the mitochondria of pyramidal neurons in hippocampal CA1, but it decreased mean volume (V) and increased the Surface Density (Sv) and the Numerical Density (Nv) in MT-4 group. MT-7 group showed little effect on it. Moreover, both Sv and Nv was higher in MT-4 group compare to MT-7 group (Fig. 4, Table 1).

DISCUSSION

The results of the present work clearly showed that administration of melatonin resulted in a significant improvement learning and memory obstruction of SAMP8 through diminishing the deposition of A-beta, increasing the hippocampal pyramidal neurons number and protecting the mitochondria in the hippocampal CA1 region. Furthermore, the impact of melatonin in preventing learning and memory deficits was higher in 4-months age initiated treatment than 7-months age initiated treatment SAMP8 mice.

The SAMP8 mice exhibits accelerated age-related decline in performance in many tasks of learning and memory (Flood & Morley, 1998; Miyamoto, 1997). It has been proposed as a model for the study of aging of the brain and of defects in learning and memory. The impairment of spatial learning and memory in SAMP8 mice becomes apparent after 4 months of age (Ikegami *et al.* 1992). Aversive memory, hippocampus-dependent place learning, was evaluated by Y maze and eight arm radial maze in present study. In the Y maze test, the SAMP8 mice treated with melatonin either from middle age (4 months old) or senile age (7 months old) showed a significant improvement retention compare to the non-treatment mice. In the eight arm radial maze, the more complex spatial memory task, only the middle-aged treatment group decreased significantly the escape latency. Surprisingly, the performances of the middle-aged treatment group were better than the senile treatment group. Animal performances in mazes are related to the integrity of hippocampus and spatial memory function (Means *et al.* 1971; Roberts *et al.* 1962). Thus the results observed in both mazes suggest-

ed that melatonin may improve the short-term spatial memory capacities of SAMP8 mice. The improvement of the middle-age initiated treatment was much more obvious than that of the senile initiated treatment.

Hippocampal neurons normally play a crucial role in the processing of spatial memory and learning (Morris *et al.* 1982). Hippocampal neurogenesis decreases drastically during aging in rodents, and this decline in neurogenic capacity has been suggested to underlie cognitive impairments that accompany senescence (Kempermann *et al.* 1998; Kuhn *et al.* 1996). There is conflicting evidence regarding SAMP8 brain cell death. Wu *et al.* (2006) has reported insignificant apoptosis in the hippocampus of SAMP8 while Karasawa *et al.* (1997) and Sureda *et al.* (2006) have found cell death in other parts of the brain of P8. Moreover, the neurogenesis of the P8 brain is still to be elucidated. In current study, we counted the hippocampal pyramidal neurons in the CA1 region and the CA3 region. Treatment with melatonin from middle age significantly increased the pyramidal neurons. However, in hippocampal CA3 region, treatment with melatonin from senile age had no significant effect. The neuroprotective effect of melatonin treatment from 4 month old SAMP8 mice might be as a result of antioxdative property, which has been proved by many other researches (Okatani *et al.* 2002). Moreover, its metabolites, e.g., AFMK, AMK, etc, are also an excellent antioxidant (Manda *et al.* 2007; Tan *et al.* 2007). This finding would also support the possibility of a neurogenesis mechanism previously proposed by De Butte *et al.* (2007) in which melatonin supplementation in pinealectomized rats seemingly promotes the genesis of CA1 cells. Interestingly, a recent study detects melatonin receptors in neural stem cells suggesting a potential role of melatonin in promoting a stem cell response (Niles *et al.* 2004). It seems important to further explore the possibility that melatonin causes the hippocampal neurogenesis in SAMP8 by using appropriate markers (e.g., 5-bromo-2-deoxyuridine) to selectively label newborn cells in vivo.

The differential effect of melatonin on the hippocampal neuroprotective effect between middle-aged and senile SAMP8 was also relative to the A-beta level on the brain. Recent studies demonstrate that overproduction and subsequent aggregation of Aβ can severely limit the survival of newborn hippocampal neurons and that hippocampal neurogenesis is dramatically impaired in transgenic mice overproducing Aβ (Verret *et al.* 2007). The SAMP8 mouse has a spontaneous mutation and has an age-related overexpression of APP, elevated levels of Aβ, and cognitive defects (Morley, 2002). The increase in cerebral cortical and hippocampal APP mRNA expression has been shown in SAMP8 at 6 months. The Aβ level of SAMP8 mice increases 100% from 4 to 12 months (Kumar *et al.* 2001). Melatonin has been found to have regulatory effects on APP metabolism. Additionally, administration of melatonin can efficiently reduce Aβ generation and deposition *in vivo* (Matsubara *et al.* 2003) and *in vitro* (Olivieri *et al.* 2001).

However, Quinn *et al.* (2005) recently shows that, despite achieving high plasma concentrations of melatonin, chronic melatonin therapy in old Tg2576 mice initiated at 14 months of age not only fail to remove existing plaques, but also fail to prevent additional Aβ deposition. The age at initiation of melatonin treatment may be the key difference that accounts for the discrepancy between the studies of Matsubara *et al.* (2003) and Quinn *et al.* (2005), in which the same transgenic Tg2576 mouse model was used. Melatonin treatment in the study of Matsubara *et al.* (2003) is started at 4 months of age (prior to the appearance of hippocampal and cortical plaques), an earlier pathological stage compared with 14 months of age in the study of Quinn *et al.* (2005). In present study, we compared the effect of melatonin on the A-beta load in hippocampus CA1 region of SAMP8 at different initiation treatment age. Our results showed that there also existed the discrepancy between the middle-aged treatment and the senile treatment SAMP8 mice. Treatment with melatonin from 4 months old decreased obviously the Gomori's methenamine silver-positive granular structures in the hippocampus CA1 region of SAMP8. The senile initiated treatment showed little effect on it, although their treatment time and concentration were equal. Since the amyloid plaque has emerged in 7 months old SAMP8 mice, our results indicated that melatonin had little effect on the established hippocampal amyloidosis in SAMP8.

Mitochondrial structural and functional abnormalities are associated with aging which is characterized by a decrease in oxidative phosphorylation and ATP (Adenosine 5'-[tetrahydrogen] triphosphate) synthesis, an increase in mtDNA (mitochondrial deoxyribonucleic acid) mutations, an increase in abnormal mitochondrial cristae structures and a marked rise in free radical production (Cortopassi & Wong, 1999; Pallotti *et al.* 1996). Decreasing brain metabolism is a well-documented cause of cognitive abnormalities of the aging and neurodegenerative disease (Blass & Gibson, 1999). A recent study demonstrates that the hippocampal ATP content of SAMP8 at 6 and 9 months old become significantly lower than SAMR1 of the same ages (Xu *et al.* 2007). The actions of melatonin on mitochondria may be mediated via at least three mechanisms. First, antioxidant and free radical scavenging properties of the indoleamine protect the organelle from oxidative damage. Secondly, its actions at the mtDNA level increase the expression of complex IV. Thirdly, a direct interaction of melatonin with the MTP was found recently. In addition, some of the products that are produced when melatonin detoxifies reactive species, especially AMK and AFMK, are also both efficient antioxidants that may be found in mitochondria; these metabolites can also act at the mitochondrial genomic level, resulting in a cascade of protective reactions (León *et al.* 2005). In

current study, we carried out a morphometric study on perikaryal mitochondria of hippocampal CA1 pyramidal cells to compare the effect of melatonin on the mitochondrial structure of SAMP8 at different initiation treatment age. Our results showed that treatment with melatonin from middle-aged exerted beneficial effects on mitochondrial structure in the hippocampal CA1 region of SAMP8 seemingly by increasing the mitochondrial number and diminishing the mitochondrial swelling. While the senile treatment showed little effect on it. Whether treatment with melatonin from senile age could improve the mitochondrial function remains to be further confirmed.

In conclusion, our results demonstrate that there existed discrepancy about the hippocampal neuroprotective effect of melatonin in SAMP8 mice because of the different initiation treatment age. The improvement of the middle-aged initiated treatment was much more obvious than that of the senile initiated treatment.

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