the following conditions were more likely to receive parenteral nutrition than enteral nutrition: sepsis on admission, catheter trouble, benign tumor, benign hematologic disease, or gastrointestinal disease including noninfectious inflammatory bowel disease, ileus, postoperative intestinal problems, ischemic intestinal disease, malabsorption, cholelithiasis and cholangitis, pancreatic diseases, and chronic liver disease. After propensity score matching, the absolute standardized differences were all <10, indicating that the baseline patient characteristics were well balanced between the groups.

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TABLE 2

Characteristics of patients in the unmatched and propensity-matched groups receiving parenteral or enteral nutrition[1]

The daily calorie and amino acid intake was similar in the 2 groups. In the parenteral and enteral groups, the median (IQR) calorie intake was 840 (410) vs. 820 (640) kcal/d, respectively, and that of amino acids was 30.0 (20.0) vs. 30.0 (24.0) g/d, respectively. In the parenteral and enteral groups, median (IQR) values for body height and weight were 155.0 (15.0) vs. 154.0 (15.0) cm and 44.2 (13.6) vs. 44.1 (14.0) kg, respectively.

Table 3 shows the outcomes for the propensity score-matched patients in the parenteral and enteral groups with regard to mortality at 14, 30, and 90 d after the start of the procedure. The incidence of postprocedural pneumonia was 11.9% vs. 15.5% ($P < 0.001$), whereas the incidence of postprocedural sepsis was 4.4% vs. 3.7% ($P = 0.164$) for parenteral and enteral groups, respectively. Table 4 shows the results of logistic regression analyses. In comparison with the parenteral group, the OR of mortality at 90 d after the start of the procedure for the enteral group was 0.78 (95% CI: 066, 0.92).

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TABLE 3

Outcomes of the 2912 pairs of propensity-matched patients in the parenteral and enteral nutrition groups[1]

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TABLE 4

Logistic regression analyses for outcomes in 2912 pairs of propensity-matched patients[1]

The Kaplan-Meier curve is presented in Figure 1. The log-rank test showed a significant difference between the parenteral and enteral nutrition groups ($\chi^2 = 46.639$, $P \leq 0.001$). In the Cox regression analysis, the HR for the enteral group relative to the parenteral group was 0.62 (95% CI: 0.54, 0.71; $P < 0.001$). After excluding patients with gastrointestinal, liver, gallbladder, or pancreatic disease, the subgroup analysis showed results similar to those of the all-patient analysis (Supplemental Table 1).

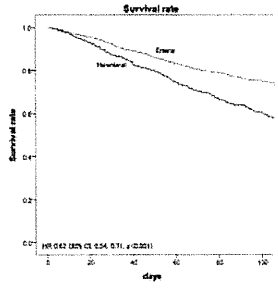

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FIGURE 1

Kaplan-Meier analysis of the propensity-matched groups for parenteral and enteral nutrition. Initial numbers of the parenteral and enteral nutrition groups were 2912 each. In the Cox regression analysis, the HR for the enteral group relative to the parenteral group was 0.62 (95% CI: 0.54, 0.71; $P < 0.001$)

Among the deceased patients, approximately half had pneumonia and approximately one-fourth had heart failure on admission (Supplemental Table 2). The proportions of urinary tract infection, ileus, or postintestinal operation

problems were higher in the enteral group; the proportion of schizophrenia was higher in the parenteral group. With regard to postprocedural complications (Supplemental Table 3), the proportions of pneumonia, respiratory failure, and circulatory failure were higher in the enteral group and the proportion of gastrointestinal hemorrhage was higher in the parenteral group.

DISCUSSION

This study compared short-term mortality and morbidity after parenteral or enteral nutrition in adult patients without cancer. It used a large sample from a national inpatient database and a robust analytic approach with propensity score matching to adjust for potential confounding factors. Patients with gastrointestinal diseases were more likely to receive parenteral nutrition, whereas those with neurological diseases were more likely to undergo enteral nutrition. The results showed lower mortality in the enteral than in the parenteral nutrition group.

To improve the comparability of these groups, we excluded patients with cancer because intestinal cancer and intestinal metastasis of various cancers represent one of the largest causes of intestinal failure. We also used propensity score matching to balance the patient backgrounds among the groups. We additionally performed a subgroup analysis excluding gastrointestinal, liver, gallbladder, or pancreatic disease, which showed a better survival rate in the enteral group than in the parenteral group.

The better survival rate with enteral nutrition than with parenteral nutrition is controversial. Previous studies comparing these 2 procedures were limited by small sample sizes. Comparisons may also have been confounded by imbalanced distributions of patient characteristics and, in particular, differences in background diseases, such as gastrointestinal diseases vs. neurological diseases (9-11). In this study, we attempted to overcome these limitations by using a large sample size and propensity score-matched pairs. We provide further support for the better survival rate with enteral nutrition than with parenteral nutrition: enteral nutrition was associated with lower short-term mortality than was parenteral nutrition.

The geriatric patients in our Japanese study had relatively low body weights (~44 kg) and had chronic illness with bedridden status. A previous study in geriatric patients showed that their mean resting energy expenditure was 18.8 kcal/kg per day (26). In the present study, the patients received ~20 kcal/kg per day of energy at 7 d postoperatively, which would be sufficient.

The differences reported here may be due to the nutritional and metabolic benefits of the use of the intestine (1). In a randomized controlled study in patients with ulcerative colitis, patients receiving enteral nutrition had a higher concentration of serum albumin than did those receiving parenteral nutrition (27). The physiologic use of the intestine may prevent its atrophy and inhibit bacterial translocation, both of which can lead to a chronic inflammation state and promote catabolism (28-30). Metabolic advantages, including glycemic control with incretin, nitrogen metabolism, and prevention of liver diseases, may also be associated with enteral nutrition (31). These factors may have had the synergistic effect of reducing short-term mortality in the enteral group. Sepsis rates did not differ between the enteral and parenteral nutrition groups, whereas the rate of pneumonia was higher in the enteral group than in the parenteral group.

Several prospective randomized controlled trials (PRCTs) related to parenteral and enteral nutrition in an intensive care unit setting were reported recently and produced divergent results (32-38); however, those trials did not compare the outcome of parenteral and enteral nutrition directly. In addition, some meta-analyses of PRCTs comparing parenteral and enteral nutrition in adults found that there were no differences in mortality rate (39-41). A recent PRCT that compared parenteral and enteral nutrition in intensive care unit patients did not find any difference in mortality (42). With respect to the difference in mortality found between these previous studies and our study, several reasons may be suggested. First, in the previous studies, the patients were young, critically ill, and nonfrail, which is in contrast to our study, which examined old, frailer patients with chronic diseases. Second, the previous studies were based on combined heterologous subjects derived from small studies; they did not have as much power as our study, with its large sample size, in providing the mortality rate. Third, our study focused mainly on a comparison between the outcomes of gastrostomy, jejunostomy, percutaneous transesophageal gastrotubing, and central venous port insertion in patients who were thought to be incapable of eating over the long term by attending doctors. The benefits of enteral feeding in acute and chronic care are different.

Several PRCTs reported the reduced pulmonary complications in an enteral nutrition group compared with a parenteral nutrition group (43), which differs from our findings. This difference could be because, unlike previous studies, the present study included older patients. Enteral nutrition carries the risk of gastroesophageal reflux, aspiration, and nosocomial pneumonia, especially in

older patients with disordered pharyngeal function (44-52).

Several limitations of this study deserve acknowledgment. First, although the study population was large, this investigation was based on a retrospective observational design without randomization. Although a propensity score method was used to adjust for differences in baseline characteristics and severity of conditions, the results may still have been biased by unmeasured confounders, such as the severity of each disease and performance status. The AUC of the ROC analysis for goodness-of-fit of the model used for propensity score matching indicated a fair, but not good, level of fit. This would reflect not only the random aspect of assignment but also the unmeasured confounder of decision making. Second, the database lacked records on vital signs, blood tests, and other graphical tests.

In conclusion, the present propensity-matched analysis with the use of data from a large national database showed a better survival rate with enteral nutrition than with parenteral nutrition in reducing short-term mortality for adults needing artificial nutrition but not afflicted with cancer. Further studies with a PRCT design are required.

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Protective effects of NMDA receptor antagonist, memantine, against senescence of PC12 cells: A possible role of nNOS and combined effects with donepezil

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ABSTRACT

Alzheimer disease (AD) is a neurodegenerative disorder characterized by cognitive dysfunction. The pathology of AD is mainly related to amyloid ß (Aß)-peptides, but glutamate-mediated toxicity is also one of the main processes of memory impairment in AD. Glutamate is the main excitatory neurotransmitter in the central nervous system (CNS) and is particularly involved in synaptic plasticity, memory, and learning. Memantine is a low-affinity voltage-dependent noncompetitive antagonist at glutamatergic NMDA receptors. Here, we investigated whether memantine protects against glutamate-induced senescence. In PC12 cells, treatment with glutamate induced senescent phenotypes as judged by the cell appearance and senescence-associated ß-galactosidase (SA-ßgal) in parallel with decreased SIRT1 and increased p53 expression. However, treatment with memantine decreased glutamate-induced senescent PC12 cells and reversed the changes in SIRT1 and p53 expression. Glutamate is known to stimulate the production of NO and O_2^- and has the capacity to generate $ONOO^-$ in the CNS. Therefore, we investigated whether glutamate activates nNOS and memantine reverses it. Treatment with glutamate increased nNOS expression, activity, and production of NO, whereas memantine blocked them. Next, the in vivo effects of memantine on cognitive function in senescence-accelerated mouse prone 8 (SAMP8), as a model of AD, were investigated. In the Morris water maze test, SAMP8 showed a marked decline in performance, but memantine administration improved it. Moreover, neuronal senescence and the level of oxidative stress in the hippocampus were decreased by memantine. Finally, the effects of combination treatment with memantine and donepezil, a cholinesterase inhibitor, were investigated. We observed additive effects of memantine and donepezil on the senescent phenotype of PC12 cells and the hippocampus of SAMP8. These results indicate that inhibition of the NMDA receptor by memantine leads to a decrease in nNOS activity and results in a reduction of glutamate-induced senescence. Thus, our present study suggests a critical role of memantine in the prevention of neuronal aging, and supports that donepezil has a combined effect with memantine.

1. Introduction

Alzheimer disease (AD) is a progressive, neurodegenerative disease characterized by a gradual decline in cognitive function. The etiology of AD is mainly attributable to Aß peptides or tau aggregates, and evidence also exists for both cholinergic and glutamatergic involvement in AD (Ingram et al., 1996). Acetylcholine (Ach), a neurotransmitter essential for processing memory and learning, is decreased in both concentration and function in patients with AD. Similarly, glutamate is the main excitatory neurotransmitter in the CNS and plays a pivotal role in learning and memory. Unlike Ach, glutamate leads to over-activation of N-methyl-D-aspartate (NMDA) receptors and results in neuronal damage. Since over-activation of NMDA receptors increases the amount of intracellular Ca^{2+}, glutamate activates neuronal nitric oxide synthase (nNOS), which produces nitric oxide (NO) and leads to production of reactive oxygen species (ROS; $ONOO^-$), which may trigger neuronal damage (Doucet et al., 2015).

Oxidative stress is known to be closely related to cellular senescence and age-related diseases associated with AD (Tacutu et al., 2011). An increase in oxidative stress has been suggested to be one of the earliest pathological changes in the brain in cognitive impairment due to AD (Mattson, 2004). Cellular senescence of neuronal cells, as well as of

Abbreviations: Aß, amyloid ß; Ach, acetylcholine; AD, Alzheimer disease; CNS, central nervous system; DAF-2, diaminofluorescein-2; DAPI, 4′, 6-diamidino-2-phenylindole; e, i, nNOS, endothelial, inducible, neuronal nitric oxide synthase; HUVEC, human umbilical vein endothelial cells; L-VNIO, N^5-(1-amino-3-butenyl)-L-ornithine; MTS, 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium; NMDA, N-methyl-D-aspartate; PBS, phosphate-buffered saline; SAMP8, senescence-accelerated mouse prone 8; SAMR1, senescence-accelerated mice resistant 1; SA-ßgal, senescence-associated ß-galactosidase; ROS, reactive oxygen species.

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peripheral cells, has been described in AD and causes cellular dysfunction (Naylor et al., 2013).

Memantine (1-amino-3, 5-dimethyladamantane hydrochloride), a noncompetitive NMDA receptor antagonist, was approved about ten years ago for the treatment of moderately severe to severe AD (2002 EU, 2003 USA, since 2011 in Japan). Its effectiveness for cognition has been shown in randomized placebo-controlled trials in patients with AD (Areosa et al., 2005). Since memantine is able to prevent pathogenic Ca^{2+} influx caused by stimulation with glutamate, we considered that the effects of memantine may reduce oxidative damage to neuronal cells and inhibit cellular senescence. In the present study, we showed that glutamate induces senescence of PC12 cells and memantine inhibits nNOS activity, reduces oxidative damage and results in protection against glutamate-induced senescence.

Furthermore, donepezil, a cholinesterase inhibitor, has been used widely in combination with memantine for the treatment of AD patients. Thus, we also examined the combined effects of memantine and donepezil.

2. Materials and methods

2.1. Materials

Glutamate, MK-801, donepezil, and N^{ω}-propyl-L-arginine were purchased from Sigma (St. Louis, MO, USA). Memantine was provided by Daiichi Sankyo Company, Limited (Tokyo, Japan).

2.2. Cells

PC12 cells were purchased from ATCC (Manassas, VA, USA). They were cultured and treated with 2.5S nerve growth factor (NGF, 50 ng/ml, Alomone Labs Ltd., Jerusalem, Israel) to induce differentiation into neuronal cells (Ogawa et al., 1984). Human umbilical vein endothelial cells (HUVEC) were purchased from Cambrex (Walkersville, MD, USA).

2.3. NOS activation assay

NOS activity was determined using an NOS assay kit (Calbiochem) according to the manufacturer's instructions. NO production was observed using fluorescent dye diaminofluorescein-2 (DAF-2) (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). Briefly, PC12 cells were loaded with DAF-2 (5 μM for 30 min at 37 °C) and then washed three times with phosphate-buffered saline (PBS). Green fluorescence intensity (DAF-2) was visualized with 4′, 6-diamidino-2-phenylindole (DAPI) (blue) (Dojindo Molecular Technologies, Inc., Tokyo, Japan) for nuclear staining. Fluorescent images were analyzed using a fluorescence microscope (BZ-9000, Keyence, Osaka, Japan).

2.4. Animal experiments

Animal experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. Senescence-accelerated mice prone (SAMP) 8 and control senescence-accelerated mice resistant (SAMR) 1 male mice were all housed and maintained in a room at 22 ± 2 °C with automatic light cycles (12 h light/dark) and relative humidity of 40–60%. Mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). Food and tap water were provided ad libitum throughout the study. In the water maze test of this study, groups of male SAMR1 (N = 5) and SAMP8 (N = 5) were first tested. Male mice of 12 weeks of age were treated daily for 3 weeks with memantine (10, 20 mg/kg), MK-801 (10 mg/kg), or donepezil (0.3 mg/kg) by subcutaneous injection (s.c) in the neck before the water maze test. Mice were anesthetized, and killed by cervical dislocation. The brain was removed for histological examination, after systemic perfusion with PBS.

2.5. Morris water maze test

The procedure of the Morris water maze test was described previously (Cao et al., 2007). Briefly, SAMR1 (N = 5) and SAMP8 mice (N = 5) were trained to find a visible platform with three trials on the first day, and then tested to find the hidden platform for 10 consecutive days. In each trial, the mice were allowed to swim until they found the hidden platform, or until 2 min had passed, and the mouse was then gently guided to the platform. On the test days, the platform was hidden 1 cm beneath the water. Probe tests were performed on the 10th day. The maze was conceptually divided into I, II, III, and IV, four equal quadrants by four poles along the perimeter of the pool. After the place navigation test was finished on the 10th day, the platform was removed from the water tank. The time was spent in the target quadrant where the platform located was recorded for analysis. Mice were started in a position opposite the location of the platform position and allowed to swim for 60 s. During the test for 10 days, mice were treated daily with memantine (10, 20 mg/kg), MK-801 (10 mg/kg), or donepezil (0.3 mg/kg).

2.6. Open field test

The open field test fear response to novel stimuli was used to assess locomotion, exploratory behavior, and anxiety. Open field test protocols were modified (Lukacs et al., 1995). The open field test consisted of a wooden box (50 × 50 × 50 cm). A 10 cm area near the surrounding wall was delimited and considered the periphery. The rest of the open field was considered the central area. The distance traveled, the ratio of the distance traveled in the central area/total distance traveled, and the time in the center of the open field were analyzed as measures of anxiety-like behavior. During the test, mice were allowed to move freely around the open field and to explore the environment for 15 min.

2.7. Senescence-Associated ß-Galactosidase (SA-ßgal) Staining

PC12 cells and HUVEC were grown in 60-mm collagen-coated dishes to 80% confluence. PC12 cells were pretreated with vehicle (0.05% DMSO), memantine (100 μM), MK-801 (100 μM), or N-propyl-L-arginine (100 μM) diluted in RPMI 1640 medium for 1 day. HUVEC were pretreated with vehicle (0.05% DMSO) or memantine (100, 200 μM) diluted in EGM-2 medium for 1 day. PC12 cells and HUVEC were washed three times with the medium and then treated for 10 h with 10 mM glutamate diluted in medium. After treatment, PC12 cells were cultured with medium containing these compounds for 10 days. At 10 days after the start of treatment with glutamate, PC12 cells and HUVEC were fixed, and the proportion of SA-ßgal-positive cells was determined as described (Dimri et al., 1995).

2.8. Antibodies and immunoblotting

Cells were lysed on ice for 1 h in buffer (50 mM Tris–HCl, pH 7.6, 150 mM NaCl, 1% NP-40, 0.1% SDS, 1 mM dithiothreitol, 1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 10 μg/mL leupeptin and 10 mM sodium fluoride). After blocking, the filters were incubated with the following antibodies: anti-nNOS (BD Biosciences, San Jose, CA, USA), anti-p53, anti-SIRT1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), anti-NMDAR 2A, 2B (Abcam PLC, MA, USA), and anti-ß-actin (Sigma-Aldrich). After washing and incubation with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG (GE Healthcare Life Sciences, Pittsburgh, PA, USA) for 1 h, the antigen-antibody complexes were visualized using an enhanced chemiluminescence system (GE Healthcare Life Sciences).

2.9. Measurement of Ach

The concentration of acetylcholine was measured with a choline/Ach quantification kit (BioVision, CA, USA) according to the manufacturer's instructions.

2.10. Detection for carbonylation of proteins

Carbonylation of proteins was detected using an Oxyblot protein oxidation detection kit (Millipore, MA, USA) according to the manufacturer's instructions.

2.11. Cell viability assays

Cell viability with glutamate (0–20 mM) for 5 h was assessed by using the 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2 H-tetrazolium (MTS) assay (Promega, Madison, WI) in 96-well plates (20,000 cells/well) following the instructions of the manufacturer.

2.12. Data analysis

Values are shown as mean ± S.E.M in the text and figures. Differences between the groups were analyzed using one-way analysis of variance, followed by the Bonferroni test. Probability values less than 0.05 were considered significant.

3. Results

3.1. Glutamate-induced senescence of PC12 cells and memantine inhibited it

To investigate whether glutamate treatment induced cellular senescence, PC12 cells with neuronal differentiation induced by NGF were used. When PC12 cells were treated with 10 mM glutamate for 10 h, a large and flattened senescent appearance was observed and the number of SA-ßgal-positive cells was increased after 10 days of treatment (Fig. 1A and B). These results indicate that glutamate has the capacity to induce the senescence of PC12 cells. Next, when 200 μM memantine pretreatment for 1 day was performed before glutamate induction of senescent PC12 cells, the senescent appearance and the number of SA-ßgal-positive cells were decreased (Fig. 1A and B). To verify the changes in other proteins related to senescent phenotypes, SIRT1 and p53 expression were examined. Treatment with glutamate decreased SIRT1 expression and increased p53 expression; however, pretreatment with 100 or 200 μM memantine prevented these changes (Fig. 1C). These results indicate that pretreatment with memantine inhibited glutamate-induced the senescence of PC12 cells.

Memantine is a noncompetitive NMDA receptor antagonist. Therefore, to clarify the protective effect of memantine against cellular senescence via the NMDA receptor, PC12 cells were treated with another potent, selective noncompetitive NMDA receptor antagonist, MK801 (Benveniste et al., 1984). Similarly to memantine, pretreatment with 100 μM MK801 decreased the number of SA-ßgal-positive cells (Fig. 1D). These results indicate that memantine inhibited the senescence of PC12 cells through the NMDA receptor.

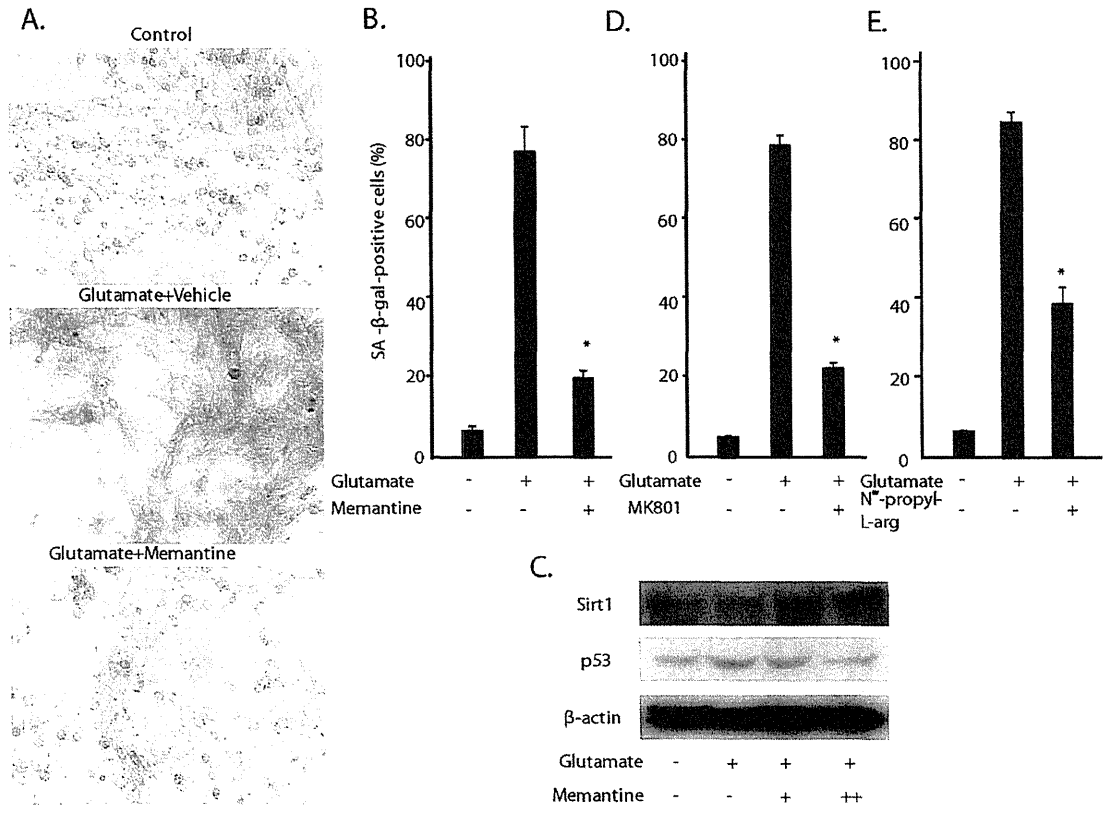

Fig. 1. A. Memantine (200 μM) inhibited SA-βgal activity and senescent morphological appearance induced by glutamate (10 mM). Percentage (%) of SA-βgal-positive PC12 cells with treatment with memantine (100 μM) (B), MK801 (100 μM) (D), or N^{ω}-propyl-L-arginine (100 μM) (E). ($^{*}p < 0.05$, N = 3). C. Expression of SIRT1 and p53 in glutamate (10 mM)-treated PC12 cells under treatment with memantine (+: 100 μM, ++: 200 μM). (N = 3, representative shown).

3.2. Glutamate increased production of NO and memantine decreased it

Glutamate leads to over-activation of NMDA receptors, and the excessive influx of Ca^{2+} into neuronal cells changes nNOS activity. Treatment with 10–40 mM glutamate for 10 h increased nNOS expression in a dose-dependent manner (Fig. 2A). Production of NO was also increased by treatment with 10 mM glutamate in the culture medium. However, pretreatment with 100 μM memantine, 100 μM MK801, or 100 μM N^{ω}-propyl-L-arginine, a potent selective inhibitor of nNOS, inhibited the production of NO (Fig. 2B, C, and D). To investigate whether memantine decreased the content of intracellular NO through nNOS, DAF-2 was used. Pretreatment with 100 or 200 μM memantine decreased the number of DAF-2-stained PC12 cells compared with glutamate-treated cells for 10 h (Fig. 3A and B). Furthermore, the number of SA-ßgal-positive PC12 cells was decreased by pretreatment with 100 μM N^{ω}-propyl-L-arginine (Fig. 1E). These results indicate that memantine inhibits the activation of nNOS and decreases NO induced by glutamate, leading to a reduction of senescence.

3.3. Memantine treatment improved cognitive decline in SAMP8

In order to assess the effects of memantine on cognitive function, we used an in vivo model of AD, SAMP8, and a control counterpart strain, SAMR1. SAMP8 was originally derived from the AKR/J strain, which is characterized by cognitive decline. SAMP8 showed a marked age-related deterioration of memory and learning as early as 2 months of age compared to SAMR1 (Yagi et al., 1988; Flood and Morley, 1998; Miyamoto et al., 1992). These mice exhibit age-related deficits in learning and memory at an early age, and are considered a suitable animal model to study the pathology of AD. By determining the time required to find the platform (escape latency) as a function of the number of days of training in the Morris water maze, we observed a marked decline in performance in SAMP8 (N = 5) compared with that in SAMR1 (N = 5) (Fig. 4A). SAMP8 treated with 20 mg/kg memantine for 3 weeks showed significantly reduced escape latency time compared with that in untreated SAMP8 on training day 10 (Fig. 4A). However, SAMP8 treated with 10 mg/kg MK801 did not show a change in escape latency time compared with untreated SAMP8 (Fig. 4A). The spatial probe trial helps to determine whether the mice would use a spatial learning strategy to locate the platform in the target quadrant. There was no difference in swim speed between the groups (Fig. 4B); the time was spent in the target quadrant where the platform was located was greatly reduced in SAMP8 compared to SAMR1 on day 10 (Fig. 4C). Memantine-treated SAMP8 showed a markedly longer time searching for the platform in the target quadrant (Fig. 4C). These results indicate that memantine, but not MK801, ameliorated spatial learning in SAMP8. The water maze is appropriate for hippocampus-dependent paradigms. However, memantine and MK801 administration may affect behavior and how animals respond to different stimuli. Therefore, we performed an open field test to examine locomotion, exploratory behavior, and anxiety. No significant effect of memantine (20 mg/kg) and MK801 (10 mg/kg) on locomotor performance was observed in SAMR1 and SAMP8, whereas SAMR1 showed significantly more movement compared with SAMP8 (Fig. 4D). The ratio of the distance traveled in the central area to that in the total area in the open field, an indirect measure of exploratory behavior and anxiety, was also determined. In SAMP8, memantine (20 mg/kg) and MK801 (10 mg/kg) increased this ratio (Fig. 4E), suggesting that both memantine and MK801 promoted exploratory behavior and diminished anxiety.

3.4. Memantine treatment inhibits senescence in hippocampus of SAMP8

Next, we assessed the number of SA-ßgal-positive cells in the CA1 and CA3 areas of the hippocampus in these mice. The number of SA-βgal-stained cells was significantly increased in SAMP8 compared with SAMR1, but treatment with memantine (10, 20 mg/kg) prevented this change in SAMP8 (Fig. 5A and B).

Glutamate stimulation increases the production of NO and O_2^- and has the capacity to generate $ONOO^-$ in the CNS (Eliasson et al., 1999). Therefore, we investigated the level of oxidative stress, using the SAMR1 and SAMP8 hippocampus at 12 weeks of age. SAMP8 hippocampus showed an increase in the level of oxidative stress compared with SAMR1 as judged by detection of carbonylated proteins. Memantine treatment (10 or 20 mg/kg) decreased carbonylated proteins in the

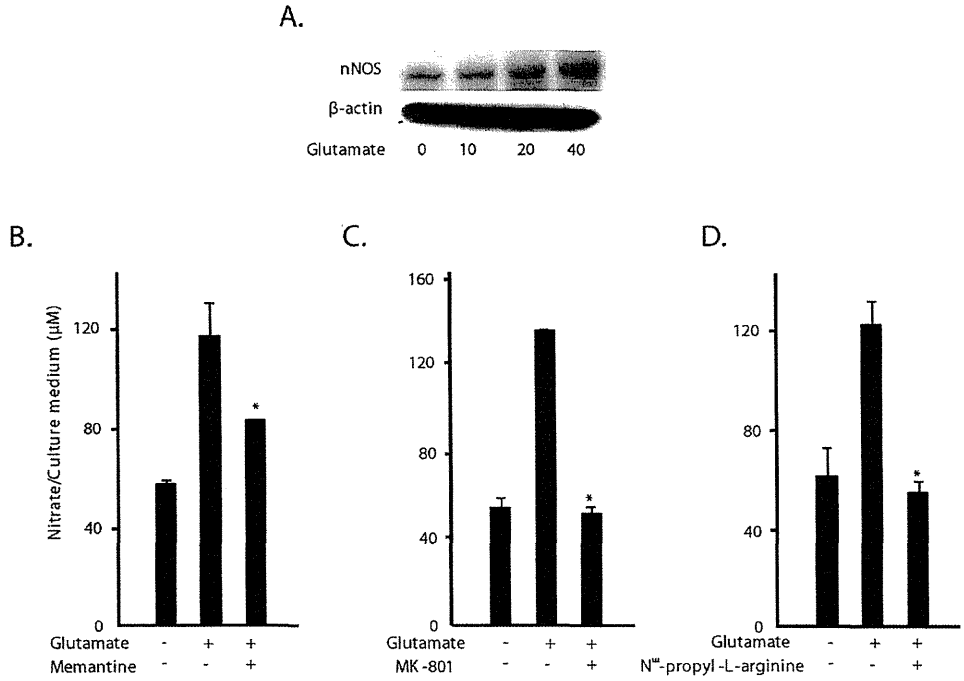

Fig. 2. A. Expression of nNOS in glutamate (10–40 mM)-treated PC12 cells. (N = 3, representative shown). Production of nitrate in PC12 cells treated with memantine (100 μM) (B), MK801 (100 μM) (C), or N^{ω}-propyl-L-arginine (100 μM) (D). (*$p < 0.05$, N = 3).

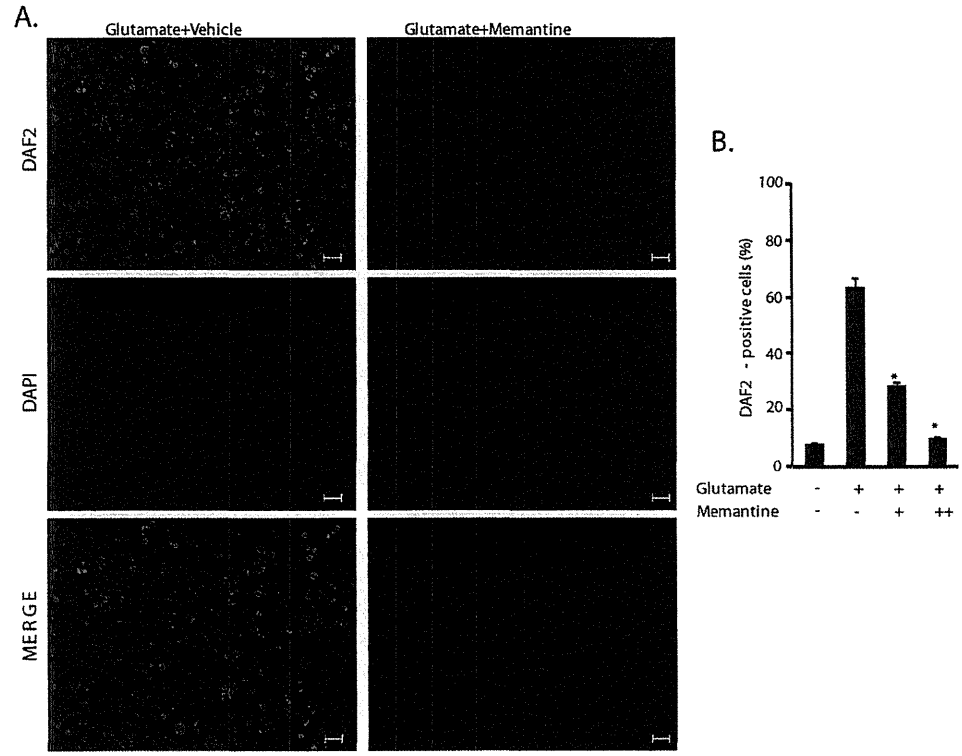

Fig. 3. A. NO production detected using DAF-2 (green) in glutamate (10 mM)-treated PC12 cells under treatment with or without memantine (200 μM). DAPI (blue) staining for nuclei. B. Number of DAF-2-stained PC12 cells treated with memantine (+: 100 μM, ++: 200 μM). ($^{*}p < 0.05$, N = 3).

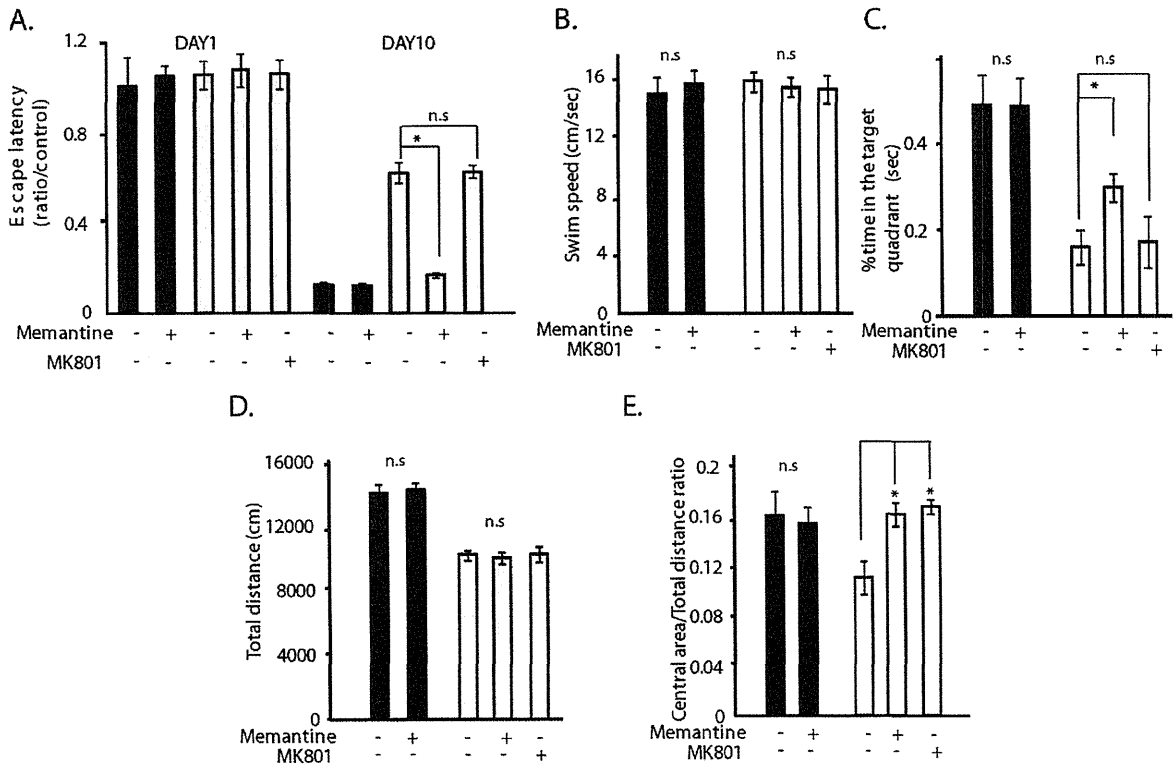

Fig. 4. A. Escape latency ratio on day 1 and 10 in SAMR1 (black bar) and SAMP8 (gray bar) mice (N = 5). Male mice were treated daily for 3 weeks with memantine (20 mg/kg s.c) or MK801 (10 mg/kg s.c) before trials. B. Swim speed during quadrant test on day 10. C. % time in target quadrant in memantine (20 mg/kg s.c)- and MK801 (10 mg/kg s.c)- treated mice on day 10. Total distance (D) and ratio of central/total distance (E) were measured in open field tests. ($^{*}p < 0.05$, n.s: not significant).

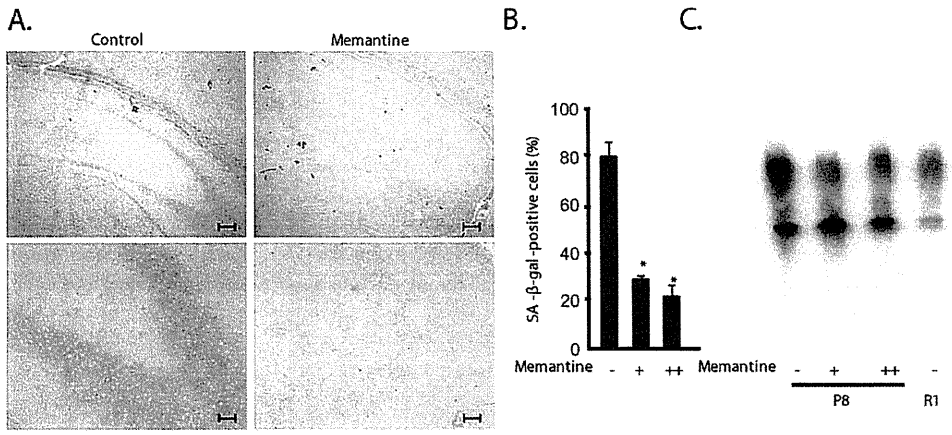

Fig. 5. A. B. SA-βgal-stained cells in CA1 and CA3 areas of hippocampus in SAMP8 with or without memantine (+: 10 mg/kg, ++: 20 mg/kg) treatment. (*$p < 0.05$, $N = 3$) (size scale 50 μm). C. Oxidative stress level was measured by detection of carbonyl groups introduced into proteins (R1: SAMR1, P8: SAMP8).

SAMP8 hippocampus (Fig. 5C). These results indicate that memantine inhibits senescence in the hippocampus of SAMP8 and suggest that reduction of oxidative stress involving NO may play an important role in the protective effect of memantine against senescence of the hippocampus.

3.5. Memantine and donepezil showed combined protective effect against senescent phenotype

Recently, the usefulness of combination therapy with memantine and donepezil has been investigated in many clinical studies. Because memantine and donepezil have different mechanisms of action, donepezil has been used together with memantine in many patients with AD. Therefore, we investigated the combined effect of memantine and donepezil. Combination treatment with memantine (50 μM) and donepezil (1 μM) significantly decreased the number of SA-ßgal-positive PC12 cells compared with only memantine or donepezil treatment (Fig. 6A). Moreover, SAMP8 treated with the combination showed significantly reduced escape latency at earlier days (Fig. 6B), and the time spent in the target quadrant was increased compared with that in memantine (10 mg/kg)- or donepezil (0.3 mg/kg)-treated SAMP8

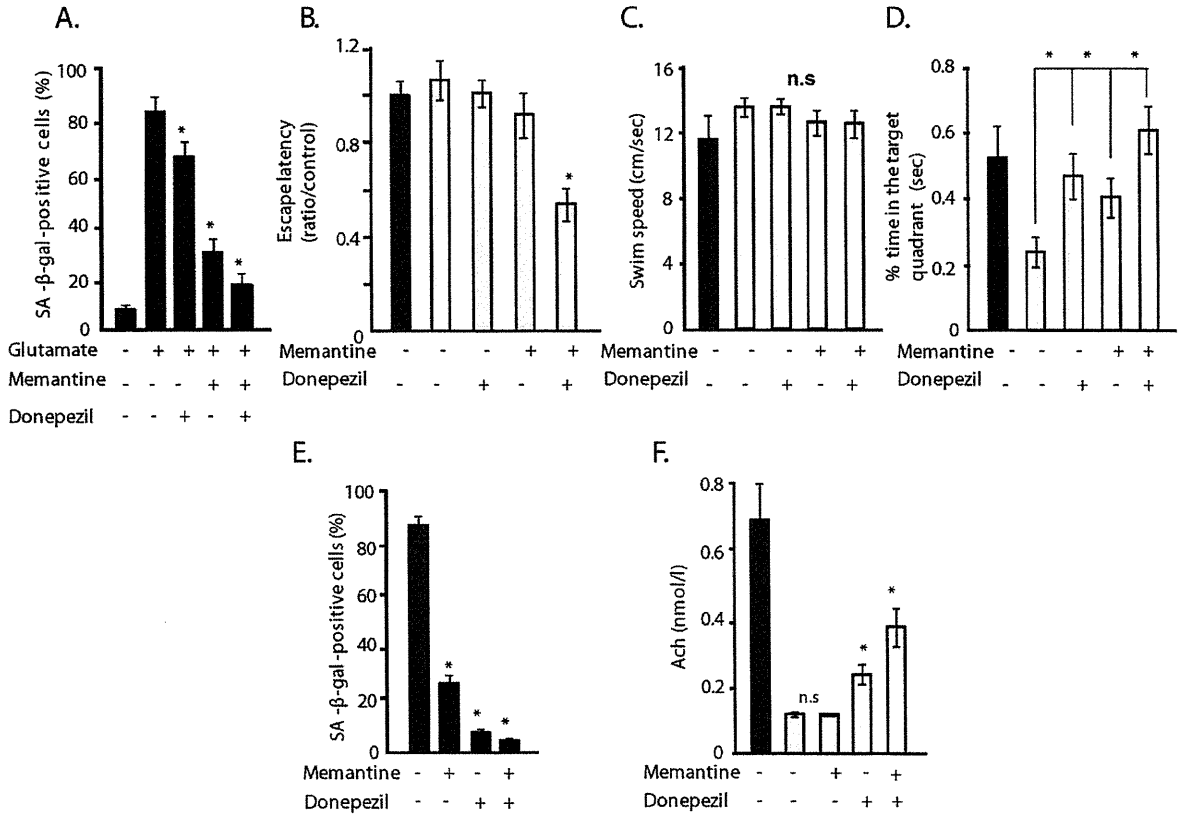

Fig. 6. A. A combination of memantine (50 μM) and donepezil (1 μM) more markedly reduced SA-βgal activity of PC12 cells compared with memantine or donepezil alone (*$p < 0.05$, $N = 3$). B. Escape latency ratio on day 1 in SAMR1 (black bar) ($N = 5$) and SAMP8 (gray bar) mice ($N = 5$) treated with donepezil (0.3 mg/kg), memantine (10 mg/kg), or donepezil + memantine. C. Swim speed during quadrant test on day 10. D. % time in target quadrant in donepezil, memantine, and donepezil + memantine-treated mice. E. Number of SA-βgal-stained cells in CA1 and CA3 areas of hippocampus (*$p < 0.05$, $N = 3$). F. Acetylcholine concentration was measured by a colorimetric method (*$p < 0.05$, $N = 3$).

(Fig. 6D). There was no difference in swim speed between these groups (Fig. 6C). The number of SA-βgal-stained cells in the CA1 and CA3 areas of the hippocampus was also significantly decreased in combination-treated compared with memantine- or donepezil-treated SAMP8 (Fig. 6E). Finally, the concentration of Ach in hippocampal lysates was increased in SAMP8 treated with donepezil but not memantine, but it was further increased by memantine in combination with donepezil (Fig. 6F).

4. Discussion

In this study, we showed that glutamate induced the senescence of PC12 cells and treatment with memantine inhibited it in accordance with restoration of the expression of SIRT1 and p53. Furthermore, memantine treatment reduced hippocampal senescence and led to improved spatial memory in SAMP8.

The Morris water maze results are often confounded by the fact that it is a potent anxiety/stress test (Morley et al., 2012). Therefore, there is a possibility that SAMP8 could show less anxiety than SAMR1, and the alteration in protein carbonyls could be due to stress.

Axonopathy observed in AD is considered to be the result of accumulation of Aß-peptide in glutamatergic synaptosomes, which leads to excessive release of glutamate with consequently more axonal degeneration in neurons through NMDA receptors (glutamate hypothesis) (Harrison, 1986). Indeed, recent findings indicate that increased levels of Aß-peptide and glutamate have a detrimental impact on neurons via interactions with NMDA receptors (Hiruma et al., 2003; Miguel-Hidalgo et al., 2002; Rammes et al., 2008), resulting in large Ca^{2+} entry into neurons and subsequent axonal and neuronal damage (Parsons et al., 2007). NMDA receptors are glutamate-gated Ca^{2+} channels that play pivotal roles in fundamental aspects of neuronal function. Glutamate treatment caused intracellular Ca^{2+} concentration to remain elevated up to 1 h in vitro (Dubinsky, 1993). Glutamate causes failure of the Ca^{2+} homeostasis early, and the degeneration primarily involves the activation of catabolic enzyme such as endonucleases, endopeptidases, and phospholipases which lead to production of ROS and $ONOO^-$ (Choi, 1992; Lazarewicz et al., 1990; Chan and Fishman, 1978). Therefore, we examined the cell viability in the course in early time. We found that when treated with 10 mM glutamate for 5 h, cell viability was not altered (Supplementary Fig. 1A). In accordance with this, it was reported that glutamate levels between 1 μM and 10 mM did not affect cell viability over a 24 h period in brain endothelial cells (Scott et al., 2007). In this study, we decided to use a glutamate concentration of 10 mM mainly because at this concentration it was easy to detect senescence phenotype at 10 days. However, glutamate, at mM concentrations, is known to exert toxic effect on CNS-derived preparation, including endothelial cells (Sharp et al., 2003; Parfenova et al., 2006). Because senescent cells were not detectable at an early time (24 h) and were detected at least 72 h after the start of treatment with glutamate, accumulation of the oxidative stress may occur, causing in cell damage, and it is thought that it gradually results in cellular senescence.

Moreover, it has been well documented that glutamate induces nNOS over-activation, secondary to elevation of extracellular glutamate, and leads to neuronal damage (Darra et al., 2009). Therefore, glutamate shows excitotoxicity at an early time, but treatment with memantine antagonized the NMDA receptor and resulted in attenuation of the senescence of PC12 cells via inhibition of nNOS activation. N^5-(1-amino-3-butenyl)-L-ornithine (L-VNIO), a selective nNOS inhibitor, was applied to examine the involvement of NOS in native testosterone-deficient SAMP8 with supplementation. L-VNIO did not show any effect on cognitive function (Ota et al., 2012). Moreover, we investigated cognitive function in aged endothelial NOS (eNOS) KO mice (96 weeks old). They did not show a cognitive decline compared with wild mice of the same age (data not shown). Unlike our in vitro experiments, these results suggest the possibility of no participation of NOS in vivo. However, it was reported that NOS inhibitors of nNOS, inducible NOS (iNOS), and eNOS had favorable effects on cognitive function in several AD mouse models (Maher et al., 2014; Santhanam et al., 2015). Given the differences observed in vivo and in vitro, because nNOS expression is naturally downregulated in the hippocampus of SAMP8 (Han et al., 2010), it could be difficult to evaluate the effect of nNOS as shown in vitro. Further studies are needed to clarify the participation of NOS.

In this study, similarly to memantine, treatment with MK-801 attenuated NO production and inhibited the senescence of PC12 cells. However, administration of MK-801 to SAMP8 did not alleviate learning deficit. Memantine has moderate affinity for NMDA receptors, with rapid blocking/unblocking kinetics and strong voltage dependency. In contrast, MK-801 has strong affinity for NMDA receptors and inhibits the induction of long-term potentiation at a concentration blocking NMDA receptors and worsens the impairment of spatial memory (Zajaczkowski et al., 1996). As described (Olney et al., 1989), noncompetitive NMDAR antagonists may by themselves induce excitotoxicity through inducing receptor hypoactivity. That is, blocking NMDA receptors on GABAergic/other inhibitory neurons leads to hypoactivity, resulting in overactivation/excitotoxicity of neurons downstream of these inhibitory neurons.

In another study, treatment of rats with memantine at the same concentrations as those used in this study (10, 20 mg/kg/day) showed serum levels of 52–64 ng/ml and 150–199 ng/ml respectively (Nakamura et al., 2006). These serum concentrations of memantine are similar to the levels seen in the serum of dementia patients treated with memantine, and the concentration of memantine in mice in our study may have been higher, assuring inhibition of the NMDA receptor based on in vitro data.

Memantine has been approved for the treatment of moderate to severe AD in Europe since 2002, in USA since 2003, and in Japan since 2011 (Allgaier and Allgaier, 2014), and has been shown in clinical trials to be a safe and effective treatment for vascular dementia (Orgogozo et al., 2002). Because memantine showed a protective effect on PC12 cells, we then examined its effect on vascular endothelial cells. Although the effect was weaker than that on neuronal cells, memantine inhibited glutamine-induced endothelial senescence (Supplementary Fig. 1B), and NMDA receptors were found in endothelial cells (Supplementary Fig. 1C). These results suggest that memantine may have a protective effect on not only neuronal function, but also vascular function.

In this study, memantine and donepezil showed an additive protective effect against neuronal senescence and improved cognitive function at an earlier time compared with monotherapy. Clinically, the combination of memantine and donepezil has demonstrated efficacy for treating the symptoms of AD (Gareri et al., 2014). Consistent with our results, several preclinical studies also have indicated cognition-enhancing effects of memantine and donepezil with repeated administration in other mouse models of AD (Nagakura et al., 2013). Because memantine and donepezil have different and complementary mechanisms of action, together they potentially offer additional benefits in relation to the etiology of AD. The detailed mechanism of the combined effect needs further study.

Memantine combined with environmental enrichment improves spatial memory in SAMP8 (Dong et al., 2012). Moreover, it has been recently reported that memantine can prevent Aß-peptide production (Miguel-Hidalgo et al., 2002). Although other mechanism may exist, the findings of the present study indicate that neuronal senescence may constitute an important target for AD and memantine may prevent it. Our results appear to support arguments justifying the presence of a "glutamate-associated excitotoxic insult" as a mechanism of the neurodegenerative changes observed in AD. We believe that the application of memantine as anti-excitotoxic treatment chronically may act as a defense against brain/neuronal aging and senescence causing AD.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.exger.2015.09.016.

Disclaimer

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Original Study

Favorable Effect of Sympathetic Nervous Activity on Rehabilitation Outcomes in Frail Elderly

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A B S T R A C T

Objectives: Previous studies have suggested the relationship between physical function, mortality, and autonomic nervous activity in frail elderly and that maintaining sympathetic nervous activity might lead to improved physical function and mortality in the elderly population. The aim of this study was to investigate the utility of sympathetic nervous activity measured by heart rate variability in frail elderly patients undergoing inpatient rehabilitation, further focusing the nervous activity on the effect of rehabilitation therapy.
Design: Prospective cohort study.
Participants: Sixty-one subjects aged 75 years or older were recruited after treatment of acute phase illness.
Measurements: Before undergoing rehabilitation, data of 24-hour Holter monitoring and a blood venous sample were obtained. From RR intervals in the electrocardiogram, heart rate and SDs of all NN intervals in all 5-minute segments of the entire recording, power spectral density, low frequency (LF), high frequency (HF), and low frequency/high frequency (LF/HF) were calculated. Functional Independence Measure (FIM) and Barthel index were used to measure physical function.
Results: FIM score and Barthel index were 46.8 ± 25.4 and 32.8 ± 31.7, respectively. Serum total protein, albumin, hemoglobin, and total cholesterol were all significantly related to FIM score and Barthel index before rehabilitation. Heart rate variability indices did not show a significant relationship with physical function, whereas the high LH/HF group showed significant improvement in physical function compared with the low LH/HF group. Moreover, LF/HF frequency was a predictive factor for improvement of physical function after 2 months of rehabilitation.
Conclusion: A favorable effect of preserved LF/HF on rehabilitation outcome was observed in elderly undergoing rehabilitation. Preservation of sympathetic nervous activity may lead to improved physical function in the elderly.

The number of disabled elderly is increasing in Japan, and the number of elderly needing long-term care reached more than 5 million in 2011.[1] It is important for disabled elderly to maintain or improve their physical function. Rehabilitation is a well-established approach to improve physical function after treatment of acute phase illness.[2] In Japan, the underlying causes of physical dysfunction necessitating rehabilitation are cerebrovascular disease, fracture, disuse syndrome, and other diseases that cause physical dysfunction.[3] It is our most urgent task to prevent and treat these diseases, and also to improve physical function after the occurrence of these diseases. Recent studies have indicated a relationship between these underlying diseases and sympathetic nervous activity.[4–8]

With regard to cerebrovascular disease, which is the most frequent cause of elderly becoming frail or needing long-term care, Muslumanoglu et al[4] demonstrated that low sympathetic nervous

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activity measured by skin sympathetic reactivity reflected greater severity of paralysis and severe limited motor function in the chronic phase of ischemic cerebrovascular disease in the elderly. In addition, RR intervals in the electrocardiogram are used to evaluate heart rate variability (HRV),[9] and low frequency/high frequency (LF/HF), which was reported to be a marker of sympathovagal balance or sympathetic modulation, showed a positive correlation with both respiratory and skeletal muscle strength in chronic obstructive pulmonary disease.[5] Moreover, it was reported that low LF/HF value was related to overall mortality in not only frail elderly and elderly in long-term care, but also community-dwelling elderly.[6–8] In addition to these previous reports, we recently showed that elderly in long-term care aged 75 years or older had lower LF/HF than in physically intact elderly controls. Moreover, in only long-term care elderly, the low LF/HF group was significantly associated with high mortality after adjustment for age, sex, cardiovascular risk factors, and physical function. These findings suggest that preserved sympathetic nervous activity might have some favorable effects in long-term care elderly.[8]

Dementia is known to be a limiting factor in rehabilitation.[10–12] Patients with Lewy body dementia and Parkinson disease dementia show low sympathetic activity.[13,14] The same relationship is observed in fractures. β-blockers are widely accepted for the treatment of hypertension and chronic heart failure through reducing sympathetic nervous activity in middle age.[15,16] However, most studies concerning the benefit of β-blockers targeted only middle age, and there have been few studies of elderly aged 75 years or older.[17] In contrast to the benefit of β-blockers in middle age, they were found to have a relationship with incident fracture in elderly people aged 80 years or older.[18]

These findings suggest that it might be necessary to maintain sympathetic nervous activity in very elderly people, especially in frail or long-term care elderly. However, few studies have examined the relationship between sympathetic nervous activity and the effect of rehabilitation therapy in very elderly people. Therefore, we investigated whether sympathetic nervous activity affects physical function increment in elderly aged 75 years or older undergoing rehabilitation therapy. The aim of this study was to determine whether the high sympathetic nervous activity group would show greater improvement in physical function, and whether sympathetic nervous activity could predict the effect of rehabilitation.

Methods

Setting and Participants

This observational study analyzed 61 consecutive elderly persons aged 75 years or older who were admitted to a rehabilitation unit. The hospital was located in Nagano prefecture, Japan. Inclusion criteria were elderly aged 75 years or older undergoing rehabilitation. Exclusion criteria were treatment of acute phase disease within the last 2 weeks, arrhythmia, administration of anti-arrhythmia drugs or β-blockers, malignancy, and neurodegenerative disease.[9] Medical records were reviewed to obtain information on history of cardiovascular disease, such as hypertension, diabetes mellitus, hyperlipidemia, chronic heart failure, or ischemic heart disease, which was confirmed by the patient or family. This study protocol was approved by the institutional review board of the Keijinkai Kikyougahara Hospital. Written informed consent was obtained from all participants or their families.

Heart Rate Variability

Ambulatory Holter recording was performed for 24 hours using QR2100 (Fukuda ME, Kogyo, Tokyo, Japan) and processed with HS1000VL (Fukuda ME Kogyo). For time domain analysis, the SDs of all NN intervals in all 5-minute segments of the entire recording (SDANN) were calculated, and frequent domain analysis was performed with fast Fourier transform. From the power spectral density, LF (0.04∼0.15 Hz), HF (0.15∼0.40 Hz), and LF/HF were determined.

Anthropometric, Physical Function, and Hematologic Measures

Height, weight, and body mass index (BMI) were measured before Holter monitoring. FIM score[19] and Barthel index[20] were determined to assess physical function. Venous blood samples were obtained from individuals in the morning after an overnight fast. Blood cell counts and serum levels of chemical parameters were determined by a commercial laboratory (Health Science Research Institute, Yokohama, Japan).

Statistical Analysis

Data were analyzed using SPSS software (Ver.11.0.1J; SPSS Japan Inc., Tokyo, Japan). Pearson correlation coefficient was calculated to determine the relationship between physical function and blood nutritional data and HRV indices. Standardized multiple regression analysis of FIM and Barthel index was performed with age, sex, and the disease necessitating rehabilitation as covariates. One-way analysis of variance was used for the effect of 2 months of rehabilitation on each HRV index. Age, sex, BMI, the disease necessitating rehabilitation, FIM before undergoing rehabilitation (pre-FIM), blood nutritional data, and HRV indices, including heart rate, SDANN, LF, HF, and LF/HF were used as covariates in stepwise regression analysis to determine independent predictors of increment of FIM after 2 months of rehabilitation (ΔFIM). For selected predictive factors, the standardized regression coefficient (β) was calculated by multiple regression analysis.

Results

We registered 61 elderly people who received rehabilitation, and assessed physical function and HRV from 24-hour Holter monitoring. The background data of this study are shown in Table 1.

The underlying diseases necessitating rehabilitation were cerebrovascular disease (n = 37, 60.7%), disuse syndrome (n = 14, 23.0%), and fracture (n = 10, 16.3%). Mean age was 86 ± 5 years, blood nutritional data including total protein, albumin, total cholesterol,

Table 1
Characteristics of Elderly Individuals

Category	Measurement Items	Results
Background data	Number	61
	Age	86 ± 6 (75–100)
	Sex, male (%)	20 (32.8)
	BMI, kg/m^2	19.7 ± 3.2
Type of disease, n (%)	Cerebrovascular disease	37 (60.7)
	Disuse syndrome	14 (23.0)
	Fracture	10 (16.3)
Blood nutritional data	Total protein, g/dL	6.4 ± 0.7
	Albumin, g/dL	3.4 ± 0.5
	Hemoglobin, g/dL	12.1 ± 1.7
	Total cholesterol, mg/dL	177 ± 44
Physical function	FIM	46.8 ± 25.4
	Barthel index	32.8 ± 31.7
HRV indices	Heart rate (beats per minute)	74.8 ± 12.8
	SDANN, ms	85.7 ± 35.5
	LF, ms^2	35.8 ± 27.5
	HF, ms^2	63.8 ± 57.8
	LF/HF	0.70 ± 0.26

Values are mean ± SD.

and hemoglobin were at the lower limit of the normal range. FIM and Barthel index, representing physical function, were 46.8 ± 25.4 and 32.8 ± 31.7, respectively. Scores for each FIM item were as follows: eating 3.8 ± 2.2, grooming 2.7 ± 1.8, bathing 1.5 ± 1.2, upper body dressing 2.5 ± 1.7, lower body dressing 2.3 ± 1.5 toileting 2.4 ± 1.7, bladder management 2.8 ± 2.2, bowel management 2.8 ± 2.1, bed-to-chair transfer 3.2 ± 1.8, toilet transfer 2.7 ± 1.9, shower transfer 1.2 ± 0.9, locomotion (ambulatory or wheelchair level) 1.7 ± 1.5, stairs 1.2 ± 0.7, cognitive comprehension 3.7 ± 1.8, expression 3.8 ± 2.1, social interaction 3.3 ± 2.2, problem solving 2.8 ± 1.8, and memory 2.8 ± 1.8. In terms of HRV indices, heart rate, SDANN, LF, HF, and LF/HF were 74.8 ± 12.8 beats per minute, 85.7 ± 35.5 ms, 35.8 ± 27.5 ms^2, 63.8 ± 57.8 ms^2, and 0.7 ± 0.26, respectively. Of these indices, heart rate was higher and the others were lower than those in healthy controls matched for age, sex, and cardiovascular risk factors, which we previously reported.

Pearson correlation coefficient and multiple regression analysis revealed an association among total protein, albumin, total cholesterol, hemoglobin, and physical function (both FIM and Barthel index). Although low heart rate was significantly related to high Barthel index, we did not find a significant relationship between other HRV indices and physical function in multiple regression analysis (Table 2). After 2 months of rehabilitation, FIM was significantly increased from 47 to 54 points. We investigated the relationship between HRV indices and change in physical function (ΔFIM). Two months of rehabilitation did not show a significant difference in SDANN, heart rate, LF, and HF between the high-value group and low-value group (Figure 1A–D). Only the high LF/HF group showed significantly improved ΔFIM compared with the low LF/HF group; 9.0 versus 4.5 points (Figure 1E). FIM is constructed of 13 physical items and 5 cognitive items, and physical items were significantly improved in the high LF/HF group compared with the low LF/HF group after 2 months of rehabilitation (Table 3).

In age, sex, pre-FIM, BMI, cardiovascular disease risk factors, blood nutritional data, and HRV indices, stepwise regression analysis revealed that high ΔFIM was significantly related to high pre-FIM ($\beta = 0.341$, $P = .006$) and high LF/HF ($\beta = 0.296$, $P = .015$) (ΔFIM = [pre-FIM] × 0.127 + [LF/HF] × 11.054 − 6.994) (Table 4).

Discussion

The present longitudinal study demonstrated that the frail elderly patients undergoing inpatient rehabilitation with high LF/HF represented a greater increase in FIM than those in the low LF/HF group, and that ΔFIM was predicted by LF/HF as well as pre-FIM at baseline. Previous studies have shown that increment of physical function with rehabilitation was predicted by pre-FIM and age.[21–24] Based on our findings, it was also suggested that FIM was the strongest predictive factor for increment of physical function in frail elderly, which was consistent with several studies about clinical characteristics of rehabilitation therapy.[21–23]

Sarcopenia[25] and frailty[26] in the elderly, whose criteria include low extremity muscle strength, such as low walking speed and low handgrip strength, have been shown to be associated with high mortality. Slow walking speed is one of the criteria of frailty that was reported by Fried et al.[26] Although we did not measure walking speed in this study, we evaluated the locomotion score in FIM. The average score of locomotion in the study population was 1.7 ± 1.5 (range 1–6) and 59 of 61 participants gained fewer than 5 points, suggesting that most participants were mobility-dependent. In this point of view, it is suggested that the individuals in our study might belong to a frail population. Frailty index was also reported by Searle et al,[27] in which procedures for selecting variables related to frailty were applied to yield 40 deficits. We measured 15 variables (help bathing, help dressing, help getting in/out of chair, help walking around house, help eating, help grooming, help using toilet, help up/down stairs, high blood pressure, heart attack, chronic heart failure, stroke, cancer, diabetes, BMI) among a total of 40 deficits, and the average deficit score in our study was 9.45, corresponding to frailty index as 0.24 ± 0.04 at least. Based on these observations, it was suggested that individuals in this study might meet the criteria of frailty. These concepts and findings of sarcopenia and frailty suggest that it is important to improve physical function and musculoskeletal property in the elderly population. Sympathetic nervous activity has the potential to improve physical function in the elderly, and activation of the sympathetic nervous system might lead to an increase in blood pressure, muscle blood flow, and muscle strength by inducing muscle protein synthesis. In practice, it has been suggested that high sympathetic nervous activity is related to improvement of physical function.[28–30] Taken together with our findings and previous studies, it would be important to maintain sympathetic nervous activity in frail elderly whose physical performance is not preserved. Age was also reported to predict increment of physical function in younger individuals,[21–23] whereas the effect of age on rehabilitation has been considered to be not so strong.[11,12,24] In addition, studies limited to only elderly individuals have not shown a significant relationship between age and increment of physical function.[11,12] This relationship was not observed in our study, and one possible explanation might be that inclusion criteria of this study were limited to age of 75 years or older.

It was also demonstrated in this study that serum levels of total protein, albumin, hemoglobin, and total cholesterol were all related to both FIM and Barthel index at baseline, whereas these blood nutritional data were not correlated with the increment of FIM in a longitudinal analysis. Although previous cross-sectional studies suggested that low serum albumin level was related to low skeletal muscle mass, grip strength, and leg power,[31–33] this relationship remains controversial in other longitudinal studies. In practice, it was reported that lower serum albumin was associated with muscle strength decline over 3 and 6 years,[32] whereas another did not represent this association.[34] Instead, the combination of low serum albumin and low total cholesterol could predict future decline of functional performance.[34,35] The prognostic role of total cholesterol varies among types of cerebrovascular disease, such as hemorrhagic stroke, noncardiac ischemic stroke, and cardiac ischemic stroke.[36,37] Although low total cholesterol at baseline was reported to be able to predict future low physical performance,[34,36] the relationship was not revealed in this study, and the discrepancy might be derived from differences in age and underlying diseases of individual study. Serum

Table 2
Relationship Among FIM, Barthel Index, and Blood Nutritional Data and HRV Indices

	FIM (r)	Barthel Index (r)	FIM (β)	Barthel Index (β)
Total protein	0.44‡	0.48‡	0.49‡	0.50‡
Albumin	0.54‡	0.56‡	0.55‡	0.55‡
Total cholesterol	0.46‡	0.46‡	0.52‡	0.49‡
Hemoglobin	0.33†	0.37†	0.34*	0.38†
BMI	0.16	0.16	0.26	0.23
Heart rate	−0.29*	−0.29*	−0.25	−0.27*
SDANN	0.27*	0.25	0.22	0.22
LF	0.02	−0.08	0.02	−0.06
HF	0.02	−0.11	0.04	−0.07
LF/HF	−0.07	0.10	−0.09	0.04

Pearson correlation coefficient (r) and standardized regression coefficient (β) were calculated. Standardized regression coefficient (β) was adjusted for age, sex, and disease necessitating rehabilitation.

*$P < .05$.

†$P < .01$.

‡$P < .001$.

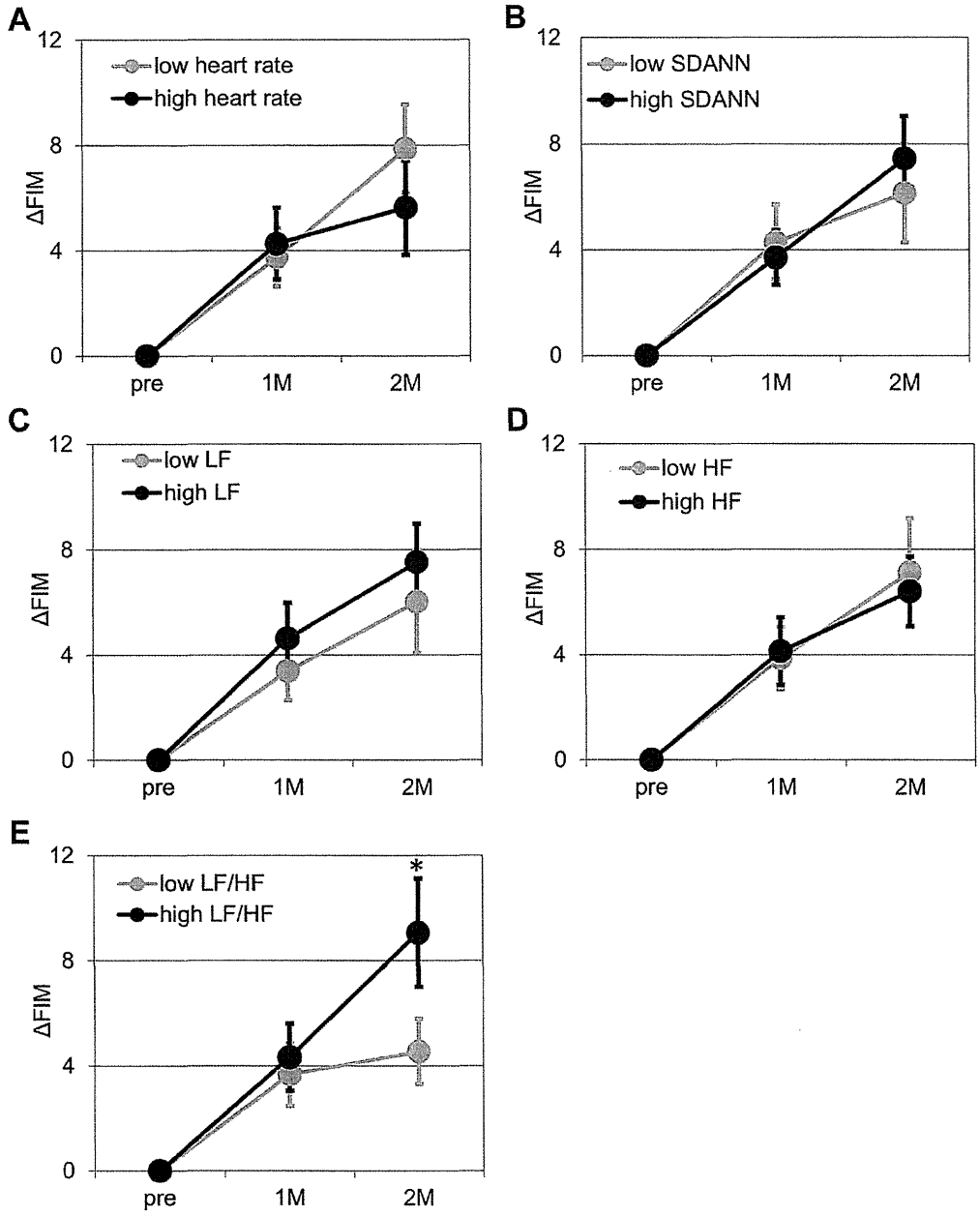

Fig. 1. HRV indices and ΔFIM before and after 2 months of rehabilitation. Data are mean ± SE. All HRV indices were divided into a high group (n = 31) and low group (n = 30) by the median. Standardized regression coefficient (β) was adjusted for age, sex, and disease necessitating rehabilitation. 1M, 1 month after start of rehabilitation; 2M, 2 months after start of rehabilitation. A, Heart rate and ΔFIM; B, SDANN and ΔFIM; C, LF and ΔFIM; D, HF and ΔFIM; E, LF/HF and ΔFIM. *$P < .05$.

hemoglobin level was related to physical function in a cross-sectional study, but no relationship was found in a longitudinal study in fracture patients,[38] which was also supported by our study results. Taken together, blood nutritional data were correlated with physical function in cross-sectional studies in the elderly based on both our results and previous studies, whereas in a longitudinal study, they could not predict the improvement of physical performance at least after 2 months of rehabilitation. Hence, it might be important to consider long-term nutritional support of elderly patients with regard to the underlying diseases as well.

To maintain or increase sympathetic nervous activity, a recent study has suggested that daylight exposure is the most simple and powerful rhythmic regulator in the environment.[39] A sitting or standing position also activates sympathetic nervous activity compared with the supine position.[40] These findings indicate that it may be possible to improve physical and cognitive function in elderly patients hospitalized in rehabilitation units by incorporating these environmental settings.

Drug intervention to induce sympathetic nervous activation has been applied to aging, disuse, and sarcopenic model rats.[41–47] Administration of β2-adrenergic agents, such as clenbuterol and formoterol, was reported to prevent muscle weakness and aging. In addition, β2-agonist administration was reported to improve not only myodystrophy[48] but also heart failure.[49] At the same time, further investigation is necessary for its clinical application because few studies have demonstrated a favorable effect of β-agonist administration in elderly people. Development of more specific drugs for skeletal muscle is expected to lead to application of such drugs in humans.

Table 3
Each Item of ΔFIM Increment in the High LF/HF Group and Low LF/HF Group

	Δ High LF/HF	Δ Low LF/HF
Physical items	8.37*	4.29
Eating	0.44	0.27
Grooming	0.44	0.44
Bathing	0.59	0.32
Upper body dressing	0.70	0.47
Lower body dressing	0.78*	0.27
Toileting	0.74	0.59
Bed to chair transfer	0.56	0.47
Toilet transfer	0.74	0.59
Shower transfer	0.70*	0.12
Bladder management	0.59*	0.12
Bowel management	0.59	0.18
Locomotion	1.00	0.35
Stairs	0.48	0.12
Cognitive items	1.07	0.35
Cognitive comprehension	0.11	0.09
Expression	0.19	0.09
Social interaction	0.15	−0.03
Problem solving	0.19	0.09
Memory	0.44	0.12

*$P < .05$.

Several limitations of this study should be considered. First, only HRV was conducted before rehabilitation therapy. The improvement of sympathetic nervous activity and physical and cognitive function needs to be shown simultaneously. Intervention therapy to improve sympathetic nervous activity should be conducted to support our study finding that increment of sympathetic nervous activity relates to good outcome. Second, as the sample size was not enough to perform multiple regression analysis with these covariates in this study, we performed a stepwise selection method to specify the explanatory variables first, and investigated subsequent multiple regression analysis with 2 selected covariates, pre-FIM and LF/HF.[50] Statistical power of the study might still not be enough to conclude the relationship between ΔFIM and LF/HF, and a large-scale study as well as direct evidence for a causal relationship should be investigated in the near future. Third, this study did not consider the effect of diseases that would affect HRV. Although the effect of LF/HF on stroke is still controversial, stroke might alter LF/HF.[51–53] Some studies also demonstrated that LF/HF was higher in patients with ischemic stroke compared with healthy controls,[51,52] but others showed it was lower in patients with stroke.[53] Multiple regression analysis in this study demonstrated no significant relationship between HRV and underlying diseases subject to rehabilitation therapy, and future study would clarify the contribution of each disease to HRV indices.

Conclusion

In summary, our study revealed that LF/HF was one of the strongest predictive factors of physical and cognitive functional recovery in rehabilitation, and that high LF/HF was related to good outcome in terms of physical function among frail elderly patients undergoing rehabilitation, further suggesting that appropriate sympathetic nervous activity may lead to well-being and successful aging.

Table 4
Independent Predictive Factors for ΔFIM

	Standardized Regression Coefficient (β)	*P*
Pre-FIM	0.341	.006
LF/HF	0.298	.015

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RESEARCH ARTICLE

The State of Health in Older Adults in Japan: Trends in Disability, Chronic Medical Conditions and Mortality

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Abstract

Both life expectancy and healthy life expectancy in Japan have been increasing and are among the highest in the world, but the gap between them has also been widening. To examine the recent trends in old age disability, chronic medical conditions and mortality in Japan, we retrospectively analyzed three nationally representative datasets: Comprehensive Survey of Living Conditions (2001–2013), Patient Survey (1996–2011) and Vital Statistics (1995–2010). We obtained the sex- and age-stratified trends in disability rate, treatment rates of nine selected chronic medical conditions (cerebrovascular diseases, joint disorders, fractures, osteoporosis, ischemic heart disease, diabetes mellitus, hypertension, pneumonia and malignant neoplasms), total mortality rate and mortality rates from specific causes (cerebrovascular diseases, heart diseases, pneumonia and malignant neoplasms) in both sexes in four age strata (65–69, 70–74, 75–79, 80–84 years). Disability rates declined significantly in both sexes. Treatment rates of all selected medical conditions also decreased significantly, except for fractures in women and pneumonia. Both total mortality rate and cause-specific mortality rates decreased in both sexes. We concluded that the recent decline in disability rates, treatment rates of chronic medical conditions and mortality rates points toward overall improvement in health conditions in adults over the age of 65 years in Japan. Nonetheless, considering the increase in the number of older adults, the absolute number of older adults with disability or chronic medical conditions will continue to increase and challenge medical and long-term care systems.

Introduction

Japan's life expectancy has increased steadily over the past several decades and is one of the highest in the world [1–3]. Despite the gradual and continuous rise in life expectancy, the definition of old age has not changed and the age of 65 has been considered the beginning of old age [3, 4]. Consequently, the increase in life expectancy, coupled with the falling birth rate, has resulted in a dramatic increase in the proportion of the "old age" population, from 14.6% in 1995 to 25.1% in 2013 [3]. The shift in demography to older ages has had a large impact on