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Indications and practice for tube feeding in Japanese geriatricians: Implications of multidisciplinary team approach

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Aim: The aim of this study was to examine how geriatricians decide the indication of tube feeding in the elderly with eating difficulty as a result of several disorders, and to determine the factors associated with their decision making and interventions for dysphagia.

Methods: The design was a cross-sectional study. All board-certified geriatricians in the Japan Geriatrics Society were recruited to this study in September 2010. We sent questionnaires to 1469 geriatricians. Among them, 629 agreed to participate. The survey consisted of self-administered questionnaires regarding demographic information, indications of tube feeding and interventions for dysphagia before tube feeding.

Results: We analyzed the remaining 555 questionnaires after excluding incomplete ones. Over 90% of geriatricians answered that "neurological disorder" and "stroke" are indications, whereas 46.8% of them answered that "dementia" is an indication for tube feeding. Geriatricians who organize a multidisciplinary team conference tended to carry out more "interventions for dysphagia before the prescription of tube feeding" compared with the reference group (odds ratio 2.1–8.7) after multivariate adjustment.

Conclusions: The results show that approximately half of the geriatricians prescribe tube feeding when the patient has dementia with loss of appetite or apraxia for eating. There is no consensus among Japanese geriatricians about the indication of tube feeding for demented people. We suggest that guidelines for tube feeding in the elderly should be established. Furthermore, a multidisciplinary approach would be desirable for decision making for tube feeding. **Geriatr Gerontol Int 2012; 12: 643–651.**

Keywords: elderly, geriatrician, multidisciplinary team, percutaneous endoscopic gastrostomy, tube feeding.

Introduction

Many older patients have nutritional problems caused by eating difficulties as a result of stroke, cancer, dementia and other conditions. When the patients have a functional gastrointestinal tract and they cannot take sufficient nutrition orally, tube feeding is an option. Percutaneous endoscopic gastrostomy (PEG) is the preferential route when enteral nutrition is expected to last for a longer period of time, because it is associated with better nutritional status and a lower incidence of aspiration than nasogastric tube (NGT).[1] PEG was originally developed for pediatric use by Gauderer in 1980.[2] However, thereafter PEG has become the most

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common way to supply artificial enteral nutrition in the elderly, including dementia patients. The number of people on PEG is increasing because of the improved simplicity and safety. Approximately 5–30% of the advanced dementia patients in nursing homes are on tube feeding in Europe and the USA; whereas, in Japan, approximately 50% of those are on tube feeding.[3–6] Thus, the percentage of tube feeding including PEG for dementia patients is higher in Japan than that in Western countries. However, recent studies have questioned the appropriateness of tube feeding in these patients. The decision of the practice or the withholding of tube feeding in patients with dementia is a difficult challenge among geriatricians and many other health-care professionals, as they need to make a decision with clinical ethical dilemmas. Furthermore, the quality of life (QOL) in the elderly with tube feeding and its effect on long-term survival have not yet been clarified,[7–13] and neither has a guideline for tube feeding in the elderly, especially in dementia patients. Accordingly, tube feeding is the focus of some extremely complex legal and ethical questions. Therefore, it is important to study the current situation of tube feeding for the elderly in Japan.

When we make a decision on tube feeding, comprehensive assessment of the patient, such as nutrition, cognition and swallowing function, is important and the assessment should be based on a multidisciplinary team approach. Previous studies showed the effectiveness of inpatient geriatric evaluation and management; that is, comprehensive geriatric assessment (CGA).[14] A multidisciplinary approach might be required for medical and nursing care of elderly patients, especially when we need to make a complicated decision, such as that of tube feeding. However, it is unknown whether the team approach can affect the decision making for tube feeding and interventions for dysphagia.

Therefore, the aim of the present study was to examine how geriatricians decide on the indication of tube feeding in the elderly with eating difficulty as a result of various disorders, and to determine whether the team approach can affect their decision making and interventions for dysphagia.

Methods

The design was a cross-sectional study. All board-certified geriatricians in the Japan Geriatrics Society were recruited to the present study in September 2010. We separately sent self-administered questionnaires to 1469 geriatricians by post and collected them from October to December 2010. These geriatricians were chosen because of their experience in taking care of patients who require tube feeding, and carry out CGA by organizing multidisciplinary team conferences. The present study was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine (no. E984, 2010).

The questionnaires included demographic information, such as age, sex, place of employment, and clinical experience, reference guidelines for tube feeding, aims and indications of tube feeding in geriatrics, interventions for dysphagia before tube feeding, and multidisciplinary team approach if tube feeding is indicated. It was explained in the questionnaires that the term "elderly" was defined as people over the age of 75 years and those who require nursing care, and tube feeding included NGT, PEG and enterostomy tube.

We carried out descriptive analyses for each item in the questionnaire. The χ^2-test or t-test was used to compare the differences of place of employment and clinical experience. Logistic regression analyses were carried out to evaluate the differences of the frequencies and conference members according to the indication for tube feeding, and the interventions for dysphagia before tube feeding. Each item in the indication for tube feeding or interventions for swallowing disorder was adjusted for sex, working place and clinical experience of geriatricians. The frequency and number of members in a multidisciplinary conference were divided into five categories: not at all, occasional and less than five different health-care professionals, occasionally and ≧5 different health-care professionals, every time and less than five different health-care professionals, and every time and ≧5 different health-care professionals. The Statistical Package for Social Sciences version 18.0J (SPSS Japan, Tokyo, Japan) was used for statistical analysis. All probability values were two-tailed with a significant level of $P < 0.05$, and all confidence intervals were estimated at the 95% level.

Results

We sent a questionnaire to 1469 board-certified geriatricians, and 51 were returned as a result of being undeliverable because of wrong address. Among the rest, 629 agreed to participate in the present study. The response rate was 44.4%. After excluding the questionnaires with missing data, we analyzed the remaining 555 questionnaires. The prevalence of doctors aged over 60 years and male doctors was 34.6% and 89.2%, respectively. We found that 43.8% of the geriatricians had a clinical experience of more than 30 years, and 63.7% were working in acute hospitals, 30.7% in a clinic and 3.9% in long-term care facilities.

Table 1 shows the percentage of geriatricians who follow the guidelines and the purpose for tube feeding according to the geriatrician's place of employment and clinical experience. A total of 68% of geriatricians did not use any guideline for tube feeding. Among geriatricians following guidelines for tube feeding, 137 used "Guideline of Parenteral and Enteral Nutrition (EN) in

Table 1 Use of guidelines and the aims of tube feeding according to place of employment and clinical experience

Questions	Characteristics of geriatricians								Total
	Place of employment					Clinical experience			
	Hospital $n = 360$	Clinic $n = 166$	Long-term care $n = 20$	Other[†] $n = 9$	*P*-value	<30 years $n = 317$	≧30 years $n = 238$	*P*-value	$n = 555$
Do you use any guidelines for TF in geriatrics?[‡]									
Guideline of Parenteral and EN in Japan*1	84 (23.3)	48 (28.9)	4 (20.0)	1 (11.1)	ND	87 (27.4)	50 (21.0)	0.082	137 (24.7)
Guideline of PEG in Japan*2	51 (14.2)	21 (12.7)	4 (20.0)	1 (11.1)	ND	41 (12.9)	36 (15.1)	0.460	77 (13.9)
Guideline of Parenteral and EN in America*3	13 (3.6)	11 (6.6)	0 (0.0)	0 (0.0)	ND	11 (3.5)	13 (5.5)	0.253	24 (4.3)
Guideline of Parenteral and EN for elderly in Europe*4	9 (2.5)	11 (6.6)	0 (0.0)	1 (1.1)	ND	9 (2.8)	12 (5.0)	0.178	21 (3.8)
Not using guideline for TF	253 (70.3)	106 (63.9)	10 (50.0)	7 (77.8)	ND	209 (65.9)	167 (70.2)	0.291	376 (67.7)
What are the aims of TF in geriatrics?[§]									
Improvement of survival	63 (17.5)	29 (17.5)	6 (30.0)	0 (0.0)	ND	54 (17.0)	44 (18.5)	ND	98 (17.7)
Improvement of general condition and prevention of complications	201 (55.8)	93 (56.0)	12 (60.0)	3 (33.3)	–	163 (51.4)	146 (61.3)	–	309 (55.7)
Improvement of activities of daily living	17 (4.7)	9 (5.4)	0 (0.0)	1 (11.1)	–	22 (6.9)	5 (2.1)	–	27 (4.9)
Improvement of quality of life	24 (6.7)	9 (5.4)	2 (10.0)	2 (22.2)	–	24 (7.6)	13 (5.5)	–	37 (6.7)
Satisfaction of patient	15 (4.2)	13 (7.8)	0 (0.0)	2 (22.2)	–	19 (6.0)	11 (4.6)	–	30 (5.4)
Burden of caregiver	5 (1.4)	9 (5.4)	0 (0.0)	0 (0.0)	–	6 (1.9)	8 (3.4)	–	14 (2.5)
Length of hospital stay	3 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	–	3 (0.9)	0 (0.0)	–	3 (0.5)
Living will	27 (7.5)	3 (1.8)	0 (0.0)	1 (11.1)	–	20 (6.3)	11 (4.6)	–	31 (5.6)
Other	5 (1.4)	1 (0.6)	0 (0.0)	0 (0.0)	–	6 (1.9)	0 (0.0)	–	6 (1.1)

Number (%). *P*-values were tested by χ^2-test. [†]Other included part-time doctors, retired doctors, researchers and so on. [‡]Multiple answers were allowed. [§]Simple answer was allowed for nine items. *1 From Japanese Society for Parenteral and Enteral Nutrition *2 From Japan Gastroenterological Endoscopy Society *3 From American Society for Parenteral and Enteral Nutrition *4 From European Society for Gastroenterological Endoscopy Society. EN, enteral nutrition; ND, not determined; PEG, percutaneous endoscopic gastrostomy; TF, tube feeding.

Japan" from the Japanese Society for Parenteral and EN. For the purpose for tube feeding, more than half of the geriatricians chose "improvement of general condition or prevention of complications." However, a few geriatricians chose "improvement of QOL," "satisfaction of patient" or "living will." The working place or clinical experience did not affect the aims of tube feeding placement.

Table 2 shows the indication for tube feeding and the interventions for dysphagia before tube feeding according to place of employment and clinical experience. Among the seven target indications for tube feeding in the elderly, over 90% of the geriatricians answered that "neurological disorders other than dementia" and "stroke" are indications for tube feeding. Over 80% of the geriatricians answered that "head injury or facial trauma" and "oropharyngeal malignancy" are also an indication. In contrast, 46.8% of the geriatricians answered that "dementia" is an indication for tube feeding, and 65.9% of the geriatricians answered that "aspiration-prone frail elderly without comorbidites" is an indication. The place of employment was not associated with the judgment for the indication. The percentage of geriatricians who answered that "head injury or facial trauma" and "neurological disorders other than dementia" were an indication for tube feeding was significantly higher in those with less than 30 years of clinical experience than in those with more than 30 years of clinical experience" (head injury or facial trauma; $P = 0.012$, neurological disorder; $P = 0.049$). However, following guideline for tube feeding did not affect the decision making of tube feeding for these disorders (data not shown). We also asked about the life expectancy of the patient after PEG placement, and 79.5% answered that at least more than 12 weeks were expected.

Next, we asked how many interventions they carried out for swallowing disorder before tube feeding. The mean number of interventions was 6.22, and geriatricians with less than 30 years of experience carried out significantly more interventions than those with more than 30 years (6.49 ± 3.2 *vs* 5.86 ± 2.8, $P = 0.015$). The number of interventions was not significantly different between geriatricians working in an acute hospital and those working in a clinic. Among 15 items of interventions for swallowing disorder, over 70% of geriatricians answered that "thickening agent" and "using semi-solid and liquid foods" were afforded to patients with swallowing disorder.

Figure 1 shows the percentage of geriatricians organizing a multidisciplinary conference for tube feeding. A total of 63% of geriatricians discussed with other health-care professionals every time or occasionally. They also answered that physicians including themselves (95.4%), primary nurses (84.9%), dieticians (49.7%) and speech therapists (42.0%) were the members of the conference. The place of employment was not associated with the number of conference members (Table 3).

Table 4 shows the multiple logistic regression analysis for the frequencies and conference members according to the indication for tube feeding and interventions for dysphagia before tube feeding. More "interventions for dysphagia before introducing tube feeding" were carried out in geriatricians organizing a multidisciplinary team conference than the reference group after multivariate adjustment (odds ratio 2.1–8.7). We also found that geriatricians who always organize a conference with many types of health-care professionals (multidisciplinary) carried out more tests for the assessment of swallowing function and interventions for dysphagia before introducing tube feeding, such as oral ice massage, than the reference group. However, the indications for tube feeding were not affected by a multidisciplinary conference.

Discussion

In the present study, we found that approximately 70 % of board-certified geriatricians did not use any guidelines for tube feeding in their practice. We also noted that the use of guidelines was not associated with the decision making for tube feeding in the elderly, because "Guideline of Parenteral and EN in Japan" or "Guideline of PEG in Japan" does not describe the indications for tube feeding in elderly patients, especially in dementia patients.[15,16] Furthermore, more than half of the geriatricians consider that the purpose of tube feeding is to improve the general condition or to prevent complications in the elderly with eating problems. In contrast, only a few geriatricians selected living will or patient satisfaction. Decision making of geriatricians for tube feeding did not seem to be related to their working place or clinical experiences. Although the guideline describes that "respecting the wishes of the family or living will of the patient when nutrition therapy is needed for the elderly at the terminal stage or with dementia,"[15] most geriatricians who decide the indication of tube feeding might not have a chance to care for patients' living will. Although there is an ideal description in the guideline, it might be difficult for doctors to obtain a patient's living will beforehand, even if they understand the importance of respecting the living will of the patient. Therefore, comprehensive approaches not only from the field of nutrition and gastroenterology, but also from the experience and know-how from the professionals involved in medicine, nursing and care for the elderly, such as geriatricians, nurses, speech therapists, caregivers and care managers, would be expected to make a new guideline for tube feeding in the elderly.

Several studies have shown that there is no survival benefit in dementia patients who receive artificial

Table 2 Indications for tube feeding and interventions for dysphagia before introducing tube feeding according to place of employment and clinical experiences

Questions	Characteristics of geriatricians Place of employment					Clinical experience			Total
	Hospital $n = 360$	Clinic $n = 166$	Long-term care $n = 20$	Other† $n = 9$	*P*-value	<30 years $n = 317$	≧30 years $n = 238$	*P*-value	$n = 555$
Is the following disorder an indication for TF?									
Head injury or facial trauma	313 (86.9)	144 (86.7)	8 (40.0)	7 (77.8)	ND	208 (88.3)	192 (80.7)	0.012	472 (85.0)
Oropharyngeal malignancy	286 (79.4)	143 (86.1)	13 (65.0)	7 (77.8)	ND	258 (81.4)	191 (80.3)	0.736	449 (80.9)
Neurological disorder	328 (91.1)	155 (93.4)	15 (75.0)	7 (77.8)	ND	295 (93.1)	210 (88.2)	0.049	505 (91.0)
Stroke	334 (92.8)	147 (88.6)	18 (90.0)	8 (88.9)	ND	290 (91.5)	217 (91.2)	0.899	507 (91.4)
Dementia	177 (49.2)	66 (39.8)	13 (65.0)	4 (44.4)	ND	1156 (49.2)	104 (43.7)	0.198	260 (46.8)
Aspiration-prone frail elderly without comorbidity	238 (66.1)	108 (65.1)	15 (75.0)	5 (55.6)	ND	216 (68.1)	150 (63.0)	0.208	366 (65.9)
Malnutrition in frail elderly without comorbidity	115 (31.9)	58 (34.9)	9 (45.0)	5 (55.6)	ND	115 (36.3)	72 (30.3)	0.137	187 (33.7)
How long does a patient need to survive after PEG placement?‡									
2 weeks	3 (0.8)	2 (1.2)	0 (0.0)	0 (0.0)	ND	3 (0.9)	2 (0.8)	ND	5 (0.9)
4 weeks	19 (5.3)	16 (9.6)	1 (5.0)	2 (22.2)	–	18 (5.7)	20 (8.4)	–	38 (6.8)
6 weeks	4 (1.1)	2 (1.2)	1 (5.0)	1 (11.1)	–	7 (2.2)	1 (0.4)	–	8 (1.4)
8 weeks	39 (10.8)	21 (12.7)	3 (15.0)	0 (0.0)	–	37 (11.7)	26 (10.9)	–	63 (11.4)
12 weeks	295 (81.9)	125 (75.3)	15 (75.0)	6 (66.7)	–	252 (79.5)	189 (79.4)	–	441 (79.5)
Interventions for swallowing disorder before introducing TF									
No. Interventions; mean ± standard deviation (total 15 items)	6.44 ± 3.12*	5.83 ± 2.93	6.70 ± 2.00	3.67 ± 3.32*	0.010§	6.49 ± 3.20	5.86 ± 2.82	0.015	6.22 ± 3.06
No. interventions, ≧6 items¶ (total 15 items)	211 (58.6)	84 (50.6)	14 (70.0)	2 (22.2)	ND	188 (59.3)	123 (51.7)	0.073	311 (56.0)
Consultation									
To otolaryngologist	131 (36.4)	60 (36.1)	3 (15.0)	4 (44.4)	ND	123 (38.8)	75 (31.5)	0.076	198 (35.7)
To speech therapist	166 (46.1)	31 (16.7)	7 (35.0)	1 (11.1)	ND	131 (41.3)	74 (31.1)	0.013	205 (36.9)
To certified nurse of dysphagia nursing	77 (21.4)	25 (15.1)	4 (20.0)	2 (22.2)	ND	67 (21.1)	41 (17.2)	0.250	108 (19.5)
Test									
Repetitive saliva swallowing test	111 (30.8)	63 (38.0)	4 (20.0)	2 (22.2)	ND	109 (34.4)	71 (29.8)	0.257	180 (32.4)
Water swallowing test	243 (67.5)	104 (62.7)	13 (65.0)	5 (55.6)	ND	210 (66.2)	155 (65.1)	0.783	365 (65.8)
Video endoscopy	55 (15.3)	26 (15.7)	1 (5.0)	0 (0.0)	ND	50 (15.8)	32 (13.4)	0.444	82 (14.8)
Video fluorography	163 (45.3)	47 (28.3)	4 (20.0)	2 (22.2)	ND	140 (64.8)	76 (31.9)	0.003	216 (61.1)
Practice and education									
Oral ice-massage	102 (28.3)	23 (13.9)	5 (25.0)	0 (0.0)	ND	86 (27.1)	44 (18.5)	0.017	130 (23.4)
Swallowing exercise	72 (20.0)	40 (24.1)	5 (25.0)	0 (0.0)	ND	70 (22.1)	47 (19.7)	0.505	117 (21.1)
Vocalization exercise	50 (13.9)	20 (12.0)	1 (5.0)	0 (0.0)	ND	44 (13.9)	27 (11.3)	0.376	71 (12.8)
Using semi-solid and liquid foods	267 (74.2)	120 (72.3)	18 (90.0)	3 (33.3)	ND	236 (74.4)	172 (72.3)	0.565	408 (73.5)
Thickening agent	308 (85.6)	131 (78.9)	20 (100.0)	3 (33.3)	ND	267 (84.2)	195 (81.9)	0.474	462 (83.2)
Positioning	235 (65.3)	106 (63.9)	17 (85.0)	4 (44.4)	ND	215 (67.8)	147 (61.8)	0.138	362 (65.2)
Appropriate approach for swallowing	161 (44.7)	80 (48.2)	12 (60.0)	2 (22.2)	ND	153 (48.3)	102 (42.9)	0.206	255 (45.9)
Ways of coping with aspiration	161 (44.7)	85 (51.2)	17 (85.0)	4 (44.4)	ND	142 (44.8)	125 (52.5)	0.071	267 (48.1)

Number (%), *P*-values were tested by χ^2-test and Student's *t*-test, †Other included part-time doctors, retired doctors, researchers and so on. ‡Single answer was allowed for five items, and the other questions were allowed to select more than one. §*P*-values were tested by ANOVA, *$P < 0.05$ by Bonferroni. ¶Number of intervention items were divided into two groups, which used median value (≧6 *vs* <6). ND, not determined; PEG, percutaneous endoscopic gastrostomy; TF, tube feeding.

feeding by PEG.[7,8,10,12] In addition, "Guideline of parenteral and EN for elderly in Europe" does not recommend enteral nutrition to persons with severe dementia as a result of more risks than benefits for persons with severe dementia, and occasionally in early and moderate dementia to ensure energy and nutrient supply and to prevent undernutrition.[17,18] In the present study, we found that approximately 45% of the geriatricians considered that dementia patients with loss of appetite or apraxia for eating should be on tube feeding and that 65% of the geriatricians considered that aspiration-prone frail elderly without comorbidities should also be on tube feeding, which is a relatively high percentage. In a previous study, approximately 60% of physicians in the USA answered that aspiration pneumonia was the indication for PEG placement, and was the most common medical indication.[19] The present finding are consistent with other results; therefore the medical situation in Japan might be quite similar to that in the USA. Indeed, PEG placement to the elderly with repeating aspiration pneumonia or not eating voluntarily with cerebrovascular disease or dementia is indicated in "Guideline of PEG in Japan."[16] In the present study, the questions did not specify the stage of disorders or the level of conditions; therefore our results should be interpreted with caution. However, it is certain that there is no consensus among Japanese geriatricians about tube feeding for the elderly with advanced dementia and there is an urgent need to develop guidelines to decide the risk/benefit ratio in the individual patient to optimize the timing and route of nutritional support. Thus, the indication for tube feeding in the elderly should be widely discussed in the future and hence a guideline should be established to describe the indication of tube feeding in more detail.

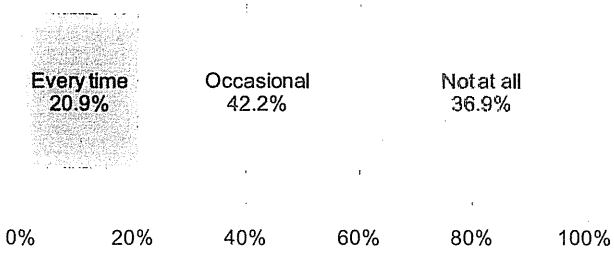

Figure 1 Do you organize a multidisciplinary conference before introducing tube feeding?

"Guideline of parenteral and EN for elderly in Europe" indicates PEG placement if EN is anticipated for longer than 4 weeks.[17,18] In contrast, the present study showed that approximately 80% of the geriatricians consider that survival more than 12 weeks should be expected for PEG placement. PEG is better than NGT for swallowing rehabilitation, and PEG placement

Table 3 Conference members for decision making of tube feeding according to place of employment

	Place of employment of geriatricians					Total
	Hospital $n = 249$	Clinic $n = 80$	Long-term care $n = 17$	Other† $n = 3$	*P*-value	$n = 350$
No. conference members; mean ± standard deviation (total 12 occupations)	4.4 ± 2.0	4.2 ± 1.8	4.3 ± 1.5	4.8 ± 4.2	0.864	4.31 ± 1.9
Conference members						
Attending physician	238 (95.2)	75 (92.6)	17 (100)	3 (100)	–	334 (95.4)
Primary nurse	224 (89.6)	54 (66.7)	15 (88)	3 (100)	–	297 (94.9)
Otolaryngologist	27 (10.8)	10 (12.3)	0 (0)	0 (0.0)	–	37 (10.6)
Certified nurse of dysphagia nursing	42 (16.8)	18 (22.2)	3 (18)	0 (0.0)	–	63 (18.0)
Physical therapist	55 (22.0)	12 (14.8)	4 (24)	1 (33.3)	–	72 (20.6)
Occupational therapist	37 (14.8)	8 (9.9)	4 (24)	1 (33.3)	–	50 (14.3)
Speech therapist	118 (47.2)	23 (28.4)	5 (29)	1 (33.3)	–	147 (42.0)
Dietician	126 (50.4)	37 (45.7)	9 (53)	2 (66.7)	–	174 (49.7)
Pharmacist	37 (14.8)	12 (14.8)	1 (5.9)	1 (33.3)	–	51 (14.6)
Discharge planning coordinator‡	26 (10.4)	14 (17.3)	2 (12)	1 (33.3)	–	43 (12.3)
Medical social worker	89 (35.6)	24 (29.6)	4 (24)	2 (66.7)	–	119 (34.0)
Care manager	46 (18.4)	39 (48.1)	5 (29)	1 (33.3)	–	91 (26.0)

Number (%), *P*-values were tested by ANOVA, $^*P < 0.05$ by Bonferroni. Of the 555 geriatricians, 350 (63.1%) carried out a conference at least once. Respectively, hospital: 249 (69.2%), clinic: 80 (48.2%), long-term care: 17 (85.0%), other: 3 (33.3%). Multiple answers were allowed. †Other included part-time doctors, retired doctors, researchers and so on. ‡They are a registered nurse and work for discharge planning and coordination in the hospital.

Table 4 Multivariate-adjusted odds ratios and 95% confidence intervals for frequency and the conference members according to the indication for tube feeding and interventions for dysphagia before using tube feeding

	Conference				
	Non	Occasional		Every time	
		Participating occupation		Participating occupation	
		Few	Multidisciplinary	Few	Multidisciplinary
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Is the following disorder an indication for TF?					
Head injury or facial trauma	Ref	1.02 (0.55–1.89)	1.15 (0.52–2.57)	0.80 (0.36–1.78)	1.52 (0.62–3.77)
Oropharyngeal malignancy	Ref	0.96 (0.56–1.66)	0.78 (0.41–1.52)	1.05 (0.48–2.31)	1.02 (0.48–2.16)
Neurological disorder	Ref	0.72 (0.34–1.52)	0.56 (0.23–1.34)	1.69 (0.46–6.16)	1.17 (0.39–3.53)
Stroke	Ref	1.41 (0.68–2.90)	1.84 (0.66–5.13)	2.35 (0.68–8.15)	4.03 (0.90–18.05)
Dementia	Ref	0.83 (0.54–1.28)	0.82 (0.48–1.42)	1.86 (1.00–3.44)	1.01 (0.56–1.83)
Aspiration-prone frail elderly without comorbidity	Ref	0.99 (0.63–1.55)	1.23 (0.69–2.19)	1.31 (0.68–2.52)	0.80 (0.44–1.46)
Malnutrition in frail elderly without comorbidity	Ref	0.77 (0.49–1.22)	0.98 (0.56–1.74)	1.30 (0.70–2.42)	1.18 (0.64–2.18)
How long does a patient need to survive after PEG placement? ≧12 weeks†	Ref	0.85 (0.50–1.43)	0.89 (0.46–1.74)	0.80 (0.39–1.63)	1.44 (0.64–3.21)
Intervention for swallowing disorder before using TF					
No. intervention items, ≧ 6 items‡	Ref	2.07 (1.33–3.20)	3.24 (1.81–5.78)	2.60 (1.39–4.85)	8.71 (3.99–19.00)
Consultation					
To otolaryngologist	Ref	1.13 (0.72–1.77)	1.36 (0.78–2.38)	0.94 (0.49–1.80)	1.48 (0.80–2.72)
To speech therapist	Ref	1.51 (0.93–2.46)	4.57 (2.52–8.29)	2.47 (1.28–4.76)	3.82 (2.01–7.27)
To certified nurse of dysphagia nursing	Ref	1.18 (0.65–2.14)	2.16 (1.11–4.23)	1.65 (0.76–3.61)	4.75 (2.43–9.32)
Test					
Repetitive saliva swallowing test	Ref	1.62 (0.98–2.66)	3.89 (2.16–6.99)	3.91 (2.05–7.44)	4.48 (2.37–8.46)
Water swallowing test	Ref	2.08 (1.32–3.28)	1.63 (0.93–2.87)	1.82 (0.96–3.44)	2.95 (1.49–5.88)
Video endoscopy	Ref	1.53 (0.83–2.82)	1.30 (0.59–2.86)	0.97 (0.37–2.53)	2.89 (1.37–6.09)
Video fluorography	Ref	1.62 (1.03–2.56)	2.08 (1.19–3.66)	3.07 (1.64–5.76)	2.28 (1.23–4.22)
Practice and education					
Oral ice-massage	Ref	1.19 (0.67–2.10)	2.19 (1.16–4.14)	2.34 (1.14–4.79)	3.59 (1.82–7.06)
Swallowing exercise	Ref	1.81 (0.97–3.39)	3.47 (1.74–6.91)	4.86 (2.34–10.09)	6.63 (3.27–13.45)
Vocalization exercise	Ref	1.55 (0.71–3.41)	2.96 (1.28–6.83)	2.70 (1.04–7.00)	6.84 (3.02–15.50)
Using semi-solid and liquid foods	Ref	1.83 (1.13–2.96)	2.12 (1.11–4.06)	1.71 (0.86–3.38)	5.96 (2.24–15.84)
Thickening agent	Ref	1.26 (0.73–2.21)	1.93 (0.85–4.39)	1.18 (0.54–2.59)	4.68 (1.36–16.12)
Positioning	Ref	1.46 (0.94–2.26)	2.36 (1.29–4.31)	1.75 (0.93–3.30)	7.22 (2.94–17.71)
Appropriate approach for swallowing	Ref	2.48 (1.59–3.88)	2.82 (1.62–4.92)	2.13 (1.15–3.95)	5.60 (2.94–10.65)
Ways to coping when the aspiration	Ref	1.48 (0.95–2.29)	2.86 (1.63–5.01)	1.24 (0.67–2.29)	5.31 (2.69–10.48)

Dependent variables: the indication for tube feeding and interventions for dysphagia before introducing tube feeding. Independent variables: frequency and the conference members (ref, non conference; 1, occasional and less than five different health-care professionals; 2, occasional and ≧5 different health care professionals; 3, every time and less than five different health-care professionals; 4, every time and ≧5 different health-care professional. Adjusted for sex, place of employment and clinical experience. †The period expected to survive after PEG was divided into two groups. (1: ≧12 weeks, 0: <12 weeks). ‡Number of intervention items were divided into two groups, which was used median value into 15 items. (1: ≧6 items, 0: <6 items). CI, confidence interval; OR, odds ratio; TF, Tube Feeding.

in patients with stroke and oropharynegeal malignancy was associated with better prognosis; therefore PEG placement is recommended for these disorders by the European guideline.[20] We did not investigate how long PEG is placed in each condition. Thus, knowledge of geriatricians for tube feeding or PEG placement was not sufficiently explored in the present study; however, a period of PEG placement should be considered in each condition.

In Japan, requests for PEG to facilitate care are prevalent, because the staff in nursing homes tend to prefer PEG to time-consuming oral feeding. A multicenter study in the USA showed that feeding tube insertion is independently associated with both clinical characteristics of residents and fiscal, organizational and demographic features of nursing homes.[4] Therefore, these situations might have affected the decision making of geriatricians for tube feeding. Unfortunately, we did not include the question whether or not the request from nursing homes might have affected the decision making for tube feeding in dementia patients. Therefore, we should ask this question next time.

Regarding interventions for swallowing disorder, the mean number of interventions for swallowing disorder before introducing tube feeding was six items, which are not so many. Among the 15 items of interventions before introducing tube feeding, over 70% of the geriatricians answered that "Thickening agent" and "Using semi-solid and liquid foods" were afforded to patients with swallowing disorder. In contrast, consultation with other specialists was not frequently carried out, and care to improve swallowing dysfunction, such as "oral ice-massage," "swallowing exercise" and "vocalization exercise" was not usually carried out either. Therefore, from these data, we think that more interventions would be necessary to care for patients with dysphagia by consulting specialists and multidisciplinary approach.

It is interesting to note the relationship between multidisciplinary conference and knowledge and practice for tube feeding for the elderly. In the present study, we showed that those who have a multidisciplinary team conference for a patient indicated for tube feeding tended to carry out more "interventions for dysphagia before tube feeding" compared with the reference group after multivariate adjustment. Furthermore, the data showed that geriatricians who organize a conference with different health-care professionals carried out more interventions for dysphagia before tube feeding, irrespective of the frequencies of conference. The present study also showed that although there were no differences in the number of conference members and interventions between the geriatricians working in an acute hospital and those in a clinic before introducing tube feeding, the percentage of geriatricians who organized a multidisciplinary conference before introducing tube feeding was higher in the hospital than in the clinic. Therefore, the characteristics of facilities, not doctors themselves, might have affected this outcome. A previous study reported that multidisciplinary CGA is effective for the care of frail older persons admitted to the hospital, because evaluation and management by a multidisciplinary team during hospitalization documented a lower rate of institutionalization after 1 year.[14] Furthermore, decision making for treatment strategy should be discussed in a multidisciplinary team. The multidisciplinary conference would provide a better answer for each elderly patient who requires tube feeding, because they tend to have a complicated background.

Several potential limitations should be considered when interpreting these results. First, a cross-sectional study does not prove any causal relationship. Second, the practice rate of tube feeding in geriatricians was not clearly determined, because the present study was carried out by self-administered questionnaires. Third, the subjects were limited to geriatricians certified by the Japan Geriatrics Society, and also the response rate was not so high. Therefore, selection bias might have occurred. Finally, we did not investigate the number of beds in their place of employment; therefore these results were not completely adjusted by hospital size.

In conclusion, the present data showed that more than half of the board-certified geriatricians consider that the purpose of tube feeding is to improve the general condition or to prevent complications in the elderly with eating problems. Furthermore, regardless of their clinical experience, approximately 40% of the Japanese geriatricians consider that demented elderly with loss of appetite or apraxia for eating should be on tube feeding. At this moment, there is no consensus among Japanese geriatricians about tube feeding for advanced demented people, and hence the guideline should be established for tube feeding in the elderly. Furthermore, a multidisciplinary team approach is expected to find a better answer for each elderly patient with eating difficulty.

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Testosterone Deficiency Accelerates Neuronal and Vascular Aging of SAMP8 Mice: Protective Role of eNOS and SIRT1

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Abstract

Oxidative stress and atherosclerosis-related vascular disorders are risk factors for cognitive decline with aging. In a small clinical study in men, testosterone improved cognitive function; however, it is unknown how testosterone ameliorates the pathogenesis of cognitive decline with aging. Here, we investigated whether the cognitive decline in senescence-accelerated mouse prone 8 (SAMP8), which exhibits cognitive impairment and hypogonadism, could be reversed by testosterone, and the mechanism by which testosterone inhibits cognitive decline. We found that treatment with testosterone ameliorated cognitive function and inhibited senescence of hippocampal vascular endothelial cells of SAMP8. Notably, SAMP8 showed enhancement of oxidative stress in the hippocampus. We observed that an NAD^+-dependent deacetylase, SIRT1, played an important role in the protective effect of testosterone against oxidative stress-induced endothelial senescence. Testosterone increased eNOS activity and subsequently induced SIRT1 expression. SIRT1 inhibited endothelial senescence via up-regulation of eNOS. Finally, we showed, using co-culture system, that senescent endothelial cells promoted neuronal senescence through humoral factors. Our results suggest a critical role of testosterone and SIRT1 in the prevention of vascular and neuronal aging.

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Introduction

Advancing age is the most significant risk factor for the development of cognitive impairment [1,2]; however, what age-related changes underlie this effect remains uncertain. With advancing age, men experience a significant decrease in the circulating level of testosterone. Although studies have shown alterations in mood, libido, and cognition resulting from testosterone deficiency [3], the full range of consequences of age-related testosterone loss remains incompletely defined. In a small clinical study of men recently diagnosed with cognitive impairment, testosterone treatment improved performance on cognitive tests [4]. In a prospective longitudinal study using subjects from the Baltimore Longitudinal Study on Aging, men who developed Alzheimer disease (AD) were observed to exhibit low testosterone levels 5 10 years prior to the clinical diagnosis of AD [5]. With a relationship between age-related testosterone decline in men and increased risk for cognitive impairment reasonably well established, a critical issue is how testosterone contributes to the pathogenesis of cognitive decline with aging. The most likely hypothesis is through the regulation of accumulation of amyloid ß (Aß) peptides, which are widely believed to be the critical initiating step in the pathogenesis of AD. However, it is becoming increasingly clear that not all aspects of cognitive decline can be explained by Aß [6,7]. Findings from such diverse lines of investigations as neuroimaging and clinical trials suggest that non-Aß factors also contribute to memory deficit in aged men.

In *S. cerevisiae*, the *Sir2* (silent information regulator-2) family of genes governs budding exhaustion and replicative life span [8,9]. *Sir2* has been identified as an NAD^+-dependent histone deacetylase and is responsible for maintenance of chromatin silencing and genome stability. Mammalian sirtuin 1 (*Sirt1*), the closest homolog of *Sir2*, regulates the cell cycle, senescence, apoptosis and metabolism, by interacting with a number of molecules such as p53. As recently reported, overexpression of SIRT1 in the brain improved the memory deficit in a mouse model of AD via activation of the transcription of α-secretase [10].

An increasing body of evidence suggests the presence of a link between cognitive decline and vascular dysfunction, especially atherosclerosis [11]. Senescence of endothelial cells is involved in endothelial dysfunction and atherogenesis, and SIRT1 has been recognized as a key regulator of vascular endothelial homeostasis, controlling angiogenesis, endothelial senescence, and dysfunction [12 14].

In the present study, we demonstrated that cognitive impairment in senescence-accelerated mouse prone 8 (SAMP8), a model of cognitive decline with aging, is associated with endothelial senescence in the hippocampus and is ameliorated by testosterone

replacement. SIRT1 plays an important role in prevention of endothelial senescence induced by oxidative stress [13]. We suggest that the protection against endothelial senescence in the hippocampus through up-regulation of testosterone and SIRT1 could contribute to a novel therapeutic strategy against cognitive decline with aging.

Results

Treatment with dihydrotestosterone ameliorated cognitive function of SAMP8

In order to assess the effects of testosterone on cognitive function, we used an in vivo model of aging, SAMP8, and a control counterpart strain, SAMR1. SAMP8 was originally derived from AKR/J strain, litters of which show the characteristic of cognitive decline with aging. These mice exhibit age-related deficits in learning and memory at an early age, and are considered a suitable animal model to study aging and memory deficit. Body weight, appearance, and plasma testosterone level of SAMR1 and SAMP8 at 12 weeks of age were determined. Body weight and appearance did not differ between SAMR1 and SAMP8, but plasma testosterone level in SAMP8 was lower than that in SAMR1 (Figure 1A). By determining the time required to find the platform (escape latency) as a function of days of training in the Morris water maze, we observed a marked decline in performance in SAMP8 compared with SAMR1 (Figure 1B). Because testosterone acts in part through aromatase-dependent conversion to estradiol, non-aromatizable dihydrotestosterone (DHT) was used to examine a direct role of androgens through androgen receptor (AR). SAMP8 treated with DHT showed significantly reduced escape latency time compared with untreated SAMP8. There was no difference in swim speed between the groups; however, % time in the quadrant was increased in DHT-treated SAMP8 (Figure 1B). These results indicate that DHT treatment ameliorated cognitive dysfunction in SAMP8. The water-maze is appropriate for hippocampal-dependent paradigms. However, DHT 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 DHT on locomotor performance was observed in SAMR1 and SAMP8, whereas SAMR1 moved significantly more compared with SAMP8 (Figure 1C). The ratio of the distance travelled in the central area to that in the total area in the open- field, an indirect measure of exploratory behavior and anxiety [15], was also observed. In SAMP8, DHT increased this ratio (Figure 1C), suggesting that DHT promoted exploratory behavior and diminished anxiety.

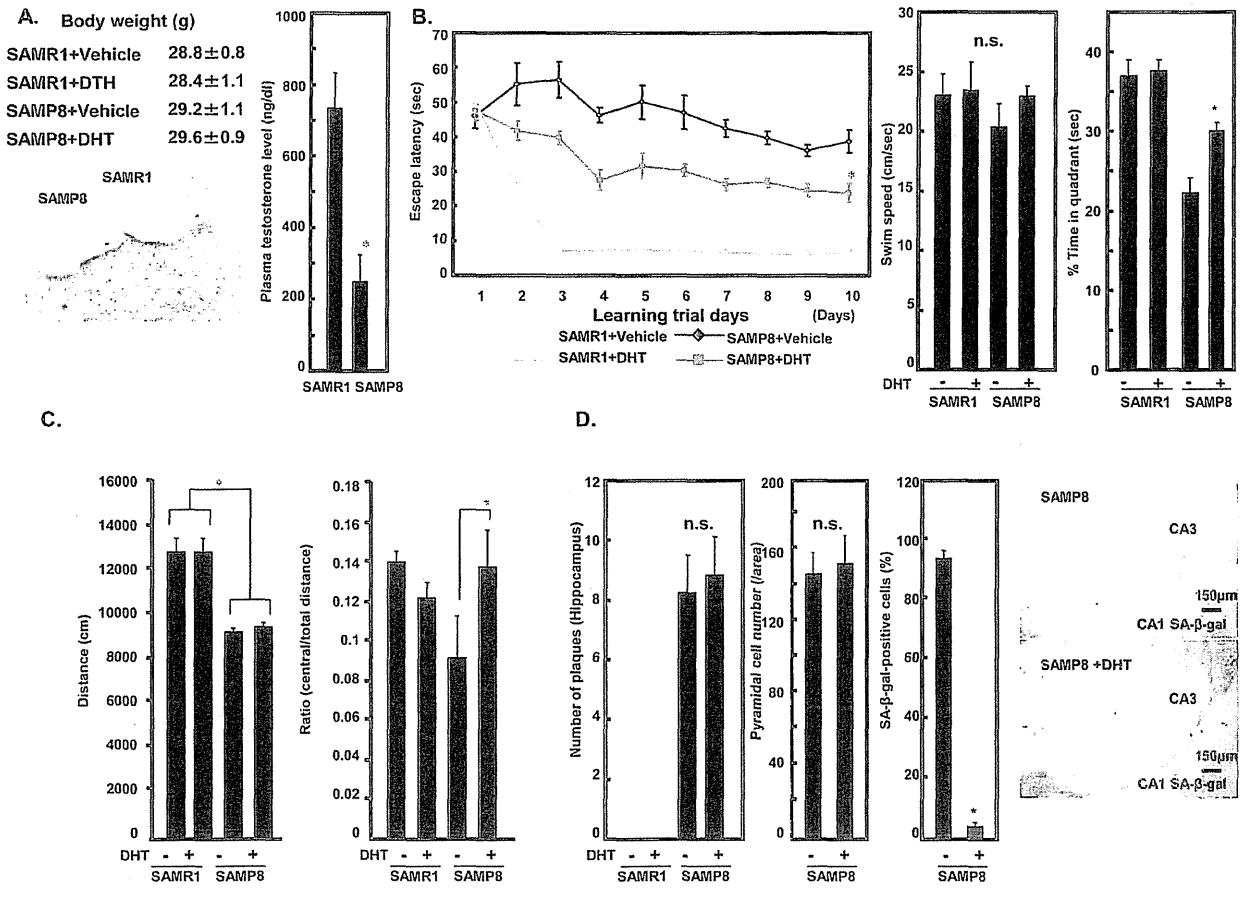

Figure 1. Testosterone deficiency causes senescence of hippocampus and cognitive impairment in SAMP8 mice. A. Body weight, appearance, and plasma testosterone level of male SAMR1 and SAMP8 mice at 12 weeks of age. **B.** Escape latency of SAMR1 (N = 10) and SAMP8 mice (N = 10). Male mice were treated daily for 2 weeks with DHT (500 μg s.c) before trials. Swim speed during quadrant test on day 10. **C.** Total distance and the ratio of central/total distance were measured in open field tests. **D.** Number of amyloid ß plaques, pyramidal cells, and SA-ßgal-positive cells in CA1 and CA3 areas of hippocampus in SAMR1 and SAMP8. (*p<0.05, n.s: not significant).
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Next, we assessed the number of amyloid ß plaques, pyramidal cells, and SA-ßgal-positive cells in CA1 and CA3 areas of the hippocampus in these mice (Figure 1D). The number of plaques was increased in SAMP8 compared with SAMR1, but was unaltered by treatment with DHT. The number of SA-βgal-stained cells was significantly increased in SAMP8 compared with SAMR1, but treatment with DHT prevented this in SAMP8 despite no difference in pyramidal cell number (Figure 1D).

DHT treatment increased protein and mRNA expression of SIRT1 in SAMP8

Furthermore, to estimate the role of testosterone deficiency in SAMP8, we examined the effect of testosterone supplementation on cognitive function in much older SAMR1 and SAMP8. Similarly to young mice, we observed a marked decline in performance in SAMP8 compared with SAMR1 at 18 months of age. SAMP8 implanted with testosterone pellets showed significantly reduced escape latency time compared with placebo-treated SAMP8 (Figure 2A). Plasma testosterone level in SAMP8 at 18 months of age was lower than that in SAMR1, but implanted mice showed recovery to the level in young mice (Figure 2A). These results indicated that similar to DHT, testosterone also showed the improvement of cognitive function in SAMP8. Next, we examined the cause of low plasma testosterone in SAMP8. SAMP8 showed no testicular atrophy (Figure S1A), but more senescent phenotypes in Leydig cells, which produce testosterone in testes, than SAMR1 (Figure 2B). Moreover, we tried to allotransplant testes from SAMR1 to SAMP8 (Figure S1B). Although performance gradually responded to treatment up to 8 10 weeks, castrated SAMR1 showed a marked decline in performance whereas recipient SAMP8 showed cognitive improvement (Figure 2C).

As recently reported, overexpression or activation of SIRT1 inhibits cellular senescence and protects cellular function in various cell lines [13,16]. Therefore, we examined SIRT1 expression in the hippocampus of SAMP8 with or without DHT treatment, at 12 weeks of age. DHT treatment increased the protein and mRNA expression of SIRT1 in SAMP8 (Figure 2D). To investigate further the involvement of AR, we examined the expression of AR in SAMR1 and SAMP8 brains. The expression of AR was more abundant in the hippocampus than in other brain regions of SAMR1 and SAMP8 (Figure 2E).

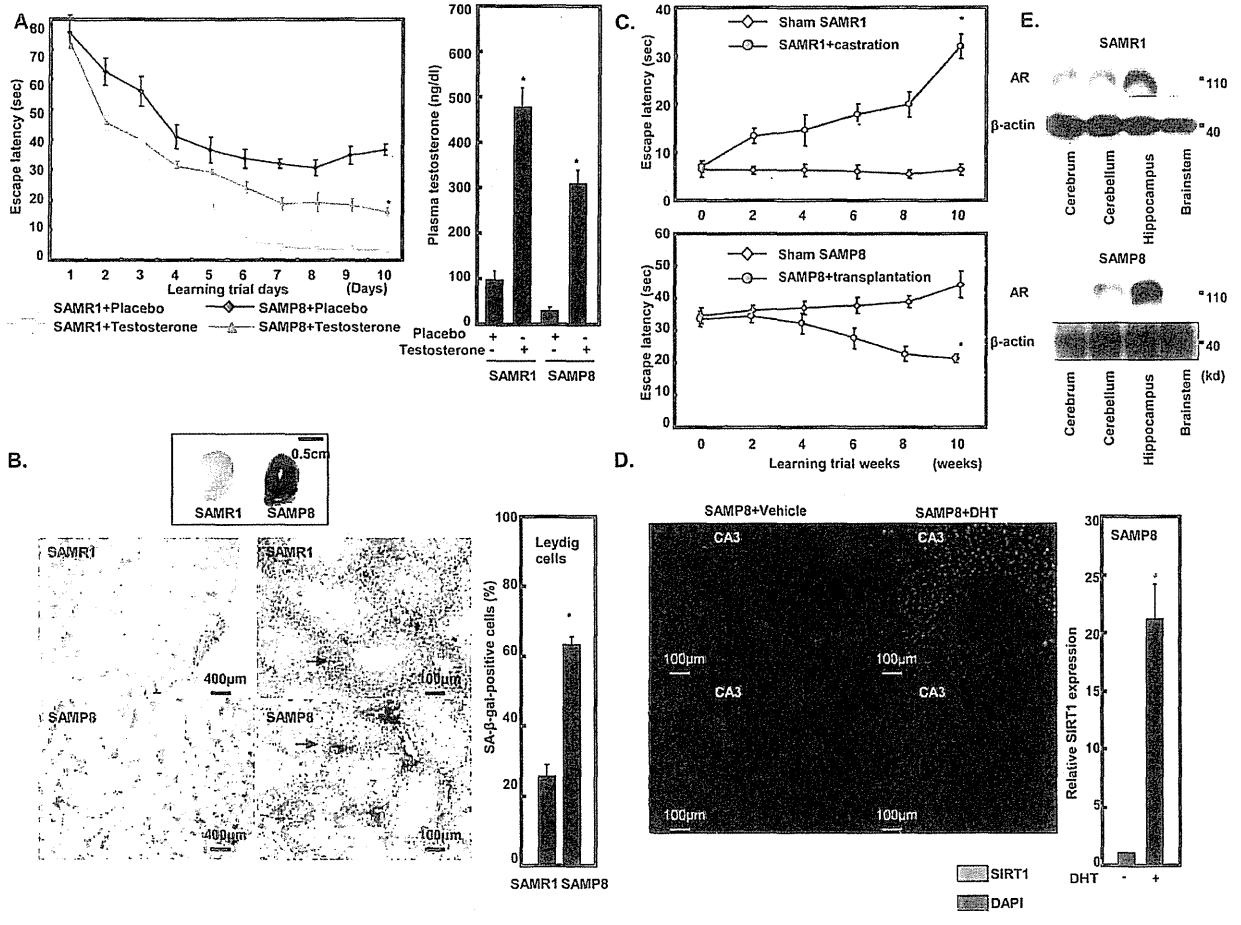

Figure 2. Supplementation of testosterone improves cognitive function in SAMP8 mice. A. Escape latency and plasma testosterone level of male SAMR1 (N = 10) and SAMP8 mice (N = 10) at 18 months of age. These mice were implanted subcutaneously with a placebo or a 21-day-release 2.5 mg testosterone pellet in the dorsal neck. **B.** Number of SA-βgal-stained Leydig cells in testes in SAMR1 and SAMP8. Arrows indicate Leydig cells. Representative SA-βgal-stained testes from SAMR1 and SAMP8. **C.** Escape latency of castrated SAMR1 (upper, N = 5) and recipient SAMP8 (lower, N = 5). Observation (0–10 weeks) was started from 3 weeks after operation. **D.** SIRT1 expression in hippocampus of SAMP8 with or without DHT treatment. Immunofluorescent staining for SIRT1 (green) and DAPI (blue). **E.** Expression of AR in SAMR1 and SAMP8 brains. (*p<0.05).
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Oxidative stress was increased in hippocampal cells of SAMP8

Oxidative stress may be closely related to senescence and age-related diseases. Also, an increase in oxidative stress has been suggested to be one of the earliest pathological changes in the brain in conditions with cognitive impairment such as AD [17]. Then, we examined 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. DHT treatment decreased carbonylated proteins in the SAMP8 hippocampus (Figure 3A). In parallel, the concentration of the neurotransmitter acetylcholine in hippocampal lysates was decreased in SAMP8 compared with that in SAMR1, and DHT treatment prevented this (Figure 3B).

Testosterone and DHT acts on vascular endothelial cells and stimulates the PI3K/Akt pathway, leading to eNOS activation through direct interaction of AR [18,19]. The eNOS/SIRT1 axis is recognized as one of the fundamental determinants of endothelial senescence, and SIRT1 acts as a driver of cellular stress resistance [20]. To examine the influence of DHT treatment on endothelial cells, we determined the degree of senescence and the expression of SIRT1 in endothelial cells around the CA3 area of the hippocampus. DHT-treated SAMP8 showed a reduction of SA-βgal-stained endothelial cells and increased SIRT1 expression compared to untreated SAMP8 (Figure 3C and D). To confirm that these cells were endothelial cells, not neuronal cells, cerebral microvessels were isolated from SAMR1 and SAMP8. In parallel with immunohistological staining, SAMP8 showed a reduction of SIRT1 expression compared to SAMR1, and DTH treatment increased SIRT1 expression compared to that in untreated SAMP8 (Figure 3E). These results suggest that vascular endothelial senescence in the hippocampus may be related to the memory deficit in SAMP8. Since testosterone and DHT activates eNOS, a NOS inhibitor, N^{G}-nitro-L-arginine methyl ester hydrochloride (L-NAME), and N^{5}-(1-Imino-3-butenyl)-L-ornithine (L-VNIO), a

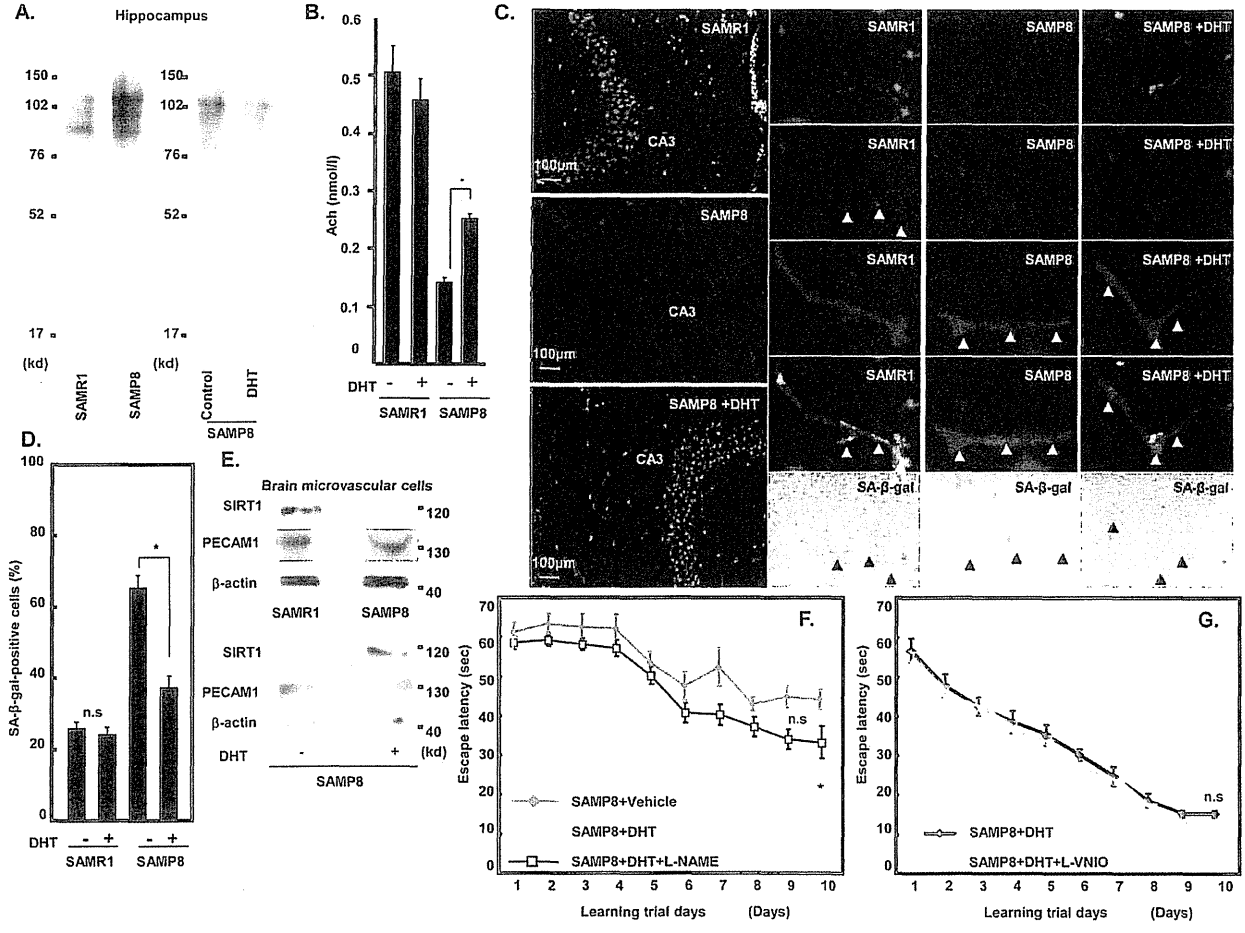

Figure 3. Senescent endothelial cells of hippocampus are decreased by treatment with DHT. A. Oxidative stress level was measured by detection of carbonyl groups introduced into proteins. **B.** Acetyl-choline concentration was measured by a colorimetric method. **C.** SA-βgal-stained endothelial cells and SIRT1 expression in CA3 area of hippocampus in SAMR1 and SAMP8 with or without DHT treatment. Immunofluorescent staining for SIRT1 (green), PECAM-1 (red), and DAPI (blue). **D.** Number of SA-βgal-stained endothelial cells in CA3 area of hippocampus in SAMR1 and SAMP8 with or without DHT treatment. **E.** Expression of SIRT1, PECAM-1, and β-actin was analyzed using cerebral micro vascular cells. **F.** Escape latency of SAMR1 (N = 10) and SAMP8 mice (N = 10). Male mice were treated daily for 2 weeks with DHT (500 μg s.c) and L-NAME (20 mg/kg gavage) before trials. **G.** Escape latency of SAMR1 (N = 5) and SAMP8 mice (N = 5). Male mice were treated daily for 2 weeks with DHT (500 μg s.c) and L-VNIO (5 mg/kg IP) before trials. (*$p<0.05$, n.s: not significant).
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selective neuronal NOS (nNOS) inhibitor, were applied to examine the involvement of NOS in this process. L-NAME abrogated the effects of DHT on cognitive function (Figure 3F). In contrast, L-VNIO did not change the effect of DHT (Figure 3G). These results suggest that eNOS/SIRT1 in endothelial cells may play an important role in the protective effect of testosterone against senescence of the hippocampus.

SIRT1 plays an important role in the protective effect of testosterone against endothelial senescence

Following the animal experiments, we examined whether testosterone inhibited endothelial senescence *in vitro* using cultured cells. We induced premature endothelial senescence by addition of H_2O_2 100 μmol/L for 1 hour. DHT or testosterone treatment inhibited SA-βgal activity and the morphological appearance of senescence (Figure 4A). We observed that oxidative stress decreased eNOS and SIRT1 and increased PAI-1 expression, and DHT or testosterone treatment prevented these changes and increased the phosphorylation of eNOS at Ser1177 (Figure 4B). Overexpression of SIRT1 significantly inhibited oxidative stress-induced senescence, and DHT accelerated the effect of SIRT1 through phosphorylation of eNOS at Ser1177 (Figure 4C). To determine the role of endogenous SIRT1, DHT-treated endothelial cells were transfected with SIRT1 siRNA or treated with sirtinol, a chemical inhibitor of SIRT1. SIRT1 siRNA or sirtinol abrogated the effect of DHT on SA-βgal activity (Figure 4D). We previously reported that testosterone activated eNOS [18], and eNOS activation promoted SIRT1 expression [21]. Accordingly, we examined the role of eNOS in the protective effect of testosterone. We observed that DHT or testosterone treatment increased NOS activity that was reduced by oxidative stress (Figure 4E). Treatment with eNOS siRNA or L-NAME decreased the inhibitory effect of DHT on a senescent phenotype in parallel with SIRT1 expression (Figure 4F and G). These results indicate that eNOS/SIRT1 play an important role in the protective effect of testosterone and DHT against a senescent phenotype.

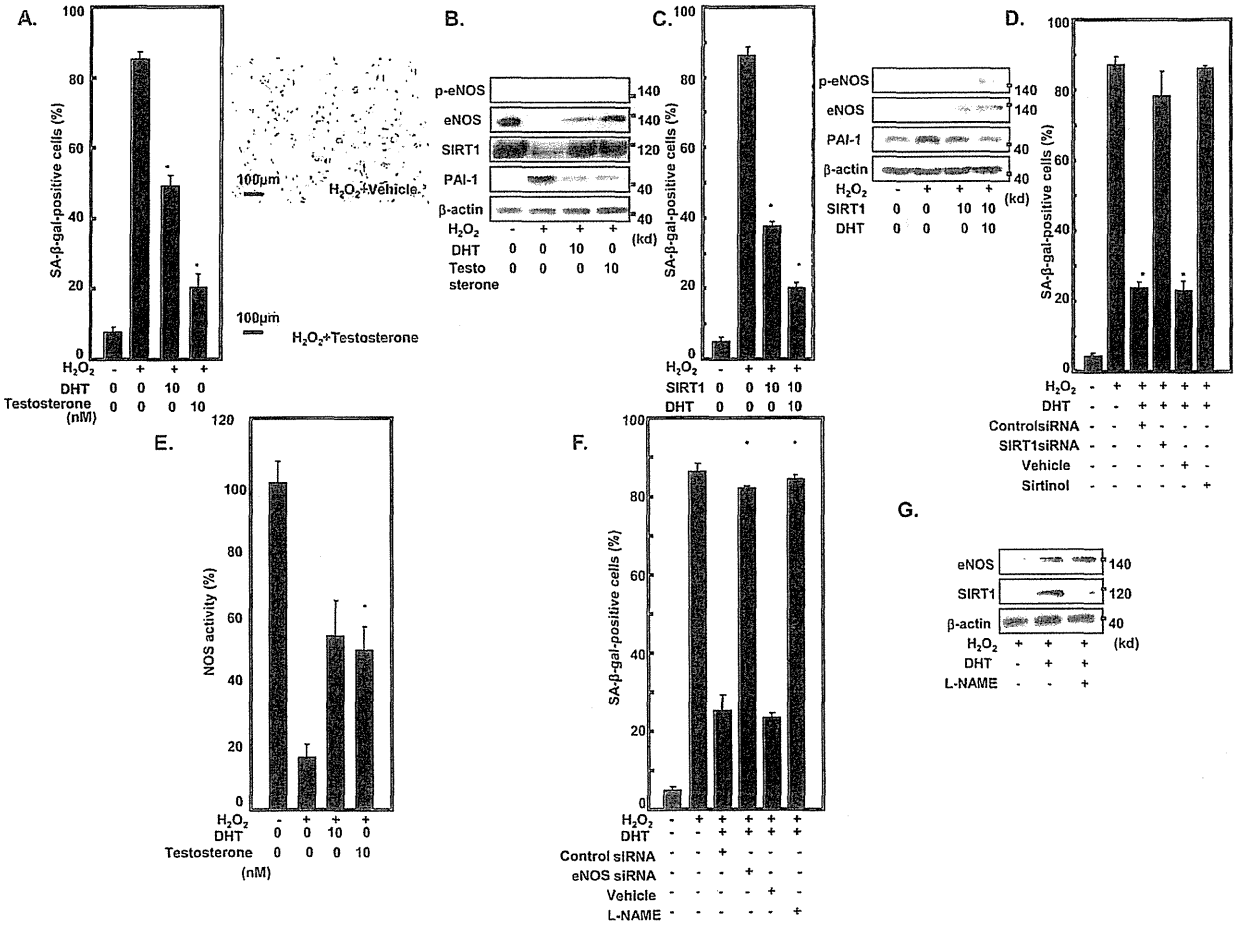

Figure 4. Testosterone inhibits oxidative stress-induced endothelial senescence through eNOS/SIRT1. A. Testosterone inhibited SA-βgal activity and senescent morphological appearance induced by hydrogen peroxide (100 μmol/L). **B.** Expression of eNOS, SIRT1, and PAI-1 in hydrogen peroxide (100 μmol/L)-treated HUVEC under treatment with DHT or testosterone. **C.** Overexpression of SIRT1 and DHT reduced SA-βgal activity. eNOS expression was increased by overexpression of SIRT1, and DHT increased phosphorylation of eNOS (Ser1177). **D.** SIRT1 inhibition by siRNA or sirtinol (100 μmol/L) abrogated the effect of testosterone on SA-βgal activity. **E.** Treatment with testosterone or DHT increased eNOS activity. **F.** eNOS inhibition by siRNA or L-NAME (10 mM) abrogated the effect of testosterone on SA-βgal activity. **G.** Treatment with L-NAME decreased SIRT1 expression in DHT-treated HUVEC. (*$p<0.05$, $N=3$).
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Senescent endothelial cells induced by oxidative stress promoted neuronal senescence

Finally, we hypothesized that endothelial senescence promotes senescence of adjacent neuronal cells. To test this hypothesis, we used a co-culture system of endothelial cells (HUVEC) with neuronal cells (mouse hippocampal neuronal cells; MHC) (Figure 5A). Both cells were co-cultured, but were separated by a microporous polycarbonate membrane, for 10 days after endothelial cells were treated with hydrogen peroxide, and the senescent phenotype of MHC was analyzed. We found that the number of SA-βgal-positive cells and the senescent appearance of MHC were increased, and the concentration of acetylcholine in cells was decreased by co-culture with senescent endothelial cells (Figure 5B). In parallel with this, MHC showed increased PAI-1 and p53, and decreased SIRT1 expression (Figure 5C). We also found that senescent endothelial cells showed increased expression of inflammatory cytokines such as IL-6, IL-8, MCP-1, and TNF-α (Figure 5D). Both MHC and HUVEC, or HUVEC alone were treated with testosterone at 3 days before HUVEC were treated with hydrogen peroxide, and both cells were co-cultured for 10 days, and the senescent phenotype of MHC was analyzed. We found that the number of SA-βgal-positive MHC was decreased by treatment of HUVEC with testosterone irrespective of the treatment of MHC with testosterone (Figure 5E). In addition, we found that a SIRT1 activator, resveratrol treatment rescued the senescent phenotype of MHC (Figure 5F). These results suggest that senescent endothelial cells exhibit a senescence-associated secretory phenotype [22], induce neuronal senescence, and testosterone rescues it through up-regulation of SIRT1 (Figure 5G).

Discussion

Testosterone level and cognitive function show a decline with age in men. A series of evidence suggests that this association is not just age related [23]. Results from cell culture and animal studies provide evidence that testosterone could have protective effects on brain function, especially in the hippocampus [24]. Here, we demonstrated that administration of testosterone restored cognitive function in male SAMP8 in association with improvement of the senescent phenotype in the hippocampus and cerebral vessels.

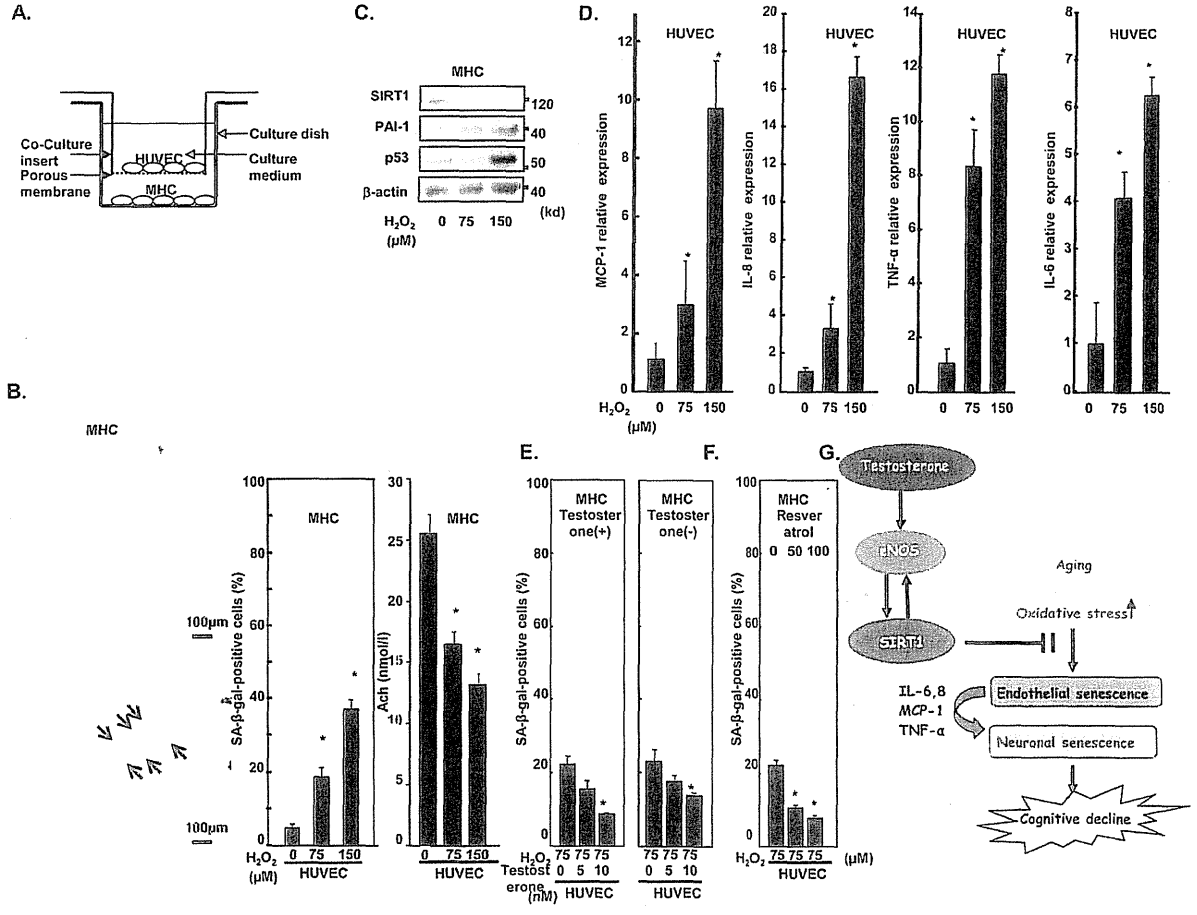

Figure 5. Oxidative stressed-induced endothelial cell senescence promotes adjacent neuronal cell senescence. A. Co-culture cell culture dish. **B.** Number of SA-βgal-stained MHC and senescent appearance of MHC were increased, and acetyl-choline concentration was decreased by co-culture with senescent endothelial cells. Senescent MHC are indicated by arrows. **C.** Expression of SIRT1, PAI-1, p53, and β-actin in MHC co-cultured with senescent endothelial cells. **D.** Expression of IL-6, IL-8, MCP-1, and TNF-α in endothelial cells were analyzed by RT-PCR. **E.** The number of SA-βgal-stained MHC was decreased by treatment with testosterone in both MHC and HUVEC (MHC, testosterone (+)), or HUVEC (MHC, testosterone (−)) alone. **F.** Resveratrol decreased the number of SA-βgal-stained MHC co-cultured with senescent endothelial cells. (*$p<0.05$, $N=3$). **G.** Hypothetical signal transduction pathways of testosterone in endothelial cells.
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We also showed that testosterone ameliorated endothelial senescence through eNOS/SIRT1-dependent mechanisms *in vitro*. The present study demonstrated that testosterone and SIRT1 interacts with each other and inhibited the senescence of hippocampal vascular and neuronal cells, suggesting that testosterone replacement therapy is a treatment option for cognitive decline with aging.

Testosterone may act in part through aromatase-dependent conversion to estradiol. To estimate a direct effect of androgens through AR, testosterone and DHT were used in this study. Both compounds showed significant protective effects on cognitive function.

In the present study, we used SAMP8 mice. SAMP is comprised of 14 strains derived from selective inbreeding of the AKR/J strain. SAMP8 exhibits age-related learning and memory deficits, as well as amyloid-like deposits in the brain [25]. Increased expression of hyperphosphorylated tau has also been detected in SAMP8 [26]. Given such features, SAMP8 has been proposed as a plausible age-associated AD animal model, and a suitable rodent model for studying the molecular mechanism underlying cognitive impairment [27]. A previous study has shown an age-related decrease in serum testosterone in SAMP8, and suggesting that impaired cognitive function in SAMP8 is due to reduced testosterone [28]. We observed that AR expression was abundant in the hippocampus of SAMR1 and SAMP8. Several studies have demonstrated that testosterone has a neuroprotective effect through AR in the hippocampus [29,30], and testosterone induced NO productions via AR-dependent activation of eNOS in endothelial cells [18,19].

Accumulating evidence suggests that NAD^+-dependent deacetylase SIRT1 play an essential role for cellular senescence and cognitive function. SIRT1 modulates endothelial cellular senescence [13], and overexpression of SIRT1 exhibits neuroprotective effects in hippocampus, and cognitive function of *Sirt1*-KO mice is markedly impaired [10,31,32].

The precise etiologic mechanism of the cognitive decline with aging is unclear, but it has been identified that cardiovascular risk factors are associated with a higher incidence of cognitive impairment [33]. In addition, age-associated vascular inflammation is an early manifestation of chronic stress responses, i.e. overloading of ROS on endothelial cells [34]. Indeed, SAMP8 showed enhancement of oxidative stress and a senescent phenotype in the hippocampus. Notably, senescent endothelial cells were increased in the hippocampus of SAMP8 accompanied by a reduction of SIRT1, and L-NAME abrogated the effect of DHT on cognitive function. Therefore, we hypothesized that testosterone influenced cerebral endothelial senescence via eNOS/SIRT1, and that pro-inflammatory cytokines, which were derived from senescent endothelial cells, promoted senescence in adjacent neuronal cells. Indeed, we observed that testosterone induced eNOS activity, and subsequently increased SIRT1 expression in endothelial cells. Inhibition of eNOS/SIRT1 abrogated the effect of testosterone on endothelial senescence. In a co-culture system, we found that senescent endothelial cells promoted senescence of adjacent neuronal cells, and treatment of endothelial cells with testosterone inhibited senescence of adjacent neuronal cells. It can reasonably be speculated, therefore, that SIRT1 may exert salutary actions against cognitive decline with aging by preventing a senescence-associated secretory phenotype of endothelial cells. Because L-NAME is a non-selective inhibitor of NOS, it is possible that the effect of L-NAME might be in part a result of inhibition of nNOS in concert with eNOS. However, a specific nNOS inhibitor, L-VNIO did not change the effect of DHT in SAMP8. In co-culture experiments, we found that treatment with resveratrol or testosterone did not change the expression or activation of nNOS in MHC (Figure S1C and D). Further studies are needed to address the differential role of eNOS and nNOS, and the exact role of SIRT1 *in vivo*.

In conclusion, supplementation of testosterone prevented cognitive impairment of SAMP8, in which testosterone secretion was decreased in association with the senescence of testis Leydig cells, through an eNOS/SIRT1-dependent mechanism. Unprecedented reversal of the senescent hippocampal changes and vascular protection may justify exploration of a neuronal rejuvenation strategy by utilizing testosterone for the prevention of cognitive decline with aging, particularly through up-regulation of eNOS/SIRT1.

Methods

Materials

Dihydrotestosterone (DHT), testosterone, and N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) were purchased from Sigma (St. Louis, MO). Hydrogen peroxide (H_2O_2) and resveratrol were purchased from Wako Pure Chemical Industries (Osaka, Japan). Testosterone and placebo pellets were purchased from Innovative Research of America (Sarasota, FL). N^5-(1-Imino-3-butenyl)-L-ornithine (L-VNIO) was purchased from Enzo Life Sciences (Plymouth Meeting, PA).

Cell culture

Human umbilical vein endothelial cells (HUVEC) were purchased from CAMBREX (Walkersville, MD). Population doubling levels (PDL) were calculated as described previously [35], and all experiments were performed at PDL of 10–11. In our preliminary experiments, HUVEC were cultured in EBM without phenol red (Clonetics, Walkersville,MD) with 10% dextran-charcoal-stripped serum to remove steroids from the culture medium. This condition, however, induced marked growth arrest and an increase in senescent cells. Consequently, we performed all experiments in EBM-2 (Clonetics) with 10% complete serum-supplemented medium.

Animal experiments

The animal experiments were approved by our institutional review board (animal experiments ethics board, Graduate School of Medicine and Faculty of medicine, The university of Tokyo (approval ID: M-P-09-056)). 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, a group of male SAMR1 (N = 10) and SAMP8 (N = 10) was first tested. Male mice of 12 weeks of age were treated daily for 2 weeks with DHT (500 μg in 0.05 ml/mouse) by subcutaneous injection (s.c.) in the neck before the water maze test. Male mice of 18 months of age underwent subcutaneously implantation of a placebo (N = 5) or a 21-day-release 2.5 mg testosterone (N = 5) pellet into the dorsal neck region. L-NAME was given by gavage once a day (20 mg/kg) [36]. L-VNIO was given by intraperitoneal injection (0.5 mg/kg) [37]. Small fragments of testis tissue fragments from SAMR1 were grafted under the back skin of castrated male SAMP8 as previously described [38]. Briefly, after removal of the capsule and obvious connective tissue, donor testes were cut into small fragments. Testis fragments were kept in Dulbecco's modified Eagle's medium

(Gibco Lab Inc., Grand Island, NY, USA) on ice until grafting. SAMR1 were anesthetized and castrated, and testicular tissue fragments were grafted under the back skin of SAMP8. Mice were anesthetized with enflurane, killed by cervical dislocation, and trunk blood collected within 1 min. The blood was centrifuged and plasma testosterone was measured by radioimmunoassay method. The brain was removed for histological examination, after systemic perfusion with phosphate-buffered saline (PBS). For immunohistochemical studies, mouse brains were processed and labeled with anti-amyloid-β antibody (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan) to visualize extracellular amyloid plaques, anti-NeuN antibody (Millipore, Billerica, MA) to assess pyramidal cell number, or DAPI (Dojindo Molecular Technologies, Inc., Tokyo, Japan) for nuclear staining. The primary antibody was purified rat anti-mouse CD31 (platelet endothelial cell adhesion molecule; PECAM-1) monoclonal antibody from Pharmingen (San Jose, CA, USA). Secondary antibodies (Alexa Fluor 488 donkey anti-rat IgG and Alexa Fluor 594 donkey anti-rat IgG) and antifade reagent were from Molecular Probes (Invitrogen). Fluorescent images were analyzed using a fluorescence microscope (BZ-9000, KEYENCE, Osaka, Japan).

Plasmids and siRNA transfection

Proliferating cells were washed three times with growth medium and exposed to the indicated concentrations of testosterone or DHT diluted in medium. pIRES-SIRT1 plasmid was provided by Dr. M. Takata [39], and Dr. R.A. Weinberg [40]. Each plasmid was overexpressed by transfection using Lipofectamine LTX and PLUS reagents (Invitrogen) for HUVEC according to the manufacturer's instructions. Proliferating cells were transfected with each siRNA using silMPORTER (Upstate Cell Signaling Solutions). siRNAs for SIRT1 (GAT GAA GTT GAC CTC CTC A [41] and TGA AGT GCC TCA GAT ATT A), and eNOS were purchased from Santa Cruz Biotechnology, Inc.

Immunoblotting and immunoprecipitation

Cells were lysed on ice for 1 hour in buffer (50 mmol/L Tris-HCl, pH 7.6, 150 mmol/L NaCl, 1% NP-40, 0.1% SDS, 1 mmol/L dithiothreitol, 1 mmol/L sodium vanadate, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 10 μg/mL leupeptin and 10 mmol/L sodium fluoride). Equal amounts of protein were separated by SDS/PAGE gel electrophoresis and transferred to nitrocellulose membranes. After blocking, the filters were incubated with the following antibodies; anti-SIRT1, anti-nNOS, anti-AR (Cell Signaling, Danvers, MA), anti-eNOS (BD Transduction Laboratories, San Jose, CA), anti-PAI-1 (Molecular Innovations, Southfield, MI), anti-PECAM-1 (Santa-Cruz Biotechnology, CA), and anti-ß-actin (Sigma). After washing and incubation with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG (Amersham, Piscataway, NJ) for 1 hour, antigen-antibody complexes were visualized by using an enhanced chemiluminescence system (Amersham).

Senescence-associated ß-galactosidase (SA-ßgal) staining

HUVEC were pretreated with diluted EGM-2 medium for 3 day. HUVEC were then washed three times with EGM-2 and treated for 1 hour with 100 μmol/l H_2O_2 diluted in EGM-2. After treatment, HUVEC were trypsinized, re-seeded at a density of 1×10^5 in 60-mm dishes, and cultured with EGM-2 containing DHT or testosterone for 10 days. The proportion of SA-ßgal-positive cells was determined as described by Dimri et al [42].

NOS activity assay

NOS activity was determined using an NOS assay kit (Calbiochem) according to the manufacturer's instructions.

Measurement of acetylcholine

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

Real-time quantitative reverse transcription PCR

Total RNA was isolated with ISOGEN (Nippon Gene Inc., Toyama, Japan). After treatment with Rnase-free Dnase for 30 min, total RNA (50 ng/μl) was reverse transcribed with random hexamers and oligo d(T) primers. The expression levels of SIRT1, IL-6, IL-8, MCP-1, and TNF-α relative to ß-actin were determined by means of staining with SYBR green dye and a LineGene fluorescent quantitative detection system (Bioflux Co., Tokyo, Japan). The following primers were used: SIRT1 F 5′-CCTGACTTCAGGTCAAGGGATGGTA-3′, R 5′-CTGATTAAAAATATCTCCTCGTACAG-3′; ß-actin F 5′-TGGGCATGGGTCAGAAGGAT-3′, R 5′-AAGCATTTGCGGTGGACCAT-3′; IL-6 F 5′-GGGAAGGTGAAGGTCGG-3′, R 5′-TGGACTCCACGACGTACTCAG-3′, IL-8 F 5′-CTGGCCGTGGCTCTCTTG-3′, R 5′-CCTTGGCAAAACTGCACCTTT-3′; TNF-α F 5′-GTAGCCCACGTCGTAGCAAAC-3′, R 5′-CTGGCACCACTAGTTGGTTGTC-3′; MCP-1 F 5′-CATTGTGGCCAAGGAGATCTG-3′, R 5′-CTTCGGAGTTTGGGTTTGCTT-3′.

Co-culture system

For these experiments, co-culture dishes were used as outlined in Figure 5A. They were obtained from BD Biosciences (Erembodegem, Belgium) with a 6-well format. HUVEC were treated with H_2O_2 (100 μM) for 1 h and cultured on the permeable microporous (0.4 μm) membrane in the insert, and mouse hippocampus neuronal cells on the base of the culture dish, kept physically separated but allowing the passage of micromolecules through the porous membrane for 10 days. Mouse hippocampus neuronal cells were purchased from DS Pharma Biomedical Inc. (Osaka, Japan).

Quantitative analysis of amyloid β

Measurement of amyloid β was performed using an amyloid β (1-40) (FL) assay kit (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan) according to the manufacturer's instructions.

Morris water maze test

The procedure of the Morris water maze test was described previously [43]. SAMR1 and SAMP8 mice 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 guided to the platform. On the test days, the platform was hidden 1 cm beneath the water. The escape latency was recorded by a video camera. The swim speed of each mouse was calculated by means of a video tracking system. Probe tests were performed on the 10^{th} day. During percent time quadrant test, the platform was removed from the pool. Mice were started in a position opposite the location of the platform position and allowed to swim for 60 seconds.

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 from that of Lukacs et al [44]. The open field test consisted of a wooden box (60×60×60 cm) and was indirectly illuminated by two fluorescent lights. A 10 cm area near the surrounding wall was delimitated and considered the periphery. The rest of the open field was considered the central area. The distance travelled, the ratio of the distance travelled in the central area/total distance travelled, and the time in the center of the open- field were analyzed as a measure 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.

Isolation of cerebral microvessels

Cerebral microvessels were isolated from the remaining brain tissue as previously described by Zhang et al [45] with minor modifications. Brain tissue, devoid of large vessels, was homogenized in ice cold PBS with Dounce homogenizer and centrifuged twice at 2000 g at 4°C. The supernatant, containing the parenchymal tissue, was discarded. The pellet was resuspended in PBS and centrifuged as described above. The resulting pellet was resuspended and layered over 15% Dextran (in PBS) (Sigma, St.Louis, MO) and centrifuged at 4500 g for 30 minutes at 4°C. The top layer was aspirated and discarded and the remaining pellet resuspended in 15% Dextran and centrifuged. The final pellet was resuspended in 1% bovine serum albumin (BSA), the suspension was then passed though a 40-µm nylon mesh (BD Falcon). Microvessels retained on the mesh were washed with BSA/PBS and collected by centrifugation at 900 g for 10 minutes at 4°C.

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 Bonferroni test. Probability values less than 0.05 were considered significant.

Supporting Information

Figure S1 Testes of SAMP8 and SAMR1 mice and role of nNOS in neuronal senescence. A. Testis weight of SAMR1 and SAMP8 with or without testosterone. **B.** Photographs of SAMR1 donor and SAMP8 recipient mice. White arrows indicate operation scar. **C.** Expression of nNOS in MHC treated with resveratrol or testosterone under the oxidative stress. **D.** Activity of nNOS in MHC treated with resveratrol or testosterone under the oxidative stress. ($^{*}p<0.05$, N = 3, n.s: not significant).
(TIF)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: HO MA YO. Performed the experiments: HO TA. Analyzed the data: HO SO KI ME MA. Contributed reagents/materials/analysis tools: TK MS. Wrote the paper: HO MA.

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Polypharmacy as a risk for fall occurrence in geriatric outpatients

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Objective: To investigate the predictors of falls, such as comorbidity and medication, in geriatric outpatients in a longitudinal observational study.

Methods: A total of 172 outpatients (45 men and 126 women, mean age 76.9 ± 7.0 years) were evaluated. Physical examination, clinical history and medication profile were obtained from each patient at baseline. These patients were followed for up to 2 years and falls were self-reported to their physicians. The factors associated with falls were analyzed statistically.

Results: A total of 32 patients experienced falls within 2 years. On univariate analysis, older age, osteoporosis, number of comorbid conditions and number of drugs were significantly associated with falls within 2 years. On multiple logistic regression analysis, the number of drugs was associated with falls, independent of age, sex, number of comorbid conditions and other factors that were significantly associated in univariate analysis. A receiver–operator curve evaluating the optimal cut-off value for the number of drugs showed that taking five or more drugs was a significant risk.

Conclusion: In geriatric outpatients, polypharmacy is associated with falls. Intervention studies are needed to clarify the causal relationship between polypharmacy, comorbidity and falls. **Geriatr Gerontol Int 2012; 12: 425–430.**

Keywords: bone/musculo-skeletal, elderly, falls, geriatric medicine, internal medicine, polypharmacy.

Introduction

Previous studies have assessed the risk factors for falls in community-dwelling elderly,[1–3] but not in geriatric outpatients, and history of falls, physical ability and living environment were found to be predictors of falls. Outpatients have different characteristics from community-dwelling elderly, and previous studies have not assessed whether medical comorbidity and therapeutic drugs might be risk factors for falls. Falls in patients on medication are complicated, because some drugs, such as aspirin, can cause serious bleeding when they have injurious falls, and others, such as antihypertensive[4] and hypoglycemic[5,6] agents, can cause falls.

Previously, we reported that polypharmacy was associated with the tendency for falls using four indices of fall tendency in a cross-sectional setting in geriatric outpatients,[7] though that study did not evaluate fall occurrences, and also not in a longitudinal manner. Therefore, we aimed at investigating whether polypharmacy was predictive of fall occurrences in a prospective fashion. For this purpose, we followed geriatric outpatients for up to 2 years, and assessed whether polypharmacy is a risk for fall occurrence, together with other risks.

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