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 siIMPORTER (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′-CTGATTAAAATATCTCCTCGTACAG-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 10th 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

We are grateful to Dr. L. Guarente (Paul F. Glenn Laboratory and Department of Biology, Massachusetts Institute of Technology, Boston, USA) for advice and discussion. We thank Dr. M. Takata (Department of Immunology and Molecular Genetics, Kawasaki Medical School, Okayama, Japan) and R.A. Weinberg (Professor of Biology, Whitehead Institute for Biomedical Research, MIT, Boston, USA) for providing the pIRES-Sirt1 plasmid.

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.

References

1. Tanzi RE, Bertram L (2005) Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. Cell 120: 545–55.
2. Evans DA, Funkenstein HH, Albert MS, Scherr PA, Cook NR, et al. (1989) Prevalence of Alzheimer's disease in a community population of older persons higher than previously reported. JAMA 262: 2551–6.
3. Kaufman JM, Vermeulen A (2005) The decline of androgen levels in elderly men and its clinical and therapeutic implications. Endocr Rev 26: 833–76.
4. Tan RS, Pu SJ (2003) A pilot study on the effects of testosterone in hypogonadal aging male patients with Alzheimer's disease. Aging Male 6: 13–7.
5. Moffat SD, Zonderman AB, Metter EJ, Blackman MR, Harman SM, et al. (2002) Longitudinal assessment of serum free testosterone concentration predicts memory performance and cognitive status in elderly men. J Clin Endocrinol Metab 87: 5001–7.
6. Small SA, Duff K (2008) Linking Abeta and tau in late-onset Alzheimer's disease: a dual pathway hypothesis. Neuron 60: 534–42.
7. Pimplikar SW (2009) Reassessing the amyloid cascade hypothesis of Alzheimer's disease. Int J Biochem Cell Biol 41: 1261–8.
8. Guarente L (2000) Sir2 links chromatin silencing, metabolism, and aging. Genes Dev 14: 1021–6.
9. Sinclair D, Mills K, Guarente L (1998) Aging in Saccharomyces cerevisiae. Annu Rev Microbiol 52: 533–60.
10. Donmez G, Wang D, Cohen DE, Guarente L (2010) SIRT1 suppresses beta-amyloid production by activating the alpha-secretase gene ADAM10. Cell 142: 320–32.
11. Hofman A, Ott A, Breteler MM, Bots ML, Slooter AJ, et al. (1997) Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam Study. Lancet 349: 151–4.
12. Potente M, Ghaeni L, Baldessari D, Mostoslavsky R, Rossig L, et al. (2007) SIRT1 controls endothelial angiogenic functions during vascular growth. Genes Dev 21: 2644–58.
13. Ota H, Akishita M, Eto M, Iijima K, Kaneki M, et al. (2007) Sirt1 modulates premature senescence-like phenotype in human endothelial cells. J Mol Cell Cardiol 43: 571–9.
14. Menghini R, Casagrande V, Cardellini M, Martelli E, Terrinoni A, et al. (2009) MicroRNA 217 modulates endothelial cell senescence via silent information regulator 1. Circulation 120: 1524–32.
15. DeFries JC, Wilson JR, McClearn GE (1970) Open-field behavior in mice: selection response and situational generality. Behav Genet 1: 195–211.
16. Tang BL (2011) Sirt1's systemic protective roles and its promise as a target in antiagingmedicine. Transl Res 157: 276–84.
17. Mattson MP (2004) Pathways towards and away from Alzheimer's disease. Nature 430: 631–9.
18. Yu J, Akishita M, Eto M, Ogawa S, Son BK, et al. (2010) Androgen receptor-dependent activation of endothelial nitric oxide synthase in vascular endothelial cells: role of phosphatidylinositol 3-kinase/akt pathway. Endocrinology 151: 1822–8.
19. Goglia L, Tosi V, Sanchez AM, Flamini MI, Fu XD, et al. (2010) Endothelial regulation of eNOS, PAI-1 and t-PA by testosterone and dihydrotestosterone in vitro and in vivo. Mol Hum Reprod 16: 761–9.
20. Ota H, Eto M, Ogawa S, Iijima K, Akishita M, et al. (2010) SIRT1/eNOS axis as a potential target against vascular senescence, dysfunction and atherosclerosis. J Atheroscler Thromb 17: 431–5.
21. Ota H, Eto M, Kano MR, Ogawa S, Iijima K, et al. (2008) Cilostazol inhibits oxidative stress-induced premature senescence via upregulation of Sirt1 in human endothelial cells. Arterioscler Thromb Vasc Biol 28: 1634–9.
22. Coppé JP, Desprez PY, Krtolica A, Campisi J (2010) The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Pathol 5: 99–118.
23. Holland J, Bandelow S, Hogervorst E (2011) Testosterone levels and cognition in elderly men: A review. Maturitas 69: 322–37.
24. Simerly RB, Chang C, Muramatsu M, Swanson LW (1990) Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. J Comp Neurol 294: 76–95.
25. Del Valle J, Duran-Vilaregut J, Manich G, Casadesús G, Smith MA, et al. (2010) Early amyloid accumulation in the hippocampus of SAMP8 mice. J Alzheimers Dis 19: 1303–15.
26. Canudas AM, Gutierrez-Cuesta J, Rodríguez MI, Acuña-Castroviejo D, Sureda FX, et al. (2005) Hyperphosphorylation of microtubule-associated protein tau in senescence-accelerated mouse (SAM). Mech Ageing Dev 126: 1300–4.
27. Tomobe K, Nomura Y (2009) Neurochemistry, neuropathology, and heredity in SAMP8: a mouse model of senescence. Neurochem Res 34: 660–9.
28. Flood JF, Farr SA, Kaiser FE, La Regina M, Morley JE (1995) Age-related decrease of plasma testosterone in SAMP8 mice: replacement improves age-related impairment of learning and memory. Physiol Behav 57: 669–73.
29. Białek M, Zaremba P, Borowicz KK, Czuczwar SJ (2004) Neuroprotective role of testosterone in the nervous system. Pol J Pharmacol 56: 509–18.

30. Ramsden M, Shin TM, Pike CJ (2003) Androgens modulate neuronal vulnerability to kainate lesion. Neuroscience 122: 573–8.
31. Gao J, Wang WY, Mao YW, Gräff J, Guan JS, et al. (2010) A novel pathway regulates memory and plasticity via SIRT1 and miR-134. Nature 466: 1105–9.
32. Michán S, Li Y, Chou MM, Parrella E, Ge H, et al. (2010) SIRT1 is essential for normal cognitive function and synaptic plasticity. J Neurosci 30: 9695–707.
33. Casserly I, Topol E (2004) Convergence of atherosclerosis and Alzheimer's disease: inflammation, cholesterol, and misfolded proteins. Lancet 363: 1139–46.
34. Mahley RW, Weisgraber KH, Huang Y (2006) Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. Proc Natl Acad Sci U S A 103: 5644–51.
35. Maciag T, Hoover GA, Stemerman MB, Weinstein R (1981) Serial propagation of human endothelial cells in vitro. J Cell Biol 91: 420–6.
36. Akishita M, Shirakami G, Iwai M, Wu L, Aoki M, et al. (2001) Angiotensin converting enzyme inhibitor restrains inflammation-induced vascular injury in mice. J Hypertens 19: 1083–8.
37. Jessup JA, Zhang L, Chen AF, Presley TD, Kim-Shapiro DB, et al. (2011) Neuronal nitric oxide synthase inhibition improves diastolic function and reduces oxidative stress in ovariectomized mRen2.Lewis rats. Menopause 18: 698–708.
38. Honaramooz A, Snedaker A, Boiani M, Schöler H, Dobrinski I, et al. (2002) Sperm from neonatal mammalian testes grafted in mice. Nature 418: 778–81.
39. Matsushita N, Takami Y, Kimura M, Tachiiri S, Ishiai M, et al. (2005) Role of NAD-dependent deacetylases SIRT1 and SIRT2 in radiation and cisplatin-induced cell death in vertebrate cells. Genes Cells 10: 321–32.
40. Vaziri H, Dessain SK, Ng Eaton E, Imai SI, Frye RA, et al. (2001) hSIR2 (SIRT1) functions as an NAD-dependent p53 deacetylase. Cell 107: 149–159.
41. Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, et al. (2004) Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. Nature 429: 771–6.
42. Dimri GP, Lee X, Basile G, Acosta M, Scott G, et al. (1995) A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci U S A 92: 9363–7.
43. Cao D, Lu H, Lewis TL, Li L (2007) Intake of sucrose-sweetened water induces insulin resistance and exacerbates memory deficits and amyloidosis in a transgenic mouse model of Alzheimer disease. J Biol Chem 282: 36275–82.
44. Lukacs H, Hiatt ES, Lei ZM, Rao CV (1995) Peripheral and intracerebroventricular administration of human chorionic gonadotropin alters several hippocampus-associated behaviors in cycling female rats. Horm Behav 29: 42–58.
45. Zhang W, Koerner IP, Noppens R, Grafe M, Tsai HJ, et al. (2007) Soluble epoxide hydrolase: a novel therapeutic target in stroke. J Cereb Blood Flow Metab 27: 1931–1940.

Geriatrics Gerontology 1st Impact Factor in June 2010 Medline Indexed

Geriatr Gerontol Int 2011; **11:** *196–203*

ORIGINAL ARTICLE: EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

Plasma sex hormone levels and mortality in disabled older men and women

Shiho Fukai,[1] Masahiro Akishita,[1] Shizuru Yamada,[2] Sumito Ogawa,[1]
Kiyoshi Yamaguchi,[1] Koichi Kozaki,[2] Kenji Toba[2] and Yasuyoshi Ouchi[1]

[1]*Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, and*
[2]*Department of Geriatric Medicine, Kyorin University School of Medicine, Tokyo, Japan*

Aim: To investigate the relationship between circulating sex hormone levels and subsequent mortality in disabled elderly.

Methods: This prospective observational study was comprised of 214 elderly subjects aged 70–96 years (117 men and 97 women; mean ± standard deviation age, 83 ± 7 years), receiving services at long-term care facilities in Nagano, Japan. All-cause mortality by baseline plasma sex hormone levels was measured.

Results: After excluding deaths during the first 6 months, 27 deaths in men and 28 deaths in women occurred during a mean follow up of 32 months and 45 months (up to 52 months), respectively. Mortality rates differed significantly between high and low testosterone tertiles in men, but did not differ significantly between middle and low tertiles. Compared with subjects in the middle and high tertiles, men with testosterone levels in the low tertile (<300 ng/dL) were more likely to die, independent of age, nutritional status, functional status and chronic disease (hazard ratio [HR] = 3.27, 95% confidence interval [CI] = 1.24–12.91). In contrast, the low dehydroepiandrosterone sulfate (DHEA-S) tertile was associated with higher mortality risk in women (multivariate adjusted HR = 4.42, 95% CI = 1.51–12.90). Exclusion of deaths during the first year and cancer deaths had minimal effects on these results. DHEA-S level in men and testosterone and estradiol levels in women were not related to mortality.

Conclusion: Low testosterone in men and low DHEA-S in women receiving care at facilities are associated with increased mortality risk, independent of other risk factors and pre-existing health conditions. **Geriatr Gerontol Int 2011; 11: 196–203.**

Keywords: dehydroepiandrosterone, disabled elderly, mortality risk, testosterone.

Introduction

Japan has the longest life expectancy at birth in the world for both men and women, although women live 8 years longer than men on average.[1,2] One explanation for this phenomenon is that estradiol production during the premenopausal years partially protects women from cardiovascular disease (CVD). In contrast, there has been a suspicion that testosterone itself is harmful; however, recent studies support the hypothesis that testosterone may be beneficial to survival in aging men.[3–8]

It is well established that endogenous androgens decline with advancing age in men.[9] Because testosterone has important physiological effects on muscle, bone, brain, erythropoietin and the vascular system, decreased testosterone levels could contribute to age-associated symptoms and diseases in older men, such as decreased muscle mass and strength,[10] impaired physical performance,[11,12] osteoporosis[13] and fractures,[12,14]

Accepted for publication 21 September 2010.

Correspondence: Dr Masahiro Akishita MD PhD, Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Email: akishita-tky@umin.ac.jp

doi: 10.1111/j.1447-0594.2010.00670.x

depressed mood,[15] cognitive impairment,[16,17] anemia[18,19]and frailty.[20] In our previous study in which older persons receiving day-care services or admitted to a facility were investigated, higher plasma testosterone levels were associated with better activities of daily living (ADL), cognitive function and vitality in men.[21] On the other hand, several epidemiological studies have demonstrated that a decline in testosterone level was associated with mortality risk in community-dwelling middle-aged or older men.[3–8] In cause-specific analyses, some studies have shown that a low testosterone level was associated with an increased risk of death due to CVD.[4,5] However, the above-mentioned studies were performed in community samples of Caucasian men, and this issue remains to be clarified in frail or disabled older men.

The majority of dehydroepiandrosterone (DHEA), an endogenous steroid precursor to testosterone and estrogen, exists as the sulfated form (DHEA-S) in the circulation, and DHEA and DHEA-S are the most abundant adrenal sex steroid hormones, with concentrations reported to be more than 100-fold higher than those of testosterone and estradiol,[22] suggesting an important physiological role of DHEA(-S). Their circulating levels also peak in young adults and decline with age in both men and women. Although the role of androgens in older women's health is not fully understood, postmenopausal women with intact ovaries continue to produce androgens, DHEA and testosterone, while their production of estradiol is minimal.[23] In our previous study,[21] in older women, higher DHEA and DHEA-S levels were related to better ADL, while estradiol and testosterone levels showed no relations. Other reports have shown a correlation between DHEA level and cognitive function,[24] depression,[25] osteoporosis[26] and frailty in older women.[27] Several studies that examined the association between DHEA-S and mortality in women have shown mixed results,[28–32] and mostly found no relation; however, both low and high levels of DHEA-S at baseline[28] and some trajectory patterns such as a steep decline or extreme variability[32] have been reported to correlate with increased mortality.

These lines of evidence suggest that endogenous androgens, including testosterone and DHEA(-S), may play a role in physical and mental function as well as longevity in older individuals. We hypothesized that low plasma androgen levels could be a mortality risk factor even in elderly with disability who are receiving facility services.

Methods

Study population

In this longitudinal observational study, 218 consecutive persons aged 70 years or older (121 men aged 70–96 years and 97 women aged 70–95 years; mean ± standard deviation [SD] age, 83 ± 6 and 83 ± 5 years, respectively) who attended health service facilities for the elderly (facilities that provide nursing care and rehabilitation services to elderly people with disability, *Mahoroba-no-Sato*) located in Nagano Prefecture, Japan were enrolled. The participants were in a chronic stable condition and receiving services under Long-term Care Insurance, which is provided by the Japanese Government, either under admission or as day care. The principal exclusion criteria were malnutrition (serum albumin <3.5 mg/dL or body mass index [BMI] <16 kg/m^2), extremely low ADL status (Barthel Index[33] <50), malignancy, acute inflammation (fever, white blood cell count >10 000/μL, or other signs of infection within 4 weeks before enrollment), severe anemia (blood hemoglobin <10.0 g/dL) and overt endocrine disease because these conditions may affect both plasma sex hormone levels and mortality. Deaths that occurred during the first 6 months of follow up (four men and no women) were also excluded to minimize the influence of comorbidity on both sex hormone levels and mortality; therefore, the remaining 214 persons were analyzed in this study. The institutional review board of *Mahoroba-no-Sato* approved the study protocol, and all participants and/or their family members gave written informed consent.

Hormone measurements

Blood samples were obtained from the participants in the morning after an overnight fast, and plasma hormone levels in addition to blood cell counts and blood chemical parameters were determined by a commercial laboratory (Health Sciences Research Institute, Yokohama, Japan). Testosterone and estradiol were assayed using chemiluminescence immunoassays with minimum detection limits of 7 ng/dL (0.2 nmol/L) and 4 pg/mL (14.7 pmol/L), respectively. DHEA-S was assayed using a sensitive radioimmunoassay with a minimum detection limit of 2.0 μg/dL (0.05 μmol/L). The intra-assay coefficients of variation for these measurements were less than 5%.

Functional and anthropometric measurements

Trained nurses and physical therapists visited the participants at the health facilities and performed comprehensive geriatric assessments. Basic ADL was assessed by Barthel Index,[33] cognitive function by Hasegawa Dementia Scale – Revised (HDS-R, 30-point scale),[34] mood by the Geriatric Depression Scale (GDS, 15 items),[35] and ADL-related vitality by Vitality Index (10-point scale).[36] BMI was calculated as weight in kilograms divided by the square of height in meters.

Comorbidity

Diseases were ascertained by experienced physicians according to pre-established criteria that combine information from self-reported physician diagnoses, medical records, current medication, clinical examinations and blood tests. Diseases included in the current analysis were hypertension, heart disease (including any of angina pectoris, myocardial infarction, congestive heart failure and arrhythmia), stroke, diabetes mellitus, osteoarthropathy (arthritis, rheumatism, osteoporosis and history of fractures), lung disease (including bronchial asthma and chronic obstructive pulmonary disease) and other chronic diseases (chronic kidney disease, gastrointestinal disease, Parkinson's disease and psychological disorders). We also obtained data on anti-androgenic treatment and intake of glucocorticoids, opiates and hormone supplements that could affect plasma hormone levels, but no subject was taking any of these.

Follow up

The subjects were followed up in 2002–2009, for a period of up to 52 months (mean ± SD, 32 ± 13 [34] months in men and 45 ± 11 [49] months in women). Time and causes of death of deceased persons were ascertained using medical records and death certificates. All deaths were registered with International Classification of Diseases, 10th version (ICD-10) codes,[37] based on the information from death certificates. We categorized deaths into the following four specific causes: (i) diseases of the circulatory system (I00–I99) including heart disease and cerebrovascular disease; (ii) diseases of the respiratory system (J00-J99); (iii) neoplasms (C00-D48); and (iv) other causes. Subjects who were alive were confirmed by checking appointment records of the facilities. Survival of 16 subjects whose records were not available was ascertained by the phone interview of each subject. Causes of death were determined for all the subjects without any missing cases.

Statistical analysis

Differences between testosterone tertiles in men and between DHEA-S tertiles in women were analyzed using ANOVA for continuous variables and χ^2-test for categorical variables. Survival was analyzed using Kaplan–Meier plots and log-rank tests. Hazard ratios (HR) for mortality were analyzed using Cox proportional hazards regression. Significance tests were two-sided, with an α-level of 0.05. Data were analyzed using SPSS statistical software.

Results

Characteristics of study subjects

Over the follow-up period, 27 men and 28 women died, yielding a mortality rate of 86.5/1000 person-years at risk in men; and 69.9/1000 person-years at risk in women. Of those, 13 deaths were due to diseases of the circulatory system (eight to ischemic and other heart disease and five to cerebrovascular disease), 10 to diseases of the respiratory system and four to cancer in men; while 14 deaths were due to diseases of the circulatory system (nine to ischemic and other forms of heart disease and four to cerebrovascular disease), eight to diseases of the respiratory system, five to cancer and two to other causes in women. Men who died were significantly older, had lower serum albumin and cholesterol, lower ADL and cognitive status, higher prevalence of heart disease, and lower testosterone level than survivors; whereas in women, subjects who died were older, had lower hemoglobin, higher prevalence of heart disease and lower plasma DHEA-S level than survivors (data not shown).

Table 1 shows the baseline characteristics of the male subjects by tertile of plasma testosterone. A significant difference was observed in serum albumin and hemoglobin levels, ADL and cognitive status among tertiles of testosterone in men. Table 2 shows the baseline characteristics of the female subjects by tertile of plasma DHEA-S. A significant difference was found in age and ADL status among DHEA-S tertiles in women, while other variables did not differ between the tertile groups.

Mortality and plasma sex hormone levels in men

As shown in Figure 1(a), Kaplan–Meier survival analysis by tertile of plasma testosterone level revealed that testosterone level was associated with mortality in men. After adjusting for age, Cox proportional hazards models showed that there was an inverse relation between testosterone level and mortality. Mortality rate differed significantly between the high and low testosterone tertiles, but not significantly between the middle and low tertiles: tertile 3 (high), reference; tertile 2 (middle), HR = 2.51 (95% confidence interval [CI] = 0.66–9.50); and tertile 1 (low), HR = 6.63 (95% CI = 1.92–23.21). Accordingly, we investigated the increased mortality in tertile 1 versus tertiles 2–3 (Table 3). Compared with subjects within tertiles 2–3,

Table 1 Association between potential confounding variables and testosterone tertiles in men

Characteristic	Testosterone tertiles			*P*-value
	T1 <10.4 nmol/L (<300 ng/dL), *n* = 39	T2 10.4–16.3 nmol/L (300–470 ng/dL), *n* = 40	T3 >16.3 nmol/L (>470 ng/dL), *n* = 38	
Age, years	83 ± 7	83 ± 6	81 ± 6	0.11
Nutritional parameters				
Body mass index, kg/m^2	21.3 ± 3.4	22.8 ± 3.8	21.7 ± 3.0	0.21
Hemoglobin, g/dL	12.7 ± 1.9	13.8 ± 1.3	14.0 ± 1.7	<0.01
Albumin, g/dL	4.0 ± 0.3	4.1 ± 0.2	4.2 ± 0.3	<0.01
Total cholesterol, mg/dL	173 ± 38	195 ± 36	176 ± 28	0.05
Prevalent diseases, *n* (%)				
Hypertension	17 (44)	16 (40)	12 (32)	0.53
Heart disease	10 (26)	5 (13)	7 (18)	0.32
Stroke	12 (31)	15 (38)	8 (21)	0.34
Diabetes mellitus	8 (21)	5 (13)	8 (21)	0.31
Osteoarthropathy	8 (21)	9 (23)	7 (18)	0.94
Lung disease	2 (5)	3 (8)	3 (8)	0.52
Other chronic diseases	17 (44)	19 (48)	18 (47)	0.95
Functional parameters				
Barthel Index	79 ± 12	82 ± 11	87 ± 13	0.04
HDS-R	18 ± 7	19 ± 6	22 ± 5	0.02
Vitality Index	9.2 ± 1.1	9.3 ± 0.9	9.5 ± 0.9	0.46
GDS	5.0 ± 3.1	5.6 ± 3.7	5.6 ± 2.9	0.66
Sex hormone levels				
Testosterone, nmol/L	7.6 ± 2.5	13.3 ± 1.6	20.9 ± 3.9	<0.01
(ng/dL)	(219 ± 73)	(382 ± 43)	(602 ± 112)	
DHEA-S, μmol/L	1.7 ± 1.1	1.8 ± 1.6	1.7 ± 1.2	0.94
(μg/dL)	(64 ± 42)	(69 ± 57)	(63 ± 45)	

Values are shown as mean (standard deviation). Differences between the groups were analyzed using ANOVA for continuous variables and χ^2-test for categorical variables. DHEA-S, dehydroepiandrosterone sulfate; GDS, Geriatric Depression Scale; HDS-R, Hasegawa Dementia Scale – Revised.

a testosterone level within tertile 1 was associated with approximately fourfold higher mortality risk. Adjustment for age, nutritional parameters (BMI, albumin, hemoglobin, total cholesterol) and functional parameters (Barthel Index, HDS-R, Vitality Index, GDS), and prevalent diseases showed no major influence on the result. In order to examine how follow-up time and cancer impacted on the results, assuming that the subjects may have had subclinical cancer or a fatal illness at baseline, we performed further analyses excluding deaths that occurred in the first 12 months ($n = 9$) and deaths from cancer ($n = 4$). However, the significant associations remained after these exclusions (Table 3). On the other hand, DHEA-S level was not associated with mortality when DHEA-S was entered as tertiles (data not shown).

Although the statistical power was not strong enough, we studied the risk for cause-specific mortality by tertiles of testosterone level in men. Neither deaths from diseases of the circulatory system nor those from non-circulatory causes showed a significant association with testosterone tertiles (tertile 1 vs tertile 2–3, HR = 3.18, 95% CI = 1.87–11.6, $P = 0.17$; HR = 3.46, 95% CI = 0.29–7.29, $P = 0.64$, respectively).

Mortality and plasma sex hormone levels in women

As shown in Figure 1(b), a low DHEA-S level was associated with higher mortality by Kaplan–Meier survival analysis. Age-adjusted Cox proportional hazards models revealed that the association was not significant when each tertile of DHEA-S was entered as a continuous variable; however, a significant association was observed when tertile 1 was compared with tertiles 2–3 (Table 3). The association remained significant after excluding deaths that occurred in the first 12 months ($n = 2$) and deaths from cancer ($n = 5$). Moreover, further adjustment had no major influence on the result. In women, testosterone and estradiol levels were not associated with mortality when they were entered as tertiles (data not shown).

In cause-specific mortality analysis, compared with tertiles 2–3, the low tertile of DHEA-S level was associated with higher risk of death from diseases of the

Table 2 Association between potential confounding variables and DHEA-S tertiles in women

Characteristic	DHEA-S tertiles			*P*-value
	T1 <1.17 µmol/L (<43 µg/dL), *n* = 33	T2 1.17–1.49 µmol/L (43–55 µg/dL), *n* = 32	T3 >1.49 µnmol/L (>55 µg/dL), *n* = 32	
Age, years	83 ± 6	82 ± 6	80 ± 6	0.08
Nutritional parameters				
Body mass index, kg/m^2	22.3 ± 2.7	22.5 ± 3.2	23.7 ± 2.7	0.31
Hemoglobin, g/dL	12.6 ± 1.4	12.6 ± 1.2	13.1 ± 1.1	0.16
Albumin, g/dL	4.1 ± 0.3	4.2 ± 0.3	4.3 ± 0.2	0.18
Total cholesterol, mg/dL	205 ± 30	204 ± 35	205 ± 35	0.99
Prevalent diseases, *n* (%)				
Hypertension	10 (30)	14 (44)	15 (47)	0.47
Heart disease	4 (12)	7 (22)	8 (25)	0.46
Stroke	5 (15)	4 (13)	6 (19)	0.79
Diabetes mellitus	5 (15)	4 (13)	5 (16)	0.90
Osteoarthropathy	8 (24)	11 (34)	13 (40)	0.47
Lung disease	3 (9)	2 (6)	2 (6)	0.56
Other chronic diseases	17 (52)	19 (59)	18 (56)	0.90
Functional parameters				
Barthel Index	90 ± 7	93 ± 8	95 ± 8	0.04
HDS-R	23 ± 6	22 ± 7	25 ± 5	0.39
Vitality Index	9.2 ± 1.4	9.1 ± 2.2	8.8 ± 2.9	0.35
GDS	6.8 ± 2.6	5.9 ± 3.4	6.9 ± 3.3	0.16
Sex hormone levels				
DHEA-S, µmol/L	0.8 ± 0.2	1.3 ± 0.1	2.0 ± 0.3	<0.01
(µg/dL)	30 ± 7	49 ± 4	73 ± 12	
Testosterone, nmol/L	1.2 ± 0.6	1.2 ± 0.6	1.3 ± 0.5	0.81
(ng/dL)	35 ± 17	36 ± 17	37 ± 13	
Estradiol, pmol/L	56 ± 32	57 ± 37	67 ± 46	0.41
(pg/mL)	15.3 ± 8.6	15.5 ± 10.2	18.3 ± 12.5	

Values are shown as mean (standard deviation). Differences between the groups were analyzed using ANOVA for continuous variables and χ^2-test for categorical variables. DHEA-S, dehydroepiandrosterone sulfate; GDS, Geriatric Depression Scale; HDS-R, Hasegawa Dementia Scale – Revised.

circulatory system (HR = 13.1, 95% CI = 2.39–72.3, $P < 0.01$), while there was no association with deaths from non-circulatory causes (HR = 0.93, 95% CI = 0.86–1.02, $P = 0.14$).

Discussion

In this small prospective study of Japanese elderly who were receiving care in facilities, a low testosterone level was associated with mortality in men independent of multiple risk factors and pre-existing health conditions. In addition, a low DHEA-S level in older women was related to increased mortality. In contrast, DHEA-S level in men and testosterone and estradiol levels in women were not related to mortality.

Recent prospective cohort studies in Western countries have yielded inconsistent findings about the use of a low total testosterone level as a predictor of all-cause and cardiovascular mortality in middle-aged to older men.[4,5,38,39] In the two studies that found no significant prediction of mortality,[38,39] the populations were younger (mean or median ages were in the early 50s), testosterone levels were higher and mortality rates were lower (11.6 and 15.4/1000 person-years, respectively) compared to those in studies that found positive results. In the present study, although the sample size was small, the subjects were frail and older than those in any previously reported studies, with a relatively small age range and higher mortality rate. Therefore, the relation between testosterone level and mortality might have been easier to detect in our study than in other studies with healthy middle-aged and older men.

There could be several mechanisms by which endogenous testosterone affects mortality in men. Although the number of subjects was too small to perform cause-specific analysis in the present study, other studies have reported that a low testosterone level predicted increased risk of death due to CVD.[4,5] Further, in addition to the relation to muscle strength, physical performance and ADL,[10–12,21] some but not all reports have

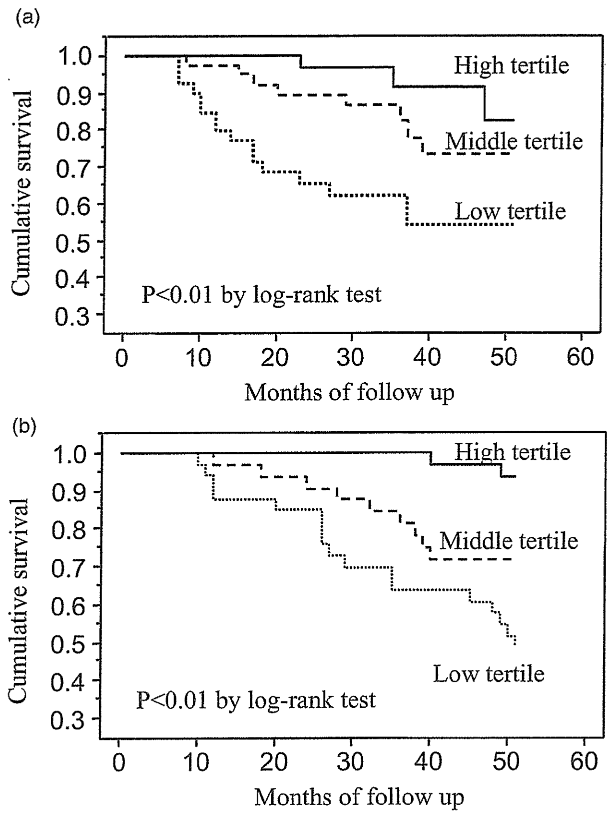

Figure 1 (a) Survival curves by tertile group of plasma testosterone level in men. (b) Survival curves by tertile group of plasma dehydroepiandrosterone sulfate level in women.

demonstrated an association between low testosterone level in older men and risk of a fall or fracture and frailty.[12–14,20] It is noteworthy that in the 10 men who died of respiratory infection, four had a history of a fall and fracture, which resulted in worse disability. Accordingly, a low testosterone level may contribute to frailty, which influences men's susceptibility to illness and falls and the capability to recover from disease or fractures, and thereby affects mortality.

Other than aging, systemic illness can result in decreased testosterone levels; therefore, low testosterone levels in older men could be attributable to acute and chronic diseases,[40] and the possible reverse causality should be considered. To evaluate this possibility, we excluded the first 12 months of observation and still found that in 12–52 months of observation, men in the low testosterone tertile had a greater risk of mortality from all causes than those in higher tertiles. We carefully excluded subjects with critical diseases and conditions at baseline, although our subjects were old with multiple chronic diseases, and it is difficult to exclude the possibility that men with subclinical critical conditions might have been included. Moreover, at baseline, there was a significant difference in functional status (ADL and cognition) and nutritional parameters (serum albumin and hemoglobin levels) between testosterone tertiles, as reported previously;[21] thus, our results need to be confirmed in a cohort with no difference in these factors between testosterone groups to exclude the influence of these biases on mortality. Also, it needs to be explored whether low testosterone in older men plays a pathogenic role, such as affecting the immune system, developing physical frailty and depression, or simply serves as a marker for biological vulnerability and poor prognosis. Long-term studies also need to test whether testosterone treatment should yield clinically significant improvements in mortality in appropriately selected older men, with consistent symptoms and signs and unequivocally low serum testosterone levels.

Low DHEA-S has been associated with increased all-cause and cardiovascular mortality in older men;[26,27,41] however, no association was found in the present study. Because DHEA(-S) is an inactive prohormone and we and others have found an association between testosterone and mortality,[3–8] it is suggested that testosterone could be a stronger predictor of mortality in older men.

On the other hand, a low DHEA-S level in older women was associated with a poor prognosis after adjusting for multiple factors related to mortality. Other previous reports showed an inconsistent relationship between DHEA-S level and mortality in older women,[29–31] possibly due to differences in the cohorts including age, DHEA-S level, heterogeneity of health status and mortality rate, and the method of statistical analysis used to demonstrate the relationship, regression models with linear/non-linear assumption.

Previous studies support a potential physiological role of DHEA-S, which could contribute to reduced mortality, an anti-inflammatory action and immune regulatory activity.[42] However, there are still many unanswered questions regarding DHEA's role in aging, and there is insufficient evidence to support DHEA replacement for increasing longevity in older women. It also needs to be explored whether the DHEA-S level contributes to mortality or is merely a biomarker of the underlying health condition of older women.

Our study has some limitations. First, the sample size was too small to reach a clear conclusion with strong statistical power, thus limiting the precision of the estimates, which is reflected in the broad range of HR for mortality. Second, the results are based on single measurements of sex hormones, which do not allow assessment of changes in levels over time; therefore, they may overestimate or underestimate the association between hormone levels and mortality. Third, we did not measure estradiol levels in men, although it would have been helpful to see whether the effects of testosterone on mortality are mediated by testosterone itself or by aromatization to estradiol in older men. Finally, active forms of testosterone such as bioavailable and

Table 3 Hazard ratios for low tertile 1 vs tertiles 2–3 of plasma sex hormone levels for all-cause mortality in men and women

	Unadjusted	Model 1	Model 2
Men (*n* = 117)			
HR of low testosterone for mortality	3.83 (1.74–8.40)**	3.71 (1.54–8.04)**	3.27 (1.24–12.91)*
Excluding first-year deaths (*n* = 108)	3.81 (1.53–6.93)**	3.49 (1.14–7.39)**	3.08 (1.11–13.62)*
Excluding deaths from cancer (*n* = 113)	4.18 (1.77–9.86)**	4.03 (1.70–9.58)**	5.02 (1.51–15.41)*
Women (N = 97)			
HR of low DHEA-S for mortality	3.77 (1.77–8.07)**	3.86 (1.79–8.32)**	4.42 (1.51–12.90)*
Excluding first-year deaths (*n* = 95)	3.38 (1.55–7.37)**	3.43 (1.56–9.54)**	3.58 (1.12–11.46)*
Excluding deaths from cancer (*n* = 92)	3.82 (1.69–8.60)**	3.55 (1.54–8.19)**	3.92 (1.28–11.98)*

*$P < 0.05$; **$P < 0.01$ vs reference group (tertile 2–3). Values are expressed as HR (95% CI). Model 1, adjusted for age; Model 2, adjusted for age, nutritional parameters, functional parameters and prevalent disease. DHEA-S, dehydroepiandrosterone sulfate; HR, hazards ratio.

calculated free testosterone were not measured, because a direct assay of bioavailable testosterone or an assay of sex hormone binding globulin, which is necessary for free testosterone calculation, is not available in Japan. However, because most of the above-mentioned previous reports have shown an association of total testosterone with mortality, the fundamental findings might not have differed if active forms of testosterone had been analyzed.

In conclusion, a low testosterone level in men and a low DHEA-S level in women are associated with increased mortality risk, independent of multiple risk factors and several pre-existing health conditions in disabled elderly. To our knowledge, the present study is the first that showed testosterone as a predictor of mortality in Asian men. Also, this is the first study that investigated frail or disabled older persons receiving care at facilities. Our results imply the clinical importance of measuring plasma androgen levels even in disabled elderly to estimate their prognosis.

Acknowledgments

This study was supported by Health and Labor Sciences Research Grants (H17-Choju-046, H18-Choju-031) from the Ministry of Health, Labor and Welfare of Japan, Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Culture and Sports of Japan (21390220, 20249041), and by grants from the Mitsui Sumitomo Insurance Welfare Foundation.

References

1 UN. World population prospects: the 2008 revision. [Cited 1 Mar 2010.] Available from URL: http://www.un.org/esa/population/

2 Statistics and Information Department Minister's Secretariat Ministry of Health, Labour and Welfare Japanese Government. Abridged life tables for Japan. 2008 Ministry of Health, Labour and Welfare. [Cited 1 Mar 2010.] Available from URL: http://www.mhlw.go.jp

3 Shores MM, Matsumoto AM, Sloan KL, Kivlahan DR. Low serum testosterone and mortality in male veterans. *Arch Intern Med* 2006; **166**: 1660–1665.

4 Khaw KT, Dowsett M, Folkerd E *et al.* Endogenous testosterone and mortality due to all causes, cardiovascular disease, and cancer in men: European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) Prospective Population Study. *Circulation* 2007; **116**: 2694–2701.

5 Laughlin GA, Barrett-Connor E, Bergstrom J. Low serum testosterone and mortality in older men. *J Clin Endocrinol Metab* 2008; **93**: 68–75.

6 Shores MM, Moceri VM, Gruenewald DA, Brodkin KI, Matsumoto AM, Kivlahan DR. Low testosterone is associated with decreased function and increased mortality risk: a preliminary study of men in geriatric rehabilitation unit. *J Am Geriatr Soc* 2004; **52**: 2077–2081.

7 Tivesten A, Vandenput L, Labrie F *et al.* Low serum testosterone and estradiol predict mortality in elderly men. *J Clin Endocrinol Metab* 2009; **94**: 2482–2488.

8 Vikan T, Schirmer H, Njølstad I, Svartberg J. Endogenous sex hormones and the prospective association with cardiovascular disease and mortality in men: the Tromsø Study. *Eur J Endocrinol* 2009; **161**: 435–442.

9 Muller M, den Tonkelaar I, Thijssen JH, Grobbee DE, van der Schouw YT. Endogenous sex hormones in men aged 40–80 years. *Eur J Endocrinol* 2003; **149**: 583–589.

10 Schaap LA, Pluijm SM, Smit JH *et al.* The association of sex hormone levels with poor mobility, low muscle strength and incidence of falls among older men and women. *J Clin Endocrinol* 2005; **63**: 152–160.

11 O'Donnell AB, Travison TG, Harris SS, Tenover JL, McKinlay JB. Testosterone, dehydroepiandrosterone, and physical performance in older men: results from the Massachussetts Male Aging Study. *J Clin Endocrinol Metab* 2006; **91**: 425–431.

12 Orwoll E, Lambert LC, Marshall LM *et al.* Endogenous testosterone levels, physical performance, and fall risk in older men. *Arch Intern Med* 2006; **166**: 2124–2131.

13 Fink HA, Ewing SK, Ensrud KE *et al.* Association of testosterone and estradiol deficiency with osteoporosis and rapid bone loss in older men. *J Clin Endocrinol Metab* 2006; **91**: 3908–3915.

14 Meier C, Nguyen TV, Handelsman DJ *et al.* Endogenous sex hormones and incident fracture risk in older men: the Dubbo Osteoporosis Epidemiology Study. *Arch Intern Med* 2008; **168**: 47–54.

15 Almeida OP, Yeap BB, Hankey GJ, Jamrozik K, Flicker L. Low free testosterone concentration as a potentially treatable cause of depressive symptoms in older men. *Arch Gen Psychiatry* 2008; **65**: 283–289.

16 Yaffe K, Lui LY, Zmuda J, Cauley J. Sex hormones and cognitive function in older men. *J Am Geriatr Soc* 2002; **50**: 707–712.

17 Moffat SD, Zonderman AB, Metter EJ, Blackman MR, Harman SM, Resnick SM. Longitudinal assessment of serum free testosterone concentration predicts memory performance and cognitive status in elderly men. *J Clin Endocrinol Metab* 2002; **87**: 5001–5007.

18 Yeap BB, Beilin J, Shi Z *et al.* Serum testosterone levels correlate with haemoglobin in middle-aged and older men. *Intern Med J* 2009; **39**: 532–538.

19 Ferrucci L, Maggio M, Bandinelli S *et al.* Low testosterone levels and the risk of anemia in older men and women. *Arch Intern Med* 2006; **166**: 1380–1388.

20 Cawthon PM, Ensrud KE, Laughlin GA *et al.* Osteoporotic Fractures in Men (MrOS) Research Group. Sex hormones and frailty in older men: the osteoporotic fractures in men (MrOS) study. *J Clin Endocrinol Metab* 2009; **94**: 3806–3815.

21 Fukai S, Akishita M, Yamada S *et al.* Association of plasma sex hormone levels with functional decline in elderly men and women. *Geriatr Gerontol Int* 2009; **9**: 282–289.

22 Labrie F. Adrenal androgens and intracrinology. *Semin Reprod Med* 2004; **22**: 299–309.

23 Arlt W. Androgen therapy in women. *Eur J Endocrinol* 2006; **154**: 1–11.

24 Davis SR, Shah SM, McKenzie DP, Kulkarni J, Davison SL, Bell RJ. Dehydroepiandrosterone sulfate levels are associated with more favorable cognitive function in women. *J Clin Endocrinol Metab* 2008; **93**: 801–808.

25 Barrett-Connor E, von Mühlen D, Laughlin GA, Kripke A. Endogenous levels of dehydroepiandrosterone sulfate, but not other sex hormones, are associated with depressed mood in older women: the Rancho Bernardo Study. *J Am Geriatr Soc* 1999; **47**: 685–691.

26 Greendale GA, Edelstein S, Barrett-Connor E. Endogenous sex steroids and bone mineral density in older women and men: the Rancho Bernardo Study. *J Bone Miner Res* 1997; **12**: 1833–1843.

27 Voznesensky M, Walsh S, Dauser D, Brindisi J, Kenny AM. The association between dehydroepiandosterone and frailty in older men and women. *Age Ageing* 2009; **38**: 401–406.

28 Cappola AR, Xue QL, Walston JD *et al.* DHEAS levels and mortality in disabled older women: the Women's Health and Aging Study I. *J Gerontol A Biol Sci Med Sci* 2006; **61**: 957–962.

29 Glei DA, Goldman N. Dehydroepiandrosterone sulfate (DHEAS) and risk for mortality among older Taiwanese. *Ann Epidemiol* 2006; **16**: 510–515.

30 Mazat L, Lafont S, Berr C *et al.* Prospective measurements of dehydroepiandrosterone sulfate in a cohort of elderly subjects: relationship to gender, subjective health, smoking habits, and 10-year mortality. *Proc Natl Acad Sci U S A* 2001; **98**: 8145–8150.

31 Trivedi DP, Khaw KT. Dehydroepiandrosterone sulfate and mortality in elderly men and women. *J Clin Endocrinol Metab* 2001; **86**: 4171–4177.

32 Cappola AR, O'Meara ES, Guo W, Bartz TM, Fried LP, Newman AB. Trajectories of dehydroepiandrosterone sulfate predict mortality in older adults: the Cardiovascular Health Study. *J Gerontol A Biol Sci Med Sci* 2009; **64**: 1268–1274.

33 Mahoney FI, Barthel DW. Functional evaluation: Barthel Index. *Md State Med J* 1965; **14**: 61–65.

34 Kato S, Shimogaki M, Onodera H *et al.* Revised Hasegawa Dementia Scale (HDS-R). *Jpn J Geriatr Psychiatr* 1991; **2**: 1339–1347.

35 Yesavage JA. Geriatric Depression Scale. *Psychopharmacol Bull* 1988; **24**: 709–711.

36 Toba K, Nakai R, Akishita M *et al.* Vitality Index as a useful tool to assess elderly with dementia. *Geriatr Gerontol Int* 2002; **2**: 23–29.

37 World Health Organization. *International Statistical Classification of Diseases*, 10th Revision (ICD-10). Geneva, Switzerland: World Health Organization, Version for 2007.

38 Smith GD, Ben-Shlomo Y, Beswick A, Yarnell J, Lightman S, Elwood P. Cortisol, testosterone, and coronary heart disease: prospective evidence from the Caerphilly study. *Circulation* 2005; **112**: 332–340.

39 Araujo AB, Kupelian V, Page ST, Handelsman DJ, Bremner WJ, McKinlay JB. Sex steroids and all-cause and cause-specific mortality in men. *Arch Intern Med* 2007; **167**: 1252–1260.

40 Matsumoto AM. Andropause: clinical implications of the decline in serum testosterone levels with aging in men. *J Gerontol A Biol Sci Med Sci* 2002; **57A**: M76–M99.

41 Barrett-Connor E, Khaw KT, Yen SS. A prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease. *N Engl J Med* 1986; **315**: 1519–1524.

42 Dillon JS. Dehydroepiandrosterone, dehydroepiandrosterone sulfate and related steroids: their role in inflammatory, allergic and immunological disorders. *Curr Drug Targets Inflamm Allergy* 2005; **4**: 377–385.

Geriatr Gerontol Int 2011; **11:** *328–332*

ORIGINAL ARTICLE: EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

Relationship between interleukin-6 and cerebral deep white matter and periventricular hyperintensity in elderly women

Kumiko Nagai,[1] Koichi Kozaki,[1] Kazuki Sonohara,[1] Masahiro Akishita[2] and Kenji Toba[1]

[1]*Department of Geriatric Medicine, Kyorin University School of Medicine, and* [2]*Department of Geriatric Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan*

Aim: We evaluated the relationships between serum levels of high-sensitivity C-reactive protein (hsCRP) and interleukin (IL)-6 with the severity of leukoaraiosis.

Methods: One hundred and thirty-seven elderly women who attended the Center for Comprehensive Care on Memory Disorders at Kyorin University Hospital were enrolled in this study. Leukoaraiosis was assessed by periventricular hyperintensity (PVH) score and deep white matter hyperintensity (DWMH) score.

Results: Serum log IL-6 level correlated with PVH and DWMH scores, but hsCRP did not. By multinomial logistic analysis, IL-6 was significantly related to DWMH score, independent of age and systolic blood pressure.

Conclusion: IL-6 is presumably an important marker of leukoaraiosis, as is the case with silent cerebral infarction. **Geriatr Gerontol Int 2011; 11: 328–332.**

Keywords: interleukin-6, leukoaraiosis, white matter hyperintensity.

Introduction

Leukoaraiosis, an isointense lesion on T_1-weighted images and hyperintense lesion on T_2-weighted images of magnetic resonance imaging (MRI), is considered to be a type of ischemic change in the brain on the basis of decreased blood flow in the area of leukoaraiosis.[1] In addition, leukoaraiosis is likely to have a relationship with vascular risk factors such as hypertension and diabetes.[2] On the other hand, the severity of leukoaraiosis also has a relationship with symptoms of the geriatric syndromes such as dementia, gait disturbance and functional disability.[3–5] Hence, leukoaraiosis is regarded as a significant brain lesion linking vascular risk factors and the occurrence of geriatric syndromes. Previous research on leukoaraiosis showed that women tended to have more white matter lesions than men,[6] and progression of deep white matter hyperintensity (DWMH) lesion was grater in women than men.[7] Furthermore, Gouw *et al.* showed that leukoaraiosis tended to develop greater in women than men and lacunes were vice versa.[8] Recently, many studies have focused on the relationships between brain ischemia and inflammation. Above all, Hoshi *et al.* demonstrated that serum high-sensitivity C-reactive protein (hsCRP) and interleukin (IL)-6 levels correlated with silent brain infarction.[9] They suggested an involvement of inflammation in cerebral infarction. However, few studies have examined the relationships between inflammatory markers and other cerebral ischemic changes such as leukoaraiosis.Therefore, we investigated whether serum levels of hsCRP and IL-6 have a relationship with leukoaraiosis in elderly women.

Accepted for publication 14 December 2010.

Correspondence: Dr Koichi Kozaki MD PhD, Department of Geriatric Medicine, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka, Tokyo 181-8611, Japan. Email: kozaki-tky@umin.ac.jp

doi: 10.1111/j.1447-0594.2010.00686.x

Methods

Patients

One hundred and thirty-seven women who attended the Center for Comprehensive Care on Memory Disorders at Kyorin University Hospital were included in this study. This study was approved by the Ethics Committee of Kyorin University School of Medicine. Accordingly, written informed consent was obtained from all patients.

MRI

Magnetic resonance imaging (MRI) was performed on 1.5-T scanners (Toshiba Medical Systems, Tochigi, Japan). T_1-weighted images (repetition time [TR] = 496 msec, echo time [TE] = 12 msec), T_2-weighted images (TR = 4280 msec, TE = 105 msec) and fluid attenuated inversion recovery-weighted images (TR = 8000 msec, TE = 105 msec, 5 mm slice thickness) were obtained in the axial planes.

Periventricular hyperintensity and DWMH Score

Leukoaraiosis was classified as periventricular hyperintensity (PVH) adjacent to the lateral ventricle, and DWMH located in the deep white matter apart from the lateral ventricles. PVH was evaluated in six regions in three slices. Each region was rated as five grades (0–4) according to the systematic quantification method developed by Junque *et al.*[3] The sum of all grades in the six regions was defined as the PVH score (range 0–40).[4] DWMH was evaluated in the frontal, temporal, parietal and occipital lobes and in the basal ganglia in both hemispheres. Each lesion was rated as three grades according to the diameter, as described by de Groot *et al.*[5] The sum of all grades in five regions in both hemispheres was defined as the DWMH score.[4]

Laboratory tests

Blood samples were obtained in the morning after an overnight fast. Serum levels of hsCRP and IL-6 were measured using nephelometry and enzyme-linked immunosorbent assay, respectively. The intra-assay coefficients of variation for the measurements of hsCRP and IL-6 were 1.3% and 2.9%, respectively.

Statistical analysis

Because the distribution of hsCRP and IL-6 levels appeared to be left-skewed, they were normalized by logarithmic transformation. We used Spearman's ρ to investigate correlations between parameters and PVH score or DWMH score. Also, to test independently the effect of the inflammatory markers associated with the severity of leukoaraiosis, multinomial logistic regression analysis was performed with the grade of PVH (tertiles of PVH score) or DWMH (tertiles of DWMH score) as the dependent variable; and hsCRP or IL-6, together with age and systolic blood pressure (SBP) as independent variables. $P < 0.05$ was considered statistically significant. All data were analyzed using SPSS ver. 17.0.

Results

The characteristics of the study subjects are shown in Table 1. They were non-obese normolipidemic elderly persons, however, SBP was elevated. The distribution of PVH score and DWMH score of these subjects were 1–24 and 0–209, respectively. In Spearman's correlation coefficient, IL-6 correlated with PVH score ($\rho = 0.340$, $P \leq 0.05$) and DWMH score ($\rho = 0.299$, $P \leq 0.05$) (Fig. 1), whereas hsCRP showed no relation to PVH score or DWMH score (Table 2). PVH score and DWMH score also correlated with age and SBP. When log IL-6 and log hsCRP were grouped by tertile (see legend to Fig. 2), it was found that the average PVH score and DWMH score were higher in the highest tertile of IL-6 level than in the lowest tertile according to the Kruskal–Wallis test (Fig. 2a,b). On the other hand, this increment was not found in hsCRP (Fig. 2c,d).

Because leukoaraiosis can be observed on MRI even in normal elderly persons,[10] and hypertension is known to be a risk factor for leukoaraiosis,[11] we performed multinomial logistic regression analysis using PVH or DWMH severity (tertiles of PVH and DWMH score) as the dependent variable, and age, SBP and inflammatory

Table 1 Clinical characteristics of study subjects (women, $n = 137$)

Age (years)	76 ± 7
BMI (kg/m^2)	20.8 ± 3.3
SBP (mmHg)	142 ± 26
DBP (mmHg)	80 ± 14
PVH score (points)	8.2 ± 4.0
DWMH score (points)	61.4 ± 51.0
Total cholesterol (mmol/L)	5.38 ± 0.91
HDL cholesterol (mmol/L)	1.50 ± 0.36
LDL cholesterol (mmol/L)	3.23 ± 0.65
Triglyceride (mmol/L)	1.08 ± 0.46
Log IL-6 (ng/L)	0.35 ± 0.46
Log hsCRP (μg/L)	2.58 ± 0.58

All parameters are expressed as mean ± standard deviation. IL-6 and CRP are shown as log transformed. BMI, body mass index; DBP, diastolic blood pressure; DWMH, deep white matter hyperintensity; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; LDL, low-density lipoprotein; PVH, periventricular hyperintensity; SBP, systolic blood pressure.

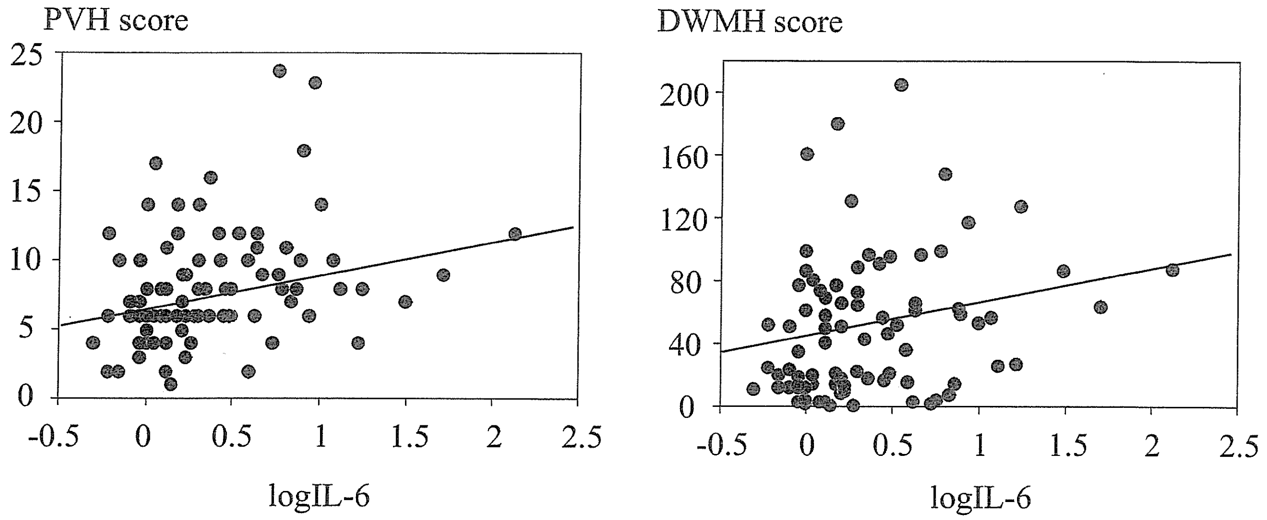

Figure 1 Relations between periventricular hyperintensity (PVH) score and log interleukin (IL)-6 (left panel; $\rho = 0.340$, $P \leq 0.05$, $n = 137$), and deep white matter hyperintensity (DWMH) score and log IL-6 (right panel; $\rho = 0.299$, $P \leq 0.05$, $n = 137$).

Table 2 Spearman's correlation coefficient between leukoaraiosis and parameters

	PVH score		DWMH score	
	ρ	*P*	ρ	*P*
Age	0.411	<0.001	0.271	0.002
BMI	−0.156	0.085	−0.124	0.179
SBP	0.215	0.014	0.232	0.009
Total cholesterol	−0.128	0.192	−0.149	0.134
HDL cholesterol	−0.053	0.595	−0.205	0.041
LDL cholesterol	−0.093	0.349	−0.025	0.802
Triglyceride	−0.014	0.885	0.080	0.421
Smoke	0.337	0.005	0.443	0.000
Log IL-6	0.340	0.002	0.299	0.006
Log hsCRP	−0.018	0.867	0.019	0.855

BMI, body mass index; DBP, diastolic blood pressure; DWMH, deep white matter hyperintensity; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; LDL, low-density lipoprotein; PVH, periventricular hyperintensity; SBP, systolic blood pressure.

markers as independent variables. As shown in Table 3, it was confirmed that the level of IL-6 was significantly associated with the progression of PVH grade (from lowest to middle and middle to highest) and DWMH score (from middle to highest). However, this trend was not found in hsCRP.

Discussion

In this study, we showed relationships between IL-6 and PVH score and IL-6 and DWMH score. It is assumed that IL-6 has an association with cerebral ischemic changes such as leukoaraiosis as well as silent brain infarction.[9] Additionally, PVH and DWMH were correlated with IL-6, but not with hsCRP. With respect to this point, Schmidt *et al.* suggested that CRP is a marker of active carotid atherosclerosis, but not of a small vessel disease-related brain lesion.[12] On the other hand, it is envisaged that elevated hsCRP levels generally reflect large vessel atherosclerosis. Because leukoaraiosis is regarded as one of the brain changes caused by small vessel disease, our results support the idea of Schmidt *et al.*

Interleukin-6 is one of the principal acute-phase reactants, playing a significant role in the activation of the coagulation–fibrinolysis system. On the other hand, leukoaraiosis has been associated with a hypercoagulable condition. Endothelium-derived adhesion molecules have been reported to be elevated in patients with great leukoaraiosis or lacunar infarcts. Leukocyte-mediated injury of the small vessels and ensuing upregulation of endothelial adhesion molecules are implicated in the pathogenesis of leukoaraiosis.[13]

The Rotterdam Scan Study showed that higher hsCRP levels were associated with presence and progression of leukoaraiosis after adjustment for cardiovascular risk factors and carotid atherosclerosis.[14] The subjects in the Rotterdam Scan Study were a population-based cohort ($n = 1033$), while the subjects in the present study were outpatients in the memory clinic ($n = 137$). In this respect, the difference in characteristics and numbers of the subjects may have given rise to the different results in terms of hsCRP in the present study and the Rotterdam Scan Study.

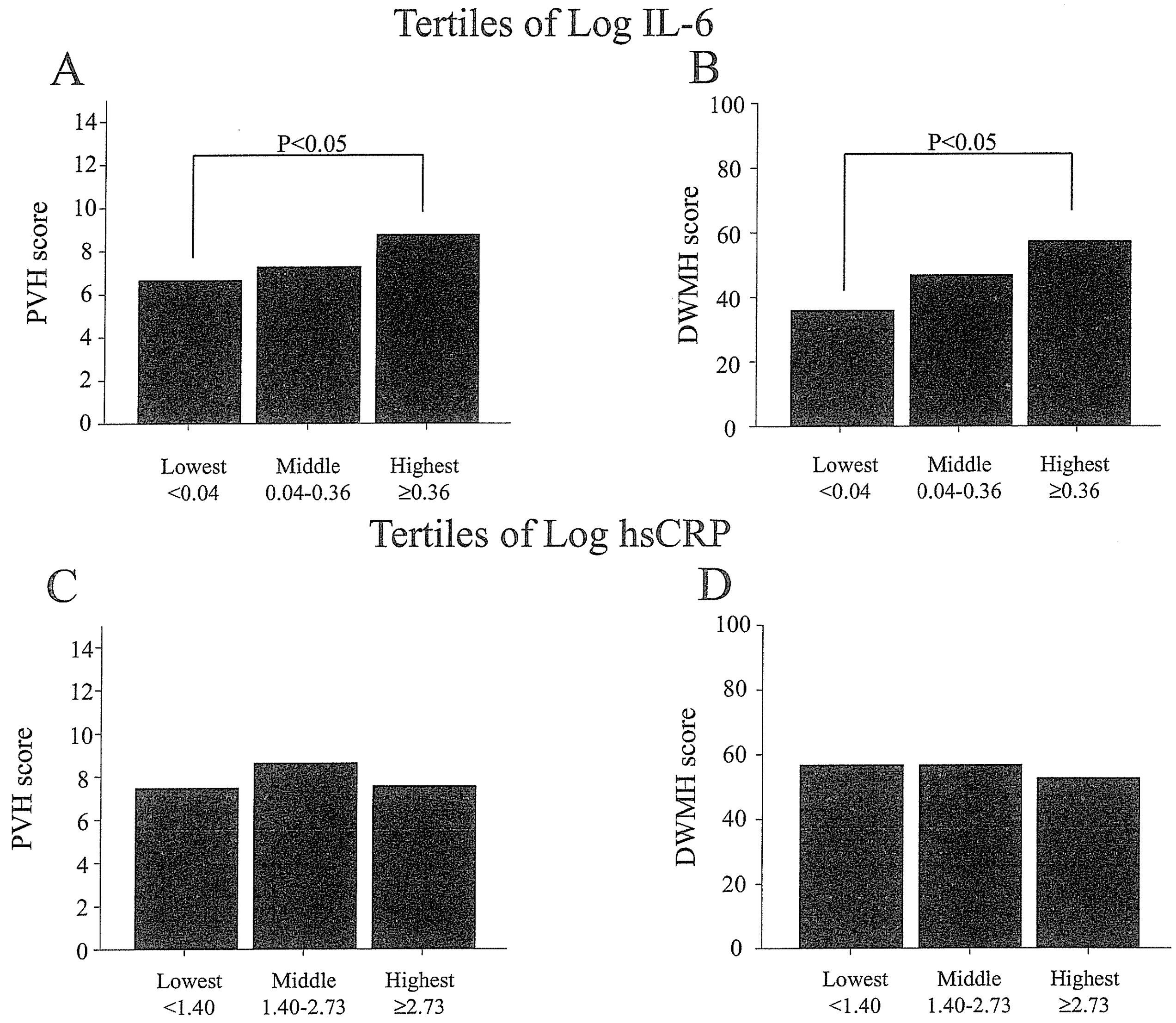

Figure 2 Average of periventricular hyperintensity (PVH) score and deep white matter hyperintensity (DWMH) score by tertile of interleukin (IL)-6 (a,b) and tertile of high-sensitivity C-reactive protein (hsCRP) (c,d). Log IL-6 tertile; lowest, <0.04 pg/mL, *n* = 55, 73.4 ± 7 1 years old (y/o); middle, 0.04–0.36 pg/mL, *n* = 38, 76.9 ± 6.8 y/o; highest, ≥0.36 pg/mL, *n* = 44, 79.5 ± 5.3 y/o. Log hsCRP; lowest, <1.40 ng/mL, *n* = 44, 73.9 ± 7.0 y/o; middle, 1.40–2.73 ng/mL, *n* = 46, 77.6 ± 7.1 y/o; highest, ≥2 73 ng/mL, *n* = 41, 77.8 ± 6.3 y/o.

In the Framingham Heart Study, no association was found between hsCRP and leukoaraiosis on MRI.[15] In the Cardiovascular Health Study, hsCRP level was modestly associated with semi-quantified leukoaraiosis volume, but the effect attenuated after excluding prevalent cerebrovascular and coronary disease cases.[13] In addition, Wright *et al.* was not able to find an association between hsCRP and leukoaraiosis volume.[16] Together, the relationships between leukoaraiosis and hsCRP varied depending upon different reports. This may come from the difference in study subjects and analytical methods. Further investigation is necessary to hold more definite opinion about which inflammatory biomarker represents the presence and development of leukoaraiosis.

Several lines of evidence suggest a relationship between IL-6 and symptoms of the geriatric syndromes, unique features of common health problems associated with poor morbidity in elderly people, such as dementia,[17] functional disability[18] and frailty.[19] On the other hand, the severity of leukoaraiosis also has a relationship with symptoms of geriatric syndromes such as dementia, falls, gait disturbance and functional disability.[3–5] Therefore, IL-6 may be an important biomarker linking the severity of leukoaraiosis to the geriatric syndromes. Because the present study is

Table 3 Associations between inflammation markers and the severity of leukoaraiosis according to tertiles (PVH score or DWMH score) adjusting for age and systolic blood pressure (logistic regression analysis)

	Log hsCRP, µg/L Odds ratio (95% CI)	Log IL-6, ng/L Odds ratio (95% CI)
PVH grade (tertiles)		
Lowest to middle	1.84 (0.78–4.31)	5.80 (1.43–23.60)
Middle to highest	0.39 (0.12–1.32)	4.39 (1.02–18.85)
DWMH grade (tertiles)		
Lowest to middle	0.81 (0.333–1.99)	3.18 (0.78–12.95)
Middle to highest	1.25 (0.48–3.29)	7.85 (1.69–36.38)

Grade of leukoaraiosis according to tertiles of PVH score or DWMH score. CI, confidence interval; DWMH, deep white matter hyperintensity; IL-6, interleukin-6; hsCRP, high-sensitivity C-reactive protein; PVH, periventricular hyperintensity.

cross-sectional, a longitudinal study would corroborate the associations of IL-6 with leukoaraiosis, and IL-6 with the geriatric syndromes.

In conclusion, we demonstrated that IL-6 level is significantly associated with the severity of PVH and DWMH lesions. The results of the present study, together with the previous studies, suggest that IL-6 is an important marker of the progression of cerebral ischemic disease, linking to the presence of geriatric syndromes.

Acknowledgments

This study was supported by a Health and Labor Sciences Research Grant (H18-Choju-031) from the Ministry of Health, Labor and Welfare of Japan, by the Mitsui Sumitomo Insurance Welfare Foundation (2006), and by the Japan Health Foundation (2007). We thank Yukiko Yamada and Ayako Machida for their technical assistance.

References

1 Oishi M, Mochizuki Y. Regional cerebral blood flow and cerebrospinal fluid glutamate in leukoaraiosis. *J Neurol* 1998; **245**: 777–780.

2 Munoz DG. Leukoaraiosis and ischemia: beyond the myth. *Stroke* 2006; **37**: 1348–1349.

3 Junque C, Pujol J, Vendrell P *et al.* Leuko-araiosis on magnetic resonance imaging and speed of mental processing. *Arch Neurol* 1990; **47**: 151–156.

4 Sonohara K, Kozaki K, Akishita M *et al.* White matter lesions as a feature of cognitive impairment, low vitality, and other symptoms of the geriatric syndrome in the elderly. *Geriatr Gerontol Int* 2008; **8**: 93–100.

5 de Groot JC, de Leeuw FE, Oudkerk M *et al.* Cerebral white matter lesions and cognitive function: the Rotterdam Scan Study. *Ann Neurol* 2000; **47**: 145–151.

6 de Leeuw FE, de Groot JC, Achten E *et al.* Prevalence of cerebral white matter lesions in elderly people: a population based magnetic resonance imaging study. The Rotterdam Scan Study. *J Neurol Neurosurg Psychiatry* 2001; **70**: 9–14.

7 van den Heuvel DM, Admiraal-Behloul F, ten Dam VH *et al.* Different progression rates for deep white matter hyperintensities in elderly men and women. *Neurology* 2004; **63**: 1699–1701.

8 Gouw AA, van der Flier WM, Fazekas F *et al.* Progression of white matter hyperintensities and incidence of new lacunes over a 3-year period: the Leukoaraiosis and Disability study. *Stroke* 2008; **39**: 1414–1420.

9 Hoshi T, Kitagawa K, Yamagami H *et al.* Relations of serum high-sensitivity C-reactive protein and interleukin-6 levels with silent brain infarction. *Stroke* 2005; **36**: 768–772.

10 Fazekas F. Magnetic resonance signal abnormalities in asymptomatic individuals: their incidence and functional correlates. *Eur Neurol* 1989; **29**: 164–168.

11 Basile AM, Pantoni L, Pracucci G *et al.* Age, hypertension, and lacunar stroke are the major determinants of the severity of age-related white matter changes. The LADIS (Leukoaraiosis and Disability in the Elderly) Study. *Cerebrovasc Dis* 2006; **21**: 315–322.

12 Schmidt R, Schmidt H, Pichler M *et al.* C-reactive protein, carotid atherosclerosis, and cerebral small-vessel disease: results of the Austrian Stroke Prevention Study. *Stroke* 2006; **37**: 2910–2916.

13 Fornage M, Chiang YA, O'Meara ES *et al.* Biomarkers of inflammation and MRI-defined small vessel disease of the brain: the cardiovascular health study. *Stroke* 2008; **39**: 1952–1959.

14 van Dijk EJ, Prins ND, Vermeer SE *et al.* C-reactive protein and cerebral small-vessel disease: the Rotterdam Scan Study. *Circulation* 2005; **112**: 900–905.

15 Jefferson AL, Massaro JM, Wolf PA *et al.* Inflammatory biomarkers are associated with total brain volume: the Framingham Heart Study. *Neurology* 2007; **68**: 1032–1038.

16 Wright CB, Moon Y, Paik MC *et al.* Inflammatory biomarkers of vascular risk as correlates of leukoariosis. *Stroke* 2009; **40**: 3466–3471.

17 Dziedzic T. Systemic inflammatory markers and risk of dementia. *Am J Alzheimers Dis Other Demen* 2006; **21**: 258–262.

18 Ferrucci L, Harris TB, Guralnik JM *et al.* Serum IL-6 level and the development of disability in older persons. *J Am Geriatric Soc* 1999; **47**: 639–646.

19 Leng S, Chaves P, Koenig K, Walston J. Serum interleukin-6 and hemoglobin as physiological correlates in the geriatric syndrome of frailty: a pilot study. *J Am Geriatric Soc* 2002; **50**: 1268–1271.

Geriatr Gerontol Int 2011; **11:** *438–444*

ORIGINAL ARTICLE: EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

Association of polypharmacy with fall risk among geriatric outpatients

Taro Kojima,[1] Masahiro Akishita,[1] Tetsuro Nakamura,[2] Kazushi Nomura,[1]
Sumito Ogawa,[1] Katsuya Iijima,[1] Masato Eto[1] and Yasuyoshi Ouchi[1]

[1]Department of Geriatric Medicine, Graduate School of Medicine, University of Tokyo, and [2]Research Institute of Aging Science, Tokyo, Japan

---

Aim: To investigate the association of fall risk with comorbidities and medications in geriatric outpatients in a cross-sectional design.

Methods: A total of 262 outpatients (84 men and 178 women, mean age 76.2 ± 6.8 years) were evaluated. Physical examination, clinical histories and medication profile were obtained from each patient. History of falls in the past year, 22-item fall risk index, 13-point simple screening test for fall, and time interval of one-leg standing test were examined as markers of fall risk.

Results: On univariate analysis, older age, female sex, hypertension, osteoporosis, history of stroke, number of comorbidities, use of antihypertensives, aspirin, bisphosphonates, hypnotics and number of prescribed drugs were significantly associated with either of four indices. On multiple regression analysis, the number of drugs was associated with all of the four indices, independent of other factors associated in the univariate analysis. The association of number of drugs with fall risk indices was stepwise.

Conclusion: In geriatric outpatients, polypharmacy rather than number of comorbidities was associated with fall risk. Prospective and intervention studies are needed to clarify the causal relationship between polypharmacy, comorbidities and fall risk. **Geriatr Gerontol Int 2011; 11: 438–444.**

Keywords: elderly, fall, polypharmacy, risk factors.

Introduction

Falls occur in more than 10% per year of community-dwelling elderly people,[1–3] and approximately 10% of falls lead to bone fracture. Also, falls are reported to be the third leading cause of a bedridden state among the elderly.[4] Previous studies assessed the risk factors of falls in community-dwelling elderly,[5–7] and history of falls, physical ability and living environment were found to be predictors of fall risk. However, these studies have not sufficiently assessed medical comorbidities and therapeutic drugs as risk factors of falls, although many elderly subjects have chronic illness such as hypertension, diabetes, cardiovascular diseases, osteoporosis and insomnia. Falls in patients on medications are more complicated, because some drugs such as aspirin could cause serious bleeding when they have injurious falls, and others such as antihypertensives[8] and hypoglycemic agents[9,10] could cause falls. Therefore, it is important to evaluate the association between fall risk and medical comorbidities or therapeutic drugs. Multiple drug use or polypharmacy is frequently seen in elderly patients because most of them have multiple chronic diseases to be treated. Moreover, inappropriate drug use is frequently seen in patients with polypharmacy.[11]

In Japan, a 22-item fall risk index questionnaire covering physical, cognitive, emotional and social aspects of

Accepted for publication 3 March 2011.

Correspondence: Dr Masahiro Akishita MD PhD, Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. Email: akishita-tky@umin.ac.jp

doi: 10.1111/j.1447-0594.2011.00703.x

functioning and environmental factors was established.[7] Also, by evaluating the validity of this questionnaire in community-dwelling older people, a simple screening test consisting of five items and total of 13 points was constructed.[2] Using these questionnaires and one-leg standing test[12] as indices of fall risk, we investigated the association of fall risk with comorbidities and medications in geriatric outpatients.

Methods

Patients

A total of 262 consecutive outpatients aged 65 years or older were enrolled who were referred for the treatment of chronic diseases such as hypertension, dyslipidemia, diabetes and osteoporosis every 2–4 weeks at a geriatric clinic located in Tokyo, Japan. All the patients were able to walk independently and were in stable conditions. Patients who had acute illness or overt dementia were excluded. Anthropometric and medical information were obtained including past history of stroke, myocardial infarction and malignancy. All the medical information including diagnoses and the prescribed drugs were obtained from the medical chart recorded by their physicians in charge. The patients whose prescriptions were changed within 1 month before enrollment were excluded. Accordingly, the included subjects had been taking the same drugs for at least 1 month before enrollment.

Ethical consideration

This study was approved by the Institutional Review Board of the Research Institute of Aging Science. We obtained written consent from all participants and/or their guardians.

Four indices of fall tendency

On the day of the enrollment, all patients were examined for four indices to investigate the fall risk: (i) history of fall in the past year (no or yes); (ii) a 22-item portable fall risk index questionnaire developed by the working group of the Ministry of Health, Labor and Welfare (see Appendix I);[7] (iii) 13-point simple screening test to assess the risk of fall which was also developed by the same working group (see Appendix II);[2] and (iv) duration time of open-eye one-leg standing test.

Table 1 Characteristics of study subjects

Age			76.2 ± 6.8 years old
Male	32.1%	(*n* = 84)	75.3 ± 6.6 years old
Female	67.9%	(*n* = 178)	76.6 ± 6.8 years old
Comorbidities			
Hypertension	64.1%	(*n* = 168)	
Dyslipidemia	47.7%	(*n* = 125)	
Diabetes	18.7%	(*n* = 49)	
Osteoporosis	24.0%	(*n* = 63)	
History of stroke	6.5%	(*n* = 17)	
History of myocardial infarction	3.4%	(*n* = 9)	
History of cancer	5.3%	(*n* = 14)	
Number of comorbidities	1.90 ± 1.09		
Drug use			
Antihypertensive use	57.6%	(*n* = 151)	
Calcium channel blockers	39.3%	(*n* = 103)	
Angiotensin-II receptors blockers	34.7%	(*n* = 91)	
Beta-blocker	6.9%	(*n* = 18)	
Angiotensin converting enzyme inhibitors	5.7%	(*n* = 15)	
Diuretics	5.0%	(*n* = 13)	
Statins	24.4%	(*n* = 64)	
Sulfonylureas	6.5%	(*n* = 17)	
Aspirin	20.6%	(*n* = 54)	
Vitamin D	4.6%	(*n* = 12)	
Bisphosphonates	6.5%	(*n* = 17)	
H_2-blockers	9.9%	(*n* = 26)	
Proton pump inhibitors	6.5%	(*n* = 17)	
Hypnotics	18.3%	(*n* = 48)	
Number of drugs	3.4 ± 2.8		

Values are expressed as mean ± standard deviation.

Experience of falls in the past year is an established and powerful tool for assessing fall risk,[2] and was reported by the patient and/or his or her family members. Duration time of one-leg standing test, which can be carried out in a narrow limited space of the outpatient office, was measured using the leg with the eyes open, until the raised leg was put down on the floor. We examined both right and left legs once for each, and the longer of the two measurements was used for statistical analysis.[12]

Data analysis and statistical methods

Values are expressed as means ± standard deviation. In order to analyze the relationship between each fall risk index and comorbidities or drugs, variables were compared using Student's t-test or the χ^2-test as appropriate. The correlations between the two continuous variables were analyzed using Pearson's r coefficient. In multivariate analysis, logistic regression analysis was performed for history of falls and multiple regression analysis for the remaining three indices, to determine the association of fall risk with the variables. Differences between the groups of number of drugs and three indices of fall tendency were analyzed using one-factor ANOVA followed by Tukey–Kramer test. Data were analyzed using JMP version 8.0.1.

Results

The characteristics of the study subjects are shown in Table 1. Calcium channel blockers, angiotensin-II receptor blockers (ARB), statins and aspirins were prescribed in more than 20% of all the patients. Calcium channel blockers prescribed in this study were all long-acting agents, and aspirin dosage prescribed were all 100 mg. Less than 10 patients received insulin therapy, took non-steroidal anti-inflammatory drugs or anticoagulants. No patients were taking neuroleptics, nor antiparkinsonian drugs. Patients prescribed five drugs or more were 36.3%.

On univariate analyses, the number of drugs was the only factor which was significantly associated with history of falls in the past year (no/yes 3.2 ± 2.6/4.0 ± 3.1 drugs, $P < 0.05$). Older age, female, hypertension, osteoporosis, history of stroke, the number of comorbidities, use of ARB, aspirin, bisphosphonates, hypnotics and number of prescribed drugs were significantly associated with either one of three indices of fall risk (Table 2). Number of drugs was significantly correlated with three scores excluding the

Table 2 Univariate analysis of association between risk factor variables and three fall indices: fall-predicting score, simple screening test, one-leg standing test

		Fall risk index (points)	Simple screening test (points)	One-leg standing test (seconds)
Age		0.23***	0.23***	–0.46***
Female	No/Yes	7.0 ± 3.1/8.4 ± 4.0**	3.8 ± 3.3/4.7 ± 3.6*	19.7 ± 11.7/16.2 ± 11.7*
Hypertension	No/Yes	7.2 ± 3.6/8.4 ± 3.8*	3.7 ± 3.3/4.8 ± 3.5*	18.9 ± 11.1/16.2 ± 12.1
Osteoporosis	No/Yes	7.6 ± 3.7/8.9 ± 4.0*	4.3 ± 3.6/4.8 ± 3.1	17.9 ± 11.7/15.6 ± 11.9
History of stroke	No/Yes	7.8 ± 3.7/9.7 ± 4.1*	4.3 ± 3.4/5.6 ± 4.1	17.9 ± 11.8/8.5 ± 8.7**
Number of comorbidies		0.27***	0.17*	–0.24***
Antihypertensives	No/Yes	7.3 ± 3.6/8.5 ± 3.8*	3.7 ± 3.3/4.9 ± 3.5*	18.8 ± 11.4/15.9 ± 12.0
Angiotensin-II receptor blockers	No/Yes	7.6 ± 3.7/8.7 ± 3.8*	3.9 ± 3.4/5.2 ± 3.5**	17.6 ± 11.5/16.3 ± 12.2
Calcium channel blockers	No/Yes	7.6 ± 3.7/8.5 ± 3.7	4.1 ± 3.5/4.8 ± 3.5	18.8 ± 11.6/14.3 ± 11.6**
Aspirin	No/Yes	7.7 ± 3.8/8.9 ± 3.8*	4.1 ± 3.5/5.5 ± 3.7*	18.0 ± 11.8/13.5 ± 11.5*
Bisphosphonates	No/Yes	7.8 ± 3.8/9.9 ± 2.5*	4.3 ± 3.5/6.5 ± 2.7*	17.3 ± 11.8/14.9 ± 11.7
Hypnotics	No/Yes	7.6 ± 3.6/9.7 ± 4.1***	4.2 ± 3.6/5.2 ± 3.1	17.6 ± 11.9/15.2 ± 11.3
Number of drugs		0.30***†	0.27***†	–0.35***

*$P < 0.05$; **$P < 0.005$; ***$P < 0.0005$, compared to "No" by simple Student's t-test. For age, number of comorbidities and number of drugs, Pearson's correlation coefficient between each indices of fall tendency are shown. †For analysis of number of drugs, a questionnaire asking "whether taking five or more drugs" were excluded for analysis. Therefore, fall risk index was analyzed by a total of 21 items, and a simple screening test by a total of 11 points. For other risk factor variables shown in the table, mean ± standard deviations are expressed. Other risk factor variables not shown in this table showed no statistically significant relationship with either one of three indices.

[Table 2 amended after online publication date September 27, 2011]