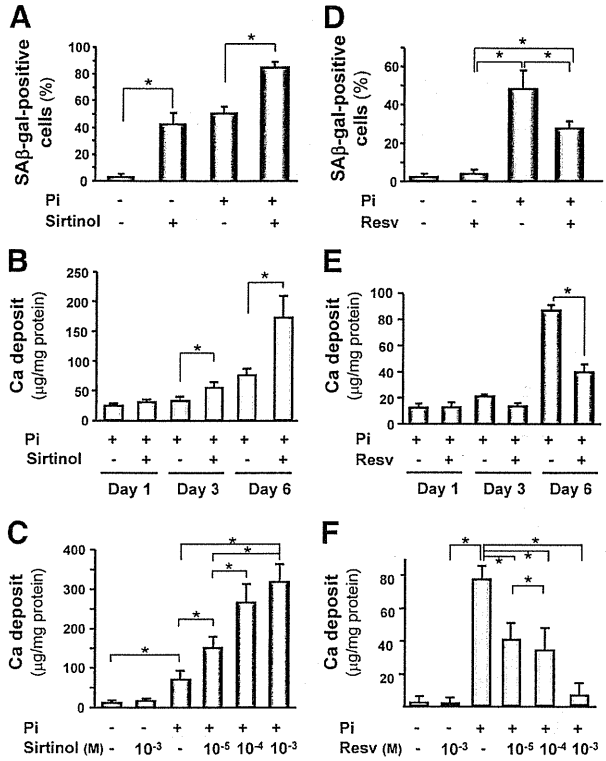

Figure 4. Modulation of sirtuin 1 (SIRT1) affects inorganic phosphate (Pi)–induced senescent phenotypic change and calcification in smooth muscle cells (SMCs). The effects of sirtinol (a chemical inhibitor of SIRT1 activity; A to C) and resveratrol (an activator of SIRT1; D to F) on Pi-induced senescent phenotypic change and calcification were examined (n=6). A, SIRT1 inhibition by sirtinol (10 μmol/L) showed an increase in the number of senescence-associated β-galactosidase (SAβ-gal)-positive cells even without Pi stimulation. The increase in Pi-induced senescence was significantly augmented by sirtinol. Sirtinol augmented Pi-induced calcium deposition in human aortic SMCs (HASMCs) in a time-dependent (B) and dose-dependent manner on day 6 (C). Conversely, treatment with resveratrol (Resv; 10 μmol/L) showed a reduction of the Pi-induced senescent phenotype (D) and calcification (E). The inhibitory effect of resveratrol on calcification was dose dependent (F).

teoblastic phenotypic change, suggesting that modulation of SIRT1 is associated with osteoblastic phenotypic change in SMCs.

Inhibition of Senescence-Related Calcification in SMCs by p21 Knockdown

To address the association of p21 with senescence-related calcification, knockdown of p21 using siRNA was performed. Treatment of p21 siRNA (up to 200 pmol/L) completely inhibited p21 (Figure 5D). p21 knockdown completely inhibited Pi-induced senescence and subsequent calcification (Figure 5E).

Regulation of NPC-Mediated Runx2 Expression by SIRT1/p21 Pathway

As the next step, the role of SIRT1 in NPC-mediated Runx2/Cbfa1 expression was examined. First, complete knockdown of SIRT1 did not show any change in both osteoblastic markers, Runx2 and alkaline phosphatase, in a normal Pi (Supplemental Figure I). As shown in Figure 5F, Pi-induced Runx2 was significantly blunted by PFA, an NPC inhibitor. SIRT1 activation by resveratrol inhibited Pi-induced Runx2 activation. The Runx2 induction was augmented by knockdown of SIRT1 by siRNA, and the activation was completely inhibited by PFA. Surprisingly, Runx2 activation was strongly inhibited by knockdown of p21 alone. In addition, the inhibition of Runx2 induction by double knockdown of SIRT1 and p21 was less than that by single knockdown of SIRT1.

To address a difference in senescent induction by Pi or Ang II, immunohistological assessment of SIRT1 in HASMCs was examined (Supplemental Figure II). Although SIRT1 was predominantly localized in nucleus without Pi, the translocation of SIRT1 to cytoplasm was observed after Pi stimulation for 24 hours, and its expression disappeared in both areas on day 6. In contrast, Ang II stimulation did not show the dynamic translocation.

High Sensitivity of SMCs With Replicative Senescence to Pi-Induced Calcification

Not only Pi-induced "premature senescence" in HASMCs but also the effects of Pi on "replicative senescence" were evaluated. Senescent cells (passage 18) were more sensitive to Pi-induced calcification compared with young cells (passage 7) (Figure 6A). SIRT1 expression was downregulated in senescent cells compared with young cells, and the downregulation was significantly augmented by Pi stimulation (Figure 6B, top). In parallel with this finding, senescent cells showed an increase in Ac-p53 and p21 expression. Statistical analyses using densitometric measurement showed that (1) downregulation of SIRT1 and upregulation of Ac-53 and p21 were augmented by replicative senescence, and (2) Pi inhibited the SIRT1-p21 pathway even in cells with replicative senescence (passage 18) (Figure 6B, bottom).

Discussion

Vascular aging, leading to cardiovascular disease, manifests complex and diverse vascular changes (eg, impairment of distensibility due to loss of arterial elasticity).[1,16] Arterial wall stiffness resulting from ectopic calcification is a complication of advanced atherosclerosis and makes the management of hemodynamics more difficult in the elderly. Few reports have addressed whether cellular senescence is associated with SMC calcification. This study showed the importance of SIRT1, a longevity gene, in arterial calcification in association with cellular senescence.

First, our data obtained from animal experiments clearly showed the association of senescent SMCs with aortic medial calcification in the renal failure rats with hyperphosphatemia. Senescent cells showed significant colocalization with calcium deposition. Intriguingly, numerous senescent cells could be detected before microscopic calcification occurred at 4 weeks after the start of renal failure induction (data not shown), suggesting that the transition to a senescent phenotype in medial SMCs may be associated with the initiation and progression of calcification. Therefore, hyperphosphatemia, a potent uremic factor, may be a stimulator to induce senescent phenotypic transition of medial SMCs.

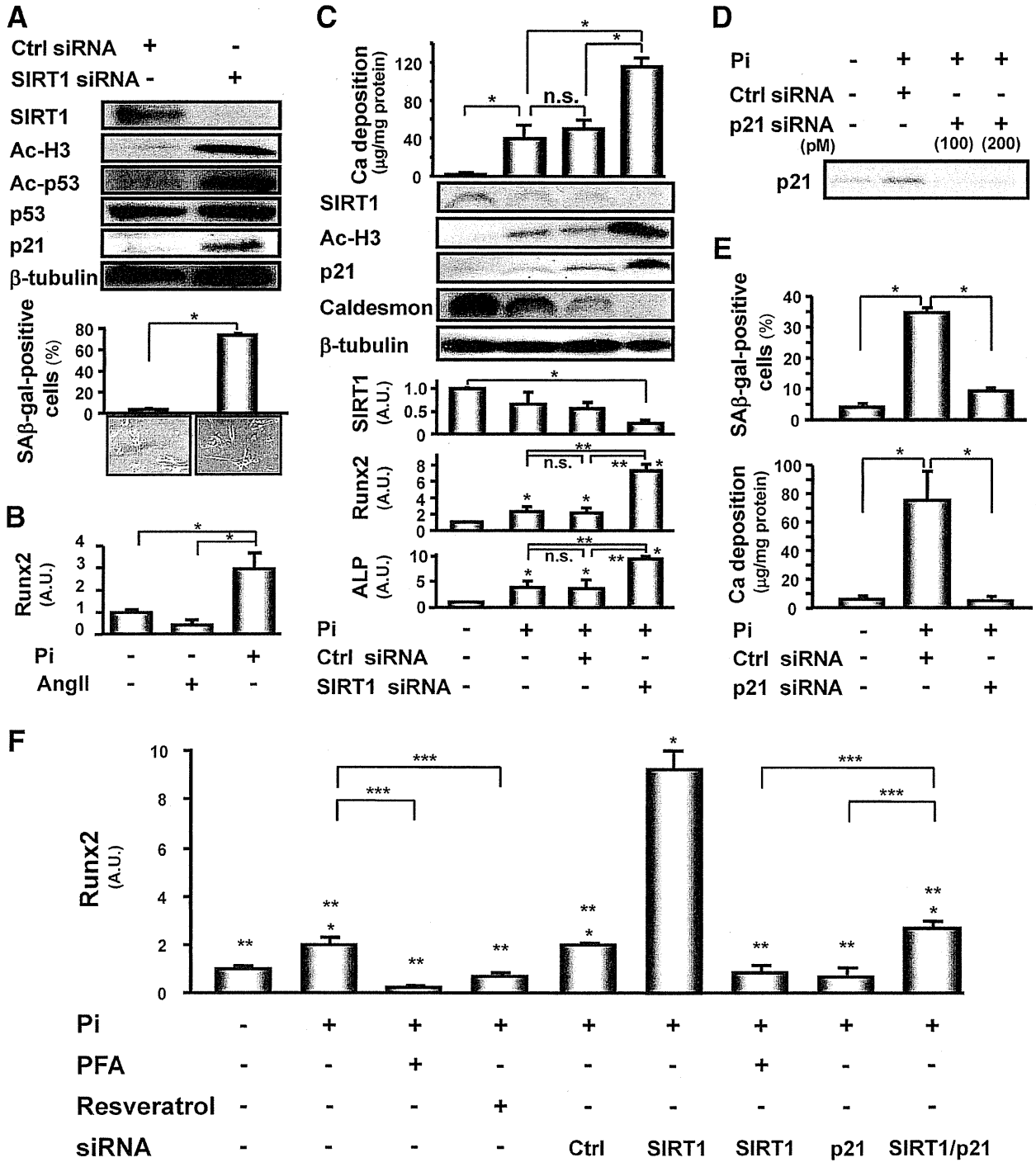

Figure 5. Augmentation of senescence-related smooth muscle cell (SMC) calcification by sirtuin 1 (SIRT1) knockdown in association with osteoblastic phenotypic change and prevention of inorganic phosphate (Pi)–induced changes by p21 knockdown. A, To achieve SIRT1 knockdown in human aortic SMCs (HASMCs), small interfering RNA (siRNA) was simultaneously administered at the start of Pi stimulation (2.6 mmol/L). Complete inhibition of SIRT1 showed a significant increase in acetylation of both substrates (acetylated [Ac]-H3 and Ac-p53), p21 expression and senescence-associated β-galactosidase (SAβ-gal)-positive cells. B, Angiotensin II (Ang II) alone (10 pmol/L) did not increase the expression of Runx2 in the absence of Pi stimulation, unlike Pi stimulation. C, top: SIRT1 knockdown by siRNA significantly accelerated Pi-induced calcification (n=6), whereas control (Ctrl) siRNA did not. C, middle and bottom: Western blots showed that Pi partially inhibited the expression of a differentiated SMC marker, caldesmon, and complete knockdown of SIRT1 by siRNA augmented its downregulation. Real-time polymerase chain reaction analysis showed that Pi induced the expression of Runx-2 and alkaline phosphatase (ALP). Complete knockdown of SIRT1 significantly accelerated the Pi-induced osteoblastic markers. A.U. indicates arbitrary units. *$P<0.05$. D and E, Knockdown of p21 by siRNA (200 pmol/L) significantly reduced the senescent phenotypic change and subsequent calcification (n=6). F, The role of SIRT1/p21 axis in Na-dependent phosphate cotransporter-mediated Runx2 expression was evaluated. Augmentation of Pi-induced Runx2 expression by SIRT1 knockdown was significantly inhibited by double knockdown of SIRT1 and p21. *$P<0.05$ vs control without Pi stimulation (left column), **$P<0.05$ vs Pi-stimulated cells with SIRT1 siRNA (sixth column from left).

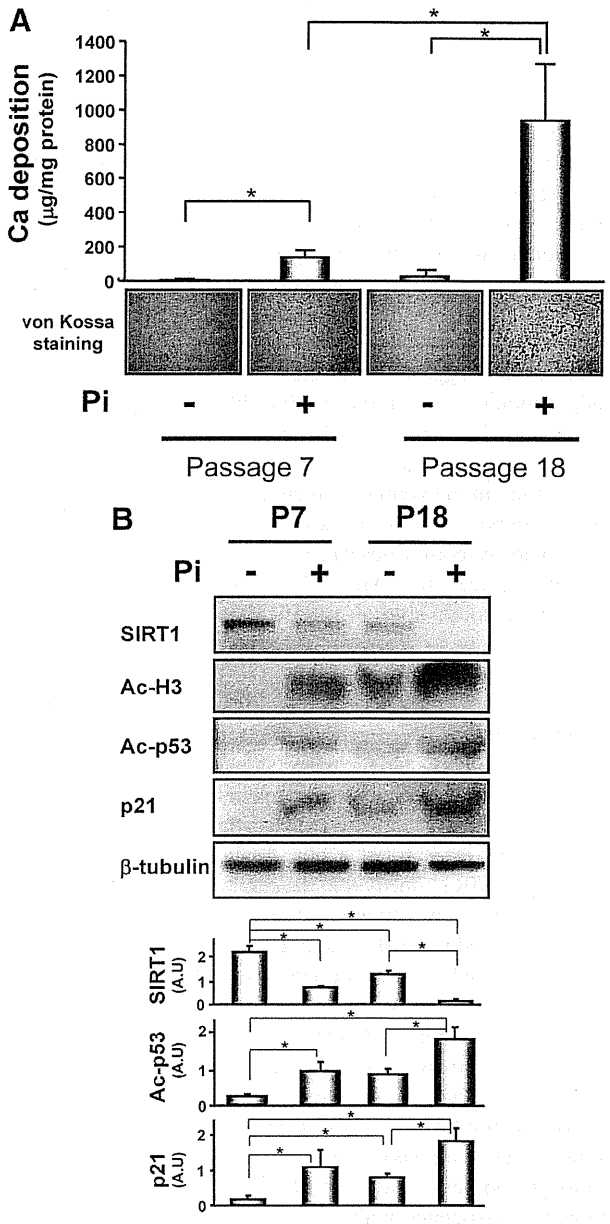

Figure 6. High sensitivity of smooth muscle cells (SMCs) with replicative senescence to inorganic phosphate (Pi)–induced calcification. The effects of replicative senescence in human aortic SMCs (HASMCs) on Pi-induced calcification (A) and sirtuin 1 (SIRT1)-related molecules (B) were also evaluated. A, Senescent cells (passage 18 [P18]) were more sensitive to Pi-induced calcification compared with young cells (passage 7 [P7]) (n=6). Representative photographs of von Kossa staining (bottom) show strong induction of calcium deposition by Pi (2.6 mmol/L). B, Senescent HASMCs (P18) showed a decline in SIRT1 expression and an increase in p21 expression compared with young cells (P7). Pi stimulation of senescent cells significantly inhibited SIRT1 expression and accelerated the increase in p21 and acetylated (Ac)-p53. Densitometric analysis confirmed these more sensitive responses in senescent cells. A.U. indicates arbitrary units. *$P<0.05$.

Second, we also confirmed the association of Pi-induced SMC senescence with calcification in in vitro experiments. Senescent SMCs were significantly increased by Pi even on day 1, although calcium deposition was not markedly increased at the same time point. A statistically significant increase in calcium deposition was found from day 3 and later. Considering these data, we hypothesize that (1) calcium deposition may be more readily induced in senescent cells compared with nonsenescent cells, and (2) Pi-induced senescent change is observed earlier than calcium deposition. In other words, senescent transition associated with Runx2 induction may lead to progressive calcification.

Senescent SMCs were associated with the SIRT1-related p53/p21 pathway, based on the findings that SIRT1 knockdown augmented not only cellular senescence but also calcification. In addition, p21 knockdown completely inhibited senescence-related calcification induced by Pi. This raises the question of how cellular senescence in SMCs is associated with calcification. Our experiments to understand the detailed mechanisms by which SIRT1 modulates senescence-related calcification showed that Pi-induced SIRT1 downregulation led to the phenotypic change from a differentiated state to osteoblast-like cells in SMCs. It has been reported that Pi induces osteoblastic change, in which NPC plays a role in inducing Runx2/Cbfa-1 expression, in SMCs.[17] As the next step, to determine how SIRT1 regulates NPC-mediated Runx2 expression, we examined the effects of knockdown of SIRT1, p21, or both by siRNA on Pi-induced Runx2 expression. Our data shown in Figure 5F suggested that (1) NPC plays an essential role in Pi-induced Runx2 expression, (2) SIRT1 has an inhibitory effect on NPC-mediated Runx2 expression, (3) knockdown of p21 alone ameliorates Runx2 induction, and (4) p21-related osteoblastic change is at least in part dependent on SIRT1.

There is now the new question of how SIRT1 regulates Runx2 regulation. A report by Jeon[18] has shown that acetylation of Runx2 itself is important in osteoblast differentiation, and it is downregulated by HDAC activities. Based on this evidence, SIRT1, 1 of the HDACs, may be able to deacetylate Runx2, leading to inhibition of Runx2-related osteoblastic transition in SMCs. Therefore, the inhibition of SIRT1 by hyperphosphatemia may lead to Runx2 activation via its hyperacetylation. Further investigation of the detailed mechanism of the SIRT1/p21/osteoblastic gene axis is needed. These data clearly suggest that SIRT1 activation may inhibit the hyperphosphatemia-induced osteoblastic phenotypic change of SMCs, and the degree of change may be dependent on SIRT1 expression level. It is possible that the inhibition of SIRT1 expression by Pi alone is "partial," because complete downregulation of SIRT1 by siRNA worsened the dynamic phenotypic change compared with Pi only. We have already shown that tumor necrosis factor-α, a potent atherogenic cytokine, augmented Pi-induced SMC calcification, as previously described.[19] In addition, tumor necrosis factor-α significantly decreased Pi-induced SIRT1 downregulation further (data not shown). According to these results, we currently hypothesize that hyperphosphatemia induces SIRT1 downregulation and subsequent osteoblastic phenotypic change in SMCs, leading to calcification, and these changes are worsened by some harmful atherogenic factors, which decrease SIRT1 expression/activity further. These results provide a new insight, showing that SIRT1 plays an essential role in the prevention of arterial calcification and that the beneficial effect may be associated with an inhibition in Pi-induced SMC senescent transition.

In addition, Ang II did not increase calcium deposition, although the stimulation increased the number of senescent cells. Of note, Ang II alone did not increase Runx2 expression in the absence of Pi (Figure 5B). This result suggests that SMC senescence shows two different features: one is SAβ-gal-positive cells with an increase in Runx2 and the other is SAβ-gal-positive cells without. First, it has recently been reported that SMCs with replicative senescence, rather than the cells without senescence, show hypersensitivity in response to induction of calcification with the more induction of osteoblastic markers,[20] suggesting that the induction of osteoblastic transdifferentiation is strongly associated with the senescent change in SMCs. In addition, the translocation of SIRT1 to cytoplasm was observed after Pi stimulation for 24 hours, although SIRT1 predominantly localized in nucleus without Pi. In contrast, Ang II did not show the dynamic translocation. Thinking about the mechanism for regulating the activity of HDACs, including SIRT1, recent several reports show the importance of their coordinated shuttling between nucleus and cytoplasm. A report demonstrates that HDAC7, an HDAC, represses the transcriptional activity of Runx2 and that osteogenic stimuli induce export of HDAC7 from nucleus, leading to a decline in the repressive potentials of HDAC7 for Runx2.[21] On the basis of our findings and a previous report, the reason that stimulation with Ang II alone did not induce Runx2 expression and subsequently SMC calcification may in part depend on the difference of SIRT1 translocation after stimulation. Therefore, we strongly hypothesize that in the senescent SMCs with upregulation of p21, Pi stimulation, but not Ang II stimulation, may activate Runx2 via at least two phenomena, the hyperacetylation of Runx2 by SIRT1 downregulation and the dynamic SIRT1 translocation, leading to marked osteoblastic transdifferentiation and subsequent calcification. In addition, we have another hypothesis. In general, it has been shown that high-dose Pi navigates release of matrix vesicles from SMCs in parallel with osteoblastic transdifferentiation. The vesicles play an essential role in the initiation of hydroxyapatite aggregation, so-called nucleation. Accumulating recent reports show that the nanocrystal formation as an initial step under hyperphosphatemia accelerates the harmful cascade of osteoblastic transdifferentiation in SMCs via endocytosis.[22,23] Maybe Ang II alone does not induce the nanocrystal formation and the cascade of osteoblastic change. Therefore, we explain that the difference of senescent phenotypic changes in SMCs between both stimulations, Pi and Ang II alone, may depend on (1) SIRT1 translocation and (2) nanocrystal formation to accelerate calcification. Further investigation to address the detailed mechanisms by which SIRT1 regulates osteoblastic transdifferentiation in SMCs under the cellular senescence is needed.

Are SIRT1 downregulation-related SMC senescence and subsequent calcification reversible or not? To answer this question, the effects of continuation or termination of high-dose Pi were examined. As shown in Figure 3B, termination (on day 6) of Pi showed no progression of senescence-related calcification in association with the restoration of SIRT1, whereas continuation (up to day 10) of Pi stimulation showed further progression of calcification. It is suggested that a therapeutic strategy to manage hyperphosphatemia to the normal range of serum phosphate concentration may lead to at least termination of progressive calcification via reversal of SIRT1 activity.

Cellular senescence has been shown to have two features: not only stress-induced premature senescence but also replicative senescence, indicating a limited number of divisions in culture.[24] In fact, both endothelial cells and SMCs derived from human atherosclerotic plaques show a senescent phenotype earlier than do cells from normal vessels.[25] Notably, we found that senescent HASMCs were significantly more sensitive to Pi-induced calcification compared with young cells. These results suggest that calcium deposition may be more readily induced in arterial medial SMCs with replicative senescence. This insight may explain the mechanisms by which arterial calcification occurs in the elderly more frequently than in the young population. Therefore, these observations support our hypothesis that arterial calcification is accelerated by both senescent types (premature and replicative senescence) in SMCs. To explore new therapeutic strategies against arterial calcification, it is essential to investigate how to maintain a higher SIRT1 level in the vasculature, leading to prevention of medial SMC senescence and which drug is capable of achieving it.

How does SIRT1 exert protective effects against SMC calcification? This study clearly showed that inhibition of SIRT1 was associated with increases in both Ac-p53 and p21 expression. These findings were significantly induced by not only replicative senescence but also Pi-induced premature senescence. SIRT1-mediated deacetylation of p53 inhibits p53-dependent transactivation of target genes, including p21. A report showed that a decline in cellular deacetylase activity increases the half-life of endogenous p53,[26] suggesting that p53 acetylation is also associated with p53 stabilization. Therefore, the increased Ac-p53 by Pi-induced SIRT1 downregulation may induce SMC senescence because of a decline in degradation of p53, leading to calcification. In addition, p53 itself can inhibit SIRT1 transcription because the SIRT1 promoter has two response elements to p53.[27] Further investigation to address how the SIRT1-p53 negative regulatory pathway is associated with SMC calcification is needed.

On the other hand, regarding p21 activation, it is reported that inhibition of p21 expression in the vasculature significantly attenuates cellular senescence, leading to prevention of atherosclerosis.[28] This evidence suggests a pivotal role of p21 in the development of atherosclerosis. p21 activation has been shown to be regulated by a pathway that is p53 dependent, p53 independent, or both. Okamoto et al have demonstrated that inhibition of HDAC by trichostatin A showed activation of p21 promoter activity by the Sp1 site even in vascular SMCs, and the induction of p21 was independent of the p53 pathway.[29] The p21 transcriptional activation in response to HDAC inhibitors was mediated by histone hyperacetylation in its promoter region. Based on these findings, Pi-induced p21 activation via SIRT1 downregulation may be in part involved in a p53-independent pathway, leading to a senescent phenotype of SMCs. Further investigation exploring which molecule activates the p21 promoter under hyperphosphatemia is needed.

Conclusion

We showed that SIRT1 exerts a protective role in hyperphosphatemia-based arterial calcification via inhibition of osteoblastic transdifferentiation, in association with cross-talk between calcification and cellular senescence. This ability of SIRT1 may orchestrate an analogous protective/longevity paradigm even in vascular SMCs, leading to maintenance of healthy elasticity of the arterial wall. Strategies to maintain a higher level of SIRT1 activity may provide novel therapeutic opportunities for the prevention of arterial calcification.

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Disclosures

None.

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

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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 2010; 10: ••–••.**

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]

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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 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), $n = 38$	*P*-value
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 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	*P*-value
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

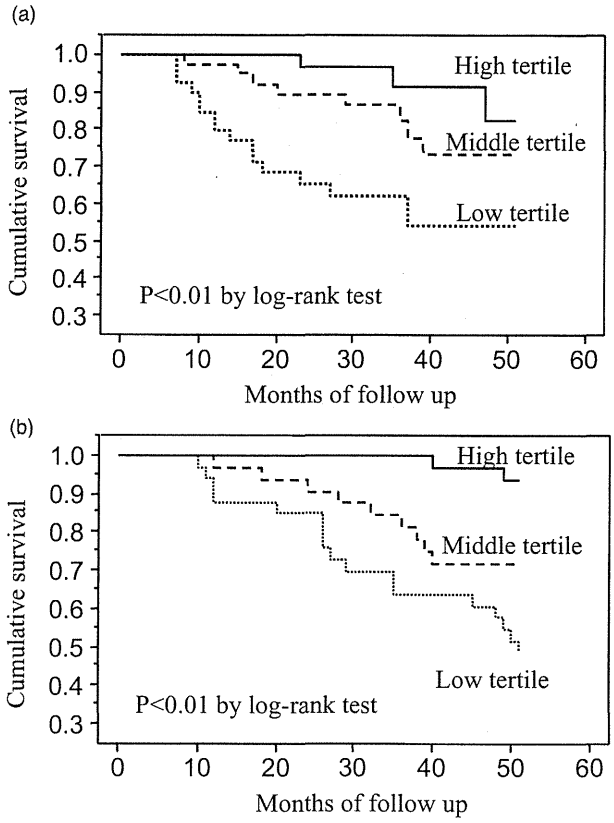

Figure 1 (a) Survival curves by tertile group of plasma testosterone level in men. (b) Survival curves by tertile group of plasma dehydroepiandrosterone sulfate level in women.

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.

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

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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: ••–••.**

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.

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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.

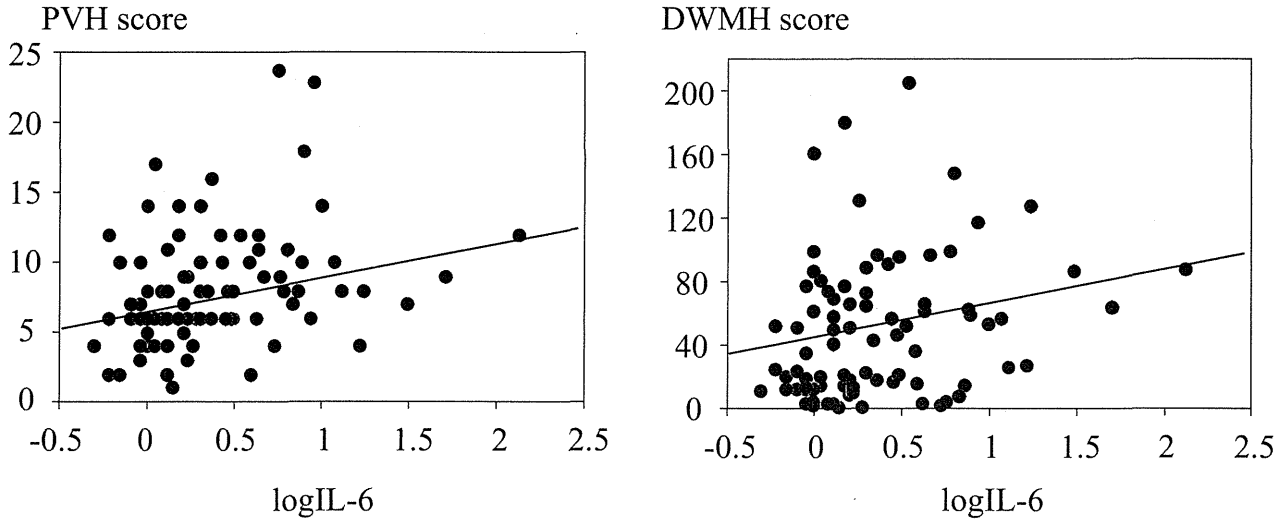

Figure 1 Relations between periventricular hyperintensity (PVH) score and log interleukin (IL)-6 (left panel; $\rho = 0.340$, $P \leq 0.05$, $n = 137$), and deep white matter hyperintensity (DWMH) score and log IL-6 (right panel; $\rho = 0.299$, $P \leq 0.05$, $n = 137$).

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.

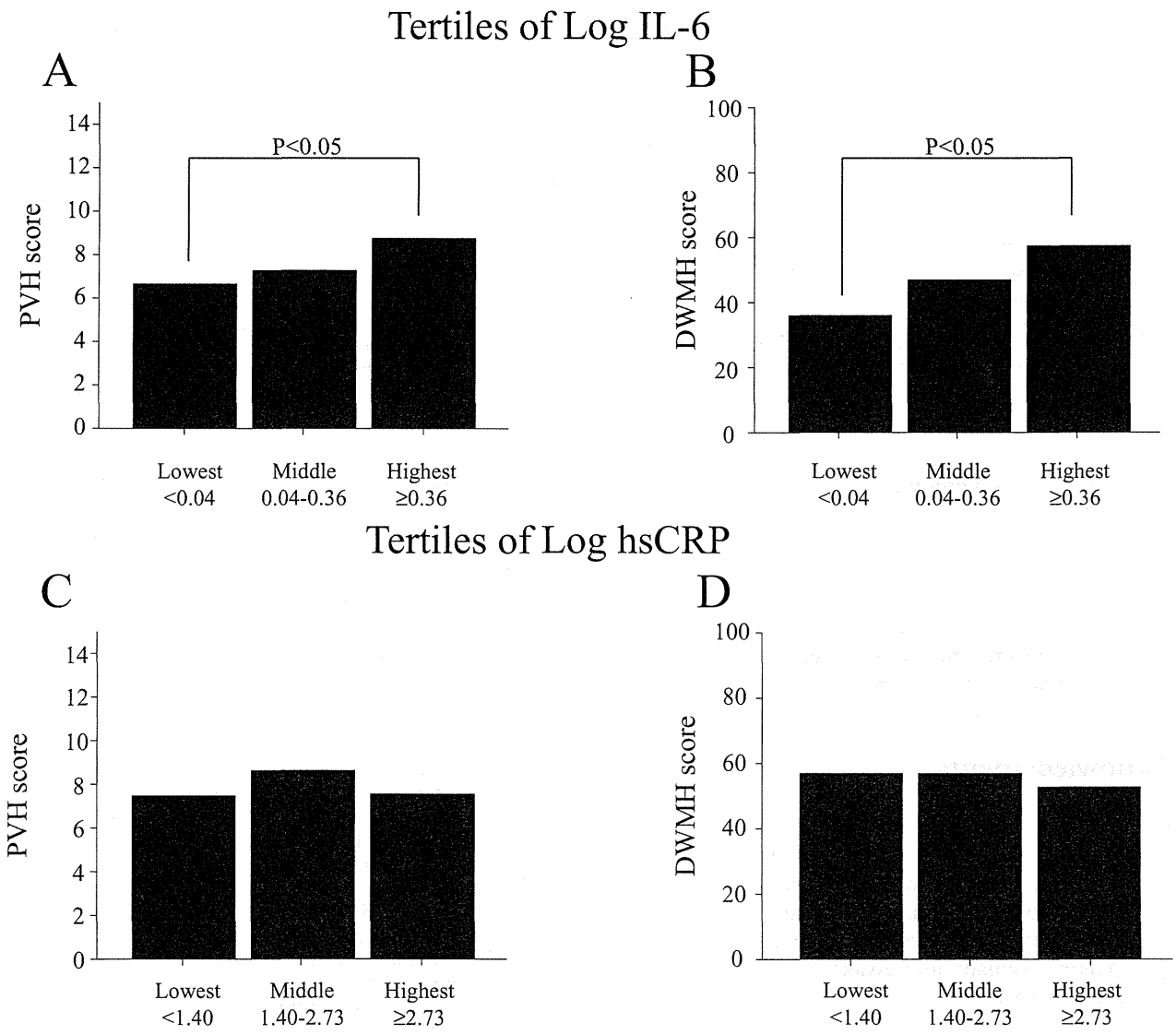

Figure 2 Average of periventricular hyperintensity (PVH) score and deep white matter hyperintensity (DWMH) score by tertile of interleukin (IL)-6 (a,b) and tertile of high-sensitivity C-reactive protein (hsCRP) (c,d). Log IL-6 tertile; lowest, <0.04 pg/mL, $n = 55$, 73.4 ± 7 1 years old (y/o); middle, 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.

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Survey on geriatricians' experiences of adverse drug reactions caused by potentially inappropriate medications: Commission report of the Japan Geriatrics Society

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Aim: The Japan Geriatrics Society (JGS) developed the guidelines for medical treatment and its safety in the elderly and the list of potentially inappropriate medication use, a Japanese version of the Beers list, in 2005. The JGS working group in collaboration with the Japan Broadcasting Corporation conducted the survey to geriatricians to investigate their experiences of adverse drug reactions (ADR) caused by potentially inappropriate medications.

Methods: In September 2008, the survey mails were sent to all the JGS certified geriatricians ($n = 1492$). The questionnaire consisted of 1 year of experiences of ADR of any type, past experiences of ADR by the use of antipsychotic benzamides, hypnotic benzodiazepines, digoxin (≥0.15 mg/day), vitamin D_3 (alfacalcidol ≥1.0 μg/day) and additional drugs, and their attitudes to reduce the dose/number of drugs for the prevention of ADR.

Results: A total of 425 geriatricians responded (response rate 29%). Seventy-two percent experienced ADR within 1 year. Past experiences of ADR were reported by 79% for antipsychotic benzamides, 86% for hypnotic benzodiazepines, 70% for digoxin and 37% for vitamin D_3. Free responses included frequent ADR by non-steroidal anti-inflammatory, antihypertensive, antiplatelet, anti-arrhythmic, antidiabetic and antidepressant drugs. Reduction of drugs for ADR prevention was attempted by 93%.

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Conclusion: This survey showed that most geriatricians experience ADR and take preventive measures for ADR. The results can be used for education and the development of new guidelines. **Geriatr Gerontol Int 2011; 11: 3–7.**

Keywords: adverse drug reactions, Beers list, geriatrician, polypharmacy, side-effect.

Introduction

Adverse drug reactions (ADR) are more frequent and severe in the elderly than in young adults. A recent systematic review[1] of prospective observational studies reported that 10.7% of hospital admissions were associated with ADR in elderly patients, while 6.3% were so in young adults. Surveys performed in acute care hospitals in Japan also showed that inpatients aged 70 years or older were 1.5-fold more likely to develop ADR than those under 60 years of age,[2] and that the ADR incidence among elderly inpatients was 6–15%.[3] It has been reported from Western countries that ADR occur in more than 10% of outpatients or nursing home residents.[4]

Although many factors relate to the high ADR incidence in the elderly, overdoses resulting from age-related changes of pharmacokinetics/pharmacodynamics and polypharmacy may be of critical importance.[2–4] Because the evidence for the elderly is limited, practical guidelines to medical treatment and its safety are required in the field of geriatric medicine.

The Japan Geriatrics Society (JGS) has conducted educational activities through scientific sessions and official journals to reduce ADR. As part of activities, the ad hoc committee "Working group on guidelines for medical treatment and its safety in the elderly" was set up in 2003, and the JGS guidelines for medical treatment and its safety in the elderly were published in 2005.[5] In the guidelines, the list of medications that should be prescribed with special attention to elderly patients was reported and was put on the JGS website. This list, a Japanese version of the Beers list,[6,7] consists of 45 drugs or drug classes that may be harmful or less efficient, thus potentially inappropriate for elderly patients, and can be applied to reduce ADR and polypharmacy in clinical settings of geriatric medicine and nursing-care facilities.[5]

Although the mass media expressed an interest in these activities, the JGS should increasingly accumulate the evidence and make a proposal on pharmacotherapy of the elderly for public education. For this purpose, the JGS working group in collaboration with the Japan Broadcasting Corporation (NHK) conducted the survey to JGS certified geriatricians to investigate their experiences of ADR caused by potentially inappropriate medications. This commission report of the working group shows the survey results.

Methods

Mailing and collection of the questionnaire

In September 2008, the questionnaire was mailed by the NHK to all the JGS certified geriatricians ($n = 1492$) who appeared on the JGS website. In the cover letter, a brief introduction including the background and aim of the survey was described, followed by the statement that this survey was carried out in collaboration with the NHK and the JGS working group. The JGS version of the Beers list (Table 1 and detailed explanation) was included in the mail for options of additional drugs. The responder was asked to return the questionnaire to the NHK by fax without his/her name.

Questionnaire item

The questionnaire consisted of 1-year experiences of ADR of any type (yes/no question), past experiences (frequent, occasional or none) of ADR by the use of antipsychotic benzamides (sulpiride, sultopride), hypnotic benzodiazepines (flurazepam, haloxazolam, quazepam, triaxolam), digoxin (≥0.15 mg/day), vitamin D_3 (alfacalcidol ≥1.0 μg/day) and free additions, and their attitudes to reduce the dose/number of drugs for the prevention of ADR (yes/no question). In addition, free comments on the problems and approaches related to pharmacotherapy in the elderly were asked. The above four classes of drugs were chosen from the JGS version of the Beers list (Table 1) because these drugs were considered frequently prescribed to elderly patients.

Statistical analysis

The data are shown as the number and the percent of subjects. The χ^2-test was performed to analyze the associations between ADR experiences.

Results

A total of 425 geriatricians responded, resulting in a response rate of 28.5%. The response rate would have been 29.1% if the 30 subjects to whom the mails were not successfully delivered were excluded.

The summary of the results is shown in Table 2. Seventy percent of the geriatricians reported