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LETTERS TO THE EDITOR

EFFECTS OF PHYSICAL EXERCISE ON PLASMA CONCENTRATIONS OF SEX HORMONES IN ELDERLY WOMEN WITH DEMENTIA

To the Editor: Physical exercise may slow the functional decline in elderly people and has been associated with a low incidence of dementia.[1] Physical activities have shown favorable effects on cognitive function as well as on neuropsychiatric symptoms and behavioral disturbance in demented subjects,[1,2] the mechanism of which is currently unknown. Because low plasma levels of sex hormones have been implicated in dementia,[3] it is reasonable to hypothesize that physical exercise could elevate plasma sex hormone levels. Here, we report a preliminary study in which daily physical exercise for 3 months increased the plasma levels of sex hormones, including dehydroepiandrosterone (DHEA) and testosterone, in elderly women with dementia. Thirteen women (aged 74–91, mean age ± standard deviation 84 ± 5) living in group homes for the elderly (small-scale facilities providing communal living) located in Nagano Prefecture, Japan, were enrolled. They were diagnosed as having Alzheimer's disease according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*, but did not have malnutrition, malignancy, or endocrine disease. Blood sampling and functional assessment were performed at baseline, at the end of a 3-month exercise program, and at the end of a 3-month follow-up period, during which the subjects returned to ordinary sedentary living. The exercise program consisted of stretching and mild resistance training using a chair and a 0.5-kg weight. The exercise was performed as a group, with training for 30 minutes daily under the instruction of a physical therapist twice a week and by other caregiver staff five times a week. Care other than exercise was comparable throughout the study. Fasting blood samples were collected early in the morning before exercise. A commercial laboratory determined plasma levels of estradiol, testosterone, DHEA, DHEA sulfate, and sex hormone–binding globulin, in addition to blood cell counts and blood chemical parameters. Basic activities of daily living (ADLs) were assessed using the Barthel Index and cognitive function using the Mini-Mental State Examination.

At baseline, the subjects showed moderate cognitive impairment and dependency and relatively low sex hormone levels (Table 1). After 3 months of exercise, significant increases were found in plasma levels of testosterone of 18%, estradiol of 38%, and DHEA of 37%, all of which returned to the baseline levels 3 months after cessation of the exercise program. A similar alteration was found in plasma DHEA sulfate level, but the increase by exercise was not statistically significant (mean ± standard error 452 ± 62 ng/mL at baseline, 508 ± 72 ng/mL after exercise, and 464 ± 77 ng/mL after discontinuation. Sex hormone–binding globulin, albumin, and other blood parameters did not change throughout the study (Table 1 and data not shown). Despite the increases in sex hormones after the exercise program, neither Barthel Index nor Mini-Mental State Examination scores changed significantly during the study.

Previous studies[4,5] have shown stimulatory effects of endurance or resistance exercise on circulating hormones in healthy postmenopausal women; metabolic alterations and increased blood flow of endocrine organs via nitric oxide and cyclic adenosine monophosphate production may play a causal role, but hormonal responses in frail or demented women have not been examined. In the present study, plasma levels of estradiol, testosterone, and DHEA were higher after 3 months of physical exercise in elderly women with dementia, whereas cognitive function and basic ADLs did not improve. Given the protective effect of exercise and sex hormones on cognitive impairment, a control sedentary group should be included to examine whether this exercise program might delay cognitive decline. Nevertheless, the finding that exercise can increase plasma sex hormone levels in demented women provides a mechanistic insight into the effect of exercise or physical activities on cognitive impairment. The results of this preliminary study need to be confirmed using larger randomized, controlled trials with longer follow-up periods.

Table 1. Effects of Daily Physical Exercise on Plasma Concentrations of Sex Hormones in Elderly Women with Dementia (N = 13)

Measurement	Baseline	Exercise (3 Months)	Discontinuation (3 Months)
	Mean ± Standard Error of the Mean		
Testosterone, ng/dL	51.4 ± 3.3	60.8 ± 3.3†	47.9 ± 3.9
Estradiol, pg/mL	15.2 ± 1.2	21.0 ± 1.2†	19.4 ± 2.9
Dehydroepiandrosterone, ng/mL	1.84 ± 0.29	2.52 ± 0.41*	1.95 ± 0.27
Sex hormone binding globulin, nmol/L	75.0 ± 6.1	69.1 ± 8.1	68.3 ± 8.3
Barthel Index	75.0 ± 5.4	70.0 ± 7.1	66.5 ± 9.4
Mini-Mental State Examination score	13.9 ± 1.9	13.8 ± 2.0	12.4 ± 2.5

$P <$ *.05; †.01 versus baseline using paired t test.

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REFERENCES

1. Colcombe S, Kramer AF. Fitness effects on the cognitive function of older adults: A meta-analytic study. Psychol Sci 2003;14:125–130.
2. Cummings JL. Alzheimer's disease. N Engl J Med 2004;351:56–67.
3. Almeida OP, Barclay L. Sex hormones and their impact on dementia and depression: A clinical perspective. Expert Opin Pharmacother 2001;2:527–535.
4. Copeland JL, Consitt LA, Tremblay MS. Hormonal responses to endurance and resistance exercise in females aged 19 to 69 years. J Gerontol A Biol Sci Med Sci 2002;57A:B158–B165.
5. Kemmler W, Wildt L, Engelke K et al. Acute hormonal responses of a high impact physical exercise session in early postmenopausal women. Eur J Appl Physiol 2003;90:199–209.

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LETTER TO THE EDITOR

Successful aging with constant physical training

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Dear Editor,

On 22 May 2003, the press reported that Yuichiro Miura, the 70-year-old Japanese adventure skier, became the oldest person to reach the peak of Mt. Everest. He, being well known internationally as the man who skied down Mt. Everest in 1970, also skied down Mont Blanc this year with his son and his 99-year-old-father, indicating that his family represents a case of successful aging. In April 2003, we had a chance to perform a checkup on Mr Yuichiro Miura, and we here report, with his informed consent, his age-related changes.

He does not have any specific history of illness, and has been a professional adventure skier for more than 40 years. He has constantly performed hard physical training to maintain his muscle strength and alertness. Specifically, his daily training consisted of walking around and climbing the nearest mountains with a 12-kilogram weight on, in addition to skiing in winter. He has taken regular food, and has not paid special attention to his diet. He has never smoked but drinks two bottles of beer four times per week. Body mass index was 27.9 kg/m^2 with waist/hip circumference ratio of 0.93. Blood pressure was 119/89 mmHg. Physical examination and laboratory tests indicated no abnormal findings except for a slightly low plasma HDL-cholesterol level of 39 mg/dL. Unsurprisingly, he showed excellent physical performance measures; e.g. timed up-and-go test of 6.3 s and one-leg standing time with eyes open of more than 10 s. Non-invasive measurements of subclinical atherosclerosis showed that endothelium-dependent flow-mediated dilatation of the brachial artery was 6.7% and brachial-ankle pulse wave velocity was 1279 cm/ s, which both corresponded to the average values in middle-aged healthy controls.[1,2] Abdominal CT (Fig. 1) revealed that the areas of visceral and subcutaneous fat were 97 cm^2 and 141 cm^2, respectively, indicating that subcutaneous fat was predominant, but calcification was found in the abdominal aorta.

Overall, he was obviously healthy and had outstanding physical function and vascular function that was young for his age, although some aspects of aging such as aortic calcification were found. Given the preventive effect of physical training on aging,[3] the influence may differ according to organ and function. The present case suggests that even a superman cannot avoid aging.

References

1 Hashimoto M, Kozaki K, Eto M *et al.* Association of coronary risk factors and endothelium-dependent flow-mediated dilatation of the brachial artery. *Hypertens Res* 2000; **23**: 233–238.

2 Tomiyama H, Yamashina A, Arai T *et al.* Influences of age and gender on results of noninvasive brachial-ankle pulse wave velocity measurement – a survey of 12 517 subjects. *Atherosclerosis* 2003; **166**: 303–309.

3 Burke GL, Arnold AM. Bild DE *et al.* Factors associated with healthy aging: the cardiovascular health study. *J Am Geriatr Soc* 2001; **49**: 254–262.

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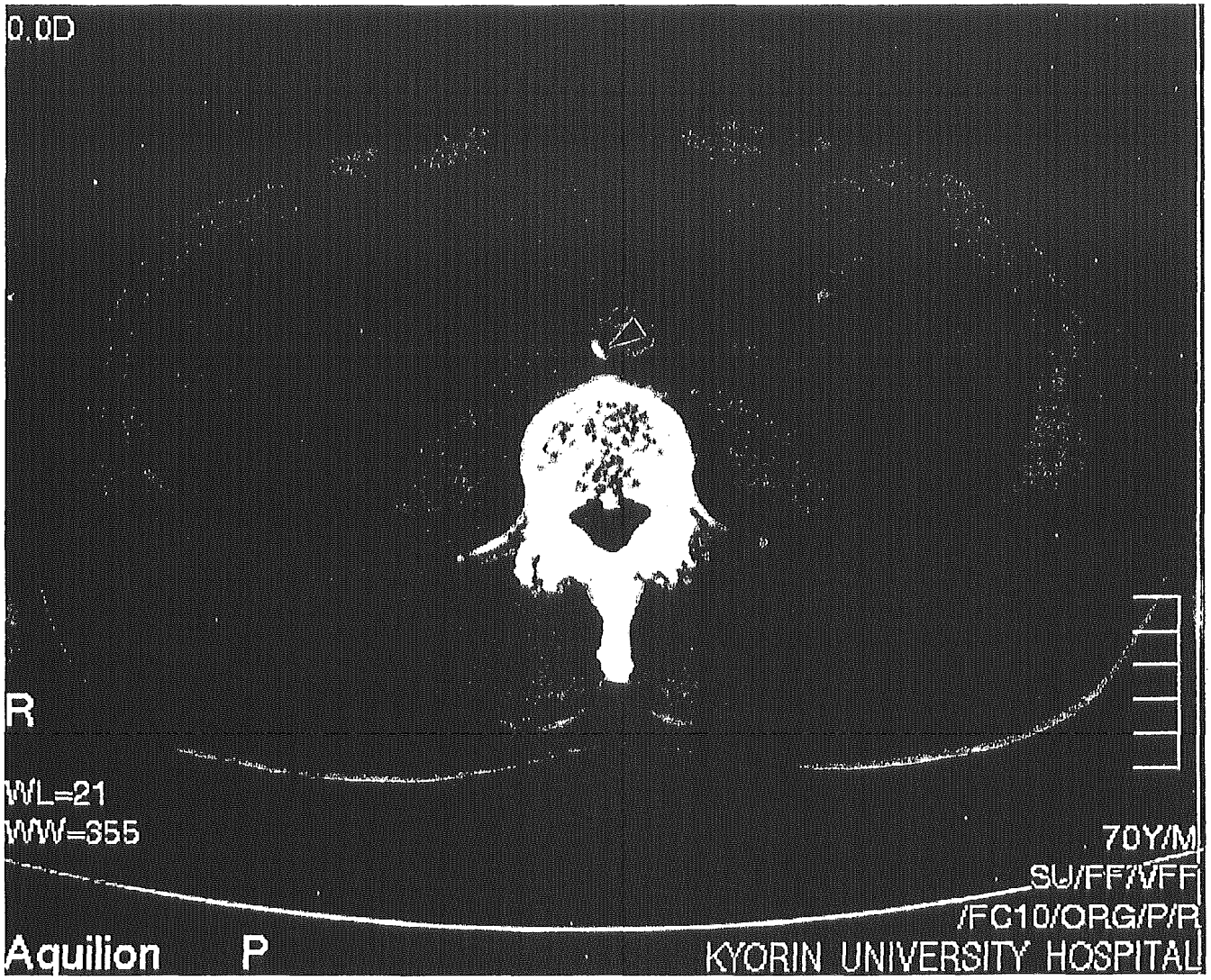

Figure 1 Abdominal plain computed tomographic image at the umbilical level showing that subcutaneous fat is predominant. The arrowhead indicates calcification of the abdominal aorta.

5. Joutel A, Dodick DD, Parisi JE et al. De novo mutation in the NOTCH3 gene causing CADASIL. Ann Neurol 2000;47:388–391.
6. O'Riordan S, Nor AM, Hutchinson M. CADASIL imitating multiple sclerosis. The importance of MRI markers. Multiple Sclerosis 2002;5:430–432.
7. Engelter ST, Ruegg S, Kirsch EC et al. CADASIL mimicking primary angiitis of the central nervous system. Arch Neurol 2002;59:1480–1483.
8. van Den Boom R, Lesnick Oberstein SA, van Duinen SG et al. Subcortical lacunar lesions: An MR imaging finding in patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Radiology 2002;224:791–796.
9. Haley EC. Encephalopathy following arteriography: A possible toxic effect of contrast agents. Ann Neurol 1984;15:100–102.
10. Dichgans M, Petersen Angiographic complications in CADASIL. Lancet 1997;349:776–777.

CORRELATION BETWEEN PULSE WAVE VELOCITY AND COGNITIVE FUNCTION IN NONVASCULAR DEMENTIA

To The Editor: We read with interest the paper by Shimoda et al.[1] showing that pulse wave velocity (PWV), an indicator of arterial stiffness, was higher in patients with vascular dementia than in patients with Alzheimer's disease and nondemented control subjects. Vascular factors such as smoking, hypertension, diabetes mellitus, and apolipoprotein E ε4 allele have also been implicated in the development of nonvascular dementia, including Alzheimer's disease,[2] but there has been no quantitative study of the relationship between the stage of arteriosclerosis and the severity of nonvascular dementia. In this study, PWV was measured in patients with mild to moderate nonvascular dementia, and greater arterial stiffness was associated with cognitive impairment.

Patients who were referred to the Memory Clinic of our department were enrolled. Patients with definite vascular dementia such as poststroke patients and patients with multiple cerebral infarcts were excluded. Twenty-seven subjects (12 men and 15 women, mean age ± standard deviation = 76 ± 7) were analyzed, including 14 patients with Alzheimer's disease diagnosed using the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*, and others with mild cognitive impairment. PWV was measured using the automated device Form PWV/ABI (Colin Co. Ltd, Komaki, Japan), and two measurements, heart-brachial (hb) PWV and brachial-ankle (ba) PWV, were analyzed.[3] Cognitive function was assessed using the Hasegawa Dementia Scale Revised (HDSR; 20 ± 7 points out of 30). Basic activities of daily living (ADLs), instrumental ADLs, mood, and volition were also measured using the Barthel index, Lawton-Brody instrumental ADLs, Geriatric Depression Scale, and Vitality Index,[4] respectively.

In the analysis including all the subjects, HDSR correlated with hbPWV ($r = -0.450$, $P < .05$) (Figure 1) and baPWV ($r = -0.433$, $P < .05$), whereas other indices of the comprehensive geriatric assessment did not correlate with hbPWV or baPWV. Multiple regression analysis using HDSR as a dependent variable and hbPWV, age, sex, mean blood pressure, and use of antihypertensive agents as independent variables showed that hbPWV ($\beta = -0.535$, $P < .05$) was a significant determinant of HDSR. Analysis using systolic blood pressure instead of mean blood pressure showed a comparable result, but analysis using baPWV instead of hbPWV did not reach statistical significance.

Subjects were excluded because they had obvious vascular factors (n = 9), extensive white-matter lesions on brain magnetic resonance imaging scans (n = 5), or a history of hypertension (n = 8) as determined by the use of antihypertensive agents or blood pressure of 140/90 mmHg or higher. These subjects showed higher hbPWV than the other 18 subjects (665 ± 139 vs 561 ± 98 cm/s, $P < .05$) and lower HDSR score (15.6 ± 5.4 vs 21.9 ± 6.7, $P < .05$), whereas age was not significantly different (79 ± 9 vs 76 ± 7, $P = .29$). Then, the correlation between PWV and cognitive function was analyzed in the 18 subjects without vascular factors. In simple regression analysis, HDSR correlated with hbPWV ($r = -0.615$, $P < .01$) (Figure 1) and baPWV ($r = -0.618$, $P < .01$). Multiple regression analysis using HDSR as a dependent variable and hbPWV, age, sex, and mean blood pressure as independent variables revealed that hbPWV ($\beta = -0.700$, $P < .05$) was independently related to HDSR.

The present study demonstrated that subjects with extensive white-matter lesions or a history of hypertension had higher PWV than others, consistent with a previous report,[1] even though subjects with typical vascular dementia were excluded. Multivariate analysis and analysis using the subjects without obvious vascular factors showed that arterial stiffness as measured using PWV was independently related to cognitive function. These results suggest that arteriosclerosis, even in a subclinical state, plays a role in cognitive impairment and that PWV serves as a useful tool to assess the vascular contribution in subjects with mild to moderate nonvascular dementia. Recent papers have shown that PWV can predict the future occurrence of cardiovascular disease.[5] Furthermore, a new paradigm—vascular cognitive impairment—in which vascular factors play a variety of roles in the pathogenesis of dementia has been proposed.[2] It is necessary to perform a large-scale study to confirm our preliminary results and a prospective

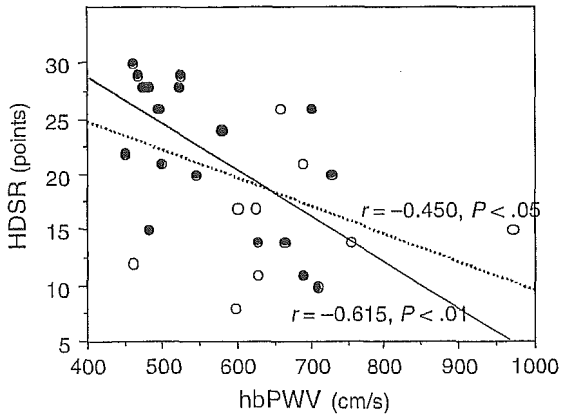

Figure 1. Correlation between heart-brachial pulse wave velocity (hbPWV) and Hasegawa Dementia Scale Revised (HDSR) in subjects with (open circles, n = 9) and without (closed circles, n = 18) vascular factors such as extensive white-matter lesions and history of hypertension. Dotted line and solid line indicate regression lines in all the subjects and the subjects without vascular factors, respectively.

longitudinal study to examine whether high PWV could be a risk factor for cognitive impairment.

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REFERENCES

1. Shimoda H, Mizushima Y, Oobasawa H et al. Pulse wave velocity in persons with vascular dementia. J Am Geriatr Soc 2003;51:1329–1330.
2. O'Brien JT, Erkinjuntti T, Reisberg B et al. Vascular cognitive impairment. Lancet Neurol 2003;2:89–98.
3. Yamashina A, Tomiyama H, Takeda K et al. Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement. Hypertens Res 2002;25:359–364.
4. 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.
5. Boutouyrie P, Tropeano AI, Asmar R et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: A longitudinal study. Hypertension 2002;39:10–15.

GERIATRIC TRAINING IN PROBLEM-BASED LEARNING: AN ASIAN PERSPECTIVE

To the Editor: Problem-based learning (PBL) is gradually becoming popular in medical schools in Asian countries.[1] It is an integrated, student-centered educational approach, which uses problems (triggers) as the key units for stimulating and structuring relevant student learning. Such learning is largely dependent on the quality of the problems[2] and the areas tested in PBL.[3] Aligning PBL activities with subsequent student assessment often proves to be difficult, because it is different from the assessment conducted in the traditional curriculum. A study was conducted to analyze the PBL problems and examination questions used in the School of Medical Sciences, Universiti Sains Malaysia (USM) to examine the demographic characteristics of the people featured and the level of acuity of case scenarios presented.

All PBL problems (n = 51) used in Phase II (Years 2 and 3) of USM PBL curriculum, 95 modified essay questions (MEQ), and 169 objective-structured clinical examination (OSCE) questions (in which age and presenting illness were mentioned) of five academic sessions (1998–2003) were analyzed. The findings revealed that problems and examination questions mostly included acute and rapidly resolving illnesses in young people and underemphasized elderly people (aged ≥ 60) with chronic, irreversible diseases. Only nine (17%) problems and 34 examination questions (MEQ 19%, OSCE 10%) featured older people. Moreover, those problems and questions mainly involved the early elderly (aged 60–74). Only one problem and one MEQ featured advanced elderly (aged ≥ 70). In the problems and questions, where the presenting illness was mentioned, it was of one month's duration in 78% of problems, 69% of MEQs, and 41% of OSCEs. Conversely, only in 4% of problems, 8% of MEQs, and 22% of OSCEs, was the presenting complaint of more than 1 year's duration. In 41 PBL problems, the outcome was mentioned; this occurred within 1 year in 11%, within 1 month in 28%, and within 1 week in 61%.

Adequate exposure to geriatric-related issues is provided to the students in the different phases of the USM curriculum. As the PBL is the main teaching–learning strategy in Phase 2 that facilitates the integration of basic and clinical sciences, such emphasis may contribute to the development of negative attitudes among the students toward elderly patients and people with chronic diseases, as mentioned in other studies.[4,5] Studies also showed that this type of emphasis might also deter students from careers that focus on the elderly[6] and chronically sick.[7] This has wider implications when there is a clear demographic trend toward a rapid increase of the elderly population in Malaysia and worldwide.[8] According to United Nations estimates, the population of elderly in the world will reach 1.2 billion by 2025, the majority of whom will be in developing countries.[9] This is also important because health care is shifting away from the diagnosis and management of acute diseases toward caring for increasingly elderly people with chronic illnesses.[8]

As a subject, geriatric medicine is not well established in the schools of Asian countries. The World Health Organization[8] strongly advocated including relevant aging- and geriatric-related issues in the medical curriculum. Medical schools should provide opportunities for their students to be exposed to older patients with adequate positive experiences in hospital, community, and long-term care settings. Some problems of the PBL segment and examination questions could be designed to focus exclusively on the elderly with chronic diseases.[10] Curriculum planners should regularly analyze the demographic and pedagogical characteristics of problems and examination questions to determine whether aging- and geriatric-related content is adequately covered in PBL curriculum. Emphasis given to such content significantly improves attitudes and knowledge of students toward the elderly.[4] Reorientation of medical education is necessary to promote more concern among physicians about the needs of the elderly and people who are chronically ill.

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REFERENCES

1. Khoo HE. Implementation of problem-based learning in Asian medical schools and students' perceptions of their experience. Med Educ 2003;37:401–409.
2. Schmidt HG, Dolmans D, Gijselaers WH et al. Theory-guided design of rating scale for course evaluation in problem-based curricula. Teach Learn Med 1995;7:82–91.
3. Connolly C, Seneque M. Evaluating problem-based learning in multilingual student population. Med Educ 1999;33:738–744.
4. Shahidi S, Devlen J. Medical students' attitudes and knowledge of the aged. Med Educ 1993;27:286–288.

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ORIGINAL ARTICLE

Improvement of inappropriate prescribing and adverse drug withdrawal events after admission to long-term care facilities

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Background: The objectives of this study were to determine whether medications, particularly inappropriate prescribing, would be reduced after admission to long-term care facilities, and whether adverse drug withdrawal events (ADWEs) would occur in relation to discontinuation of medications.

Methods: The study consists of a retrospective survey using medical chart review in five health service facilities for the elderly in Japan. All the patients who were admitted to the facilities between January 2001 and December 2002 ($N = 627$) were participants in the study. Medications taken on admission, at 1 month and 3 months after admission, and events (significant worsening of the disease status, accidents, new symptoms and signs, and other acute events) during a 3-month period were recorded. Inappropriate prescribing was determined using Beers' criteria with some modification. ADWEs were determined using the Naranjo causality algorithm.

Results: On admission, the patients were taking 3.5 ± 2.5 (mean ± SD) drugs. One month later, the number of prescribed drugs was decreased by 17% ($P < 0.01$ vs on admission), but did not show an additional reduction 3 months later. Inappropriate prescribing was found in 10% of the patients taking drugs on admission, but the number of inappropriately prescribed medications was reduced by 33% after 1 month. Of 105 events recorded, only five (2% of the patients with drug reduction) were considered ADWEs; three cases of confusion, a case of depression, and a case of hyperglycemia, following discontinuation of psychotropic drugs, antidepressants and a sulfonylurea, respectively.

Conclusion: Adverse drug withdrawal events were not frequent despite the significant reduction of medications after admission to long-term care facilities. This might be because the rate of reduction was relatively high for inappropriately prescribed medications.

Keywords: adverse drug reaction, long-term care, medical expense, medical injury, pharmacotherapy.

Introduction

Adverse drug reactions in elderly people increase with age,[1–3] with most being attributable to medication errors that are preventable.[3,4] Age-dependent changes in pharmacokinetics and pharmacodynamics, polypharmacy and non-compliance related to patients' functional

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decline may play a role.[1,3] In particular, polypharmacy resulting from multiple pathology in elderly people is a critical problem leading to adverse drug reactions.[1–3] To prevent polypharmacy, review of prescriptions is essential according to evidence-based medicine and criteria for inappropriate prescribing.[5,6] In fact, inappropriate use of medication in elderly people has been reported to be as frequent as 16% to 25%.[7–9]

Conversely, discontinuation of medications to improve polypharmacy or inappropriate prescribing may induce adverse drug withdrawal events (ADWEs),[10] although the net effect on adverse drug reactions can be favorable in elderly outpatients.[11] Fixed payment insurance systems restrict medication use, possibly leading to a reduction of inappropriate prescribing and/or an increase of ADWEs. In health service facilities for the elderly in Japan, where functional training and nursing/personal care are provided under long-term care insurance,[12] a fixed payment system including prescribing of medication is applied. Accordingly, it is hypothesized that prescribed drugs, particularly inappropriate prescribing, would be reduced after admission to the facilities, and that ADWEs would occur in relation to discontinuation of medications. To test this hypothesis, we performed a retrospective chart review of a total of 627 patients in five health service facilities for the elderly, and found that prescribed drugs can be reduced with few ADWEs in such a frail elderly population with chronic diseases.

Methods

Sample and data collection

The data were derived from five health service facilities for the elderly (Mahoroba-no-Sato, Nagano; Moeu-no-Sato, Nagano; Himawari-En, Fukuoka; Millenium-Sakuradai, Tokyo; Mizukusaki-En, Tokyo) in Japan. Institutional medical charts were reviewed for all the patients admitted between January 2001 and December 2002. Diagnoses of each patient were not recorded because they were unclear from the institutional charts, but Alzheimer's disease, cerebrovascular disease and osteoporosis were the main causes of disability in each institution. The average basic activities of daily living, as measured by the Barthel index, were 70–80 points out of 100 points according to the institutions. Medications that the patients were taking on admission and prescribed drugs 1 month and 3 months after admission were recorded. Similarly, all the events (significant worsening of the disease status, accidents, new symptoms and signs, and other acute events) during a 3-month period were recorded. The institutions that managed the patients before admission were categorized as acute care hospitals, outpatient clinics (home), sanitarium-type wards, special nursing homes for the elderly and health service facilities for the elderly. Patients with voluntary discharge within 3 months excluding cases of death or transfer to another hospital were excluded, and a total of 627 patients were analyzed. The director of each institution gave written approval to the participation in this study. The study protocol was approved by the committee on ethics and the institutional review board of Kyorin University School of Medicine.

Analysis

Inappropriately prescribed medications were determined using an updated version of the list developed by Beers with some modification.[5] Basically, we followed the list by Sloane *et al.* in which several drugs were excluded from Beers' list in consultation with Dr Beers,[5,9] reflecting changes in pharmacotherapy, but we included digoxin at more than 0.125 mg/day and oral iron at more than 325 mg/day in the list because these dosages were recorded in the medical chart. In this study, diagnosis-related inappropriate prescribing was excluded,[3] as in the study by Sloane *et al.* because the institutional chart did not include all the diagnoses of the patients.[9]

All the events were reviewed by a consultant geriatrician, and ADWEs were determined using the Naranjo causality algorithm.[13] Because detailed information, such as the effect of re-administration was lacking in most cases, a probability scale ≥ 1 (possible, probable or definite) was considered to indicate an ADWE.

The data in the text and the tables are expressed as means ± SD unless otherwise specified. Changes in the number of prescribed drugs after admission were analyzed using paired *t*-test. Differences between the groups were analyzed using ANOVA followed by Newman-Keuls' test.

Results

Number of prescribed drugs

The patients were taking 3.5 ± 2.5 drugs when admitted to the facilities (Table 1). Forty-six patients (7.3%) were not taking any drug, while 50 patients (8.0%) were on eight or more drugs. Women were taking fewer drugs than men. This sex difference seemed independent of age, although a statistically significant difference was found only at 80–89 years of age when the patients were categorized by age groups (Table 1). Interestingly, patients of 80 years or older were taking fewer drugs than those younger than 70 years, in contrast to a previous finding that the number of prescribed drugs increased according to age.[2,14,15]

As shown in Table 2, the mean number of prescribed drugs had decreased by 0.6 (17%) 1 month

Table 1 Number of drugs taken on admission according to sex and age

	All	Men	Women	*P* for sex difference
Total	3.5 ± 2.5 (627)	4.2 ± 2.8 (177)	3.3 ± 2.4 (450)	< 0.01
≤ 69 years	4.4 ± 3.1 (36)	4.6 ± 3.5 (19)	4.2 ± 2.6 (17)	0.70
70–79 years	4.0 ± 2.6 (131)	4.6 ± 3.0 (43)	3.7 ± 2.3 (88)	0.08
80–89 years	3.3 ± 2.3* (316)	4.0 ± 2.6 (81)	3.0 ± 2.2 (235)	0.02
≥ 90 years	3.5 ± 2.7* (144)	4.2 ± 2.4 (34)	3.2 ± 2.8 (110)	0.08

*$P < 0.05$ versus ≤ 69 years by Newman-Keuls' test.
Data are expressed as mean ± SD. Number of subjects is indicated in parentheses.

Table 2 Changes in number of prescribed drugs after admission to health service facilities for the elderly

	No. of subjects	On admission	After 1 month	After 3 months
Total	627	3.5 ± 2.5	2.9 ± 2.2*	3.0 ± 2.1*
Type of institution before admission				
Acute care hospital	115	4.8 ± 3.3†	4.2 ± 2.9*†	4.1 ± 2.7†
Outpatient	200	3.6 ± 2.3	2.8 ± 1.8*	2.9 ± 2.0
Special nursing home	24	3.3 ± 2.1	2.5 ± 1.7*	2.6 ± 1.8*
Sanitarium-type ward	188	3.1 ± 2.3	2.6 ± 1.9*	2.6 ± 1.9*
Health service facility	100	2.6 ± 1.8	2.4 ± 1.6*	2.5 ± 1.7*
Facility				
A	83	4.9 ± 3.4	4.6 ± 3.0	4.6 ± 2.4
B	80	4.2 ± 2.8	3.9 ± 2.4*	4.0 ± 2.5
C	39	4.1 ± 2.7	2.4 ± 1.7*	2.2 ± 1.4*
D	172	3.2 ± 1.9	2.4 ± 1.5*	2.4 ± 1.5*
E	253	3.0 ± 2.2	2.6 ± 1.9*	2.5 ± 1.9
Event				
No	517	3.5 ± 2.5	2.8 ± 2.0*	2.8 ± 2.1*
Yes	104	3.6 ± 2.7	3.4 ± 2.5***	3.7 ± 2.2**‡

*$P < 0.01$ versus on admission by paired *t*-test; **$P < 0.01$ versus after 1 month by paired *t*-test; †$P < 0.01$ versus other types of institution by Newman-Keuls' test; ***$P < 0.05$; ‡0.01 versus Event (–) by Newman-Keuls' test.
Data are expressed as mean ± SD.

after admission ($P < 0.01$ versus on admission), but did not show an additional reduction 3 months after admission. A significant reduction was seen at 1 month irrespective of the type of institution that had managed the patients before admission, although the number of drugs on admission and the degree of reduction differed between the types of institutions. However, there was a large variation in the reduction of prescribed drugs between the facilities, presumably due to differences in the overall philosophy of the attending physicians and the disease and/or functional status of the patients. Patients with and without events during a 3-month period were analyzed separately (Table 2). They were taking a comparable number of medications on admission. The number of drugs in the patients with events was not significantly decreased at 1 month, and was rather increased at 3 months after admission because in many cases additional drugs were prescribed for treatment of events.

Discontinued drugs and inappropriate prescribing

Categorized by therapeutic class, discontinuation was frequent with neuropsychologic (121 cases), gastrointestinal (116 cases) and cardiovascular (94 cases) drugs, followed by metabolic/endocrine drugs (36 cases). Anti-ulcer drugs (44 cases) including H2 blockers and prostaglandin analogs, antipsychotics (35 cases), antihypertensives (33 cases) including calcium channel blockers, β blockers and angiotensin converting enzyme inhibitors, hypnotics (31 cases), laxatives (31 cases) and non-steroidal anti-inflammatory drugs (22 cases) were frequently withdrawn.

On admission, inappropriate prescribing was seen in 58 patients (10.0% of 581 patients taking drugs). Ticlopidine, digoxin at more than 0.125 mg/day and oxybutynin were prescribed in five or more cases (Table 3). Inappropriately prescribed medications were reduced by 33% 1 month after admission, and did not change

Table 3 Number of inappropriately prescribed drugs on admission and 1 month after admission

Medication	On admission	After 1 month
Ticlopidine	36	25
Digoxin†	11	8
Oxybutynin	5	4
Amitriptyline	4	2
Benzodiazepines‡	3	1
Disopyramide	1	1
Indomethacin	1	1
Total	61	41

†More than 0.125 mg/day; ‡Flurazepam, Chlordiazepoxide and Diazepam

thereafter (data not shown). The reduction was not restricted to specific drugs.

Events during admission

A total of 104 events were seen in 16.7% of the patients during a 3-month admission period. Frequent events (nine cases or more) were new occurrences or worsening of psychological disorders (14 cases); gastrointestinal symptoms (12 cases); respiratory problems, including aspiration, pneumonia and respiratory failure (10 cases); pyrexia and infection other than pneumonia (10 cases); and falls and fractures (nine cases).

Five cases of ADWEs were found in 2.2% of 230 patients with drug reduction. These included three cases of confusion following discontinuation of psychotropic drugs, a case of depression following discontinuation of antidepressants and a case of hyperglycemia following discontinuation of a sulfonylurea.

Subgroups analyses were performed to examine the bias effect on events. The rates of events by type of institutions before admission were 24.5% in the subjects from acute care hospitals, 18.1% in those from outpatient clinics (not significant compared to other groups) and 13.1% in those from other types of institutions ($P < 0.05$ versus the subjects from acute care hospitals). Specific types of events were not related to the higher rate of events in the subjects from acute care hospitals, suggesting that unstable conditions of these patients may play a role. Of five cases with ADWEs, three were found in the subjects from outpatient clinics, one from special nursing homes and one from sanitarium-type wards. Thus, it is likely that possible non-compliance in outpatients or types of institutions before admission did not influence the principal results concerning ADWEs.

The subjects in facilities A and B (Table 2), in which significant drug reduction was not observed, showed a higher rate of events than those in other facilities (28.1% versus 12.9%, $p < 0.001$). This result indicates that adverse drug reactions associated with polypharmacy would have been included in these events, or additional drugs would have been prescribed for treatment of events, although no specific type of events was noted regarding the difference between the facilities. No ADWEs were found in the subjects in facilities A and B, presumably relating to the continuation of medications.

Discussion

The present study showed that the number of prescribed drugs was significantly decreased within 1 month after admission to health service facilities for the elderly. Discontinuation was not limited to inappropriate prescribing, but a larger proportion of inappropriately prescribed medications were discontinued compared to the total reduction of prescribed drugs (33% versus 17%). ADWEs were not frequent, being found in only 2.2% of the patients with drug reduction, while unrelated events occurred in 16.7% of the total patients.

Reflecting on the high incidence of polypharmacy and adverse drug reactions in elderly patients,[1–3,14] the principal finding of the present study that prescribed drugs can be reduced safely in frail elderly patients provides important information on pharmacotherapy. Every physician may make an effort to prescribe the minimum number of drugs, but a patient's long history of illness results in the accumulation of prescribed drugs together with the uncertain efficacy of the drugs. Consequently, the necessity of each medication should be reviewed regularly according to evidence-based medicine and criteria for inappropriate prescribing.[5,6] There is a great opportunity to reconsider prescriptions when attending physicians and/or the insurance system change, as was the case with the present study.

The number of prescribed drugs on admission in this study was smaller than that found in the geriatric ward of our university hospital and that found in residential care/assisted living facilities in the USA.[2,9] This may be because nearly half of the subjects were admitted from long-term care hospitals or facilities, and thus, prescribed drugs had already been restricted. In fact, patients from acute care hospitals were taking more drugs than those from other types of institution. It is interesting that an older age was associated with a smaller number of prescribed drugs, and this did not change when the data were analyzed according to the type of institution from which the patients had come (data not shown). This finding is inconsistent with previous observations in hospitalized or community-dwelling patients,[2,14,15] but is reasonable to prevent non-compliance and adverse drug reactions. At the same time, however, the age-related decrease in medications may involve possible discrimination towards very old people. The smaller number of prescribed drugs in women and discontinuation of medications after

admission in this study are inconsistent with previous reports,[16,17] and may imply age and sex discrimination, although discontinuation seemed successful in this study. Thus, the discrimination issue should also be taken into consideration concerning pharmacotherapy in older people.

In the present study, ADWEs were fewer than the previously reported study in which 26% of cases of discontinuation led to ADWEs in elderly outpatients during a 4-month period.[10] One of the reasons that ADWEs were rare in the present study might be that the rate of reduction was relatively high for inappropriately prescribed medications, although most of the attending physicians did not know the criteria for inappropriate prescribing such as Beers' list.[5] Another reason is that consultant physicians or geriatricians made decisions on prescriptions, based on the disease and functional status of each patient. In fact, most of discontinued drugs were not on the list of inappropriate prescribing, implying that unnecessary drugs had been prescribed before admission to long-term care facilities. In addition, it is possible that we missed ADWEs that progressed very slowly and manifested after the follow-up period of 3 months. We also failed to address the effect of prophylactic medications such as antiplatelet and lipid-lowering agents.

It should be kept in mind that the present results were obtained in a frail elderly population admitted to long-term care facilities where most of the subjects were in a stable state with chronic illness.[12] However, as a model to investigate the effect of drug reduction in elderly people, the present findings will add new insight into pharmacotherapy in the elderly, and should be confirmed in different settings such as hospitals and outpatient clinics. Obviously, medications for acute illness should neither be decreased, nor should physicians hesitate to initiate them even in very old patients, and in fact, prescribed drugs were increased in the patients with events during admission in this study. To safely apply the findings of the present study to clinical practice, knowledge of the criteria for inappropriate prescribing should be widely distributed, and blanket discontinuation of drugs must be avoided. In the present study, we used the Beers' criteria to determine inappropriately prescribed medications because corresponding criteria do not exist in Japan.[5] Consequently, we failed to check many inappropriate drugs that are used in Japan but are not on the Beers' list or sold in the USA. Future investigation using the Japanese criteria for inappropriate prescribing, which the Japan Geriatrics Society is going to establish, will add more information. In Japan, the fixed payment insurance system has begun to cover elderly patients, with the expansion of the elderly population and medical expenses. Therefore, it is essential to establish an effective and safe way to refine the use of medication in elderly people in terms of prevention of adverse drug reactions and ageism.

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References

1 Hanlon JT, Shimp LA, Semla TP. Recent advances in geriatrics: drug-related problems in the elderly. *Ann Pharmacother* 2000; **34**: 360–365.
2 Akishita M, Toba K, Nagano K, Ouchi Y. Adverse drug reactions in older people with dementia. *J Am Geriatr Soc* 2002; **50**: 400–401.
3 Rothschild JM, Bates DW, Leape LL. Preventable medical injuries in older patients. *Arch Intern Med* 2000; **160**: 2717–2728.
4 Phillips DP, Christenfeld N, Glynn LM. Increase in US medication-error deaths between 1983 and 1993. *Lancet* 1998; **351**: 643–644.
5 Beers MH. Explicit criteria for determining potentially inappropriate medication use by the elderly. An update. *Arch Intern Med* 1997; **157**: 1531–1536.
6 Hanlon JT, Schmader KE, Samsa GP *et al.* A method for assessing drug therapy appropriateness. *J Clin Epidemiol* 1992; **45**: 1045–1051.
7 Spore DL, Mor V, Larrat P, Hawes C, Hiris J. Inappropriate drug prescriptions for elderly residents of board and care facilities. *Am J Public Health* 1997; **87**: 404–409.
8 Hanlon JT, Schmader KE, Boult C *et al.* Use of inappropriate prescription drugs by older people. *J Am Geriatr Soc* 2002; **50**: 26–34.
9 Sloane PD, Zimmerman S, Brown LC, Ives TJ, Walsh JF. Inappropriate medication prescribing in residential care/assisted living facilities. *J Am Geriatr Soc* 2002; **50**: 1001–1011.
10 Graves T, Hanlon JT, Schmader KE *et al.* Adverse events after discontinuing medications in elderly outpatients. *Arch Intern Med* 1997; **157**: 2205–2210.
11 Hanlon JT, Weinberger M, Samsa GP *et al.* A randomized, controlled trial of a clinical pharmacist intervention to improve inappropriate prescribing in elderly outpatients with polypharmacy. *Am J Med* 1996; **100**: 428–437.
12 Hirakawa Y, Masuda Y, Uemura K *et al.* Current admission policies of long-term care facilities in Japan. *Geriatrics Gerontol Int* 2003; **3**: 73–78.
13 Naranjo CA, Busto U, Sellers EM *et al.* A method for estimating the probability of adverse drug reactions. *Clin Pharmacol Ther* 1981; **30**: 239–245.
14 Stewart RB, Cooper JW. Polypharmacy in the aged. Practical solutions. *Drugs Aging* 1994; **4**: 449–461.
15 Linjakumpu T, Hartikainen S, Klaukka T, Veijola J, Kivela SL, Isoaho R. Use of medications and polypharmacy are increasing among the elderly. *J Clin Epidemiol* 2002; **55**: 809–817.
16 Martin I, Hall J, Gardner T. Prescribing for patients aged 65 years and over in New Zealand general practice. *N Z Med J* 2002; **115**: U221.
17 Koopmans RT, van der Borgh JP, Bor JH, Hekster YA. Increase in drug use after admission to Dutch nursing homes. *Pharm World Sci* 2003; **25**: 30–34.

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Interrelationship between non-invasive measurements of atherosclerosis: flow-mediated dilation of brachial artery, carotid intima-media thickness and pulse wave velocity

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Abstract

Flow-mediated dilation (FMD) of the brachial artery, carotid intima-media thickness (IMT) and pulse wave velocity (PWV) have been shown to be good surrogate markers of clinical atherosclerosis. We determined the interrelation between these measurements, and examined whether their combination would be of clinical significance. One hundred and thirty-five consecutive subjects (79 women/56 men) were enrolled, including 110 patients with risk factors for atherosclerosis, and 33 patients with atherosclerotic disease such as coronary heart disease, stroke or arteriosclerosis obliterans. IMT and plaque formation of the carotid artery and FMD of the brachial artery were assessed using ultrasonography. Brachial-ankle PWV (baPWV) was measured using an automated device (form ABI/PWV, Colin). Age, FMD, IMT and PWV were significantly correlated with each other. Multivariate analysis revealed an independent correlation between the parameters except for FMD, and all four parameters were independently correlated with each other in subjects <70 years. Next, we classified the subjects by tertile according to the values of FMD, IMT and PWV. Each of the worst tertiles was associated with a higher prevalence of atherosclerotic disease and carotid plaques compared to the other tertiles. Moreover, subjects with the worst tertiles of all three measurements had a markedly higher prevalence of atherosclerotic disease and carotid plaques. These results suggest that FMD, IMT and PWV are related to each other, but the combination of these measurements will be of stronger clinical relevance.

Keywords: Arteriosclerosis; Endothelium; Arterial stiffness; Elasticity

1. Introduction

A number of methods have been applied for the non-invasive assessment of cardiovascular risks. These include flow-mediated dilation (FMD) of the brachial artery, pulse wave velocity (PWV) and carotid intima-media thickness (IMT). FMD is known to be endothelium-dependent and can be measured during reactive hyperemia using high-resolution ultrasound [1,2]. Measurement of IMT also employs B-mode ultrasonography, which can detect morphological change of the carotid artery, consisting of both an intimal atherosclerotic process and medial hypertrophy [3]. PWV reflects arterial distensibility and can be measured by pressure or volume pulse wave analysis using a transducer [4]. These three methods have been widely used in clinical settings because they are shown to be good surrogate markers of clinical atherosclerosis [1–4]. Impairment of these indices is associated with coronary artery disease or cerebrovascular disease. Also, in patients with atherosclerosis risk factors such as hypertension, hyperlipidemia and diabetes mellitus, each of these three indices is impaired and can be ameliorated by treatment [1–4].

Several studies have demonstrated a significant correlation between aortic PWV and carotid IMT [5,6]. We have previously shown that FMD was negatively correlated with IMT [7]. However, little data have been published on the interrelation of these three measurements. In addition, whether their combination is clinically significant and useful has not been elucidated.

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In the present study, we demonstrated that decreased FMD in the brachial artery was related to increased brachial-ankle and heart-carotid PWV (hcPWV) as well as to increased carotid IMT. Furthermore, we showed that the combination of these three measurements was useful in predicting the presence of atherosclerotic disease.

2. Methods

2.1. Subjects

One hundred and thirty five consecutive subjects were enrolled in this study. The characteristics of the study subjects are shown in Table 1. They included 25 healthy volunteers, 110 patients with atherosclerosis risk factors such as hypertension, hyperlipidemia or diabetes mellitus, and 33 patients with atherosclerotic disease such as coronary heart disease, stroke or arteriosclerosis obliterans. They were recruited from outpatients, inpatients, and community volunteers. A history was taken, and physical examination and laboratory tests were performed in all subjects. Atherosclerotic disease was defined as follows: (1) stroke, confirmed by brain computed tomography and a documented history; (2) coronary artery disease, confirmed by coronary arteriography and/or a documented history of myocardial infarction within 5 years; (3) a clinical diagnosis of arteriosclerosis obliterans. Exclusion criteria for this study included clinical manifestations of venous thromboembolism, liver disorder and history of cancer(s). Each subject gave written informed consent before enrollment in this study, after receiving a thorough explanation of the study design and protocol.

Table 1
Clinical characteristics of subjects and classification by tertile of atherosclerotic measures

Men/women	56/79 (n = 135)
Age (years)	62 ± 16
No risk factor, n (%)	25 (19)
Hypertension, n (%)	51 (38)
Hyperlipidemia, n (%)	64 (47)
Diabetes mellitus, n (%)	35 (26)
Current smoker, n (%)	20 (15)
Atherosclerotic disease	
Stroke, n (%)	21 (16)
Coronary artery disease, n (%)	10 (7)
Arteriosclerosis obliterans, n (%)	5 (4)
Total atherosclerotic disease, n (%)	33 (24)
Atherosclerotic measurements	
FMD (%)	
Tertile 1 (≥4.0)	6.0 ± 1.7
Tertile 2 (≥1.9, <4.0)	2.9 ± 0.7
Tertile 3 (<1.9)	0.8 ± 0.9
IMT (mm)	
Tertile 1 (<0.75)	0.63 ± 0.08
Tertile 2 (≥0.75, <1.02)	0.87 ± 0.11
Tertile 3 (≥1.02)	1.17 ± 0.19
baPWV (m/s)	
Tertile 1 (<14.34)	12.37 ± 1.40
Tertile 2 (≥14.34, <18.80)	16.18 ± 1.26
Tertile 3 (≥18.80)	22.88 ± 3.99
hcPWV (m/s)	
Tertile 1 (<7.40)	6.00 ± 1.04
Tertile 2 (≥7.40, <9.80)	8.53 ± 0.73
Tertile 3 (≥9.80)	11.56 ± 1.65

Age and atherosclerotic measurements by tertile are expressed as mean ± S.D. FMD, percent flow-mediated dilation of brachial artery; IMT, intima-media thickness of common carotid artery; baPWV and hcPWV, brachial-ankle and heart-carotid pulse wave velocity, respectively.

2.2. Measurement of carotid IMT

Ultrasound measurements of IMT of the common carotid artery were performed as previously described [7] by an examiner who was unaware of the subjects' clinical background. The same examiner performed the measurements of IMT, FMD and PWV throughout the study. Briefly, IMT was measured from high-resolution, two-dimensional ultrasound images obtained with an ultrasound machine (PowerVision 6000, Toshiba) with a 7.5 MHz linear-array transducer. The subject reclined on the examination table for 15 min before the initial carotid ultrasound scanning, in a quiet, temperature-controlled (22–24 °C) room. This measurement was applied to the far wall of the right carotid artery. With the subject in the supine position, an ultrasound probe was applied longitudinally to the surface of the skin on the right side of the neck. Longitudinal scanning was performed from the common carotid artery to the bifurcation of the common carotid artery. Scanning was performed in the optimal position. An ECG monitor integrated with the ultrasound machine was also applied. The ultrasound images were recorded on S-VHS videotape. After the bifurcation of the common carotid artery was confirmed, IMT was measured from the B-mode scan with electronic calipers to within 10 mm proximal to the bifurcation. Four points were measured in one scan, which was synchronized with the R-wave peaks on the ECG to avoid possible errors resulting from variable arterial compliance. Two scans were performed for each study subject. Mean IMT was calculated from eight points. The variability of the ultrasound measurements of IMT was studied by performing five measurements over 1 month in 12 volunteers. The intraobserver coefficient of variation for measurement of IMT was 4.2 ± 0.7%.

The presence of plaque(s) in the right carotid artery was assessed by evaluating the ultrasound images of the common and internal carotid artery, and the bifurcation. A plaque was defined as a focal widening relative to adjacent segments, with protrusion into the lumen composed of either only calcified deposits or a combination of calcified and non-calcified material [5].

2.3. Measurement of FMD of brachial artery

Studies of FMD were performed according to the method described previously [7]. The diameter of the artery was

measured on high-resolution, two-dimensional ultrasound images obtained with an ultrasound machine (PowerVision 6000, Toshiba) with a 7.5 MHz linear-array transducer. Machine operating parameters were kept constant during each study.

The right brachial artery was scanned over a longitudinal section, 3–5 cm above the right elbow. Depth and gain settings were optimized to identify the lumen-to-vessel wall interface. An ECG monitor integrated with the ultrasound machine was also applied. When an adequate image was obtained, the surface of the skin was marked, and the arm was kept in the same position throughout the study. A pneumatic tourniquet placed around the forearm distal to the target artery was inflated to a pressure of 250 mmHg, and inflation was held for 5 min. Increased flow was then induced by sudden cuff deflation. A second scan was performed continuously for 60 s before and 120 s after cuff deflation. The ultrasound images were recorded on S-VHS videotape. The diameter of the brachial artery was measured from the anterior to the posterior interface between the media and adventitia ("m line") at a fixed distance. The mean diameter was calculated from four cardiac cycles synchronized with the R-wave peaks on the ECG. All measurements were made at end-diastole to avoid possible errors resulting from variable arterial compliance. Maximal vasodilatation was observed 45–60 s after cuff release. The change in diameter caused by FMD was expressed as the percent change relative to that in the initial resting scan. The velocity profile of blood flow was simultaneously recorded. Mean flow velocity was calculated by measuring the area under this velocity profile curve. Blood flow (in milliliters per minute) was then calculated by multiplying the cross-sectional area of the brachial artery, which was based on the diameter, and the mean flow velocity. Changes in diameter of 0.1–0.2 mm can be detected accurately with this method [7]. The intraobserver coefficient of variation for measurements of FMD was 5.8 ± 0.3% (10 measurements in five subjects).

2.4. Measurement of PWV

PWV measurements were performed subsequently to FMD measurements, with the subject in the supine position. PWV was measured using an automated device (form PWV/ABI, Colin Co. Ltd., Komaki, Japan) as previously reported [8,9]. The device records PWV, blood pressure, ECG and heart sounds simultaneously. ECG electrodes were placed on both wrists, and a heart sound microphone was placed on the left sternal border.

The cuffs to measure brachial-ankle PWV (baPWV) were wrapped around both upper arms and ankles, and connected to a plethysmographic sensor that determines volume pulse form. Volume waveforms were stored for a sampling time of 10 s with automatic gain analysis and quality adjustment. The time delay from the ascending point of the right brachial waveform to the ascending point of each ankle waveform (ΔTba) was determined. The distance of each segment (Lb-La) is automatically calculated based on the patient's height and was derived from statistical studies. Then, baPWV was calculated using the formula; baPWV = La-Lb/ΔTba. The average of left and right baPWV in each subject was used for the analysis. To measure heart-carotid PWV, a multi-element carotid tonometry sensor with a holder arm was placed around the neck [9]. The time delay from the foot of the second sound of the phonocardiogram to the dicrotic notch of the carotid waveform (ΔThc) was calculated. The distance from the heart to the carotid artery (Lc) was deduced based on the patient's height. Then, hcPWV was calculated using the formula; hcPWV = Lc/ΔThc. The intraobserver coefficients of variation for measurements of baPWV and hcPWV were 2.0 ± 0.5 and 4.5 ± 1.4%, respectively (five measurements in eight subjects).

2.5. Statistical analysis

All data in the text, tables, and figures are expressed as mean ± S.E.M. unless otherwise specified. Pearson's simple correlation coefficient between age, FMD, IMT and PWV was determined. Categorical difference was analyzed by Chi-squared test. Standardized regression coefficients from multiple regression analysis of baPWV in relation to age, FMD and IMT were analyzed. Logistic regression analysis was performed to evaluate the relation between the combination of FMD, IMT and PWV, and the prevalence of atherosclerotic disease and carotid plaques. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Correlation between age, FMD, IMT and PWV

Table 2 shows the Pearson correlation matrix between age, FMD, IMT, baPWV and hcPWV. All the parameters were significantly correlated with each other. However, each subject did not always belong to the same category by tertile of atherosclerotic measurements (Fig. 1); e.g., the subjects in tertile 3 of baPWV (baPWV ≥ 18.80) were widely distributed in the tertiles of FMD; conversely, the subjects in

Table 2
Pearson correlation matrix between age, FMD, IMT and PWV

	FMD	IMT	baPWV	hcPWV
Age	−0.592	0.567	0.662	0.478
FMD		−0.343	−0.493	−0.364
IMT			0.477	0.460
baPWV				0.392

FMD, percent flow-mediated dilation of brachial artery; IMT, intima-media thickness of common carotid artery; baPWV and hcPWV, brachial-ankle and heart-carotid pulse wave velocity, respectively. All correlation coefficients were statistically significant ($P < 0.0001$).

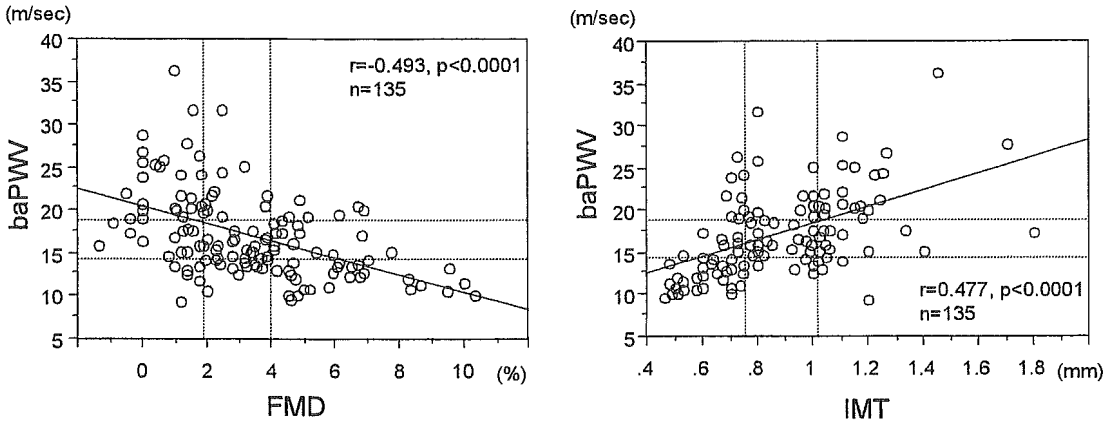

Fig. 1. Relations between brachial-ankle PWV (baPWV) and flow-mediated dilation (FMD) of brachial artery (left panel), and between baPWV and carotid intima-media thickness (IMT) (right panel). Solid lines and dotted lines indicate regression lines and tertile borders of each measurement, respectively.

tertile 3 of FMD (FMD $\leq$ 1.9) were widely distributed in the tertiles of baPWV.

Multiple regression analysis was performed with baPWV as a dependent variable and with age, mean arterial pressure of the right brachium, FMD and IMT as independent variables. As shown in Table 3, age, mean arterial pressure and IMT were independently related to baPWV. If the subjects <70 years (n = 89) were analyzed separately to diminish the effect of age, FMD as well as IMT, mean arterial pressure and age were independent determinants of baPWV. Multiple regression analysis with FMD or IMT as a dependent variable and analysis using hcPWV instead of baPWV showed comparable results.

3.2. Prevalence of atherosclerotic disease and carotid plaques in relation to FMD, IMT and PWV

We classified the subjects by tertile according to the values of FMD, IMT and baPWV (Table 1). Each of the worst tertiles, tertile 3, was associated with a higher prevalence of atherosclerotic disease and carotid plaques compared to the other tertiles. Atherosclerotic disease was found in 36, 40 and 39% of subjects in tertile 3 of FMD, IMT and baPWV, respectively, but was found in 18, 15 and 16% of subjects in the other tertiles of the corresponding parameter ($P < 0.05$ by Chi-squared test). Similarly, carotid plaques were found in 64, 70 and 69% of subjects in tertile 3 of FMD, IMT and baPWV, respectively, but in 34, 28 and 30% of subjects in the other tertiles of the corresponding parameter ($P < 0.01$ by Chi-squared test). These results suggest that each of the three measurements is comparably predictive of atherosclerotic disease and carotid plaques. As shown in Fig. 2, however, the subjects with the worst tertiles of all three measurements had a markedly higher prevalence of atherosclerotic disease and carotid plaques (67 and 89%, respectively). In logistic regression analysis unadjusted or adjusted for sex, hypertension, hyperlipidemia, diabetes and current smoking, the number of worst tertiles was signif-

Table 3
Multiple regression analysis with brachial-ankle PWV as dependent variable and age, mean arterial pressure (MAP), FMD and IMT as independent variables

	All subjects		Age <70 years	
	R	P-value	R	P-value
Age	0.406	0.000	0.245	0.022
MAP	0.261	0.000	0.215	0.009
FMD	−0.130	0.110	−0.212	0.025
IMT	0.169	0.027	0.336	0.000

R represents standardized regression coefficients. FMD, percent flow-mediated dilation of brachial artery; IMT, intima-media thickness of common carotid artery and PWV, brachial-ankle pulse wave velocity.

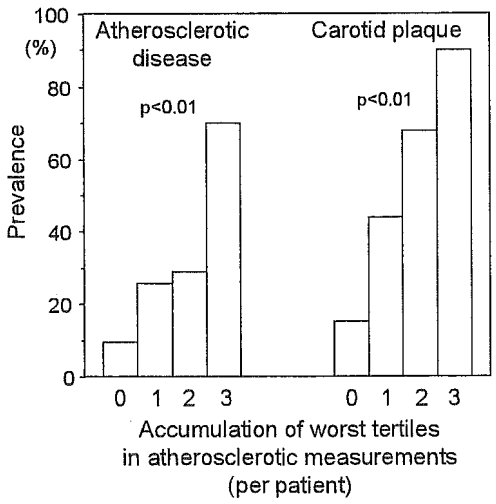

Fig. 2. Prevalence of atherosclerotic disease and carotid plaques according to the accumulation of worst tertiles in atherosclerotic measurements. Scores were recorded by counting how many measurements were in the worst tertiles in each patient. Atherosclerotic disease includes stroke, coronary artery disease and arteriosclerosis obliterans. Statistical analysis between the groups was performed by Chi-squared test and logistic regression analysis.

icantly related to the prevalence of atherosclerotic disease and carotid plaques; adjusted hazard ratio (95% confidence interval) by increment of the number of worst tertiles was 1.88 (1.13–3.13) for atherosclerotic disease and 3.37 (2.00–5.70) for carotid plaques. Accordingly, it is likely that the combination of FMD, IMT and PWV serves as a more accurate indicator of clinical atherosclerosis than any single measurement.

4. Discussion

Endothelial dysfunction is an early and potentially reversible event in atherogenesis [10], being detected as a decrease in FMD of the brachial artery [1,2]. An increase in PWV reflects arterial stiffening as a result of structural and functional changes of the vascular tree [4]. Recent reports have demonstrated that endothelial nitric oxide is also implicated in the regulation of PWV [11,12]. In contrast, carotid IMT quantitatively measures the arterial morphology consisting of intimal lesions and medial hypertrophy [3]. Consequently, brachial FMD, carotid IMT and brachial-ankle or heart-carotid PWV evaluates different aspects of atherosclerosis as well as different sites of the artery. Atherosclerosis, however, undergoes systemic progression and results in the worsening of these atherosclerotic parameters to some extent. Taken together, it is reasonable that FMD, IMT and PWV correlated with each other in this study. This result implies that each of the three measurements would be clinically useful in a cohort study investigating the effect of intervention therapy on outcomes.

When applying FMD, IMT and PWV to the clinical setting, a problem may exist concerning the variability between the three measurements on an individual basis. A good or bad result of a single measurement may mislead the clinical evaluation of a subject. An important issue is how these atherosclerotic parameters can predict the future occurrence of cardiovascular events, and should be addressed by prospective studies. Although each of FMD [13], IMT [14] and PWV [15] is reported to be predictive of cardiovascular events, the significance of their combination has not been determined. Alternatively, we used the existence of atherosclerotic disease and carotid plaques as surrogate atherosclerotic outcomes, and tested the hypothesis that the accumulation of inferior results would be associated with higher rates of these outcomes. Analysis by tertile showed that the combined evaluation of FMD, IMT and PWV highly detected the prevalence of atherosclerotic disease and carotid plaques compared to single assessment. Therefore, accuracy may be improved by combining these three measurements.

Combination of FMD, IMT and PWV may give reliable information on clinical or subclinical atherosclerosis, but bears the burden of time and manpower. Particularly, measurement of FMD requires more than half an hour, as well as skill for examination and measurement. Also, inter-observer or inter-institutional variation in FMD [2] may make it difficult to repeat measurements in a patient over years. In contrast, IMT and PWV seem less complicated in terms of examination time and procedure [3,4]. The automated device for the measurement of PWV used in the present study requires only a few minutes for the whole procedure [8]. Thus, PWV and/or IMT may be appropriate for the screening of patients with atherosclerosis risk factors and for large-scale studies. As a non-invasive assessment of endothelial function, however, FMD will remain the gold standard until alternative simple and objective methods are established. Because some subjects have endothelial dysfunction before developing atherosclerosis that can be measured by IMT and PWV, it is important to evaluate endothelial function as well.

This study contains some limitations in interpreting the data. Brachial-ankle PWV is not so familiar as conventional carotid-femoral and heart-ankle PWV, and its significance for the prediction of cardiovascular events has not been published. However, the validity and reliability of brachial-ankle PWV are defined using the same device as ours [8,16]. We also confirmed using the present samples that brachial-ankle PWV was correlated well with heart-ankle PWV ($r = 0.859$). Our device estimates the path length from the height of each subject based on the superficial measurements in a Japanese population, suggesting possible errors. However, use of the equation should not seriously harm the reliability of PWV measurements because Pearson's correlation coefficient between the estimated length and the actual surface measurement was higher than 0.9 (unpublished results). At the time of FMD measurement, we did not administer nitroglycerin to the study subjects in order to avoid adverse reactions. Accordingly, we could not separate endothelium-independent dilation from endothelium-dependent dilation. In addition, to collect a sufficient number of subjects with different stages of atherosclerosis, miscellaneous subjects were included such as healthy young volunteers and older patients with atherosclerotic disease. Most of the subjects had atherosclerosis risk factors and were taking some drugs for its treatment. Because these factors influence FMD, IMT and PWV [1–4], it is possible that the disease and/or medication may have affected the relationship between the measurements. Consequently, the results of the present study should be confirmed in subjects without medication and without risk factors. In addition, we used the presence of atherosclerotic disease and carotid plaques as surrogate atherosclerotic outcomes, and showed that the accumulation of inferior results was associated with higher rates of these outcomes. This should be confirmed by prospective studies examining the future occurrence of cardiovascular events.

In conclusion, FMD of the brachial artery, carotid IMT, and brachial-ankle and heart-carotid PWV were related to each other. Combination of the three methods was useful in predicting the burden of atherosclerosis, and is thus of clinical relevance.

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References

[1] Behrendt D, Ganz P. Endothelial function. From vascular biology to clinical applications. Am J Cardiol 2002;90:40L–8L.
[2] Raitakari OT, Celermajer DS. Flow-mediated dilatation. Br J Clin Pharmacol 2000;50:397–404.
[3] Simon A, Gariepy J, Chironi G, Megnien JL, Levenson J. Intima-media thickness: a new tool for diagnosis and treatment of cardiovascular risk. J Hypertens 2002;20:159–69.
[4] O'Rourke MF, Mancia G. Arterial stiffness. J Hypertens 1999;17:1–4.
[5] van Popele NM, Grobbee DE, Bots ML, Asmar R, Topouchian J, Reneman RS, et al. Association between arterial stiffness and atherosclerosis: the Rotterdam Study. Stroke 2001;32:454–60.
[6] Mackey RH, Sutton-Tyrrell K, Vaitkevicius PV, Sakkinen PA, Lyles MF, Spurgeon HA, et al. Correlates of aortic stiffness in elderly individuals: a subgroup of the Cardiovascular Health Study. Am J Hypertens 2002;15:16–23.
[7] Hashimoto M, Eto M, Akishita M, Kozaki K, Ako J, Iijima K, et al. Correlation between flow-mediated vasodilatation of the brachial artery and intima-media thickness in the carotid artery in men. Arterioscler Thromb Vasc Biol 1999;19:2795–800.
[8] Yamashina A, Tomiyama H, Takeda K, Tsuda H, Arai T, Hirose K, et al. Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement. Hypertens Res 2002;25:359–64.
[9] Narimatsu K, Takatani S, Ohmori K. A multi-element carotid tonometry sensor for non-invasive measurement of pulse wave velocity. Front Med Biol Eng 2001;11:45–58.
[10] Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362:801–9.
[11] Kinlay S, Creager MA, Fukumoto M, Hikita H, Fang JC, Selwyn AP, et al. Endothelium-derived nitric oxide regulates arterial elasticity in human arteries in vivo. Hypertension 2001;38:1049–53.
[12] Wilkinson IB, Qasem A, McEniery CM, Webb DJ, Avolio AP, Cockcroft JR. Nitric oxide regulates local arterial distensibility in vivo. Circulation 2002;105:213–7.
[13] Neunteufl T, Heher S, Katzenschlager R, Wolfl G, Kostner K, Maurer G, et al. Late prognostic value of flow-mediated dilation in the brachial artery of patients with chest pain. Am J Cardiol 2000;86:207–10.
[14] O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson Jr SK. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovasc Health Stud Collaborative Res Group N Engl J Med 1999;340:14–22.
[15] Boutouyrie P, Tropeano AI, Asmar R, Gautier I, Benetos A, Lacolley P, et al. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. Hypertension 2002;39:10–5.
[16] Munakata M, Ito N, Nunokawa T, Yoshinaga K. Utility of automated brachial ankle pulse wave velocity measurements in hypertensive patients. Am J Hypertens 2003;16:653–7.

Original Article

Reduced Endothelial Vasomotor Function and Enhanced Neointimal Formation after Vascular Injury in a Rat Model of Blood Pressure Lability

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Increased short-term blood pressure variability is known to be associated with hypertensive target organ damage. Sinoaortic denervation (SAD) induces a marked increase in blood pressure lability without affecting the average blood pressure level. The aim of this study was to investigate the effects of blood pressure lability on endothelial vasomotor function and neointimal formation after balloon injury in SAD rats. Direct long-term measurement of mean arterial pressure showed no significant difference in the average of mean arterial pressure between the SAD group and sham-operated control group. In contrast, the standard deviation of mean arterial pressure, as an index of blood pressure lability, was 3-fold greater in SAD rats. To study endothelial function, isometric tension of aortic rings was measured 4 weeks after SAD or sham operation. Endothelium-dependent vasorelaxation induced by acetylcholine was significantly reduced in the SAD group (20% reduction at maximum relaxation). Endothelium-independent vasorelaxation induced by sodium nitroprusside was similar in each group. Acetylcholine-induced NO release from aortic rings was significantly reduced in the SAD group. Next, we examined neointimal formation in carotid arteries in SAD and sham-operated rats at 2 weeks after balloon injury. The neointimal-to-medial area ratio in the SAD group was 50% higher than that in the sham-operated group. The percentage of proliferating cell nuclear antigen-positive cells in the intima was significantly higher in the SAD group. These findings suggest that increased blood pressure lability, independently of average blood pressure level, impairs endothelial function by inhibiting NO production, enhances neointimal formation after balloon injury, and may thereby contribute to atherogenesis. (*Hypertens Res* 2003; 26: 991–998)

Key Words: blood pressure lability, sinoaortic denervation, endothelial vasomotor function, neointimal formation, growth factor

Introduction

It has been well established that hypertension is one of the most important risk factors for cardiovascular disease (*1*). The goal of treatment of hypertensive patients is not only to lower blood pressure but also to prevent cardiovascular events. In recent years, 24-h ambulatory blood pressure monitoring has become extensively used in clinical practice, and lines of evidence supporting its clinical value to predict hypertensive target organ damage and cardiovascular events have accumulated (*2*, *3*). It has been reported that the aver-

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age 24-h blood pressure level is significantly associated with cardiovascular damage, whereas the office blood pressure level is not (*2*). It has also been reported that an absence of nocturnal blood pressure fall (non-dipper phenomenon) is associated with cerebrovascular damage (*4*), whereas blood pressure lability, which is defined as increased short-term blood pressure variability and is a feature of hypertension in the elderly (*5*, *6*), has been reported to be associated with hypertensive target organ damage (*7*, *8*). However, the causal relationship between blood pressure lability and hypertensive organ damage remains unknown. To clarify this point, the direct effects of blood pressure lability on the vascular wall independent of the average blood pressure level should be investigated.

For this purpose, we selected sinoaortic-denervated rats as an animal model of blood pressure lability. The arterial baroreflex plays a pivotal role in the neural regulation of blood pressure. The afferent fibers of this negative feedback reflex arise from the carotid sinuses and aortic arch. Denervation of the afferent fibers of the baroreflex (sinoaortic denervation; SAD) was originally reported to induce neurogenic hypertension in rats (*9*). However, several studies using long-term continuous blood pressure measurement in the conscious state have shown that SAD does not affect the average blood pressure level and induces a marked increase in blood pressure variability (*10*, *11*).

The aim of this study was thus to elucidate the effects of blood pressure lability on vascular function and remodeling in SAD rats. In the first experiment, we examined the isometric tension of isolated aortic rings to investigate endothelial vasomotor function. In the second experiment, the degree of neointimal formation after balloon injury of the carotid artery was analyzed.

Methods

SAD Procedure

Ten-week-old male Wistar rats (Japan Clea, Tokyo, Japan) were used in this study. They were kept individually in stainless steel cages in a room where lighting was controlled (12 h on, 12 h off) and room temperature was maintained at around 22°C. They were given a standard diet and water *ad libitum*. The experimental protocols were approved by the Animal Research Committee of the University of Tokyo. SAD was performed according to the method of Krieger with slight modification (*9*). Briefly, rats were anesthetized with a single intraperitoneal injection of pentobarbital (50 mg/kg). A midline neck incision was made and the sternocleidomastoid muscle was retracted laterally. The cervical sympathetic trunks, the superior laryngeal nerves and the aortic depressor nerves were bilaterally isolated and resected. The carotid sinuses were stripped of all connective tissue and treated with 10% phenol. After this procedure was completed, the incision was sutured. Sham-operated rats underwent the same procedure except that afferent fibers of the baroreflex were not denervated.

Continuous Mean Arterial Pressure (MAP) Recording

The femoral artery and vein were catheterized with polyethylene tubes (PE-50 and PE-20, respectively; Becton Dickinson, Parsippany, USA). To confirm denervation, baroreflex sensitivity was evaluated after intravenous bolus injection of phenylephrine hydrochloride (6 mg/kg). MAP and heart rate were recorded continuously *via* the arterial catheter in the conscious state over a 3-h period (2 to 5 PM). Continuous recording was performed from 1 to 3 days and again at 4 weeks after SAD operation. Data were sampled every 20 s with an analog-to-digital converter and stored on a Macintosh computer. The average value and standard deviation of MAP were calculated. The standard deviation of MAP was used as an index of blood pressure lability.

Vascular Reactivity of Aortic Rings

Four weeks after SAD or sham operation, the vasoreactivity of isolated aortic rings was evaluated as described previously (*12*, *13*) with slight modification (Fig. 1A). The rats were killed with a lethal dose of anesthetic, and the thoracic aorta was removed. The aorta was dissected free of adherent fat and connective tissue and cut into rings (3 mm in length). The aortic ring was placed horizontally between L-shaped stainless wires in an organ bath chamber filled with oxygenated (95% O_2, 5% CO_2) balanced salt solution (37°C, pH 7.4) of the following composition: NaCl 112 mmol/l, KCl 4.7 mmol/l, $CaCl_2$ 0.9 mmol/l, $MgCl_2$ 1.2 mmol/l, $NaHCO_3$ 25 mmol/l, KH_2PO_4 1.2 mmol/l, glucose 11 mmol/l, and EDTA 0.026 mmol/l. The aortic ring was connected to a force transducer for isometric tension recording. After a 60-min equilibration period, the ring was gradually stretched to an optimal resting tension of 2 g. Then the ring was contracted by addition of KCl (60 mmol/l) and washed with fresh balanced salt solution. The aortic ring was allowed to equilibrate for 30 min before the experiment.

To test the vasorelaxing reactivity, the aortic ring was precontracted with norepinephrine (100 nmol/l) and then relaxed by cumulative addition of an endothelium-dependent vasodilator, acetylcholine (1 nmol/l to 10 μmol/l), or an endothelium-independent vasodilator, sodium nitroprusside (1 nmol/l to 10 μmol/l). Relaxation was expressed as a percentage of the tension induced by norepinephrine.

NO Production by Aortic Rings

Four weeks after SAD or sham operation, the isolated aortic ring was opened longitudinally and incubated in 1 ml balanced salt solution containing acetylcholine (1 μmol/l) and L-arginine (100 μmol/l) at 37°C. After 20 or 60 min of incubation, a sample of the solution was collected to analyze ni-

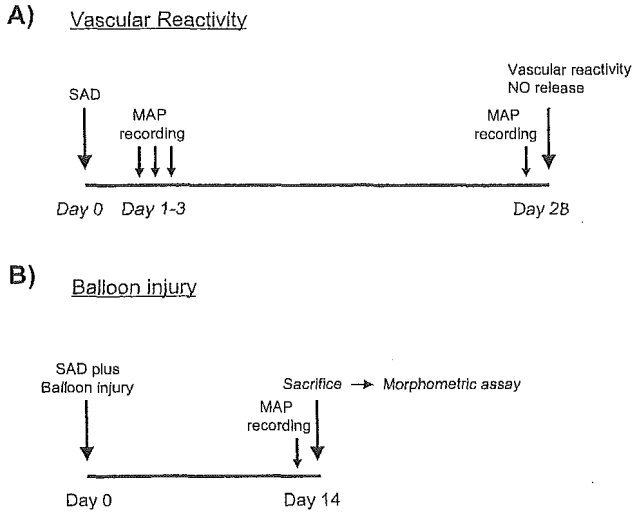

Fig. 1. *Experimental protocols for the vascular reactivity (A) and balloon injury (B). SAD, sinoaortic denervation; MAP, mean arterial pressure.*

trite/nitrate concentration as a measure of NO release. After incubation, the wet weight of the aortic ring was measured. Nitrite/nitrate concentration in the solution was determined using an automatic analyzer that employs automated flow injection analysis (TCI-NOX5000S; Tokyo Kasei Kogyo, Tokyo, Japan) as described previously (*14*). Briefly, the sample (0.1 ml) was diluted with 0.4 ml distilled water, and 0.3 ml 0.3 eq/l NaOH was added. After incubation for 5 min at room temperature, 0.3 ml 5% (w/v) $ZnSO_4$ was added, and the sample was incubated for an additional 5 min. The mixture was centrifuged at 2,800×*g* for 10 min and the supernatant was applied to the analyzer. The nitrite reacted with Griess reagent to form a purple azo compound. The absorbance at 540 nm was measured. The nitrate concentration was determined by means of reduction to nitrite through a copperized cadmium reduction column.

Balloon Injury in the Carotid Artery

In a separate experiment, balloon injury of the carotid artery and SAD (or sham operation) were performed simultaneously in order to investigate the effects of blood pressure lability on neointimal formation (Fig. 1B). Balloon injury was performed as described previously (*15*). Briefly, a 2 French Fogarty arterial embolectomy balloon catheter (Baxter, Irvine, USA) was inserted into the left common carotid artery through the left external carotid artery and advanced to the aortic arch. The balloon was inflated with saline and gradually withdrawn to the carotid bifurcation. This procedure was repeated three times, and then the balloon catheter was removed and the left external carotid artery was ligated.

The rats were killed with a lethal dose of anesthetic 14 days after balloon injury. The carotid artery was perfused and fixed with 4% paraformaldehyde at 100 mmHg and then removed for histological examination.

Morphometric Assay

Morphometric assay was performed as described previously (*16*). The middle third of the fixed carotid artery was embedded in paraffin, and multiple 5-μm cross sections were stained with hematoxylin and eosin or elastica van Gieson. After the section was photographed, the image was scanned and analyzed using NIH Image software. Then, the area of the neointima and of the media and the neointimal-to-medial area ratio were calculated. Three portions of each sample were analyzed, and the mean values were subjected to statistical analysis.

In Vivo Cell Proliferation Assay

Immunohistochemical staining of sections was carried out by the streptavidin-biotin-peroxidase method as described previously (*17*). We used anti-proliferating cell nuclear antigen (PCNA) antibody (PC10, 10 μg/ml; Boehringer Mannheim Biochemica, Mannheim, Germany) and normal mouse IgG (10 μg/ml) as the primary antibody. Specifically bound antibody was visualized by immersing the section in a substrate solution of 3,3-diaminobenzine (Vector Laboratories, Burlingame, USA). The number of positively stained nuclei within the neointima was counted, and the ratio of the number of positive cells to the total number of cells, expressed as a percentage, was calculated as an index of proliferation. Three vision fields of each sample were analyzed, and the mean values were subjected to statistical analysis.

Data Analysis

All values were expressed as the mean±SEM. In the vascular reactivity experiment, the concentration of substance (expressed as $-\log$ mol/l) evoking 50% relaxation (pD_2) and the maximum relaxation response (expressed as a percentage of precontraction response) were calculated. Unpaired Student's *t*-test was used for statistical analysis of differences between the two groups. Values of $p<0.05$ were considered to indicate statistical significance.

Results

Baroreflex sensitivity was assessed 3 days after SAD in terms of the reflex reduction in heart rate occurring in response to phenylephrine-induced increase in MAP level. When expressed as the ratio of heart rate decrease to MAP increase, the reflex response in SAD rats ($n=10$) was significantly diminished compared with that in sham-operated rats ($n=6$) (-1.09 ± 0.23 *vs.* -4.43 ± 0.96 bpm/mmHg, respectively, $p<0.01$).

The typical heart rate and MAP recordings of a SAD rat and a sham-operated rat on day 3 are shown in Fig. 2. A