Conclusions. A founder V592fs/8 mutation in the MyBPC gene was identified in 15 of 94 Japanese families with HCM. Elderly patients in particular may evolve to the "end-stage" HCM, characterized by LV systolic dysfunction, cavity dilation, and irreversible heart failure. Although the manifestation is late in onset, the clinical course in patients with this mutation is not benign in the long run with progressive LV remodeling with advancing age.

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Common carotid intima–media thickness is predictive of all-cause and cardiovascular mortality in elderly community-dwelling people: Longitudinal Investigation for the Longevity and Aging in Hokkaido County (LILAC) study

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Abstract

Several cohort studies have examined the association of carotid intima–media thickness (IMT) with the risk of stroke or myocardial infarction in apparently healthy persons. We investigated the predictive value of IMT of cardiovascular mortality in elderly community-dwelling people, beyond the prediction provided by age and MMSE, assessed by means of a multivariate Cox model. Carotid IMT and plaque were evaluated bilaterally with ultrasonography in 298 people older than 75 years (120 men and 178 women, average age: 79.6 years). The LILAC study started on July 25, 2000. Consultations were repeated every year. The follow-up ended on November 30, 2004. During the mean follow-up span of 1152 days, 30 subjects (21 men and nine women) died. Nine deaths were attributable to cardiovascular causes (myocardial infarction: two men and three women; stroke: two men and two women). The age- and MMSE-adjusted relative risk (RR) and 95% confidence interval (95% CI) of developing all-cause mortality was assessed. A 0.3 mm increase in left IMT was associated with a RR of predicted 1.647 (1.075–2.524), and a similar increase in right IMT with a RR of 3.327 (1.429–7.746). For cardiovascular mortality, the corresponding RR values were 2.351 (1.029–5.372) and 2.890 (1.059–7.891), respectively. Carotid IMT assessed by ultrasonography is positively associated with an increased risk of all-cause and cardiovascular death in elderly community-dwelling people.

Keywords: Carotid intima–media thickness; All-cause mortality; Cardiovascular mortality; Cognitive function; Elderly community-dwelling people

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1. Introduction

Several prospective population-based studies documented that carotid intima–media thickness (IMT) was positively associated with stroke and myocardial infarction in highly selected patients. Carotid IMT has also been shown to predict fatal coronary death and fatal stroke in elderly people [1–5]. It is now considered to constitute a surrogate marker of cardiovascular morbidity and mortality risk not only in patients, but also quite generally in young, middle-aged and elderly populations.

In 2000, we began a community-based study to Longitudinally Investigate the Longevity and Aging in Hokkaido County (LILAC), and to evaluate the population's neurocardiological function. Our goal is the prevention of cardiovascular events, including stroke and myocardial ischemic events, to prevent the decline in cognitive function of the elderly in a community dwelling. In this investigation, we estimated the ability of carotid IMT to predict all-cause and cardiovascular mortality in an elderly population. We already found that the cognitive function, estimated by MMSE and HDS-R, and age statistically significantly predicted cardiovascular death in this population. Herein, we examine the predictive value of all-cause and cardiovascular mortality offered by the carotid IMT, beyond the prediction provided by age and MMSE, as assessed by means of a multivariate Cox model.

2. Methods

2.1. Subjects and LILAC study design

We examined 298 subjects (120 men and 178 women) older than 75 years (average age: 79.6 years). BP was measured at the beginning of the study in a sitting position, and the brachial-ankle PWV (baPWV) was measured between the right arm and ankle in a supine position, using an ABI/Form instrument (Nippon Colin Co., Ltd., Komaki, Japan). The baPWV was measured using a volume-plethysmographic method. baPWV was measured in duplicate after at least a 5-min rest. Only baPWV measurements from participants with normal ankle/brachial pressure index (ABI) values (>0.90) were considered. The maximal value among the four readings was used for analysis. An echocardiogram and a conventional ECG record were also obtained as usual.

2.2. Carotid artery assessment

To measure the carotid intima–media thickness, ultrasonography of the common carotid artery, carotid bifurcation, and internal carotid artery of the left and right carotid arteries was performed with a 7.5-MHz linear-array transducer (SonoSite 180PLUS, Olympus, Tokyo). On a longitudinal, two-dimensional ultrasound image of the carotid artery, the anterior (near) and posterior (far) walls of the carotid artery are displayed as two bright white lines separated by a hypoechogenic space. The distance between the leading edge of the first bright line of the far-wall (lumen–intima interface) and the leading edge of the second bright line (media–adventitia interface) indicates the intima–media thickness. For the near-wall, the distance between the trailing edge of the first bright line and the trailing edge of the second bright line at the near-wall provides the best estimate of the near-wall intima–media thickness. When an optimal longitudinal image was obtained, it was frozen and the frozen images were digitized. The beginning of the dilatation of the distal common carotid artery served as a reference point for the start of measurement. The average of the intima–media thickness of each of the three frozen images was calculated. For each individual, the common carotid intima–media thickness was determined as the average of near- and far-wall measurements of both the left and right arteries. Usual lumen parameters including the common carotid artery (CCA), systolic peak velocity (VPS) and end-diastolic velocity (VED), measured by Doppler ultrasonogram, and the resistive index (RI) were also measured.

2.3. Heart rate variability

The first 1-h record of an ambulatory ECG obtained during routine medical examination conducted each year in July was processed for HRV, using a Fukuda-Denshi Holter analysis system (SCM-280-3). Time-domain (SDNN) and frequency-domain (spectral power in the "very low frequency" – VLF: 0.003–0.04 Hz, "low frequency" – LF: 0.04–0.15 Hz, and "high frequency" – HF: 0.15–0.40 Hz regions, and the LF/HF ratio) measures were determined. SDNN was calculated over the whole 1-h record, whereas the frequency-domain endpoints were computed as averages from estimates obtained over consecutive 5-min intervals. Spectral indices were obtained by the maximum entropy method (MEM) with the MemCalc/CHIRAM program (Suwa Trust Co., Ltd., Tokyo, Japan).

The Japanese version of the Mini-Mental State Examination (MMSE) and the Hasegawa Dementia Scale Revised (HDSR) were used to assess the overall cognitive function, including verbal orientation, memory, and constructional ability (Kohs block test). The Up & Go test measured, in seconds, the time it took the subject to stand up from a chair, walk a distance of 3 m, turn, walk back to the chair, and sit down again. This test is a simple measure of physical mobility and demonstrates the subject's balance, gait speed, and functional ability (Up & Go). A lower time score indicates better physical mobility. Functional Reach (FR), used to evaluate balance, represents the maximal distance a subject can reach forward beyond arm's length while maintaining a fixed base of support in the standing position. A higher score indicates better balance. Manual dexterity was assessed using a panel with combinations of 10 hooks, 10 big buttons, and five small buttons. There were three discrete measurements of time recorded for each participant (10 "hook-on"s, 10 big "button-on-and-off"s, and five small "button-on-and-off"s). The total manual dexterity time in seconds, defined as the button score (Button-S), was calcu-

lated by adding the average times for one hook-on and one big or small button-on-and-off. A lower button score indicates better manual dexterity.

2.4. All-cause and cardiovascular mortality

The follow-up span herein ended on November 30, 2004. The follow-up time was defined as the time elapsed between the date of the first (reference) examination and the date of death.

2.5. Statistical analysis

All data were analyzed with the Statistical Software for Windows (StatFlex Ver.5.0, Artec, Osaka, http://www.statflex.net). We used Cox regression analysis to calculate the unadjusted or adjusted relative risk (RR) and corresponding 95% confidence interval (CI) for all-cause and cardiovascular mortality. To identify independent predictors of mortality, we used multivariate Cox regression analyses with stepwise selection. Variables included in the multivariate models were age, gender, BMI and HR variability indices. Significance was considered at a value of $P < 0.05$.

3. Results

During the mean follow-up time of 1152 days, 30 subjects (21 men and nine women) died. Nine deaths were attributable to cardiovascular causes (myocardial infarction: two men and three women; stroke: two men and two women).

3.1. All-cause mortality

Among the variables considered herein, Cox proportional hazard models adjusted for age and MMSE found a statistically significant association with all-cause mortality only for gender, baPWV and carotid IMT, Table 1 (left). Being a man had a relative risk of 3.570 (95% CI: 1.619–7.874). A 200 or 500 cm/s increase in baPWV was associated with a relative risk of 1.122 (95% CI: 1.001–1.258) or 1.333 (95% CI: 1.002–1.774), respectively. A 0.2 or 0.3 mm increase in left carotid IMT was associated with a relative risk of 1.395 (95% CI: 1.049–1.854) or 1.647 (95% CI: 1.075–2.524), respectively. For the right carotid IMT, the relative risk was 2.228 (95% CI: 1.268–3.915) or 3.327 (95% CI: 1.429–7.746), respectively.

3.2. Cardiovascular mortality

Age- and MMSE-adjusted predictors of cardiovascular mortality were found to be baPWV and the carotid IMT, Table 1 (right). A 200 or 500 cm/s increase in baPWV was associated with a relative risk of 1.321 (95% CI: 1.120–1.558) or 2.005 (95% CI: 1.327–3.031), respectively. A 0.2 or 0.3 mm increase in left carotid IMT was associated with a relative risk of 1.768 (95% CI: 1.019–3.067) or 2.351 (95% CI: 1.029–5.372), respectively. For the right carotid IMT, the relative risk was 2.029 (95% CI: 1.039–3.963) or 2.890 (95% CI: 1.059–7.891), respectively.

4. Discussion

The findings herein indicate that in elderly community-dwelling people, independently of cognitive function, an increased common carotid IMT is associated with an elevated risk of both all-cause and cardiovascular mortality. Among the many variables considered in this study, including lumen parameters of the carotid artery, various kinds of parameters of echocardiography, heart rate variability, QT interval, behavioral activities (Up and Go, functional reach and button test), time perception and depressive mood, it is noteworthy that only carotid IMT and baPWV predicted the occurrence of all-cause mortality and cardiovascular death. It is important to realize that arterial blood flow in the common carotid artery, estimated by systolic peak velocity, end-diastolic velocity and the resistive index is virtually normal in these subjects. Kuller et al. [6] showed a considerably increased risk of cardiovascular morbidity and mortality for subjects with subclinical disease compared with subjects with no signs of subclinical disease. These results are in accordance with our finding that among subjects free from symptomatic cerebrovascular and cardiovascular disease, an increased IMT is associated with an increased risk of cardiovascular mortality.

To our knowledge, this is the first prospective study for a community-dwelling population to demonstrate statistically significant associations with cardiovascular mortality of carotid atherosclerosis, in a multivariate Cox model adjusted for cognitive function. It is also noteworthy that carotid IMT predicted not only cardiovascular mortality but also all-cause mortality. Since an impaired cognitive function was associated with all-cause mortality in several populations, our observation after adjustment for age and MMSE may have applications in clinical practice.

Several cross-sectional studies [7] have shown that increased common carotid IMT may be useful as a marker of atherosclerosis elsewhere in the arterial system, in keeping with our finding that not only carotid IMT but also baPWV conferred an increased risk of cerebro- and cardiovascular mortality. It should be noted that the relative risk of an increased IMT was higher than that of an increased baPWV, suggesting that IMT may be a better predictor than baPWV. baPWV is a novel noninvasive technique assessing pulse wave transmission between the brachial and tibial arteries [8]. It is considered to be an indicator of arterial stiffness and a marker of vascular damage [9].

Our data suggest that measurement of IMT in subclinical subjects may be useful to obtain an estimate of mortality risk that is more precise than that based on the measurement of conventional risk factors alone, and may thus have additional predictive value. In addition, using IMT as a primary outcome measure in intervention trials on the efficacy of blood pressure or lipid lowering regimens, especially from the viewpoint of chronodiagnosis and chronotherapy, may lead to major applications in clinical practice to reduce the progression of atherosclerosis.

Table 1
Age- and MMSE-adjusted relative risk of all-cause and cardiovascular mortality in elderly population

Variables	All-cause mortality				Cardiovascular mortality			
	n	RR	95% CI	*P*-value	n	RR	95% CI	*P*-value
Gender	291	3.570	1.619–7.874	0.0016	271			N.S.
BMI	279			N.S.	260			N.S.
SBP	279			N.S.	259			N.S.
DBP	278			0.0733	259			N.S.
PP	278			N.S.	259			N.S.
Postural BP change	276			0.0818	256			0.0818
Pulse rate	279			N.S.	259			N.S.
Up and Go	288			N.S.	269			N.S.
FR	287			N.S.	268			N.S.
Button	289			N.S.	269			N.S.
HDSR	291			N.S.	271			0.0762
Kohs	272			N.S.	253			N.S.
GDS	273			N.S.	254			N.S.
Time estimation (60A)	258			N.S.	244			N.S.
Time estimation (60B)	252			N.S.	240			N.S.
HR	243			N.S.	229			N.S.
VLF	191			N.S.	182			N.S.
LF	189			N.S.	180			N.S.
HF	192			N.S.	183			N.S.
LF/HF	192			0.0936	183			0.0936
SDNN	191			N.S.	182			N.S.
Lown	273			N.S.	253			N.S.
PWV (200)	242	1.122	1.001–1.258	0.0487	223	1.321	1.120–1.558	0.0010
PWV (500)	242	1.333	1.002–1.774	0.0487	223	2.005	1.327–3.031	0.0010
ABI	260			N.S.	241			N.S.
IMT Lt (0.1)	130	1.181	1.024–1.362	0.0220	128	1.330	1.010–1.751	0.0426
IMT Lt (0.2)	130	1.395	1.049–1.854	0.0220	128	1.768	1.019–3.067	0.0426
IMT Lt (0.3)	130	1.647	1.075–2.524	0.0220	128	2.351	1.029–5.372	0.0426
IMT Rt (0.1)	129	1.493	1.126–1.979	0.0053	126	1.424	1.019–1.991	0.0383
IMT Rt (0.2)	129	2.228	1.268–3.915	0.0053	126	2.029	1.039–3.963	0.0383
IMT Rt (0.3)	129	3.327	1.429–7.746	0.0053	126	2.890	1.059–7.891	0.0383
Lt CCA	131			0.0682	128			N.S.
Rt CCA	127			0.0699	124			N.S.
Lt VPS	130			N.S.	127			N.S.
Rt VPS	129			N.S.	126			N.S.
Lt VED	130			N.S.	127			N.S.
Rt VED	128			N.S.	125			N.S.
Lt RI	123			N.S.	120			N.S.
Rt RI	114			0.1069	111			N.S.
LVMI	135			N.S.	126			N.S.
Calcification of M-valve	151			N.S.	141			N.S.
Calcification of A-valve	150			N.S.	140			N.S.
LVDd	135			N.S.	126			N.S.
%FS	135			N.S.	126			N.S.
EF	135			N.S.	126			N.S.
E	146			N.S.	137			N.S.
A	146			N.S.	137			N.S.
E/A	146			0.0624	137			N.S.
DT	143			N.S.	134			N.S.
QTd	134			N.S.	125			N.S.
QT	177			N.S.	167			0.0505
QTc	175			N.S.	165			N.S.

Gender: male versus female; Lt = left; Rt = right.

We conclude that carotid IMT assessed by ultrasonography is positively associated with an increased risk of all-cause mortality and cardiovascular death in particular. This study provides supportive evidence for the use of IMT measurements as an intermediate endpoint in intervention trials.

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VERTICAL GROUND REACTION FORCE SHAPE IS ASSOCIATED WITH GAIT PARAMETERS, TIMED UP AND GO, AND FUNCTIONAL REACH IN ELDERLY FEMALES

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Objective: **The aim of this study was to evaluate the relationship between knee pain and various indicators of the combined performance of the lower extremity (including gait parameters, functional performance such as timed up and go, and functional reach test) and to determine whether the classification of vertical ground reaction forces correlates with gait parameters and functional performance.**

Subjects and Methods: **Simultaneous analysis of gait, time-distance parameters and vertical ground reaction force. Timed up and go, and functional reach test were examined in 130 elderly women. The vertical component of the ground reaction force was grouped into 2 categories: M-shaped and non-M-shaped.**

Results: **No significant association was found between knee pain and timed up and go, functional reach test, or gait parameters in elderly female participants. There were significant differences between subjects with M- and non-M-shaped vertical ground reaction forces with regard to timed up and go, functional reach test and Japan Orthopaedic Association score. There were also significant differences between the 2 groups (M shaped and non-M-shaped) in gait parameters.**

Conclusion: **Evaluation of the vertical ground reaction force to determine its shape may be a useful and simple tool in the analysis of gait and functional performance.**

Key words: knee pain, gait analysis, elderly females, ground reaction force, osteoarthritis.

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INTRODUCTION

Osteoarthritis of the knee is one of the most common diseases in elderly females. There are several ways of testing locomotor function of the lower extremity, including measures of muscle strength, gait analysis and some types of knee evaluation scales (1–3). However, there is limited evidence that these parameters are highly correlated with the functional state of the knee. Gait analysis is becoming recognized as an important clinical tool in orthopaedics, in pre-surgery planning, post-surgery monitoring and in a posterior evaluation of various corrective interventions (4, 5). However, it is sometimes difficult for clinicians to analyse the large amounts of data gathered in the assessment of gait time and distance parameters (5).

Objective quantitative assessment of mobility and balance is important for older people because problems with gait and balance can result in a restriction of activity. The Timed Up and Go (TUG) test correlates with gait speed, balance and movement of the lower extremities (6). The Functional Reach (FR) test is a simple measurement of standing balance that can predict falls in elderly people (7, 8).

There have been several reports concerning gait analysis in osteoarthritis of the knee (1, 9). The vertical ground reaction force (VGRF) has been shown to be a reliable and repeatable feature of gait (10–11). There have been numerous studies regarding ground reaction forces during walking (12–14). Gait speed significantly affects VGRF (12, 13, 16). The VGRF varies continually from the instant of initial contact until the foot leaves the supporting surface (17). Body mass, proportions, walking style and balance all affect VGRF (17).

There have been only a few reports regarding the relationship between VGRF and various gait parameters in elderly females with osteoarthritic knees. Analyses that include a classification of VGRF have also been limited. Thus, in this study, we focused on the vertical ground force component, classified into 2 groups: M-shaped, also known as a "dual-hump" shape (18) and non-M-shaped. The purpose of this study was to evaluate the relationship between knee pain and various indicators of the combined performance of the leg, including gait parameters, functional performance, TUG and FR and to determine whether the classification of VGRF is correlated with gait parameters and functional performance.

MATERIAL AND METHODS

Subjects

We defined the subjects with osteoarthritic knee as having knee pain and less than 100 points of Japan Orthopaedic Association (JOA) score. We have been performing annual medical checks of adults aged 65 years and

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Table I. *Japan Orthopaedic Association scores based on the osteoarthritic knee evaluation form*

Pain on walking (maximum 30 points)	*Score*
No pain, walking unlimited	30
Pain, walking unlimited	25
Pain, walking distance of 0.5–1 km	20
Pain, walking less than 0.5 km	15
Pain, walking only indoors	10
Cannot walk	5
Cannot stand	0
Pain on ascending or descending stairs (maximum 25 points)	
No pain	25
Pain, relieved by using handrails	20
Pain, with handrails, but no pain with each step	15
Pain, with each step, pain relieved by using handrails	10
Pain, with each step even with handrail use	5
Cannot ascend or descend	0
Range of motion (maximum 35 points)	
Kneeling	35
Sideways or cross-legged sitting	30
More than 110°	25
75°–109°	20
35°–74°	10
Less than 35°	0
Joint effusion (maximum 10 points)	
No effusion	10
Occasional puncture required	5
Frequent puncture required	0
Maximum total points	100

over who live in the community in Kahoku of Kochi prefecture since 1994. We then examined the locomotor ability of the subjects.

The mean age of the 130 participants was 80 years (range 65–94 years), with a mean height of 143.0 cm. Knee pain while walking was classified into 3 groups: no pain (45%), unilateral pain (28%) or bilateral pain (26%).

Average maximum flexion for all subjects was 140.9 ± 13.4 degrees. Average maximum extension was 5.2 ± 6.1 degrees. JOA scores determined from the osteoarthritic knee evaluation form (Table I) were used for the evaluation of knee function (19). JOA (0–100 points) scores averaged 90.1 ± 12.9 points. The distance between the medial chondyles was evaluated, and averaged 2.5 ± 1.4 fingers breadth.

Co-morbidities of the subjects included hypertension (31.6%), cardiac arrhythmia (6.1%), coronary artery disease (3.2%) and diabetes mellitus (5.7%). Eighteen subjects with the following conditions were excluded from this study: knee disorders after total knee arthroplasty (5 patients), high tibial osteotomy (2 patients), miscellaneous knee operations (2 patients), osteosynthesis (1 patient), multiple cerebral infarctions (7 patients) and Parkinson's disease (1 patient).

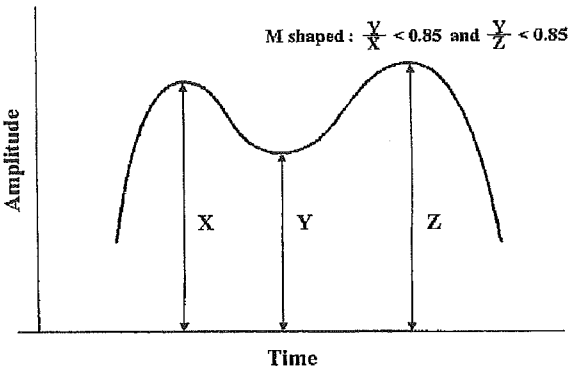

Fig. 1. Calculation of M-wave shape of vertical ground reaction force. M-shaped was defined as Y/X and Y/Z less than 0.85. All others were defined as non-M-shaped.

Gait analysis

The interviewer asked to record the gait parameters of subjects who were able to walk a distance of 10 metres. Subjects were allowed to wear their usual clothes and use their preferred (normal) speed while walking a 7-metre-long course. The first and last 2–3 metres on the walkway were not considered for measurement.

A Gait Scan® 8000 (Nitta Co. Ltd, Osaka, Japan) of gait-pattern measurement system consisting of a thin-film sensor walkway, a computer for automatic recording of the data was used in this study. This gait analysis device consists of a sensor seat (264 × 52 cm), a connector unit which fixes the sensor seat, and an interface board with a personal computer and software for data analysis.

Gait parameters, temporal distance and time factors, and ground reaction forces were measured simultaneously. Ground reaction force data for both legs was collected at a self-selected walking speed. The peak force was measured as the highest VGRF that occurred anytime during the stance phase, while the lowest VGRF occurred during the mid-stance phase.

Patients were classified into 2 groups based on the VGRF: M-shaped and non-M-shaped (Fig. 1). We defined M-shaped as lowest/highest × 100 (%) of less than 85. We assessed the shape of the VGRF for every step and classified individuals based on the result that was obtained for the greater number of steps. The mean gait variables measured in this study were walking speed (metres/sec), stride length, step width (cm), time of stride, time of single stance and time of double stance (sec). The distance parameters of stride length and step width were normalized for the height of the subject (15).

Functional performance

Timed up and go

To measure TUG, subjects were given oral instructions to stand up from

Table II. *Data (mean (SD)) for patients without pain, with unilateral and bilateral pain in elderly females*

	No pain ($n = 59$)	Unilateral pain ($n = 37$)	Bilateral pain ($n = 34$)
Body weight (kg)	45.2 (7.53)	47.2 (7.49)	52.2 (8.94)
Timed up and go (sec)	13.0 (3.0)	13.8 (4.51)	15.1 (7.28)
Functional reach (cm)	20.6 (7.2)	21.0 (7.07)	23.1 (6.89)
Stride length (cm)	63.2 (9.21)	61.1 (11.7)	61.7 (10.9)
Stride width (cm)	5.4 (2.20)	5.7 (2.14)	5.6 (1.92)
Time of stride (sec)	1.1 (0.117)	1.1 (0.179)	1.2 (0.167)
Time of single stance (sec)	0.58 (0.059)	0.59 (0.073)	0.60 (0.082)
Time of double stance (sec)	0.16 (0.037)	0.17 (0.052)	0.18 (0.069)
Gait speed (m/s)	0.6 (0.115)	0.56 (0.147)	0.54 (0.135)

Table III. *Participant characteristics given as mean (SD)*

	Height (cm)	Weight (kg)	JOA (point)	TUG (sec)	FR (cm)
Right side					
M-shaped ($n=32$)	143.8 (7.2)	46.1 (8.6)	95.2 (10.3)	11.6 (2.3)	22.5 (6.9)
Non-M-shaped ($n=47$)	142.4 (5.2)	45.9 (7.4)	86.6 (13.5)	14.6 (4.5)	18.4 (8.2)
	$p=0.187$	$p=0.96$	$p=0.0013$	$p<0.0001$	$p=0.026$
Left side					
M-shaped ($n=29$)	143.1 (8.1)	45.8 (8.1)	96.9 (6.25)	11.35 (2.25)	22.9 (7.56)
Non-M-shaped ($n=50$)	142.9 (4.7)	46.2 (7.8)	86.1 (14.1)	14.5 (4.44)	18.45 (7.74)
	$p=0.41$	$p=0.92$	$p=0.0002$	$p<0.0001$	$p=0.026$

JOA: Japan Orthopaedic Association; TUG: timed up and go; FR: functional reach

a chair, walk 3 metres as quickly and as safely as possible, cross a line marked on the floor, turn around, walk back and sit down (6).

Functional reach. FR represents the maximal distance a subject can reach forward beyond arm's length while maintaining a fixed base of support in the standing position (7, 20).

Statistics

Data were expressed as a mean and standard deviation (SD). Differences between groups were evaluated using a Kruskal Wallis test for the analysis of knee pain (Table II) and a Mann-Whitney U test for the analysis of VGRF (Tables III and IV). Statistical significance was set at $p<0.05$.

RESULTS

Occurrence of knee pain showed a significant association with body weight; however, there was no significant difference between patients with or without pain and TUG, FR, or any gait parameters (Table II).

The shape of the VGRF was associated with certain measures of functional performance, as well as the JOA score (Table III). Patients exhibiting an M-shaped VGRF on the right and left sides had shorter TUGs and longer FRs than patients with a non-M-shaped VGRF. The total JOA score was greater for the M-shaped group than for the non-M-shaped group. Within both groups, the ground reaction forces were similar on left and right sides.

Several gait parameters varied according to the shape of the VGRF (Table IV). Stride length was longer for the M-shaped VGFR group than for the non-M-shaped VGRF group. The times of stride and single and double stance were shorter in the M-shaped VGRF group than in the non-M-shaped group. The walking speed of the M-shaped group was faster than that of the non-M-shaped group. There was no significant difference between the 2 groups in the step width on both sides.

DISCUSSION

Osteoarthritis of the knee is common in elderly females and it is well-known that it is associated with gait disturbances. There have been numerous reports regarding the relationship between osteoarthritis and gait parameters. An evaluation of the relationship between gait parameters and knee pain in elderly females found no significant association between knee pain and gait parameters or functional performance. Findings such as these have suggested that numerous factors, such as the posture of the trunk, lumbar lesions, the condition of other joints (such as the hip and ankle) and mental status, all contribute to gait parameters in elderly females. Therefore, it is important to consider these factors in the analysis of people with knee pain.

An advantage of gait analysis as a diagnostic or research tool is that many factors can be assessed at one time; however, proper evaluation of the resulting data can be complex. Quantitative data of time and distance parameters of gait analysis is difficult to understand and interpret whether it is within normal or not.

One study showed no overall abnormality in the shape or amplitude of the ground reaction force measured for the natural gait of knee-pain subjects (21). The present study, which involved the evaluation of one simple aspect of the VGRF (classified as M-shaped and non-M-shaped), showed that the shape of the ground reaction force was correlated with the pain

Table IV. *Gait parameters (mean (SD)) for subjects with M-shape and non-M-shape of vertical ground reaction force*

	Stride length (cm)	Step width (cm)	Time of stride (sec)	Time of single stance (sec)	Time of double stance (sec)	Gait speed (m/s)
Right side						
M-shaped ($n=32$)	70.1 (8.7)	5.5 (2.1)	1.03 (0.09)	0.5 (0.04)	0.1 (0.02)	0.7 (0.11)
Non-M-shaped ($n=47$)	55.8 (89.9)	5.8 (2.3)	1.2 (0.15)	0.6 (0.07)	0.2 (0.047)	0.5 (0.1)
	$p<0.0001$	$p=0.712$	$p<0.0001$	$p<0.0001$	$p<0.0001$	$p<0.0001$
Left side						
M-shaped ($n=29$)	70.6 (9.2)	5.5 (2.08)	1.0 (0.087)	0.54 (0.042)	0.1 (0.02)	0.69 (0.12)
Non-M-shaped ($n=50$)	56.5 (9.9)	6.0 (2.47)	1.8 (0.15)	0.61 (0.075)	0.2 (0.046)	0.5 (0.11)
	$p<0.0001$	$p=0.146$	$p<0.0001$	$p<0.0001$	$p<0.0001$	$p<0.0001$

component of the JOA score. In another study, increased gait speed was associated with shorter force periods and larger peak forces (16).

In the present study we found that there were no differences between the right and left legs with respect to gait parameters, functional performance or the shape of the ground reaction force. Consistent with our findings, another study showed no significant differences between the right and left foot with respect to ground reaction force during walking (22).

In our study we found that both gait parameters and functional performance were significantly correlated with the shape of the VGRF. Several previous studies have examined VGRFs in normal subjects and patients with osteoarthritis; however, prior to the present study, there was little known concerning the relationship between the VGRF and gait parameters or functional performance in elderly females with knee osteoarthritis. In one study it was found that the 2 peaks in the vertical component measured for the affected side in knee-osteoarthritis patients became less apparent, with significantly lower magnitudes than in normal subjects (18). In addition, patterns of VGRFs were nearly identical during overground and treadmill walking (23) and the general waveform and its characteristic features did not seem to be affected by the sex of normal subjects (18). In the present study, we could not find a correlation between pain and the mechanism of the shape of VGRF. Further study is needed to clarify the changing mechanism of VGRF in osteoarthritic knee.

In the present study, we did not examine inter-rater reliability: future study is needed to investigate this and the validity with respect to M-shape and gait analysis.

In conclusion, our classification of VGRF is a simple and useful tool for assessment of gait function. It was correlated with many parameters of gait and functional performance, such as TUG and functional reach. Our study indicated that a change in the VGRF, from non-M-shaped to M-shaped, is crucial to the improvement of gait parameters and gait performance. Further studies are needed to seek methods for altering the shape of the ground reaction force.

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Angiotensin converting enzyme inhibitor attenuates oxidative stress-induced endothelial cell apoptosis via p38 MAP kinase inhibition

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Abstract

Background: The effects of angiotensin converting enzyme (ACE) inhibitors on oxidative stress-induced apoptosis of endothelial cells and the intracellular signaling were investigated.
Methods: Cultured endothelial cells derived from a bovine carotid artery were treated with H_2O_2 or TNF-α to induce apoptosis. Apoptosis was evaluated by DNA fragmentation and cell viability, p38 MAP kinase activity by Western blotting, and oxidative stress by formation of 8-isoprostane. The effects of ACE inhibitors were examined by adding them into the medium throughout the experiments.
Results: Apoptosis was attenuated by ACE inhibitors, temocapril and captopril, in a dose-dependent manner (1–100 μmol/l). H_2O_2 (0.2 mmol/l for 1.5 h) or TNF-α (10 ng/ml for 72 h) treatment stimulated the activities of p38 MAP kinase. Temocapril and captopril decreased the activity of p38 MAP kinase as well as 8-isoprostane formation induced by H_2O_2. A p38 MAP kinase inhibitor, SB203580, partially inhibited the effect of temocapril on apoptosis.
Conclusions: These results suggest that ACE inhibitors protect endothelial cells from oxidative stress-induced apoptosis, and that p38 MAP kinase plays a critical role in the process.

Keywords: Apoptosis; ACE inhibitor; Endothelial cell; p38 MAP kinase

1. Introduction

Stress-induced injury of vascular endothelial cells (ECs) is considered to be an initial event in the development of atherosclerosis [1]. In particular, oxidative stress has been implicated in endothelial injury caused by oxidized LDL and smoking as well as hypertension, diabetes and ischemia-reperfusion [1–3]. This notion is supported by the findings that the production of reactive oxygen species is upregulated in vascular lesions [4,5], and that lesion formation such as endothelial dysfunction is accelerated by superoxide anion [6] and, in contrast, is attenuated by free radical scavengers including vitamin E [7] and superoxide dismutase [8].

Angiotensin converting enzyme (ACE) inhibitors effectively interfere with the renin angiotensin system and exert various beneficial actions on vascular structure and function beyond their blood pressure-lowering effects [9,10]. ACE inhibitors attenuate neointimal formation after vascular injury in animals [11] and endothelial dysfunction in humans [12]. It has been demonstrated that ACE activation induces oxidative stress [13]. However, it has not been elucidated whether ACE inhibitors could attenuate oxidative stress-induced EC apoptosis, an initial and important process in atherosclerosis [14,15].

In this study, we examined the effects of ACE inhibitors, temocapril and captopril, on H_2O_2- and TNF-α-induced EC apoptosis and the pro-apoptotic intracellular signaling, p38 mitogen-activated protein (MAP) kinase, to clarify the underlying mechanism.

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2. Materials and methods

2.1. Induction of EC apoptosis

ECs derived from a bovine carotid artery [16] was cultured in Dulbecco's modified Eagle medium (Gibco) supplemented with 10% fetal bovine serum. Cells were maintained at 37 °C in a 95% air/5% CO_2 atmosphere. ECs of the 5th to 7th passage were used in the experiments. When the cells had grown to 70–80% confluence, ECs were pretreated for 24 h with culture medium containing the reagents that were tested in the experiments. Subsequently, after washing twice with Hank's balanced salt solution (Gibco), the cells were exposed to H_2O_2 (0.1–0.4 mmol/l) diluted in Hank's balanced salt solution for 1.5 h at 37 °C to induce apoptosis. The cells were washed three times with Hank's balanced salt solution, and then cultured in culture medium containing the reagents until assay. Similarly, tumor necrosis factor-α (TNF-α, 5-20 ng/ml; Sigma) was added to the medium until assay after 24-h pretreatment with the reagents tested. EC viability and apoptosis were evaluated at 24 h after H_2O_2 treatment, or at 72 h after TNF-α treatment. The effects of temocapril (1–100 μmol/l) and captopril (1–100 μmol/l) were examined by adding them into the medium throughout the experiments. The effect of a specific p38 MAP kinase inhibitor, SB203580 (10 μmol/l; Calbiochem), was examined by treating ECs with SB203580 for 1 h before H_2O_2 treatment.

2.2. Cell viability

Cell viability was estimated using an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma) assay [17]. Briefly, 1 mg/ml MTT (final concentration) was added to the well and incubated for 2 h at 37 °C. The medium was removed and cells were lysed with 2-isopropanol containing 0.04 mol/l HCl. The absorbance measured at 595 nm was used to calculate the relative cell viability ratio.

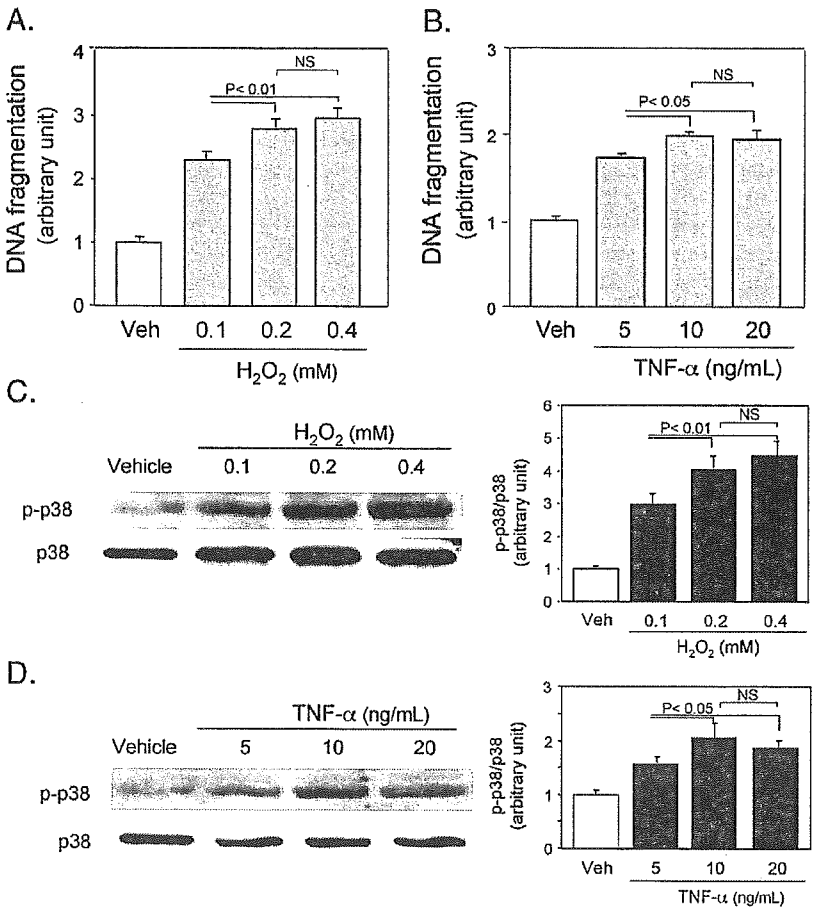

Fig. 1. Dose-dependent effects of H_2O_2 (A, C) and TNF-α (B, D) on EC apoptosis (A, B) and p38 MAP kinase activity (C, D). A and B, apoptosis was evaluated 24 h after H_2O_2 treatment (for 1.5 h) or 72 h after addition of TNF-α by means of DNA fragmentation ($n=3$). C and D, the activity of p38 MAP kinase was evaluated by immunoblotting using the specific antibody against the phosphorylated form of the kinase (p-p38) at 30 min after addition of H_2O_2 or TNF-α. Right panels show the results of densitometric analyses of immunoblotting (mean±SEM, $n=3$). NS, not significant. Values are expressed as mean±SEM ($n=3$).

2.3. Evaluation of EC apoptosis and formation of 8-isoprostane

For quantitative determination, EC apoptosis was measured as DNA fragmentation. DNA fragmentation was evaluated by histone-associated DNA fragments using a photometric enzyme immunoassay (Cell Death Detection ELISA, Roche), according to the manufacturer's instructions. Briefly, attached cells were harvested with trypsin, and the cell suspension was pelleted by centrifugation. Floating and attached cells were lysed. After centrifugation, the supernatant was measured by ELISA.

Formation of 8-isoprostane (8-*iso* prostaglandin $F_{2\alpha}$) was measured using a commercially available EIA kit (Cayman Chemical). Culture supernatants were diluted with EIA buffer when necessary, and were applied to EIA according to the manufacturer's instructions.

2.4. Immunoblotting

The cells were washed twice with ice-cold phosphate-buffered saline and lysed in lysis buffer (25 mmol/l Tris/HCl, pH 7.5, 25 mmol/l NaCl, 0.5 mmol/l EGTA, 10 mmol/l NaF, 20 mmol/l β-glycerophosphate, 1 mmol/l Na_3VO_4, 1 mmol/l

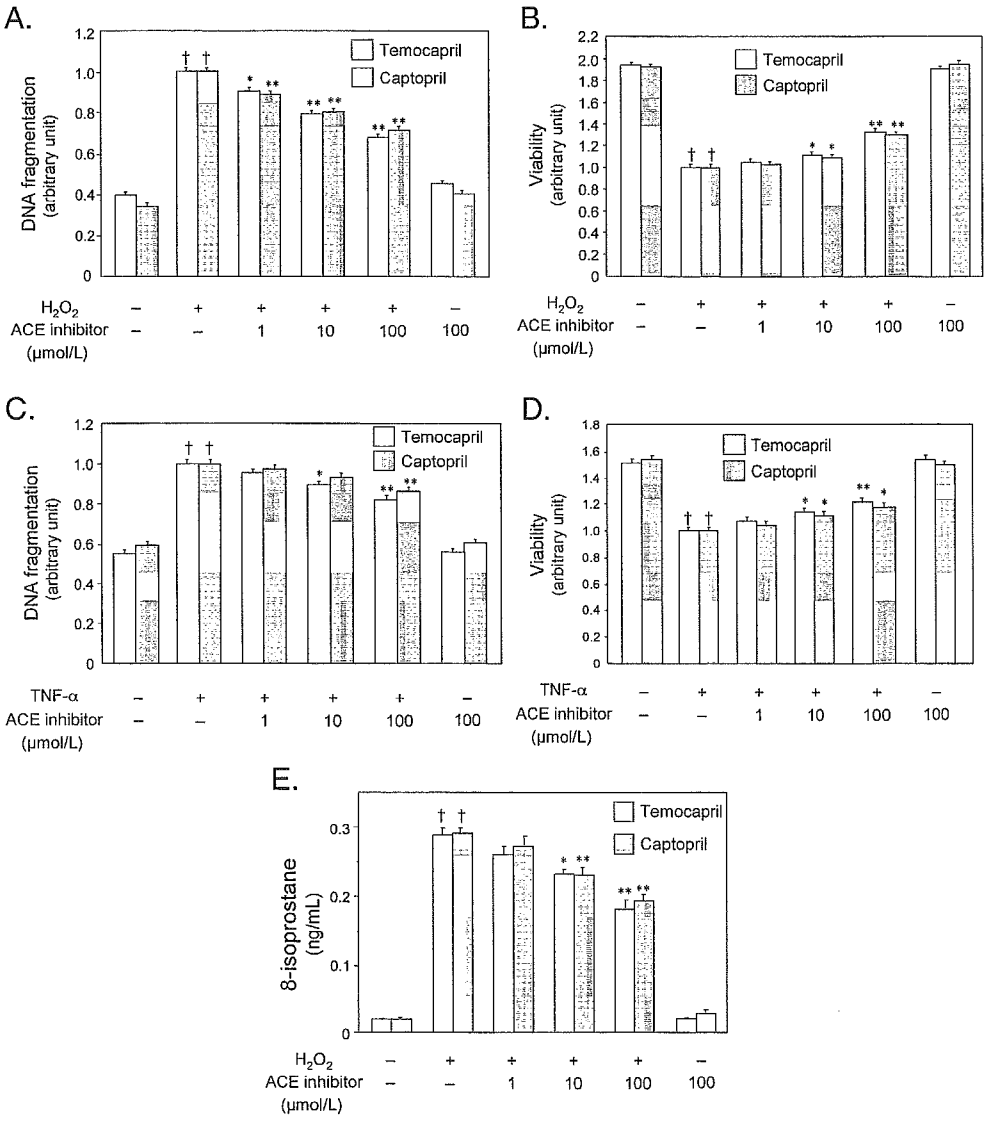

Fig. 2. Effects of ACE inhibitors on H_2O_2-induced (A, B) and TNF-α-induced (C, D) EC apoptosis and the effects of ACE inhibitors on H_2O_2-induced 8-isoprostne formation (E). Temocapril, captopril or their vehicle was added to the culture medium 24 h before H_2O_2 or TNF-α treatment until assay. Apoptosis (A, C) and cell viability (B, D) were evaluated 24 h after H_2O_2 treatment (0.2 mmol/l for 1.5 h) or 72 h after TNF-α treatment (10 ng/ml for 72 h) by means of DNA fragmentation ($n=3$) and MTT assay ($n=8$), respectively. 8-Isoprostane concentration (E; $n=3$) in the culture supernatant was measured 3 h after H_2O_2 treatment. A and B, †$P<0.01$ vs. H_2O_2 (−). *$P<0.05$, **$P<0.01$ vs. H_2O_2 (+)+ACE inhibitor (−). C and D, †$P<0.01$ vs. TNF-α (−). *$P<0.05$, **$P<0.01$ vs. TNF-α (+)+ACE inhibitor (−). Values are expressed as mean±SEM. Similar results were obtained in three independent experiments.

PMSF, and 10 μg/ml aprotinin) at 4 °C. After sonication and centrifugation at 15,000 rpm, the supernatant was used for the following immunoblotting. The lysate (20 μg protein per lane) was separated on 12% SDS-polyacrylamide gel, electroblotted onto nitrocellulose membrane, and immunoblotted with specific primary antibodies, both of which were purchased from Cell Signaling Technology (Beverly, MA). The antibodies used in this study were anti-phospho-p38 MAP kinase (phospho-p38 28B10 #9216) and anti-p38 MAP kinase (#9212). Antibodies were detected by means of a horseradish peroxidase-linked secondary antibody using an enhanced chemiluminescence system (Amersham Pharmacia Biotech). Densitometric analysis was performed using an image scanner and analyzing software (NIH image ver. 1.61). The activity of each kinase was evaluated by calculating the ratio of the amount of the phosphorylated form to that of the total form.

2.5. Data analysis

The values are expressed as mean±SEM in the text and figures. Data were analyzed using one-factor ANOVA. If a statistically significant effect was found, Newman–Keuls' test was performed to isolate the difference between the groups. Differences with a value of $P<0.05$ were considered statistically significant.

3. Results

3.1. Dose-dependent effects of H_2O_2 and TNF-α on EC apoptosis and p38 MAP kinase activity

Increasing concentrations of H_2O_2 and TNF-α were applied to examine the effects on EC apoptosis and p38 MAP kinase activity. Based on the literature [18] and time–response experiments (data not shown), EC apoptosis was evaluated at 24 h after H_2O_2 treatment for 1.5 h, or at 72 h after addition of TNF-α. The activity of p38 MAP kinase, as measured by immunoblotting using the specific antibody against the phosphorylated form of the kinase, was evaluated at 30 min after addition of H_2O_2 or TNF-α, based on time–response experiments (data not shown). As shown in Fig. 1A–D, the effects of H_2O_2 and TNF-α were

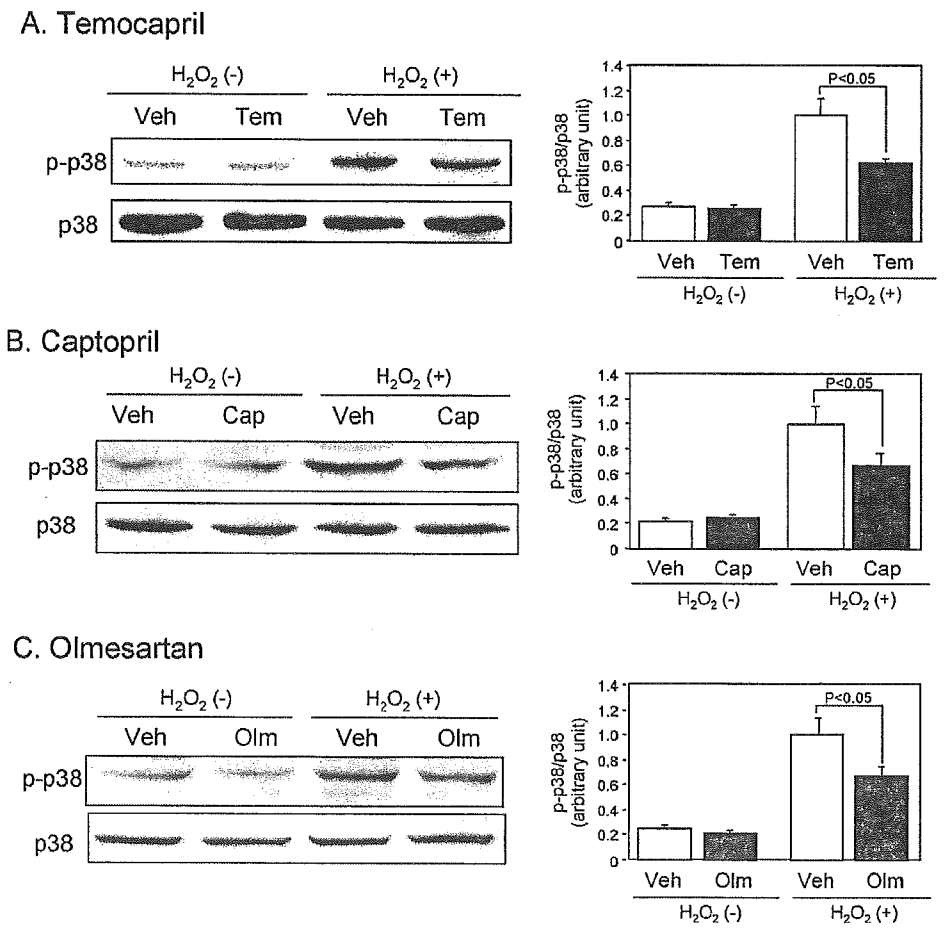

Fig. 3. Effects of temocapril (A), captopril (B) and olmesartan (C) on p38 MAP kinase activity at 30 min after exposure to H_2O_2. Temocapril (100 μmol/l), captopril (100 μmol/l), olmesartan (10 μmol/l) or its vehicle was added to the culture medium 24 h before H_2O_2 treatment until assay. Right panels show the results of densitometric analyses of immunoblotting (mean±SEM, $n=3$).

dose dependent, but there was no significant further increase in EC apoptosis and p38 MAP kinase activity by H_2O_2 of >0.2 mmol/l or by TNF-α of >10 ng/ml. Based on these data, the following experiments were examined using 0.2 mmol/l H_2O_2 or 10 ng/ml TNF-α.

3.2. Effect of ACE inhibitors on EC apoptosis

EC apoptosis, as measured by DNA fragmentation, was significantly attenuated by temocapril and captopril in a dose-dependent manner (Fig. 2A). Reflecting this effect, cell viability was ameliorated by addition of temocapril and captopril in a dose-dependent manner (Fig. 2B).

We also tested using TNF-α whether anti-apoptotic effects of ACE inhibitors would be specific to H_2O_2 or not. As shown in Fig. 2C, both temocapril and captopril effectively inhibited EC apoptosis in a dose-dependent manner. This was associated with the recovery of cell viability by the ACE inhibitors (Fig. 2D). Throughout the experiments, the effects of temocapril were comparable to those of captopril.

To confirm the antioxidant effects of temocapril and captopirl, the formation of 8-isoprostane, a marker of oxidative stress, was measured. Temocapril and captopril restrained 8-isoprostane formation induced by H_2O_2 in a dose-dependent manner (Fig. 2E).

3.3. Effect of ACE inhibitor on p38 MAP kinase activity

Next, the effects of ACE inhibitors on p38 MAP kinase activity were examined because the kinase has been implicated in the cell signaling leading to apoptosis [14,19,20]. As shown in Fig. 3A,B, temocapril and captopril decreased the activity of p38 MAP kinase at 30 min after H_2O_2 treatment by approximately 30–40% without any change in the total protein. An AT1 receptor blocker, olmesartan, showed similar effects on p38 MAP kinase activity (Fig. 3C).

Finally, the effect of a p38 MAP kinase inhibitor, SB203580, was examined. SB203580 reduced H_2O_2-induced EC apoptosis by 20%. More importantly, SB203580 partially but significantly inhibited the effect of temocapril on apoptosis (Fig. 4). Taking these results together with the pro-apoptotic action of p38 MAP kinase, it is suggested that p38 MAP kinase is involved in the effect of temocapril on EC apoptosis.

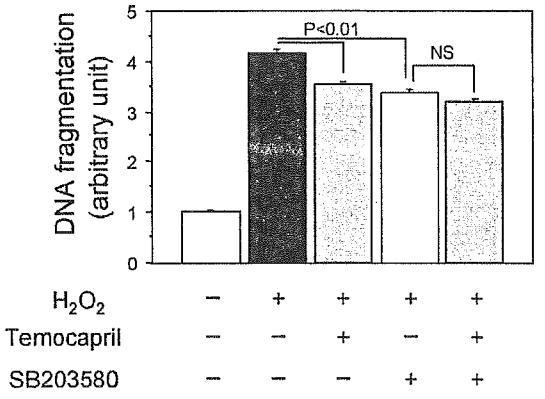

Fig. 4. Effects of temocapril and SB203580 on H_2O_2-induced EC apoptosis. Temocapril (100 μmol/l) or its vehicle was added to the culture medium 24 h before H_2O_2 treatment until assay. SB203580 (10 μmol/l) or its vehicle was added to the culture medium for 1 h before H_2O_2 treatment. EC apoptosis was determined by DNA fragmentation 24 h after H_2O_2 treatment. NS, not significant. Values are expressed as mean±SEM (n=3). Similar results were obtained in three independent experiments.

4. Discussion

A number of investigations have shown that angiotensin II induces oxidative stress in ECs. Angiotensin II stimulates the production of reactive oxygen species in ECs by upregulating the subunits of NAD(P)H oxidase, gp91 phox [21] and p47 phox [22]. It has been reported that the renin angiotensin system contributes to endothelial dysfunction in patients with renovascular hypertension [23]. Conversely, it has been shown experimentally that ACE inhibitors can reduce the production of reactive oxygen species in pathological conditions such as peripheral arteries in rats with chronic heart failure [24], rat diabetic nephropathy [25] and kidney mitochondria in aged rats [26]. In the clinical setting, 4-week treatment with ramipril, in patients with coronary artery disease, diminished the response of endothelium-dependent vasodilation to intracoronary administration of antioxidant vitamin C in parallel with improvement of basal endothelium-dependent vasodilation [27], indicating that ACE inhibitors can improve endothelial function in association with a reduction of oxidative stress.

In the present study, we investigated EC apoptosis, an important process that leads to endothelial dysfunction and atherosclerosis [14,15], and showed that ACE inhibitors, temocapril and captopril, attenuated EC apoptosis induced by H_2O_2 as well as by TNF-α. This result indicates that anti-apoptotic effects of ACE inhibitors are not specific to H_2O_2, but might be attributable to the anti-oxidant action of ACE inhibitors, because reactive oxygen species are known to be involved in TNF-α-induced EC apoptosis [28,29]. Reduction in 8-isoprostane formation by temcapril and captopril further supports the anti-oxidant effects of ACE inhibitors. It is not likely that the anti-apoptotic effects of ACE inhibitors were mediated through nitric oxide production via the inhibition of bradykinin degradation [11], because a nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester, did not influence the effect of temocapril on EC apoptosis (data not shown). Rather, the effects of ACE inhibitors are likely to be mediated through inhibition of angiotensin II production, as was demonstrated by the effect of olmesartan on p38 MAP kinase.

Reactive oxygen species activate many kinds of intracellular signaling, resulting in the transcription of numerous genes and the modulation of cellular function [30]. As

previously reported [31–33], extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and Akt in addition to p38 MAP kinase were activated in ECs by exposure to H_2O_2 (data not shown). Of these serine/threonine kinases, we focused on p38 MAP kinase because p38 MAP kinase is pro-apoptotic signaling, while ERK and Akt are anti-apoptotic, and JNK is anti- or pro-apoptotic depending on conditions [14,19,20]. We found that both temocapril and captopril inhibited the activity of p38 MAP kinase induced by H_2O_2. Although p38 MAP kinase is activated by stress and cytokines and acts on various target proteins, little is known about the downstream signaling [19,20,34]. However, EC apoptosis was effectively blocked in studies using a p38 MAP kinase inhibitor [35,36] and a dominant-negative form of p38 MAP kinase [35], indicating that activation of p38 MAP kinase leads to EC apoptosis. As a matter of fact, a p38 MAP kinase inhibitor, SB203580, partially inhibited H_2O_2-induced EC apoptosis in the present study. More importantly, SB203580 partially but significantly inhibited the effect of temocapril on apoptosis, further implying the role of p38 MAP kinase in the effect of temocapril. However, the partial effects of SB203580 also suggest the role of other pathways than p38 MAP kinase. We should perform future studies to determine the exact mechanism underlying H_2O_2-induced EC apoptosis.

In summary, we found that ACE inhibitors attenuated oxidative stress-induced EC apoptosis in culture. Furthermore, it was suggested that p38 MAP kinase was critical in the inhibitory effect of temocapril on EC apoptosis. These findings provide a mechanistic insight into the effects of ACE inhibitors, which have been used for the treatment of cardiovascular disease.

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ORIGINAL ARTICLE

Incidence of adverse drug reactions in geriatric units of university hospitals

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Background: Adverse drug reactions (ADR) in elderly people are often attributed to functional decline and polypharmacy.

Methods: In this study, a multi-institutional retrospective survey was undertaken to investigate the current status of ADR in geriatric units of university hospitals. The inpatient databases from 2000 to 2002 for five university hospitals were studied, and a total of 1289 patients were analyzed.

Results: The incidence of ADR, as determined by attending physicians, was 9.2% on average, but varied from 6.3 to 15.8% among the institutions. Factors significantly related to ADR were the number of diagnoses, the number of geriatric syndromes, the number of prescribed drugs, an increase of two or more drugs during hospitalization, longer hospital stay, emergency admission, depression and apathy.

Conclusion: These results are mostly consistent with previous reports and provide important information on drug treatment in elderly people.

Keywords: adverse drug reaction, elderly, medication error.

Introduction

Adverse drug reactions (ADR) in elderly people are common causes of admission to hospitals and are important causes of morbidity and mortality.[1,2] The risk of ADR has been shown to be related to the number of prescribed drugs and elderly people tend to receive more medications than younger people,[3] which are sometimes inappropriately prescribed.[4] Indeed, the risk of ADR is exponentially rather than linearly related to the number of medications taken.[5] Factors that predispose to pharmacological ADR include the dose, drug formulation, pharmacokinetic or pharmacodynamic abnormalities and drug interactions. Frail elderly patients may be more vulnerable because of impaired homeostatic reserve, multiple medication use, cognitive decline and impaired functional status. Drug therapy taking account of safety as well as effectiveness is still needed in the elderly, although there is accumulating evidence on drug therapy in the elderly with hypertension and hyperlipemia.[6,7]

Although the incidence of ADR for specific drugs can be obtained by large-scale examination and post-marketing surveillance studies by pharmaceutical companies, little data are available on ADR in the elderly as a whole. Previously, we reported the incidence of ADR in inpatients of the geriatric unit of the University of

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Tokyo Hospital, and showed that drug overdose and polypharmacy are important factors in ADR.[8,9] However, it is necessary to confirm whether similar results are obtained in geriatric units of other hospitals. Therefore, in this study, we analyzed the inpatient databases of five university hospitals with geriatric units, and examined the incidence of ADR and factors related to ADR.

Methods

Subjects

We performed a retrospective investigation of the hospital records of five university hospitals with geriatric units: Kyorin University Hospital, University of Tokyo Hospital, Kyoto University Hospital, Kanazawa Medical University Hospital and Tohoku University Hospital. We surveyed the records of inpatients from January 2000 to December 2002 in these hospitals, and a total of 1289 cases were used for analysis.

Investigation and analysis

We studied the incidence of ADR as judged by attending physicians during hospitalization, along with the number of medications taken on admission and on discharge. We also examined the number of final diagnoses on discharge, the length of hospital stay, age, sex and body weight of each patient, and whether or not the admission was emergent. We investigated the number of geriatric syndromes in the cases at Kyorin University Hospital and the University of Tokyo Hospital and performed comprehensive geriatric assessments (CGA). The 30 most significant of 51 geriatric syndromes are listed in Table 1. The CGA included Barthel Index on admission and discharge to evaluate activities of daily living (ADL), Hasegawa's Dementia Scale-Revised (HDS-R) to assess cognitive function, Geriatric Depression Scale 30-items (GDS-30) to assess depressive mood, and Vitality Index to assess energy.[10]

The data were expressed as means ± SD. The unpaired *t*-test was used to compare the data between two groups, and comparison among multiple groups was performed by ANOVA followed by Newman-Keuls' test. The incidences were compared using the χ^2 test. Correlation was analyzed according to Pearson's correlation coefficient. A value of $P < 0.05$ was considered statistically significant.

Results

Frequency of adverse drug reaction

In the analysis of a total of 1289 cases, the incidence of ADR was 9.2%. We analyzed the incidence at each hospital and found that the lowest incidence was 6.6%, while the highest was 15.8% among the five hospitals studied (Fig. 1).

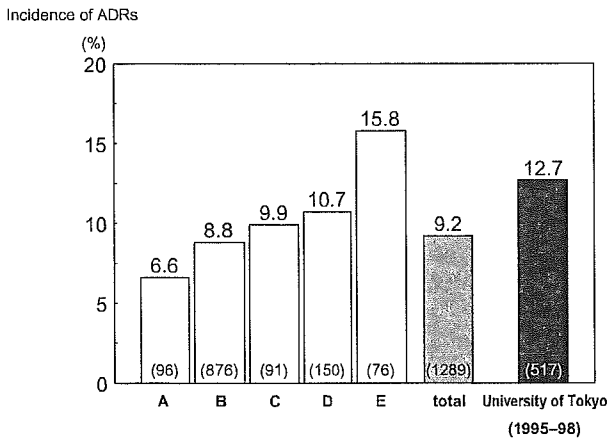

Figure 1 Incidence of ADR in inpatients of geriatric units of five university hospitals. The incidence of ADR in the geriatric unit of University of Tokyo Hospital in 1995–98 is shown as a reference.[9] The numbers of patients surveyed are shown in parentheses.

Table 1 List of major geriatric syndromes

Consciousness disturbance	Chest pain/chest oppression	Edema
Delirium	Palpitation/shortness of breath	Dehydration
Dementia	Arrhythmia	Hearing impairment
Insomnia	Abdominal pain	Motor disturbance
Depression	Constipation	Visual impairment
Dizziness/vertigo	Diarrhea	Back pain
Headache	Body weight loss	Fever
Anemia	Appetite loss	Arthralgia
Pressure ulcers	Nausea/vomiting	Osteoporosis
Falls	Malnutrition	Bleeding tendency
Hemoptysis	Dyspnea	Dysphasia
Urinary incontinence	Pollakisuria	Cough/sputum

Factors related to adverse drug reactions

Background factors related to ADR in cases with or without ADR are summarized in Table 2. There was no significant difference in sex, age or body weight between the two groups. However, patients with ADR had more diagnoses, were taking more drugs on discharge, and stayed longer in hospital than those without ADR ($P < 0.05$). They also showed a tendency to be taking more drugs on admission ($P = 0.08$). When we analyzed the relationship between ADR and the increase in medication during hospitalization, the incidence of ADR in patients with an increase of two or more drugs was 14.4%, which was significantly higher than in those with an increase of one drug (7.9%) and those without an increase (7.8%). Moreover, the incidence of ADR was higher in patients who received emergency admission than in those with scheduled admissions (12.5% vs 7.8%, $P < 0.05$).

The relationship between the factors related to ADR and the variation in ADR among the hospitals was analyzed. In hospital A, where the incidence of ADR was lowest, the number of diagnoses at discharge (2.8 ± 1.1 diseases), number of medications (4.3 ± 1.9 drugs), and the length of hospital stay (28.5 ± 6.8 days) were lowest among the five hospitals. Intriguingly, the mean age of the patients in hospital A was 82 years, while it was 67 years in hospital E, where the incidence of ADR was highest. The mean age of the patients was 71–72 years at other hospitals.

Age was positively correlated with the number of diagnoses ($r = 0.219$, $P < 0.001$) and the number of drugs at discharge ($r = 0.213$, $P < 0.001$), as previously reported.[8,9]

Geriatric syndrome and CGA were analyzed in relation to ADR in the cases at University of Tokyo Hospital and Kyorin University Hospital. The number of geriatric syndromes was significantly higher in patients with ADR than in those without ADR (Table 3). Patients with ADR showed depressed moods and apathy, as assessed by GDS and the Vitality Index, compared to those without ADR, while cognitive function and basic ADL, as assessed by HDS-R and Barthel index, did not differ between the two groups (Table 3).

Table 2 Characteristics of patients with or without adverse drug reactions (ADR)

	ADR (–)	ADR (+)
Number of patients	1170	119
Sex (female, %)	46%	50%
Age (years)	72 ± 14	73 ± 14
Body weight (kg)	56 ± 14	54 ± 14
Number of diagnoses	4.1 ± 2.0	4.9 ± 2.3*
Number of drugs on admission	5.0 ± 3.6	5.7 ± 4.1**
Number of drugs on discharge	5.3 ± 3.3	6.2 ± 3.7*
Length of hospital stay (days)	28 ± 27	38 ± 27*

*$P < 0.01$; **$P = 0.08$ by unpaired t-test.
Data are means ± SD.

Discussion

In this study, we surveyed ADR in the geriatric units of five university hospitals and found that the number of diagnoses, number of geriatric syndromes, number of prescribed drugs, an increase of two or more drugs during hospitalization, longer hospital stay, emergency admission, depression, and apathy were related to the incidence of ADR in elderly inpatients. Our study indicates that the number of diagnoses and drugs would be a better predictor for ADR in the elderly than age.

According to reports on ADR from the USA and Europe, the incidence of ADR in elderly inpatients is 6–15%.[11] The incidence was 1.5–2 fold higher in patients older than 70 years than in patients younger than 60 years. In nursing home residents, the incidence of ADR per year has been reported to be 15–20%.[11] In the outpatient setting, ADR were found in more than 10%

Table 3 Geriatric syndrome and comprehensive geriatric assessment in patients with or without adverse drug reactions (ADR)

	ADR (–)	ADR (+)
Number of geriatric syndromes	4.6 ± 3.8 (866)	6.4 ± 4.7** (85)
Barthel Index on admission	84 ± 28 (854)	80 ± 31 (82)
Barthel Index on discharge	86 ± 27 (840)	85 ± 28 (79)
HDS-R	23.0 ± 8.2 (358)	24.4 ± 6.3 (35)
GDS-30	10.2 ± 6.0 (325)	12.5 ± 6.8* (33)
Vitality index	9.0 ± 2.1 (535)	8.4 ± 2.6* (52)

*$P < 0.05$; **$P < 0.01$ by unpaired t-test. Data are mean ± SD. Numbers in parentheses indicate number of patients studied.
HDS-R, Hasegawa dementia scale-revised; GDS-30, Geriatric depression scale-30 items.