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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).

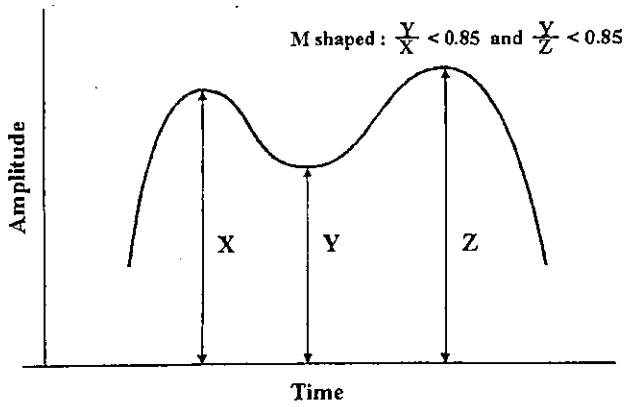

Fig. 1. Calculation of M-wave shape of vertical ground reaction force. M-shaped was defined as Y/X and Y/Z less than 0.85. All others were defined as non-M-shaped.

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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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

$P < {}^{*}.05$; ${}^{\dagger}.01$ versus baseline using paired t test.

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Inhibitory effect of low-dose estrogen on neointimal formation after balloon injury of rat carotid artery

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Abstract

The current regimens of hormone replacement therapy for postmenopausal women, estrogen combined with progestogen, have failed to show beneficial effects for the prevention of atherosclerotic disease. Although the relatively higher dose of estrogen contained in those regimens exerted adverse effects, there are few data examining a lower dose of estrogen in an atherosclerosis model. Therefore, we investigated experimentally whether lower doses of estrogen could inhibit neointimal formation after balloon injury of the rat carotid artery. Ten-week-old Wistar rats were subjected to ovariectomy or sham-operation (n=7). Four days after ovariectomy, rats were implanted with an osmotic mini-pump containing 17-β estradiol (0.2, 1, 2, 10 and 20 μg/kg/day; n=6, 4, 8, 6 and 5, respectively) or placebo (n=10). After 3 days of hormone therapy, balloon injury was performed in the left common carotid artery. Neointimal formation was histologically evaluated 2 weeks after injury. Cross-sectional intimal area and the ratio of intimal area to medial area were dose-dependently reduced by estrogen replacement compared with those in ovariectomized rats without estrogen replacement. The effects of estrogen replacement were identical to those of an angiotensin II type 1 receptor blocker, candesartan. Interestingly, the effect was significant even in rats receiving lower doses of estrogen, in which plasma estradiol concentrations were not increased and the hyperplastic response of the uterus was minimal. These results suggest the efficacy of low-dose estrogen therapy for the protection of atherosclerosis.

Keywords: Estrogen; Low-dose; Neointimal formation

1. Introduction

Previous studies have shown that estrogen administration in ovariectomized animals inhibits the process of atherosclerosis. Different doses of estrogens in combination with or without progestins have decreased the lesion formation in injured vessels or cholesterol-fed animals using rodents, rabbits and swine (Chen et al., 1996; Oparil et al., 1997; Bakir et al., 2000; Chandrasekar and Tanguay, 2000; Finking et al., 2001; Tolbert et al., 2001). Most of the studies, however, have used the estradiol doses of 20 μg/kg/day or higher, which were accompanied by the raised plasma estradiol concentration compared to intact female animals (Chen et al., 1996; Bakir et al., 2000; Tolbert et al., 2001). More importantly, these doses of estrogen (≥20 μg/kg/day of estradiol subcutaneously) elicited adverse effects such as uterine hyperplasia (Bakir et al., 2000; Tolbert et al., 2001; Xu et al., 2003) and dyslipidemia (Joles et al., 1998; Gades et al., 1998; Tomiyoshi et al., 2002). On the other hand, it has been reported that the effect of estradiol on uterine weight was dose-dependent (Kerdelhue and Jolette, 2002) and that low dose estrogen (approximately 3 μg/kg/day of estradiol) could exert its favorable effect on bone metabolism (Chen et al., 2001). Since limited information is

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available on the vascular effect of low dose estrogen therapy, it is intriguing to study whether the lower dose of estrogen could inhibit vascular lesion formation.

In the present study, we hypothesized that lower doses of estrogen could have protective effects on the process of atherosclerosis with minimal adverse effects. To test this hypothesis, we examined neointimal formation of the carotid artery after balloon angioplasty in ovariectomized female rats receiving 10 μg/kg/day or lower doses of estradiol.

2. Materials and methods

2.1. Animals

Ten-week-old female Wistar rats (Oriental Yeast, Tokyo) were used in this study. They were housed in individual cages in a room in which lighting was controlled (12 h on, 12 h off) and room temperature was kept at ≈22 °C. They were given a standard diet and water ad libitum. All the surgical procedures were performed under sterile conditions. All of the experimental protocols were approved by the Animal Research Committee of the University of Tokyo.

2.2. Experimental protocols

Rats were randomly divided into 10 groups. Nine groups of rats were subjected to ovariectomy and the other group underwent sham operation (Akishita et al., 1997). After a 4-day recovery period, six groups of ovariectomized rats were subcutaneously implanted with osmotic minipumps (Alzet 2002, 0.5 μl/h; Alza) prefilled with water-soluble 17β-estradiol (0.2, 1, 2, 10 or 20 μg/kg/day; Sigma) or its vehicle (2-hydroxypropyl-β-cyclodextrin; Sigma) under ether anesthesia. To compare the effect of estrogen with that of an angiotensin II type 1 (AT1) receptor blocker, candesartan, the remaining four groups of rats were subcutaneously implanted with an osmotic minipump containing the active metabolite of candesartan, candesartan cilexetil (2, 20 or 200 μg/kg/day; kindly donated by Takeda Chemical Industries, Tokyo) or its vehicle (0.9% saline).

Three days after minipump implantation, balloon injury was performed as previously described (Chen et al., 1996; Nakaoka et al., 1997). General anesthesia was induced by the administration of 90 mg/kg of ketamine intraperitoneally and 15 mg/kg of xylazine intramuscularly. The left carotid artery was exposed and its branches were ligated using 7–0 nylon. After intravenous injection of 75 U/kg of heparin, a portion of the external carotid artery and a portion of the internal carotid artery were cross-clipped using a microclip (2v-clip: S&T, Neuhausen, Switzerland). A 2F Fogarty embolectomy catheter (Baxter, Irvine, CA) was introduced into the artery via the external carotid artery. The common carotid artery was injured by six passes of an embolectomy catheter inflated with 0.2 ml of air. The portion proximal to the incision was ligated with 7–0 nylon, the cross-clip was released and the common carotid artery was reperfused.

2.3. Measurement of hormones and lipids

Blood sampling was performed at sacrifice, after a 16-h overnight fast, to measure serum concentrations of estradiol and progesterone, serum lipids and other biochemical parameters. Serum estradiol, estrone and progesterone concentrations were measured by sensitive radioimmunoassay (Hashimoto et al., 2002). Serum total cholesterol and triglyceride concentrations were measured enzymatically, and serum high-density lipoprotein cholesterol concentration was measured by heparin-Ca^{2+} Ni^{2+} precipitation method (Hashimoto et al., 2002).

2.4. Morphometrical analysis of the balloon-injured carotid artery

A portion of the left common carotid artery was harvested at 14 days after balloon injury. The artery was perfusion- and pressure-fixed at 100 mm Hg using 10% neutral formalin buffer and then paraffin-embedded. Five round cross-sections per 1.5-cm length of artery specimens were stained with *Elastica van Gieson staining*, and photographed. Cross-sectional areas of the intima and the media were measured using an image analyzing software package (Scion Image, shared NIH software). The average of five sections was used for analysis as the value of each animal.

2.5. Data analysis

Values are expressed as mean±S.E.M. in the text, table and figures. Data were analyzed by one-factor analysis of variance (ANOVA) followed by Newman–Keuls' multiple comparison test. Differences with a value of $P<0.05$ were considered statistically significant.

3. Results

Sixty-five rats were set up and allocated to each group. Four rats were excluded because of failure of intervention. Estrogen replacement in ovariectomized rats increased serum concentration of estradiol dose-dependently, and replacement of 2 μg/kg/day estradiol achieved a concentration comparable to that in sham-operated rats (Table 1). In all groups, the serum concentration of estrone was below the detection limit (data not shown) and that of progesterone was unchanged. With respect to the lipid profile, the concentration of total cholesterol, triglyceride and high-density lipoprotain (HDL) cholesterol were increased in rats

Table 1
Blood pressure, serum lipids, plasma hormone concentrations and body and uterus weight after balloon injury of left carotid arteries of female Wistar rats

	Sham	Ovariectomy+17β-estradiol (μg/kg/day)						Ovariectomy+TCV-116 (μg/kg/day)		
		0	0.2	1	2	10	20	0	2	20
No. of rats	7	10	6	4	8	6	5	4	4	4
SBP (mm Hg)	121±4	113±7	123±2	120±5	127±2	121±4	121±4	121±7	122±7	116±8
T.chol (mg/dl)	76±9	75±5	86±4	78±10	84±6	96±5[a]	113±3[b]	79±2	89±4	81±8
HDL-C (mg/dl)	20±2	21±3	20±2	16±3	23±2	27±1	30±1[a]	17±2	21±2	22±2
Triglyceride (mg/dl)	41±6	53±8	46±9	64±16	91±13[a]	87±10[a]	153±31[b]	64±11	25±6	35±10
Estradiol (pg/ml)	19±4[b]	8±1	9±1	12±2	20±2[b]	54±5[b]	96±3[b]	11±3	11±1	14±2
Progesterone (ng/ml)	20±5	13±2	6±3	21±5	9±2	11±3	5±2	16±4	21±6	15±6
Body weight (g)	269±6	282±8	281±8	260±6	264±6	257±5[a]	263±7	285±10	290±5	290±3
Uterus (mg)	661±102[b]	174±29	321±23	577±46[b]	511±76[b]	–	–	148±22	149±5	156±7

Values are expressed as mean±S.E.M. SBP, systolic blood pressure; T.chol, total cholesterol; HDL-C, high-density lipoprotein cholesterol; –, not examined.
[a] $P<0.05$ vs. OVX+0 μg/kg/day of 17β-estradiol.
[b] $P<0.01$ vs. OVX+0 μg/kg/day of 17β-estradiol.

receiving higher doses of estrogen, as previously reported (Gades et al., 1998; Joles et al., 1998; Tomiyoshi et al., 2002), whereas those were unchanged in rats receiving 2 μg/kg/day or a lower dose of estrogen. The body weight of rats treated with higher doses was significantly lower than that in rats without estrogen replacement. In contrast, uterine weight in rats receiving lower doses of estrogen was greater than that in rats without estrogen.

Morphometric analysis showed that the neointimal area of the carotid artery was dose-dependently decreased by estrogen replacement (Figs. 1 and 2). As shown in Fig. 2, neointimal formation was sufficiently attenuated even in rats treated with 0.2 μg/kg/day of estradiol compared to that in ovariectomized rats without estrogen replacement. The inhibitory effect of estrogen on neointimal formation was compared with that of candesartan because the effects of AT1 receptor blockers including candesartan have been established (Kim et al., 2002; Liu et al., 2002; Nozawa et al., 1999; Tazawa et al., 1999). The effect of 20 μg/kg/day estradiol was more potent than that of subdepressor dose of candesartan (20 μg/kg/day) and was as potent as that of 200 μg/kg/day candesartan; a dose that lowered blood pressure and body weight as well as neointimal formation (intima/media ratio was 0.66±0.07, data not shown). Importantly, the effect of 2 μg/kg/day or a lower dose of estradiol on neointima formation was comparable to that of 20 μg/kg/day candesartan (Fig. 2). Medial area was not different among all groups of rats. Small non-significant differences in several measurements between the control for estrogen and that for candesartan were likely to be due

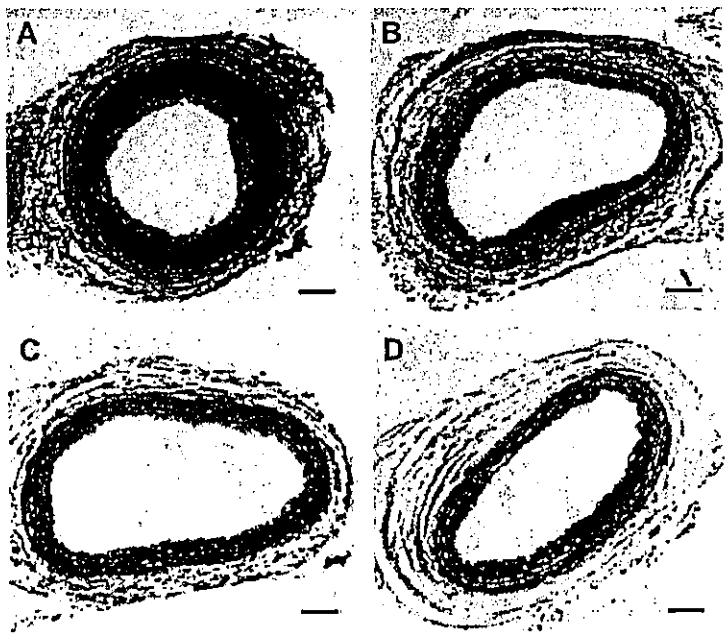

Fig. 1. Representative cross-sections of the rat carotid artery 2 weeks after balloon injury (elastica van gieson staining, magnification ×100). Rats were treated with 20% cyclodextrin vehicle (A), 0.2 μg/kg/day of 17-β estradiol (B), 20 μg/kg/day of 17-β estradiol (C) and 20 μg/kg/day of candesartan (D). Bars: 100 μm.

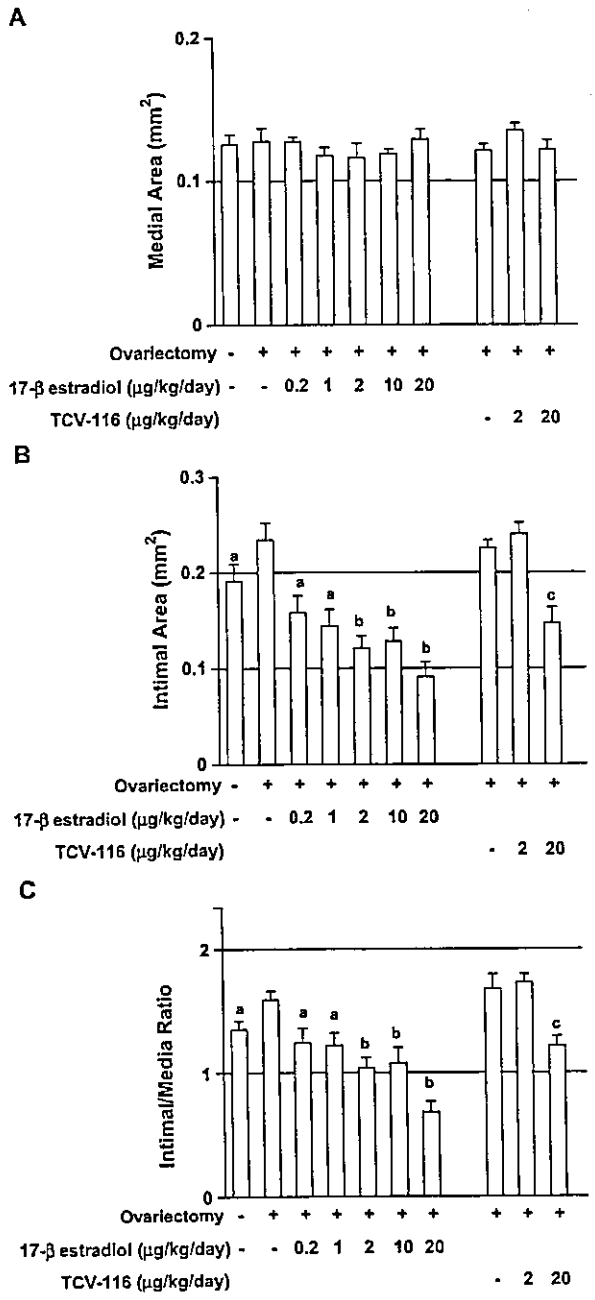

Fig. 2. Morphometric analyses of intimal area (A), medial area (B) and intima/media area ratio (C) in the carotid artery 2 weeks after balloon injury. The results are expressed as mean±S.E.M. $^{a}P<0.05$, $^{b}P<0.01$ vs. ovariectomized rats without 17-β estradiol, $^{c}P<0.01$ vs. ovariectomized rats without candesartan.

to the variation of the measurements rather than the effect of vehicle for each group.

4. Discussion

This study showed that subcutaneous administration of 2 μg/kg/day or lower doses of estradiol inhibited neointimal formation after vascular injury with minimal adverse effects on the uterus and lipid metabolism, suggesting the efficacy of lower doses of hormone replacement therapy for the prevention of atherosclerosis.

Estrogen has been reported to inhibit neointimal formation after vascular injury in rodents using balloon angioplasty of the rat carotid artery (Bakir et al., 2000; Chen et al., 1996; Oparil et al., 1997, 1999), cuff placement around the rat femoral artery (Akishita et al., 1997) and ligation of the mouse carotid artery (Tolbert et al., 2001). Oparil and her colleagues have shown using the rat carotid balloon-injury model that subcutaneous administration of 20 μg/kg/day estradiol reduced neointimal formation by more than 50% compared to that without estradiol treatment (Chen et al., 1996; Oparil et al., 1997, 1999; Bakir et al., 2000). In their studies, plasma estradiol levels in estrogen-replaced rats (135.0±5.7 pg/ml, Chen et al., 1996, or 32.0±4.8 pg/ml, Bakir et al., 2000) were higher than those in intact female rats (51.9±5.8 pg/ml, Chen et al., 1996, or 25±6.9 pg/ml, Bakir et al., 2000). In the present study, administration of 10 or 20 μg/kg/day estradiol in ovariectomized rats inhibited neointimal formation with the increased plasma estradiol concentration beyond that in sham-operated rats as well. These results suggest that the estradiol doses used in the previous studies (>10 μg/kg/day) may be relatively high although plasma estradiol concentration fluctuates in rats with the estrous cycle (ranged from 16±2 to 39±7 pg/ml, Anisimov and Okulov, 1980, or from 1±1 to 44±15 pg/ml, Hawkins et al., 1975), and changes with development and age (Meijs-Roelofs et al., 1975). In contrast, replacement of 2 μg/kg/day estradiol achieved serum estradiol concentrations comparable to those in sham-operated rats in the present study. Replacement of 1 μg/kg/day or a lower dose of estradiol did not increase the serum estradiol concentration. However, the inhibition of neointimal formation was significant at the lower doses and was comparable to the effect of 20 μg/kg/day of candesartan (Fig. 2). Moreover, 1 μg/kg/day or a lower dose of estradiol did not increase the serum triglyceride concentration, and 0.2 μg/kg/day of estradiol caused the minimal and non-significant increase of uterus weight. This could be a new finding with respect to the adverse effects on lipid profiles and uterus. Taken these findings together, a local effect of estrogen replacement on organs or cells was observed even if circulating estrogen was not elevated, providing some hints on determining the dose of hormone replacement therapy.

In the present study, we did not demonstrate the mechanisms by which estrogen inhibited neointimal formation. Previous reports have shown that re-endothelialization (White et al., 1997), preservation of endothelial survival (Sudoh et al., 2001) and function (White et al., 1997), inhibition of smooth muscle cell proliferation (Akishita et al., 1997) and inhibition of fibroblast proliferation and differentiation in the adventitia (Oparil et al., 1999) contribute to the effect of estrogen on the response to

vascular injury. Stimulation of nitric oxide synthesis as well as modulation of other vasoactive substances has been implicated in these effects, although activation of endothelial nitric oxide synthase may play a major role (Chambliss and Shaul, 2002). Further investigation is needed to elucidate the contribution and interaction of these factors in the effects of lower doses of estrogen on neointimal formation.

Recent randomized trials (Hulley et al., 1998; Rossouw et al., 2002) have suggested that hormone replacement therapy with the standard regimen should not be recommended for postmenopausal women. Improvement of the regimen, such as the dose, route (oral or subcutaneous) or schedule (continuous or cyclic), could resolve the adverse effects of hormone replacement therapy, although few data are currently available (Grodstein et al., 2000; Jick et al., 1996; Hashimoto et al., 2002; Wakatsuki et al., 2003, 2004). Direct comparisons of animal studies to clinical studies are inadequate because several major differences can be pointed including route of administration, duration of the treatment, cardiovascular risk profile of subjects and body fat distribution. However, our experimental result that lower doses of estrogen inhibited the response to vascular injury with relatively small adverse effects may imply the potential efficacy of low dose hormone replacement therapy in postmenopausal women.

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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 $\pm$ 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; Moeuno-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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Caveolin-1, Id3a and two LIM protein genes are upregulated by estrogen in vascular smooth muscle cells

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Abstract

Estrogen has diverse effects on the vasculature, such as vasodilation, endothelial growth and inhibition of vascular smooth muscle cell (VSMC) proliferation and migration. However, little is known about the genes that are regulated by estrogen in the vascular wall. Wistar rats were ovariectomized or sham-operated (Sham group), and 2 weeks after the operation, were subjected to subcutaneous implantation of placebo pellets (OVX + V group) or estradiol pellets (OVX + E group). Endothelium-denuded aortic tissue was examined 2 weeks after implantation. By applying high-density oligonucleotide microarray analysis, the expression of approximately 7000 genes was analyzed. Among the genes with different expression levels between the OVX + E group and the OVX + V group, those that have been reported to be expressed in the vasculature or muscle tissue, were chosen. Finally, four genes, caveolin-1, two LIM proteins (enigma and SmLIM) and Id3a, were identified. Microarray as well as real-time polymerase chain reaction showed that the expression levels of these genes were significantly higher in the OVX + E group than in the OVX + V group. To clarify whether estrogen directly upregulates these genes in the vascular wall, Northern blot analysis was performed using cultured rat VSMC. Addition of 100 nmol/L estradiol for 24 hours increased the mRNA levels of all four genes. Although the

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precise mechanism remains unclear, regulation of these genes by estrogen might contribute to its effect on VSMC.

Keywords: Atherosclerosis; Gene expression; Hormones; Smooth muscle

Introduction

Epidemiological studies have shown that the risk for cardiovascular disease is lower in premenopausal women than in men of the same age. Hormone replacement therapy has been reported to lower the incidence of cardiovascular disease in postmenopausal women (Colditz et al., 1987; Kannel et al., 1976), although the beneficial effects of estrogen have not been confirmed in recent randomized trials (Hulley et al., 1998; Rossouw et al., 2002). A number of animal studies have also shown estrogen's anti-atherogenic effects, including amelioration of the response to vascular injury (Sullivan et al., 1995), inhibition of endothelial cell apoptosis (Sudoh et al., 2001), and nitric oxide-mediated vasodilatation (Bell et al., 1995). Estrogen receptors (ER) are expressed in the vasculature (Hodges et al., 2000; Karas et al., 1994), supporting that estrogen can exert its effect directly on the vascular wall.

Several estrogen-responsive genes, such as pS2 (Brown et al., 1984), c-fos (Weisz and Bresciani, 1988), and efp (Inoue et al., 1993), have already been identified in reproductive tissues. In the vasculature, estrogen-regulated genes without estrogen-responsive elements in their promoter region are reported (Akishita et al., 1996; Gallagher et al., 1999; Nickenig et al., 1998). The expression of c-fos (Akishita et al., 1996), angiotensin-converting enzyme (Gallagher et al., 1999), and angiotensin receptor-1 (Nickenig et al., 1998) in the aorta was downregulated by estrogen replacement in ovarietomized rats. These changes of gene expression could explain a part of atheroprotective effects of estrogen. Recently, methods for global gene analysis have been developed, and among them, the high-density oligonucleotide microarray, has come to be used as a powerful tool by many investigators. In this study, to discover new genes that might play a role in the action of estrogen, we performed microarray analysis to identify genes that are differentially expressed in the vascular wall, especially in vascular smooth muscle cells (VSMC), before and after treatment with estrogen. To confirm the results obtained from the microarray, we performed real-time polymerase chain reaction (PCR) and Northern blotting. Finally, four genes were identified as novel estrogen-regulated genes in VSMC.

Methods

Animals

Eight-week-old female Wistar rats (Oriental Yeast, Co., Ltd., Tokyo, Japan) were used in this study. They were kept individually in stainless-steel cages in a room where lighting was controlled (12 hours on, 12 hours off) and room temperature was kept at around 22 °C. They were given a standard diet and water ad libitum. All the surgical procedures were performed under ether anesthesia. All of the experimental protocols were approved by the Animal Research Committee of the University of Tokyo.

Ovariectomy and E2 Implantation

Rats were randomly divided into three groups. Two groups of rats were ovariectomized and the other group of rats was sham-operated. After a two-week recovery period, one group of ovariectomized rats (OVX + E group, n = 5) underwent subcutaneous implantation of a three-week releasing pellet containing 0.5 mg 17β-estradiol (E2; Innovative Research of America). The other group of ovariectomized rats (OVX + V group, n = 5) and sham-operated rats (Sham group, n = 4) received placebo pellets. Two weeks after pellet implantation, blood samples were obtained from rats. Serum estradiol concentration was 5.6 ± 1.5 pg/ml in the Sham group (n = 4), 2.8 ± 1.0 pg/ml in the OVX + V group (n = 5), and 74.5 ± 12.1 pg/ml in the OVX + E group (n = 5). The thoracic aorta was obtained from rats after sacrifice. The endothelium was removed from the aorta by scraping with blade to ensure that the sample was mainly derived from VSMC.

High-density oligonucleotide microarray analysis

Total RNA was extracted from the aorta with Isogen (Wako Junyaku Ltd.) according to the manufacturer's instructions. One microgram of RNA isolated from the aorta of OVX + E group, OVX + V group and Sham group (n = 2, each group) rats was amplified up to approximately 100 μg cRNA and hybridized to the high-density oligonucleotide microarray (GeneChip Rat GenomeU34A; Affymetrix, Santa Clara, CA) as described previously (Ishii et al., 2000). This array contains probes interrogating approximately 7000 full-length rat genes. The intensity for each feature of the array was calculated by using Affymetrix Gene Chip version 3.3 software. The average intensity was made equal to the target intensity, which was set at 100, to reliably compare variable multiple arrays. In addition to the default parameters of the software, we added a criteria that >100 average intensity units per transcript was required for a gene to be considered "present" in the samples. Genes, with an intensity of around 1.5-fold higher or lower in the OVX + E group than in the OVX + V group, were identified.

Real-time PCR

Total RNA was treated with DNase (Progema) at 37°C for 1 h. One microgram of RNA was reverse transcribed into cDNA using Oligo dT primer (GIBCO) and an Ominiscript kit (GIBCO). Real-time PCR was carried out in an iCycler (BioRad) at 95°C for 15 min to activate HotStar Taq DNA polymerase, followed by 35 cycles of 94°C for 15 sec, 55°C for 30 sec and 72°C for 30 sec using a SYBR green assay kit (TAKARA). Amplicons were around 100 bp long. We selected the primer sets that amplified the sequences as close as possible to the 3′ coding region of the target genes. The sequences of the primers are shown in Table 1. The expression levels of each gene were normalized for glyceraldehyde-3-phosphate dehydrogenase expression.

Cell culture

VSMC were harvested from the aorta of Wistar rats by enzymatic dissociation, as previously reported (Watanabe et al., 2001). Cells were maintained in Dulbecco's modified Eagle's medium (Nikken Bio Medical Laboratory, Tokyo) supplemented with 10% fetal bovine serum (Intergen Co., Purchase, NY), penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37°C in a humidified atmosphere of 95% air and