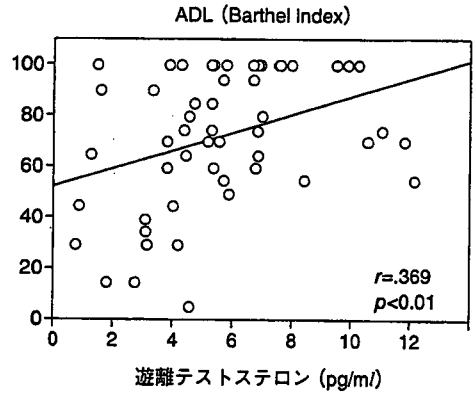

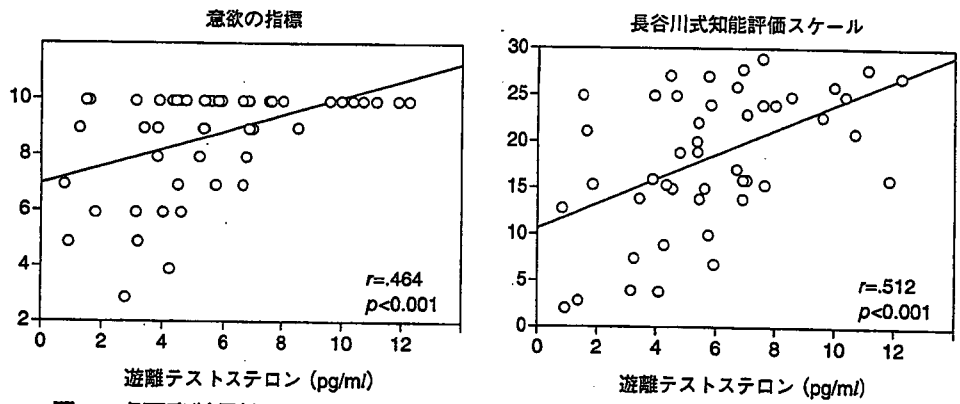

図3　虚弱高齢男性における血中遊離テストステロン濃度と日常生活機能の関係
(Akishita M, et al. Testosterone and comprehensive geriatric assessment in frail elderly men. J Am Geriatr Soc 2003；51：1324-1326 より引用)

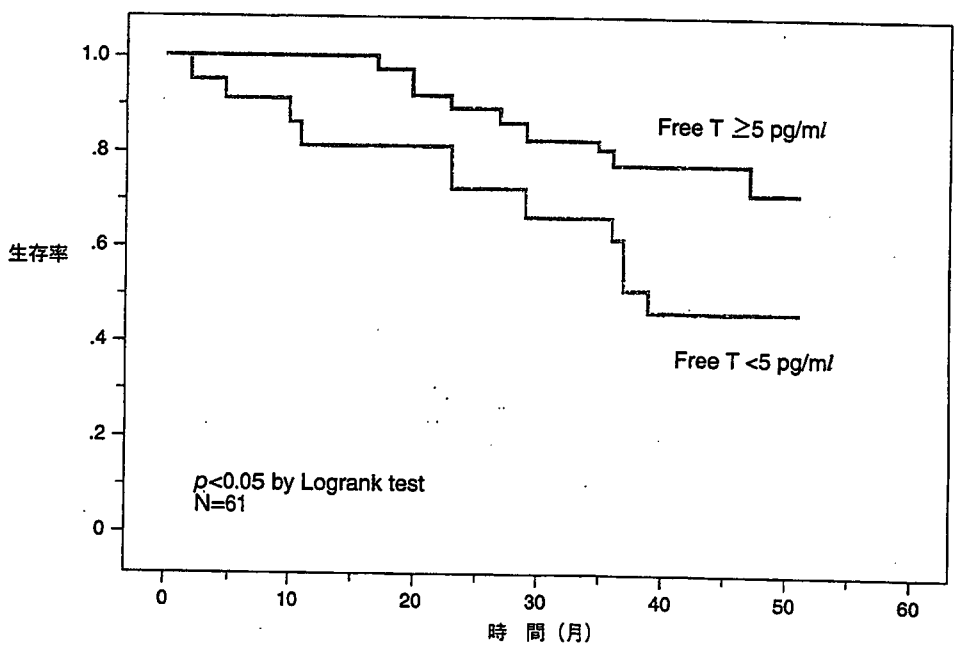

図4　虚弱高齢男性の遊離テストステロン(Free-T)濃度と生命予後
介護を要する男性(平均 82 歳)の血清 Free-T 濃度を測定し，平均 3.1 年間追跡した

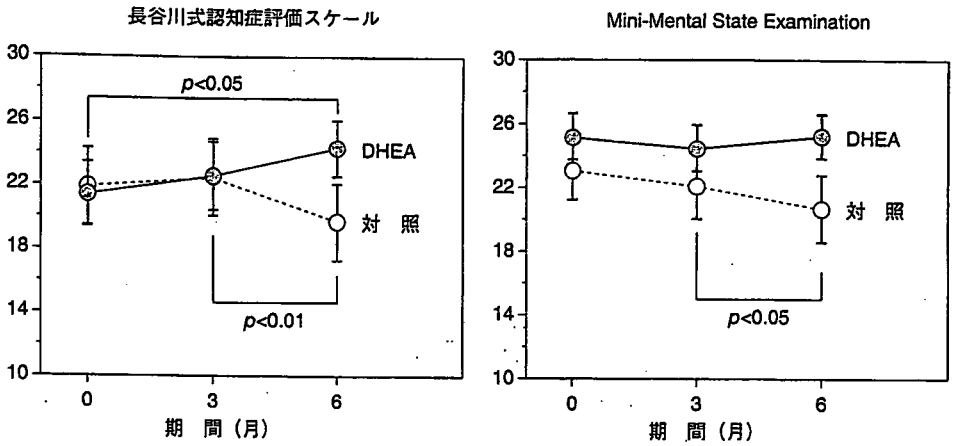

図 5 軽度認知機能障害を有する高齢女性に対する DHEA 補充療法の効果
軽度認知機能障害と診断された女性（平均 81 歳）に DHEA 25 mg/ 日を投与し，非投与群と比較した

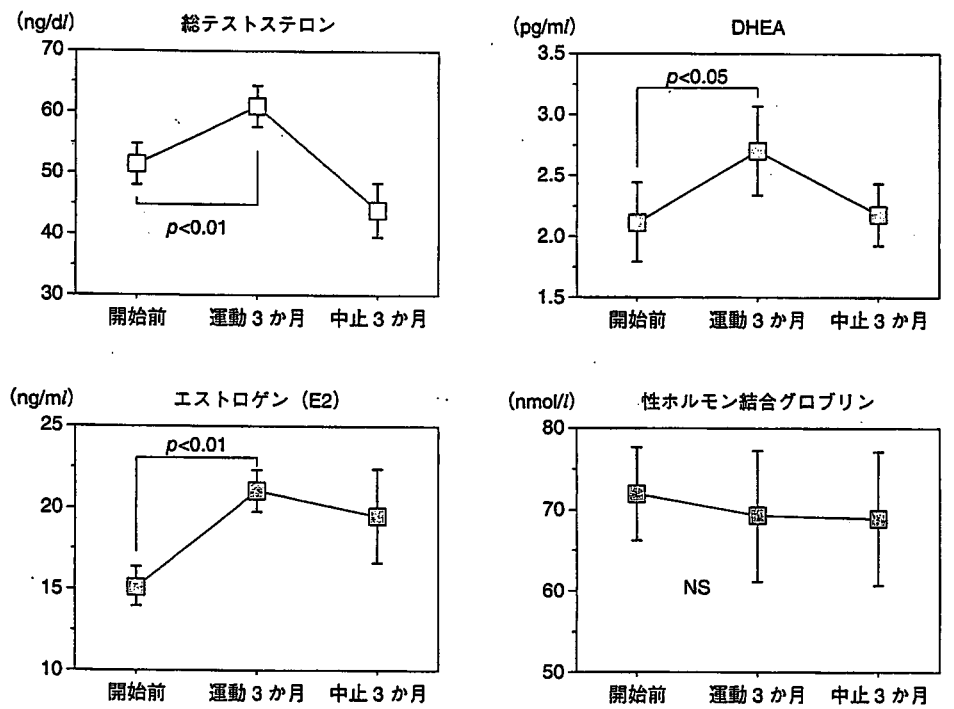

図 6 虚弱高齢女性に対する運動療法のホルモン増加効果
（Akishita M, et al. Effects of physical exercise on plasma concentrations of sex hormones in elderly women with dementia J Am Geriatr Soc. 2005；53：1076-1077 より引用）
グループホーム入所中の女性 13 名（平均 84 歳）に対し，イスおよびダンベルを用いた上下肢筋力トレーニングを週日 30 分間，3 か月間実施した

変化に加え，インスリン抵抗性，内臓脂肪蓄積が指摘されており，動脈硬化の進行に関わると考えられる．上述した Massachusetts Male Aging Study では，テストステロン低値がその後の 2 型糖尿病発症につながることを報告している．

アンドロゲン補充療法の動脈硬化とその危険因子に対する効果については，体脂肪の減少，インスリン感受性の改善，LDL コレステロールの低下といった代

謝性変化，心筋梗塞や脳卒中の引き金となる凝固系と炎症への作用が報告されているが，効果が認められなかったとする報告も同程度みられる．動脈硬化の進展，心筋梗塞や脳卒中など心血管イベントといった長期予後に対する効果は不明である．

アンドロゲンは高齢男性の生活機能にも影響している．介護を受けている男性では，総テストステロン濃度および遊離テストステロン濃度は，基本的 ADL，手段的 ADL，認知機能，意欲の指標と正相関した．特に遊離テストステロンと認知機能，意欲との関係は強く，年齢や各種栄養の指標で補正しても有意であった（図 3）．一方，DHEA-S 濃度は認知機能とのみ相関した．興味あることに，この集団で遊離テストステロン濃度 5 pg/ml 未満の男性は，5 pg/ml 以上の男性に比べてその後 3 年間の死亡率が高かった（図 4）．

2 女性におけるアンドロゲン低下と疾患

閉経後女性を 16 年間追跡した研究[3]では，骨粗鬆症の圧迫骨折に由来する身長低下に，開始時のテストステロン濃度は関連したがエストラジオールやエストロンは関連しなかった．同様に，DHEA-S 濃度が骨量と関連したことが複数報告されている．

多嚢胞性卵巣症候群の女性では高アンドロゲン血症と内臓肥満を呈するのに対し，閉経後女性ではアンドロゲン低値と関連して体脂肪（皮下脂肪＋内臓脂肪）蓄積を示す．テストステロン補充により内臓脂肪が減少したという報告もある．また，アンドロゲン低値は，男性と同様，閉経後女性における動脈硬化の進行にも関係しているようである．101 名の更年期女性（平均 47 歳）を対象に超音波で頸動脈肥厚を調べたイタリアの研究[4]では，DHEA-S 濃度が肥厚度と逆相

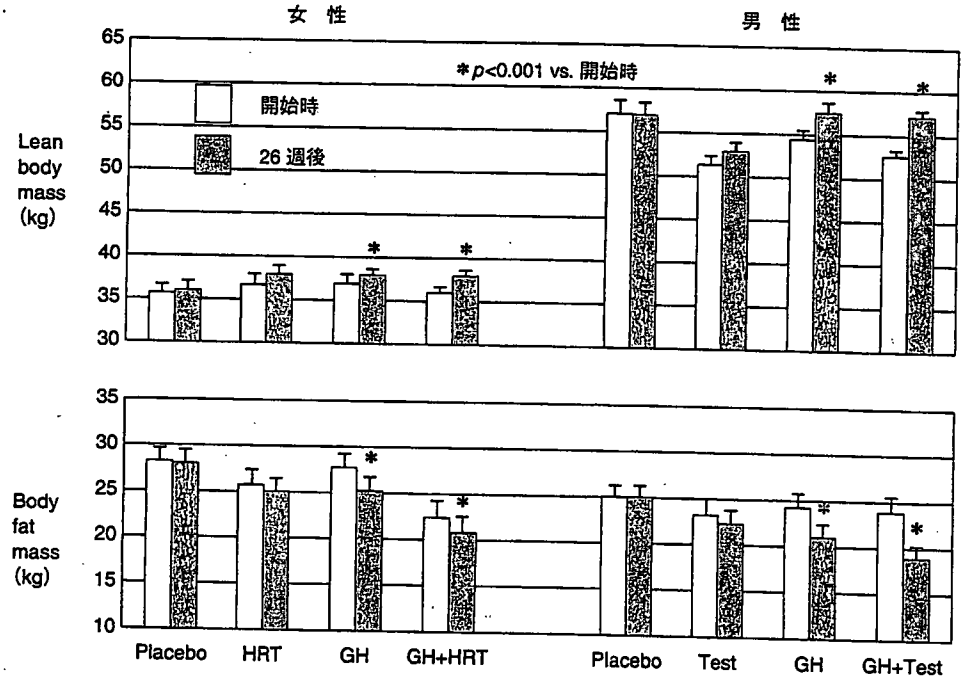

図 7 高齢者における GH 補充療法の筋肉量および体脂肪量に対する効果
(Blackman MR, et al. Growth hormone and sex steroid administration in healthy aged women and men：a randomized controlled trial. JAMA 2002；288：2282-2292 より引用)
平均 72 歳の健常男女に対し，プラセボもしくは GH 投与(20 μg/kg 皮下注射，週 3 回)と，女性には HRT(エストロゲン＋プロゲスチン)，男性にはテストステロン(Test)を組み合わせて投与した．筋肉量は lean body mass，体脂肪量は body fat mass として DXA 法で測定した

関した.

高齢女性でもDHEA-S濃度は生活機能と正相関する.また,軽度認知機能障害を有する高齢女性に対してDHEA補充療法を行ったところ,認知機能は有意に改善した(図5).運動療法は高齢者の生活機能を改善し,転倒や心血管病のリスクを減らす効果があるとされる.認知症のグループホーム入所高齢女性(図6)に運動療法を行ったところ各種ホルモンの血中濃度が増加した.在宅高齢者の転倒予防運動教室でも同様の効果を確認しており,運動療法にはホルモン補充療法の代替療法としての意義も期待できる.

GHとIGF-1

GH欠乏は小児期には成長障害(小人症)をもたらし,成人でも筋肉減少と関係するが,加齢によるGH/IGF-1低下が老化とどの程度関係しているのか明らかでない.GH/IGF-1の濃度低下が骨量減少や体脂肪蓄積と関連したという報告があるが,その関係はアンドロゲンに比べると弱い印象がある.

GH補充療法の効果も,GH欠乏症に対しては明瞭であり,成人では自覚的健康感の改善,筋肉増加,体脂肪減少,各種代謝異常の改善が示されている.一方,明らかなGH欠乏症ではない高齢者にGHを投与した場合の効果も検討されている.女性ではHRTと組み合わせて,男性ではテストステロンと組み合わせてGH投与を行った無作為比較試験では,GHによりIGF-1濃度は2倍程度に上昇し,26週後には性ホルモンの有無にかかわらず筋肉量は増加し,体脂肪は減少した(図7).同じグループの研究では,GH投与によりタンパク合成指標の増加,内臓脂肪の減少はみられたものの,骨量の有意な増加はみられなかった.また,耐糖能異常の発生がGH投与群で多くみられ,有害作用にも注意が必要である.

文献

1) 岩本晃明ほか.日本人成人男子の総テストステロン,遊離テストステロンの基準値の設定.日泌尿会誌2004;95:751-760.
2) Snyder PJ, Peachey H, Hannoush P, et al: Effect of testosterone treatment on bone mineral density in men over 65 years of age. J Clin Endocrinol Metab 1999;84:1966-1972.
3) Jassal SK, Barrett-Connor E, Edelstein SL: Low bioavailable testosterone levels predict future height loss in postmenopausal women. J Bone Miner Res 1995;10:650-654.
4) Bernini GP, Sgro' M, Moretti A, Argenio GF, Barlascini CO, Cristofani R, Salvetti A: Endogenous androgens and carotid intimal-medial thickness in women. J Clin Endocrinol Metab 1999;84:2008-2012.

keeping with the harsher American climate and influence of the Protestant ethic, cold-water wraps were the rule in the New World.

Almost 100 years after Alzheimer's death, we seem to have come full circle in our attempts to address humanely with the behavioral manifestations of dementia. Clearly, one hopes that articles such as these will prevent us from throwing out this important form of treatment along with the bath water.

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REFERENCES

1. Sloane PD, Hoeffer B, Mitchell M et al. Effects of person-centered showering and the towel bath on bathing-associated aggression, agitation, and discomfort in nursing home residents with dementia: A randomized, controlled trial. J Am Geriatr Soc 2004;52:1795–1804.
2. Naik AD, Concato J, Gill TM. Bathing disability in community-living older persons. Common, consequential, and complex. J Am Geriatr Soc 2004;52:1805–1810.
3. Evans L. The bath! Reassessing a familiar elixir in old age. J Am Geriatr Soc 2004;52:1957–1958.
4. Mauer K, Mauer U. Alzheimer: The Life of a Physician and the Career of a Disease. New York: Columbia University Press, 2004.
5. Whorton JC. Nature Cures. The History of Alternative Medicine in America. Oxford: Oxford University Press, 2002.

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.

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

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

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

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.

ACKNOWLEDGMENT

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REFERENCES

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

RACIAL DIFFERENCES IN PRESSURE ULCER PREVALENCE IN NURSING HOMES

To the Editor: We read with great interest the recent article regarding black/white differences in the rate of nursing home–acquired pressure ulcers. Indeed, the findings reported were similar to what was found using the Health Care Financing Administration's Multi-State, Case-Mix and Quality Demonstration Project, which involved all Medicare/Medicaid certified nursing homes (n = 1,492) in five U.S. states (Kansas, Maine, Mississippi, New York, South Dakota). We identified 223,448 entrants to nursing homes in these five states over a 4-year period (1992–96). Patients were evaluated using the federally mandated Resident Assessment Instrument, which includes a 300-item Minimum Data Set (MDS). At least 100 residents of each racial/ethnic category were required. A nested linear model provided estimates of state- and sex-stratified differences in pressure ulcer prevalence after adjustment for pressure ulcer risk factors. Across all state/sex strata, blacks were substantially less likely than whites to have a Grade I pressure ulcer recorded. Higher-grade ulcers (II–IV) were, alternatively, consistently higher in blacks than whites, and even greater disparities were seen when only the highest-grade (IV) ulcers were compared. These findings were slightly adjusted when physical mobility (as measured by activities of daily living) was controlled for, although not so much as to change the basic interpretation. Control by additional clinical, diagnostic, behavioral, social, facility, and area-level characteristics failed to reveal any further confounding, demonstrating that this analysis was robust to adjustment for a wide-ranging set of factors identified or hypothesized as risk factors for pressure ulcer development. Moreover, it also observed that pressure ulcer prevalence in Native Americans demonstrated a similar pattern in South Dakota and Mississippi to that of whites; rates of Grade I ulcers were generally lower, whereas higher-grade ulcers were more common in Native Americans than in non-Hispanic whites.

Underdiagnosis of low-grade pressure ulcers in racial minorities, with subsequent progression to open lesions of higher grade, is largely consistent with these findings, as well as the findings of another study.[1] It has been long noted that the definition of low-grade pressure ulcers (persistent nonblanchable erythema) could result in the underdiagnosis of these lesions on dark skin. Because detection of low-grade pressure ulcers is an important factor in preventing their progression to higher stages, underdiagnosis likely contributes to the higher rates of high-grade ulcers found in older blacks and Hispanics. The relationship between contextual factors (such as resources and staffing) related to the types of nursing homes serving predominantly people of color and the underdiagnosis of pressure ulcers needs to be explored. Facilities serving primarily African Americans may have fewer funds and consequently offer fewer services and provide less staff training and amenities than other facilities.[2,3]

Although attempts have been made to enhance the detection of low-grade ulcers in blacks and Hispanics,[4] the extent to which public reporting of quality indicators focused on pressure ulcers and other quality improvement initiatives[5] is likely to reduce or exacerbate[3] racial/ethnic differences in pressure ulcer occurrence in nursing homes remain to be evaluated.

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Renin-Angiotensin System Modulates Oxidative Stress–Induced Endothelial Cell Apoptosis in Rats

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Abstract—The role of the renin-angiotensin system in oxidative stress–induced apoptosis of endothelial cells (ECs) was investigated using a rat model and cultured ECs. EC apoptosis was induced by 5-minute intra-arterial treatment of a rat carotid artery with 0.01 mmol/L H_2O_2 and was evaluated at 24 hours by chromatin staining of *en face* specimens with Hoechst 33342. Although activity of angiotensin-converting enzyme in arterial homogenates was not increased, administration of an angiotensin-converting enzyme inhibitor temocapril for 3 days before H_2O_2 treatment inhibited EC apoptosis, followed by reduced neointimal formation 2 weeks later. Also, an angiotensin II type 1 (AT1) receptor blocker (olmesartan) inhibited EC apoptosis, whereas angiotensin II administration accelerated apoptosis independently of blood pressure. Next, cultured ECs derived from a bovine carotid artery were treated with H_2O_2 to induce apoptosis, as evaluated by DNA fragmentation. Combination of angiotensin II and H_2O_2 dose-dependently increased EC apoptosis and 8-isoprostane formation, a marker of oxidative stress. Conversely, temocapril and olmesartan reduced apoptosis and 8-isoprostane formation induced by H_2O_2, suggesting that endogenous angiotensin II interacts with H_2O_2 to elevate oxidative stress levels and EC apoptosis. Neither an AT2 receptor blocker, PD123319, affected H_2O_2-induced apoptosis, nor a NO synthase inhibitor, N^G-nitro-L-arginine methyl ester, influenced the effect of temocapril on apoptosis in cell culture experiments. These results suggest that AT1 receptor signaling augments EC apoptosis in the process of oxidative stress–induced vascular injury. (***Hypertension.*** **2005;45:1188-1193.)**

Key Words: angiotensin ■ apoptosis ■ carotid arteries ■ endothelium ■ free radicals

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]

The renin-angiotensin system (RAS) is known to play a pivotal role in the process of vascular lesion formation such as atherosclerosis and restenosis after angioplasty. The expression of RAS components renin,[9] angiotensinogen,[10] angiotensin-converting enzyme (ACE),[11,12] and angiotensin II (Ang II) receptors[13] is upregulated in vascular lesions. Also, RAS inhibitors attenuate neointimal formation after vascular injury in animals[12,14] and endothelial dysfunction in humans.[15,16] The interaction between oxidative stress and the RAS, factors essential for the development of vascular disease, needs to be addressed. It has been demonstrated that RAS activation induces oxidative stress[17–20] and can enhance EC apoptosis in vitro.[20,21] However, it has not been elucidated whether the RAS plays a role in oxidative stress–induced vascular injury in vivo, particularly in EC apoptosis, an initial and important process in atherosclerosis.[1,22,23]

In this study, we first tested whether the RAS would augment EC apoptosis induced by brief exposure to H_2O_2 and the subsequent neointimal formation using a rat model.[24] Next, we used an in vitro model of H_2O_2-induced EC apoptosis to clarify the underlying cellular mechanism.

Methods

H_2O_2 Treatment of Carotid Artery

Ten- to 12-week-old male Wistar rats (Japan Clea; Tokyo, Japan) were used in this study. Maintenance of rats and surgical procedures for H_2O_2 treatment were performed as described previously.[24] Methods are detailed in the online data supplement (available online at http://www.hypertensionaha.org). All of the experimental protocols were approved by the animal research committee of the Kyorin University School of Medicine.

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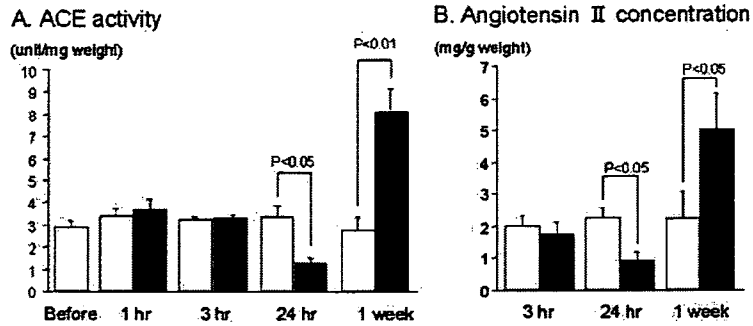

Figure 1. ACE activity and Ang II concentration in rat carotid artery after H_2O_2 treatment. Treated (closed bars) and contralateral (open bar) carotid arteries were harvested at the indicated time points after H_2O_2 treatment. ACE activity and Ang II concentration in tissue homogenates were measured using a pool of samples consisting of 6 to 10 arteries and were calibrated by the tissue wet weight. Values are expressed as mean±SEM of 5 to 6 independent pools.

Animal Groups and Blood Pressure Measurement

An ACE inhibitor, temocapril (10 mg/kg per day; donated by Sankyo Co, Ltd; Tokyo, Japan), or vehicle (40% ethanol) was administered orally using a feeding tube daily for 3 days. Separately, an Ang II type 1 (AT1) receptor blocker, olmesartan (1 mg/kg per day; donated by Sankyo Co, Ltd), or vehicle (40% ethanol) was administered orally for 3 days. Ang II was administered for 3 days using an osmotic minipump (Model 103D; Alza Corporation) prefilled with Ang II (0.7 mg/kg per day; Sigma), and implanted subcutaneously in the back. Hydralazine (25 mg/kg per day; Sigma) was orally administered alone for 5 days and subsequently with or without Ang II for 3 days before H_2O_2 treatment to abolish the effect of Ang II on blood pressure. On the last day of drug administration, blood pressure was measured with the animals in a conscious state by the tail-cuff method (BP-98A; Softron), and then H_2O_2 treatment was performed.

Measurement of ACE Activity and Ang II Concentration

At various time points after H_2O_2 treatment, the carotid arteries were dissected, weighed, and stored at −80°C. Pooled samples (n=6 to 10 for a pool) were homogenized with a polytron homogenizer in distilled water and centrifuged at 25 000*g* for 30 minutes at 4°C. ACE activity and Ang II concentration in the supernatants were measured using a colorimetric assay[12] and a sensitive radioimmunoassay, respectively. The values were calibrated by the tissue wet weight. ACE activity in the cell lysates of cultured ECs was measured using a colorimetric assay and calibrated by the protein concentration.

Evaluation of EC Apoptosis and Neointimal Formation in Carotid Artery

EC apoptosis was evaluated at 24 hours after H_2O_2 treatment as described previously.[24] Neointimal formation in the common carotid artery was evaluated 2 weeks after H_2O_2 treatment as described previously.[24] Methods are detailed in the online data supplement.

Induction of EC Apoptosis in Culture

ECs isolated from bovine carotid artery[25] were used at the fifth to seventh passage. When the cells had grown to 80% confluence, ECs were pretreated for 24 hours with culture medium containing the reagents that were tested in the experiments. Subsequently, after washing twice with Hank's balanced salt solution, the cells were exposed to H_2O_2 (0.01 to 0.2 mmol/L) diluted in Hank's balanced salt solution for 1.5 hours at 37°C to induce apoptosis. The cells were washed twice with Hank's balanced salt solution and then cultured in culture medium containing the reagents until assay.

The effects of temocapril, olmesartan, a NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME; Sigma), an Ang II type 2 (AT2) receptor blocker, PD123319 (Research Biochemical International), and Ang II (Sigma) were examined by adding them into the medium throughout the experiments.

Measurement of EC Apoptosis and Oxidative Stress Markers in Culture

For quantitative determination of apoptosis, we measured DNA fragmentation and caspase-3 activity at 24 hours after H_2O_2 treatment. DNA fragmentation was evaluated by histone-associated DNA fragments using a photometric enzyme immunoassay (EIA; Cell Death Detection ELISA; Roche) according to manufacturer instructions. Caspase-3 activity was measured using a colorimetric kit (Caspase-3 Colorimetric Activity Assay Kit; Chemicon) based on its activity to digest the substrate DVED according to manufacturer instructions.

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 manufacturer instructions. Intracellular oxidative stress levels were measured using 2′,7′-dichlorofluorescein (DCF) as described previously,[26] and the intensity values were calculated using the Metamorph software.

Real-Time Polymerase Chain Reaction

Real-time polymerase chain reaction (PCR) to quantify AT1 receptor mRNA in cultured ECs was performed using SYBR Green I (Sigma) and the ABI Prism 7000 Sequence Detection System (Applied Biosystems). Methods are detailed in the online data supplement.

Data Analysis

The values are expressed as mean±SEM in the text and figure data were analyzed using 1-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.

Results

ACE Activity in Carotid Artery After H_2O_2 Treatment

We examined whether H_2O_2 treatment would activate ACE and stimulate Ang II synthesis in the carotid artery. As shown in Figure 1A, ACE activity in tissue homogenates was not increased at 1 to 3 hours and, rather, was decreased at 24 hours, probably because of EC denudation.[24] Low ACE activity in the de-endothelialized artery is consistent with the previous finding[11,12] and was confirmed by measurement of ACE activity in the rat carotid artery, in which ECs were denuded ex vivo using a cotton swab (data not shown). In contrast, ACE activity was significantly increased at 1 week after H_2O_2 treatment, reflecting neointimal formation.[11,12,24] Ang II concentration in arterial homogenates showed similar changes to ACE activity after H_2O_2 treatment (Figure 1B).

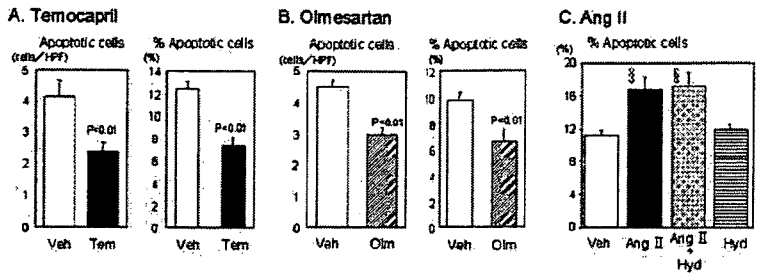

Figure 2. Effects of temocapril (A), olmesartan (B), and Ang II (C) on EC apoptosis after H_2O_2 treatment in rat carotid artery. The number of apoptotic ECs was counted per high power field (HPF; ×200), and the ratio of the apoptotic cell number to the intact cell number was calculated using *en face* specimens of the carotid artery stained with Hoechst 33342. A and B, Temocapril (Tem; 10 mg/kg per day; n=12), olmesartan (Olm; 1 mg/kg per day; n=8), or their vehicle (Veh; n=10 and n=6, respectively) was administered orally for 3 days before H_2O_2 treatment. C, Ang II (0.7 mg/kg per day) or its vehicle was administered subcutaneously for 3 days using an osmotic minipump alone (n=8 for Ang II and n=10 for vehicle) or in combination with oral administration of hydralazine (Hyd; 25 mg/kg per day; n=6 for Ang II and n=6 for vehicle; single administration for 5 days and coadministration with Ang II for 3 days) before H_2O_2 treatment. §$P<0.01$ vs vehicle. Values are expressed as mean±SEM.

Effect of RAS Inhibitors and Ang II on EC Apoptosis After H_2O_2 Treatment in Rats

The effects of an ACE inhibitor, temocapril, and an AT1 receptor blocker, olmesartan, on EC apoptosis were examined at 24 hours after H_2O_2 treatment because the peak of apoptosis was observed at 6 to 24 hours.[24] Administration of 10 mg/kg per day temocapril or 1 mg/kg per day olmesartan for 3 days before H_2O_2 treatment did not significantly change body weight, heart rate, or blood pressure, but this dose of temocapril effectively inhibited plasma ACE activity (data not shown). The number and percentage of apoptotic cells, as determined using *en face* specimens with Hoechst 33342 staining, were significantly decreased by temocapril compared with vehicle (Figure 2A; supplemental Figure I, available online at http://www.hypertensionaha.org). Olmesartan showed a comparable inhibitory effect on EC apoptosis (Figure 2B).

Ang II was administered for 3 days in combination with hydralazine to eliminate the effect of Ang II on blood pressure. Consequently, systolic blood pressure was higher in rats administered Ang II alone (161±5 mm Hg; $P<0.01$) than in the other groups of rats: 123±3 mm Hg in the vehicle group, 129±7 mm Hg in the Ang II plus hydralazine group, and 114±4 mm Hg in the hydralazine group. In contrast to RAS inhibitors, Ang II administration augmented EC apoptosis independent of the pressor effect because coadministration of hydralazine did not influence EC apoptosis (Figure 2C).

Inhibitory Effect of Temocapril on Neointimal Formation

We examined whether inhibition of EC apoptosis by temocapril would result in a reduction of neointimal formation. To do so, histological analysis of the carotid artery was performed 2 weeks after H_2O_2 treatment. Temocapril significantly decreased the neointimal area and the intima/media area ratio: intima/media area ratio was 0.18±0.02 in the vehicle group versus 0.12±0.02 in the temocapril group (n=9; $P<0.05$; supplemental Figure II). Because temocapril was administered for only 3 days before H_2O_2 treatment, it is suggested that inhibition of EC apoptosis may play a mechanistic role in attenuation of neointimal formation, although ACE inhibitors have various effects such as anti-inflammation and antimigration as well.

Effect of RAS Inhibitors on H_2O_2-Induced EC Apoptosis in Culture

To reproduce oxidative stress–induced EC apoptosis in culture, we applied 0.2 mmol/L H_2O_2 to cultured ECs derived from a bovine carotid artery for 1.5 hours based on dose- and time-response experiments. EC apoptosis, as determined by DNA fragmentation and caspase-3 activity, was induced at 24 hours after H_2O_2 treatment. Comparable to in vivo experiments, temocapril inhibited EC apoptosis in a dose-dependent manner (Figure 3A and 3B). The inhibitory effect on EC apoptosis was mimicked by 10 μmol/L olmesartan (Figure 3C), but an AT2 receptor blocker, PD123319, did not influence EC apoptosis (supplemental Figure IIIA). The involvement of NO in the effect of temocapril was examined using an NO synthase inhibitor, L-NAME, because ACE inhibitors stimulate NO production via the inhibition of bradykinin degradation.[12] However, L-NAME did not influence the effect of temocapril (supplemental Figure IIIB).

To make the interaction between H_2O_2 and Ang II clear, dose response and combined effects of both agents on EC apoptosis and 8-isoprostane formation, a marker of oxidative stress, were examined. As shown in Figures 3D and 4A, combination of Ang II and H_2O_2 dose-dependently stimulated EC apoptosis and 8-isoprostane formation. Conversely, temocapril and olmesartan restrained 8-isoprostane formation (Figure 4B) and intracellular DCF formation (Figure 4C; supplemental Figure IV) induced by H_2O_2, suggesting that endogenous Ang II also interacts with H_2O_2 to elevate oxidative stress levels.

ACE activity and the expression of AT1 receptor mRNA in cultured ECs were determined. ACE activity calibrated by the protein concentration was not changed after H_2O_2 treatment: 106±9% at 3 hours and 103±8% at 24 hours after H_2O_2 treatment compared with the values at baseline and 3 hours after vehicle treatment (100±3% and 96±13%, respectively; n=3). The relative amount of the AT1 receptor to the housekeeping gene G3PDH, as measured by real-time PCR analysis, was not significantly changed after H_2O_2 treatment: 91±2% at 1.5 hours during the treatment, 99±5% at 3 hours, and 102±4% at 6 hours after H_2O_2 treatment compared with vehicle treatment (100±6%; n=3). Considering negative regulation in vascular smooth muscle cells[27,28] together, upregulation of the AT1 receptor is not likely to occur in response to H_2O_2 treatment.

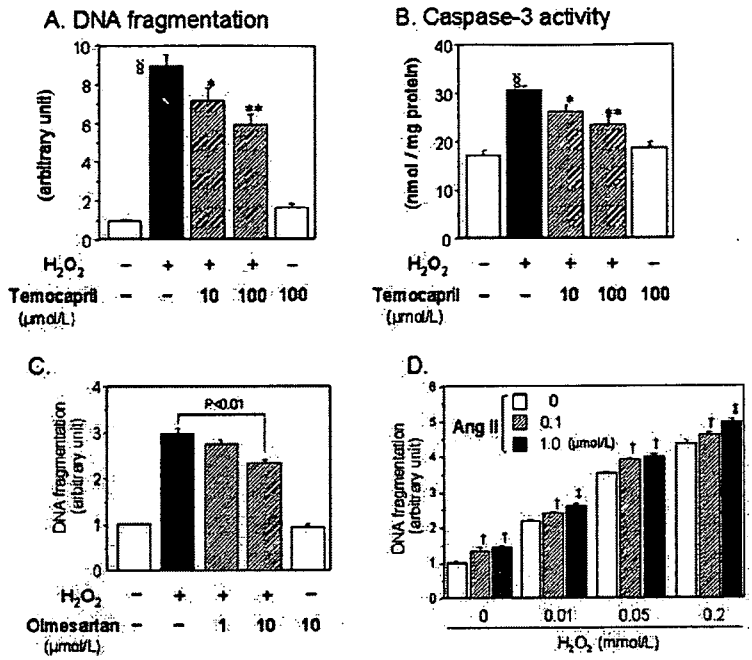

Figure 3. Effects of temocapril (A and B), olmesartan (C), and Ang II (D) on H_2O_2-induced EC apoptosis in culture. A through D, Temocapril, olmesartan, Ang II, or their vehicle was added to the culture medium 24 hours before H_2O_2 treatment until assay. EC apoptosis was evaluated 24 hours after H_2O_2 treatment (0.2 mmol/L in A through C; 0.01 to 0.2 mmol/L in D) by means of DNA fragmentation (A, C, and D; n=3) and caspase-3 activity (B; n=4). §$P<0.01$ vs H_2O_2 (−). *$P<0.05$; **$P<0.01$ vs H_2O_2 (+) + temocapril (−). †$P<0.05$ vs Ang II (−). ‡$P<0.05$ vs Ang II 0.1 μmol/L. Values are expressed as mean±SEM. Similar results were obtained in 3 independent experiments.

Discussion

This study was conducted to elucidate the role of the RAS in oxidative stress–induced EC apoptosis using a rat model and cultured ECs. Treatment with H_2O_2 did not increase ACE activity or Ang II in the rat carotid artery during the acute phase. However, administration of an ACE inhibitor, temocapril, and an AT1 receptor blocker, olmesartan, inhibited EC apoptosis in vivo. Furthermore, we demonstrated using cultured ECs that combination of Ang II and H_2O_2 dose-dependently increased EC apoptosis and 8-isoprostane formation. In addition, temocapril and olmesartan reduced but not canceled EC apoptosis and 8-isoprostane formation induced by H_2O_2, suggesting that endogenous Ang II interacts with H_2O_2 to elevate oxidative stress levels and EC apoptosis.

In vascular lesions such as atherosclerosis and intimal hyperplasia, the production of reactive oxygen species[4,5] as well as the components of the RAS[9–12] are upregulated, suggesting a possible interaction between them. A number of investigations have clarified that Ang II induces oxidative stress in vascular cells. Ang II stimulates the production of reactive oxygen species in ECs by upregulating the subunits of NAD(P)H oxidase: gp91 phox[17] and p47 phox.[18] It has been reported that the RAS enhances EC apoptosis in vitro[20,21] and contributes to endothelial dysfunction in patients with renovascular hypertension through the oxidant-dependent mechanism.[19] Conversely, it remains unknown whether oxidative stress could regulate the RAS; only 1 report has shown the modulation of ACE by oxidative stress.[29] Usui et al[29] reported that the inhibition of NO synthesis by chronic administration of L-NAME in rats augmented superoxide production and ACE activity in aortic ECs, and these effects were eliminated by treatment with

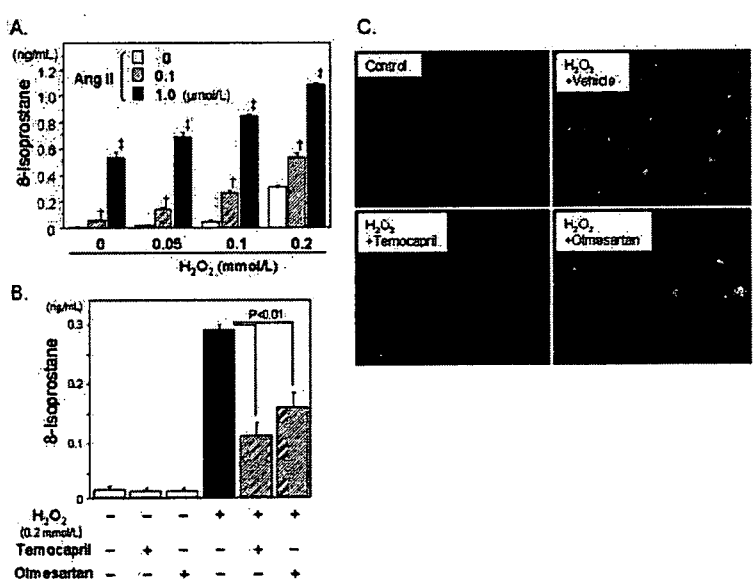

Figure 4. Effects of Ang II (A), temocapril, and olmesartan (B and C) on 8-isoprostane and DCF formation in cultured ECs. Ang II, temocapril (100 μmol/L), olmesartan (10 μmol/L), or their vehicle was added to the culture medium 24 hours before H_2O_2 treatment until assay. Then 8-isoprostane concentration in the culture supernatant and intracellular DCF intensity were measured 3 hours after H_2O_2 treatment. †$P<0.05$ vs Ang II (−). ‡$P<0.05$ vs Ang II 0.1 μmol/L. Values are expressed as mean±SEM (n=3). Similar results were obtained in 3 independent experiments.

antioxidants. In the present study, ACE activity in the carotid artery was not increased until 24 hours after H_2O_2 treatment. We also found that ACE activity was not changed after H_2O_2 treatment in cell culture experiments. Furthermore, the expression of AT1 receptor mRNA in cultured ECs, as measured using real-time PCR, was not increased after H_2O_2 treatment. Together, it is not likely that Ang II production or its receptor expression was upregulated in response to H_2O_2.

However, an ACE inhibitor, temocapril, and an AT1 receptor blocker, olmesartan, inhibited H_2O_2-induced EC apoptosis in rats as well as in cell culture experiments. No influence of L-NAME on the antiapoptotic effect of temocapril in cell culture studies indicates that the effect of temocapril was attributable to the inhibition of Ang II synthesis. An AT2 receptor blocker, PD123319, did not influence H_2O_2-induced EC apoptosis either. This result appears to be inconsistent with the previous finding[30] but suggests a minimal contribution of the AT2 receptor in H_2O_2-induced EC apoptosis or minimal expression of the AT2 receptor in the cultured ECs used in the present study. Reduction in 8-isoprostane formation by temocapril and olmesartan suggests that endogenous Ang II adds to the oxidative stress levels on top of exogenous H_2O_2; otherwise temocapril and olmesartan would have antioxidant effects independent of Ang II through currently unknown mechanisms, although the in vivo role of bradykinin/NO in the effect of ACE inhibitors and that of the AT2 receptor remain to be addressed.

Administration of Ang II provided evidence that Ang II can interact with H_2O_2 to elevate oxidative stress levels and induce EC apoptosis. In rat experiments, a high and pressor dose of Ang II was used in combination with hydralazine[31] because 3-day administration of lower doses of Ang II (0.1 to 0.2 mg/kg per day) did not show significant effects on EC apoptosis (data not shown). The cell culture experiments to examine the effect of submaximal doses of Ang II and H_2O_2 on apoptosis and 8-isoprostane formation gave us clear information that AT1 receptor signaling augments EC apoptosis by an interaction with oxidative stress. Although the doses of H_2O_2 and the time duration of exposure were optimized on the basis of the time- and dose-response experiments, the conditions in cell culture studies were different from those in animal studies. However, it has been reported that cigarette smoke, oxidized lipoproteins, and polymorphonuclear leukocytes, which play important roles in atherogenesis, can generate H_2O_2 concentrations of 0.05 to 0.2 mmol/L in vitro.[32] These reports suggest that the dosages of H_2O_2 used in the present study do not far exceed the physiological range, although direct comparison of physiological or pathophysiological conditions with those in our experiments may be inappropriate.

Considering the stimulatory effect of Ang II on free radical production,[17–19] our finding that endogenous Ang II exacerbates EC apoptosis induced by exogenous H_2O_2 is not surprising. In fact, a number of reports have shown experimentally that RAS inhibitors can reduce the production of reactive oxygen species in pathological conditions such as peripheral arteries in rats with chronic heart failure,[33] rat diabetic nephropathy,[34] and kidney mitochondria in aged rats.[35] In the clinical setting, it is reported that administration of an AT1 receptor blocker (losartan) to patients with chronic renal disease reduced urinary excretion of oxidized albumin and malondialdehyde.[36] Also, 4-week treatment with losartan or an ACE inhibitor (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,[37] indicating that RAS 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[22,23] using an in vivo model. Moreover, our finding that RAS inhibitors attenuated EC apoptosis suggests broad end-organ protective effects of RAS inhibitors, which have been used for the treatment of hypertension and heart failure.

Perspectives

We found using an in vivo model and cultured ECs that Ang II elevated oxidative stress levels and increased EC apoptosis, whereas RAS inhibitors restrained them. These findings will add new information for cardiovascular research and the clinical application of RAS inhibitors.

Acknowledgments

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References

1. Ross R. Atherosclerosis–an inflammatory disease. *N Engl J Med.* 1999; 340:115–126.
2. Griendling KK, Sorescu D, Lassegue B, Ushio-Fukai M. Modulation of protein kinase activity and gene expression by reactive oxygen species and their role in vascular physiology and pathophysiology. *Arterioscler Thromb Vasc Biol.* 2000;20:2175–2183.
3. Zalba G, San Jose G, Moreno MU, Fortuno MA, Fortuno A, Beaumont FJ, Diez J. Oxidative stress in arterial hypertension: role of NAD(P)H oxidase. *Hypertension.* 2001;38:1395–1399.
4. Sorescu D, Weiss D, Lassegue B, Clempus RE, Szocs K, Sorescu GP, Valppu L, Quinn MT, Lambeth JD, Vega JD, Taylor WR, Griendling KK. Superoxide production and expression of nox family proteins in human atherosclerosis. *Circulation.* 2002;105:1429–1435.
5. Spiekermann S, Landmesser U, Dikalov S, Bredt M, Gamez G, Tatge H, Reepschlager N, Hornig B, Drexler H, Harrison DG. Electron spin resonance characterization of vascular xanthine and NAD(P)H oxidase activity in patients with coronary artery disease: relation to endothelium-dependent vasodilation. *Circulation.* 2003;107:1383–1389.
6. Rey FE, Li XC, Carretero OA, Garvin JL, Pagano PJ. Perivascular superoxide anion contributes to impairment of endothelium-dependent relaxation: role of gp91(phox). *Circulation.* 2002;106:2497–2502.
7. Pratico D, Tangirala RK, Rader DJ, Rokach J, FitzGerald GA. Vitamin E suppresses isoprostane generation in vivo and reduces atherosclerosis in ApoE-deficient mice. *Nat Med.* 1998;4:1189–1192.
8. Fennell JP, Brosnan MJ, Frater AJ, Hamilton CA, Alexander MY, Nicklin SA, Heistad DD, Baker AH, Dominiczak AF. Adenovirus-mediated overexpression of extracellular superoxide dismutase improves endothelial dysfunction in a rat model of hypertension. *Gene Ther.* 2002;9:110–117.
9. Iwai N, Izumi M, Inagami T, Kinoshita M. Induction of renin in medial smooth muscle cells by balloon injury. *Hypertension.* 1997;29: 1044–1050.
10. Rakugi H, Jacob HJ, Krieger JE, Ingelfinger JR, Pratt RE. Vascular injury induces angiotensinogen gene expression in the media and neointima. *Circulation.* 1993;87:283–290.

11. Rakugi H, Kim DK, Krieger JE, Wang DS, Dzau VJ, Pratt RE. Induction of angiotensin converting enzyme in the neointima after vascular injury. Possible role in restenosis. *J Clin Invest.* 1994;93:339–346.
12. Akishita M, Shirakami G, Iwai M, Wu L, Aoki M, Zhang L, Toba K, Horiuchi M. Angiotensin converting enzyme inhibitor restrains inflammation-induced vascular injury in mice. *J Hypertens.* 2001;19: 1083–1088.
13. Akishita M, Horiuchi M, Yamada H, Zhang L, Shirakami G, Tamura K, Ouchi Y, Dzau VJ. Inflammation influences vascular remodeling through AT2 receptor expression and signaling. *Physiol Genomics.* 2000;2:13–20.
14. Wu L, Iwai M, Nakagami H, Li Z, Chen R, Suzuki J, Akishita M, de Gasparo M, Horiuchi M. Roles of angiotensin II type 2 receptor stimulation associated with selective angiotensin II type 1 receptor blockade with valsartan in the improvement of inflammation-induced vascular injury. *Circulation.* 2001;104:2716–2721.
15. Antony I, Lerebours G, Nitenberg A. Angiotensin-converting enzyme inhibition restores flow-dependent and cold pressor test-induced dilations in coronary arteries of hypertensive patients. *Circulation.* 1996;94: 3115–3122.
16. Prasad A, Tupas-Habib T, Schenke WH, Mincemoyer R, Panza JA, Waclawin MA, Ellahham S, Quyyumi AA. Acute and chronic angiotensin-1 receptor antagonism reverses endothelial dysfunction in atherosclerosis. *Circulation.* 2000;101:2349–2354.
17. Rueckschloss U, Quinn MT, Holtz J, Morawietz H. Dose-dependent regulation of NAD(P)H oxidase expression by angiotensin II in human endothelial cells: protective effect of angiotensin II type 1 receptor blockade in patients with coronary artery disease. *Arterioscler Thromb Vasc Biol.* 2002;22:1845–1851.
18. Landmesser U, Cai H, Dikalov S, McCann L, Hwang J, Jo H, Holland SM, Harrison DG. Role of p47(phox) in vascular oxidative stress and hypertension caused by angiotensin II. *Hypertension.* 2002;40:511–515.
19. Higashi Y, Sasaki S, Nakagawa K, Matsuura H, Oshima T, Chayama K. Endothelial function and oxidative stress in renovascular hypertension. *N Engl J Med.* 2002;346:1954–1962.
20. Dimmeler S, Zeiher AM. Reactive oxygen species and vascular cell apoptosis in response to angiotensin II and pro-atherosclerotic factors. *Regul Pept.* 2000;90:19–25.
21. Lin LY, Lin CY, Su TC, Liau CS. Angiotensin II-induced apoptosis in human endothelial cells is inhibited by adiponectin through restoration of the association between endothelial nitric oxide synthase and heat shock protein 90. *FEBS Lett.* 2004;574:106–110.
22. Choy JC, Granville DJ, Hunt DW, McManus BM. Endothelial cell apoptosis: biochemical characteristics and potential implications for atherosclerosis. *J Mol Cell Cardiol.* 2001;33:1673–1690.
23. Dimmeler S, Haendeler J, Zeiher AM. Regulation of endothelial cell apoptosis in atherothrombosis. *Curr Opin Lipidol.* 2002;13:531–536.
24. Sudoh N, Toba K, Akishita M, Ako J, Hashimoto M, Iijima K, Kim S, Liang YQ, Ohike Y, Watanabe T, Yamazaki I, Yoshizumi M, Eto M, Ouchi Y. Estrogen prevents oxidative stress-induced endothelial cell apoptosis in rats. *Circulation.* 2001;103:724–729.
25. Akishita M, Kozaki K, Eto M, Yoshizumi M, Ishikawa M, Toba K, Orimo H, Ouchi Y. Estrogen attenuates endothelin-1 production by bovine endothelial cells via estrogen receptor. *Biochem Biophys Res Commun.* 1998;251:17–21.
26. Tampo Y, Kotamraju S, Chitambar CR, Kalivendi SV, Keszler A, Joseph J, Kalyanaraman B. Oxidative stress-induced iron signaling is responsible for peroxide-dependent oxidation of dichlorodihydrofluorescein in endothelial cells: role of transferrin receptor-dependent iron uptake in apoptosis. *Circ Res.* 2003;92:56–63.
27. Nickenig G, Strehlow K, Baumer AT, Baudler S, Wassmann S, Sauer H, Bohm M. Negative feedback regulation of reactive oxygen species on AT1 receptor gene expression. *Br J Pharmacol.* 2000;131:795–803.
28. Ichiki T, Takeda K, Tokunou T, Funakoshi Y, Ito K, Iino N, Takeshita A. Reactive oxygen species-mediated homologous downregulation of angiotensin II type 1 receptor mRNA by angiotensin II. *Hypertension.* 2001; 37:535–540.
29. Usui M, Egashira K, Kitamoto S, Koyanagi M, Katoh M, Kataoka C, Shimokawa H, Takeshita A. Pathogenic role of oxidative stress in vascular angiotensin-converting enzyme activation in long-term blockade of nitric oxide synthesis in rats. *Hypertension.* 1999;34:546–551.
30. Dimmeler S, Rippmann V, Weiland U, Haendeler J, Zeiher AM. Angiotensin II induces apoptosis of human endothelial cells. Protective effect of nitric oxide. *Circ Res.* 1997;81:970–976.
31. Fukui T, Ishizaka N, Rajagopalan S, Laursen JB, Capers Q 4th, Taylor WR, Harrison DG, de Leon H, Wilcox JN, Griendling KK. p22phox mRNA expression and NADPH oxidase activity are increased in aortas from hypertensive rats. *Circ Res.* 1997;80:45–51.
32. De Bono DP. Free radicals and antioxidants in vascular biology: the roles of reaction kinetics, environment and substrate turnover. *QJM.* 1994;87: 445–453.
33. Varin R, Mulder P, Tamion F, Richard V, Henry JP, Lallemand F, Lerebours G, Thuillez C. Improvement of endothelial function by chronic angiotensin-converting enzyme inhibition in heart failure: role of nitric oxide, prostanoids, oxidant stress, and bradykinin. *Circulation.* 2000;102: 351–356.
34. Onozato ML, Tojo A, Goto A, Fujita T, Wilcox CS. Oxidative stress and nitric oxide synthase in rat diabetic nephropathy: effects of ACEI and ARB. *Kidney Int.* 2002;61:186–194.
35. de Cavanagh EM, Piotrkowski B, Basso N, Stella I, Inserra F, Ferder L, Fraga CG. Enalapril and losartan attenuate mitochondrial dysfunction in aged rats. *FASEB J.* 2003;17:1096–1098.
36. Agarwal R. Proinflammatory effects of oxidative stress in chronic kidney disease: role of additional angiotensin II blockade. *Am J Physiol Renal Physiol.* 2003;284:F863–F869.
37. Hornig B, Landmesser U, Kohler C, Ahlersmann D, Spiekermann S, Christoph A, Tatge H, Drexler H. Comparative effect of ace inhibition and angiotensin II type 1 receptor antagonism on bioavailability of nitric oxide in patients with coronary artery disease: role of superoxide dismutase. *Circulation.* 2001;103:799–805.

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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).

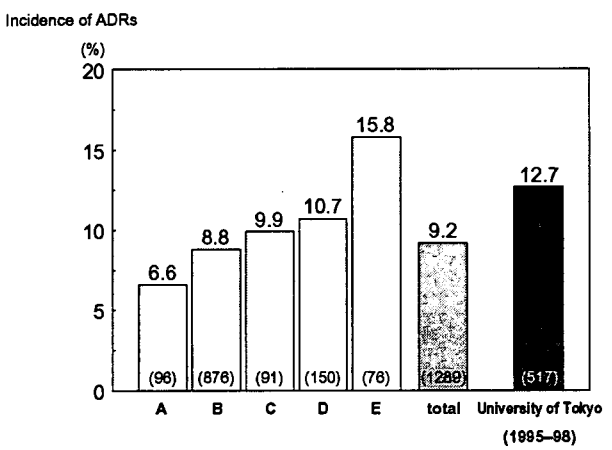

Figure 1 Incidence of ADR in inpatients of geriatric units of five university hospitals. The incidence of ADR in the geriatric unit of University of Tokyo Hospital in 1995–98 is shown as a reference.[9] The numbers of patients surveyed are shown in parentheses.

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.

of elderly patients, although the study relied on self-reporting and review of medical records.[11] Only a few studies have been reported in Japan; the incidence was 12.7% in elderly inpatients of the geriatric unit of University of Tokyo Hospital.[9] In the present survey, the average incidence was 9.2%, ranging from 6.6 to 15.8% among facilities, but was similar to that reported previously.[9] Although the incidence varied among hospitals, it is important to note that the incidence of ADR was more than 5% in all hospitals.

Adverse drug reactions were judged by attending physicians in this study, whereas they were determined by objective review of the medical records in addition to judgment by attending physicians in the previous report from the geriatric unit of University of Tokyo Hospital. In the present study, the incidence of ADR in this facility was 8.8%, which was 30% lower than that in our last survey. This difference may be attributable to underestimation by the attending physicians rather than a decrease in ADR over this short period of 3 years. Therefore, if another authorized person judged the ADR strictly, the overall incidence rate might have been slightly higher.

Our results on the incidence of ADR in elderly patients may add important information. However, all the facilities in this survey were geriatric units of university hospitals, where most of the inpatients were older than 65 years and the doctors in those units are careful in prescribing medication to elderly patients. Therefore, our data might not be directly applicable to elderly patients in other hospitals or units. In fact, ADR were found in nearly half of elderly inpatients of the neuropsychiatry unit of University of Tsukuba Hospital (unpubl. obs, Mizukami *et al.*). In addition, our data in university hospitals, which are acute care hospitals, might not be applicable to chronic care facilities such as long-term care facilities. Since the introduction of the fixed payment system, Diagnosis Procedure Combination system, to university hospitals in Japan in 2003, drug treatment in university hospitals might be changing in the future. Therefore, the incidence of ADR in various types of hospitals in Japan needs to be studied.

In this study, depression and apathy were found to be associated with ADR in addition to the accumulation of diseases and geriatric syndromes, polypharmacy, an increase of prescribed drugs during hospitalization, longer hospital stay and emergency admission. This result is consistent with other reports.[9] However, the causal relationship remains unknown. A higher number of diseases or geriatric syndromes can lead to an increase in ADR through polypharmacy[8,9] while ADR themselves may increase diseases or geriatric syndromes. Similarly, longer hospital stays can increase the risk of ADR, while ADR prolong the duration of hospitalization. The latter point is critical to medical economics as well. Age was not associated with ADR in this study, inconsistent with other studies. This might be due to effects of education on pharmacotherapy in elderly patients for several years at university hospitals. Although we did not analyze the types or classes of ADR in this survey, it has been reported that severe ADR such as neuropsychiatric disorders or cardiovascular injury occur in elderly patients.[9]

Recently, evidence has been accumulating on drug therapy in the elderly. However, there are very few data available in people aged 75 years and older or in frail elderly people. Therefore, it is necessary to establish the safety and effectiveness of drug therapy in these patients in the future. Evidence-based medicine in the elderly aims to discontinue unnecessary drugs and to avoid polypharmacy. On the other hand, a fixed payment system such as the long-term care insurance system in Japan forces doctors to reduce prescribed drugs from a business viewpoint. Indeed, it has been reported that 0.6 drugs were on average discontinued within a month after admission to long-term care facilities, although adverse drug withdrawal events were very few.[12] Because minimally prescribed drugs have not increased ADR in patients with dementia and a low capacity for medication management,[13] it is necessary to cut down unnecessary drugs in frail elderly patients based on evidence-based medicine. In the USA, Beers' criteria are available to identify potentially inappropriate medication use, in order to reduce drug-related problems.[14] In Japan, however, we do not have such guidelines for drug treatment in the elderly. Because the drugs and medical situation in Japan are different from those in the USA, we need to establish our own guidelines, which will be published this year. In addition, we need to accumulate clinical evidence to support the guidelines. We also need to utilize pharmacists more efficiently, because they are an underused resource in avoiding medication errors and can provide important safeguards for elderly patients in hospitals and nursing homes.

Elderly patients are exposed to more medications and have an increased risk of ADR, many of which are avoidable. Knowledge of pharmacological principles and age-related effects on pharmacokinetics/pharmacodynamics is essential to promote safe prescribing. Other factors related to ADR such as polypharmacy, long admission and depression should also be evaluated during hospitalization.

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References

1 Cunningham G, Dodd TR, Grant DJ, McMurdo ME, Richards RM. Drug-related problems in elderly patients admitted to Tayside hospitals, methods for prevention

and subsequent reassessment. *Age Ageing* 1997; **26**: 375–382.
2 Mannesse CK, Derkx FH, de Ridder MA, Man in't Veld AJ, van der Cammen TJ. Contribution of adverse drug reactions to hospital admission of older patients. *Age Ageing* 2000; **29**: 35–39.
3 Williamson J, Chopin JM. Adverse reactions to prescribed drugs in the elderly: a multicentre investigation. *Age Ageing* 1980; **9**: 73–80.
4 Straand J, Rokstad KS. Elderly patients in general practice: diagnoses, drugs and inappropriate prescriptions. A report from the More & Romsdal Prescription Study. *Fam Pract* 1999; **16**: 380–388.
5 Carbonin P, Pahor M, Bernabei R, Sgadari A. Is age an independent risk factor of adverse drug reactions in hospitalized medical patients? *J Am Geriatr Soc* 1991; **39**: 1093–1099.
6 Horiuchi H, Kita T, Mabuchi H *et al.* Primary cardiovascular events and serum lipid levels in elderly Japanese with hypercholesterolemia undergoing 6-year simvastatin treatment: a subanalysis of the Japan lipid intervention trial. *J Am Geriatr Soc* 2004; **52**: 1981–1987.
7 Hiwada K, Ogihara T, Morimoto S *et al.* [Guidelines on treatment of hypertension in the elderly – 1999 revised version. A comprehensive study on geriatric science by the Ministry of Health and Welfare. A Section for the 'Study on Long-Term Prognosis of Hypertension among the Aged'.] *Jpn J Geriat* 1999; **36**: 576–603. (In Japanese.)
8 Akishita M, Ouchi Y, Toba K *et al.* [Compliance with prescriptions and adverse drug reactions in the elderly.] *Jpn J Geriat* 1995; **32**: 178–182. (In Japanese.)
9 Toba K, Akishita M, Mizuno Y *et al.* [Adverse drug reaction in the elderly.] *Jpn J Geriat* 1999; **36**: 181–185. (In Japanese.)
10 Toba K. [Who watch and how to improve quality of long-term residential care in Japan? – it is a role of geriatrician.] *Jpn J Geriat* 1997; **34**: 981–986. (In Japanese.)
11 Rothschild JM, Bates DW, Leape LL. Preventable medical injuries in older patients. *Arch Intern Med* 2000; **160**: 2717–2728.
12 Mita Y, Akishita M, Tanaka K *et al.* Improvement of inappropriate prescribing and adverse drug withdrawal events after admission to long-term care facilities. *Geriatr Gerontol Int* 2004; **4**: 146–150.
13 Akishita M, Toba K, Nagano K, Ouchi Y. Adverse drug reactions in older people with dementia. *J Am Geriatr Soc* 2002; **50**: 400–401.
14 Beers MH. Explicit criteria for determining potentially inappropriate medication use by the elderly. An update. *Arch Intern Med* 1997; **157**: 1531–1536.

〈原　著〉

転倒リスク予測のための「転倒スコア」の開発と妥当性の検証

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転倒ハイリスク者の早期発見の評価方法作成ワーキンググループ

〈要　約〉　【目的】転倒は，身体的要因と環境要因によっておきるとされているが，地域において，環境要因と身体的要因を定量的に比較した研究は少ない．両者を加味した転倒リスク測定表の開発を目的とする．【方法】厚生労働省研究班，転倒ハイリスク者の早期発見のための評価方法作成ワーキンググループの会議によって過去の転倒歴と21項目の危険因子を選択し仮の「転倒スコア」とした．1）過去一年の転倒　2）つまずく　3）手摺につかまない階段の昇降　4）歩く速度が遅延　5）横断歩道を青のうちにわたりきれない　6）1km歩行できない　7）片足で5秒起立できない　8）杖の使用　9）タオルを固く絞れない　10）めまい，ふらつき　11）円背　12）膝痛　13）視力低下　14）難聴　15）物忘れ　16）転倒不安　17）5種類以上の服薬　18）屋内が暗く感じる　19）家の中の障害物　20）家の中の段差　21）家の中の階段使用　22）生活上家の近くの急な坂道歩行．対象は全国7地域住民2,439名（76.3±7.4歳）．検討項目は各項目の該当頻度，項目の該当有無と転倒の相関，過去の転倒歴を従属変数とし，21項目を独立変数とした重回帰分析を行った．有意な項目に関しては，ロジスティック回帰分析によってオッズ比を算出した．【結果】転倒歴は29%に認められた．転倒スコア項目では，物忘れ，家に段差が60%以上，つまずく，階段昇降に支障，視力障害が50%を越えた．横断歩道を青のうちにわたりきれない，一方照明が暗い，タオルがきつく絞れないは20%未満であった．転倒の有無による各因子の頻度の有意差を検定すると，段差，階段，坂道以外のすべての項目が，転倒者は非転倒者に比べ，有意に「はい」と答えた率が高かった．重回帰分析では，独立した有意な危険因子として，つまずく（$p<0.0001$），めまい（$p<0.0001$），家の中に障害物がある（$p=0.0001$），タオルがきつく絞れない（$p=0.0003$），杖を使っている（$p=0.0027$），膝が痛む（$p=0.0362$）が抽出された．この項目と横断歩道の歩行（$p=0.1$）の7項目を用いて，転倒予測を解析し，3項目以上に該当する場合に，転倒の感度，特異度とも良好な値を得た．【結論】内的要因と外的要因を加味した簡便な転倒危険度調査票「転倒スコア」を開発した．「転倒スコア」は，下位項目の殆どが転倒既往者で高く，項目選択の妥当性は高い．段差，階段などの環境バリアは過去の転倒の危険因子としては重要ではない．転倒予測因子として，7項目の短縮板の作成を試み，カットオフ値3項目該当で2/3程度の転倒の予測が可能であり「転倒スコア」の有用性が示唆された．

Key words：転倒，地域住民，内的要因，環境要因，転倒スコア

（日老医誌　2005；42：346—352）

緒　言

転倒・骨折は高齢者における寝たきり要因の第三位に位置づけられ，骨粗鬆症性骨折のなかで最も重い骨折である大腿骨頸部骨折は，その90%以上が転倒によって生ずるとされている[1]．転倒は骨折を合併しなくても，数度の転倒を経験すると，意欲や日常生活動作能力（ADL）を低下させる[2]．地域住民におけるADL依存の危険因子として，転倒は約2倍のリスクであり[2]，転倒予防は寝たきり予防にきわめて重要である．

従来，転倒危険因子は，特定のフィールドでの横断的，

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あるいは縦断的解析によってなされているが，抽出された危険因子は，身体的脆弱性，歩行機能の低下など共通の危険因子がある一方，めまいや痴呆などは成績が一致していない[2]．転倒，内的要因である身体的側面と，外的要因である環境要因による複合的症候群と捉えられるが，後者は地域や文化的，生活習慣的側面により大きく異なる可能性もある．

従来の転倒危険因子は，病歴，現症，血液検査，生活能力などの簡便な検査，専門調査員による測定検査，特殊な機器を用いた検査などが統一性なく調査され，一般健康診断に適応できるかどうかの観点に著しく欠けていた．本研究では，内外の文献的レビューをもとに，転倒ハイリスク者の早期発見の評価方法作成ワーキンググループの研究班によって簡易な「転倒リスク予測表」を作成し，その評価表の妥当性，有効性を検証する．

方　法

平成14年度厚生労働省科学研究効果的医療技術の確立推進，転倒骨折班の合同討議において，「転倒」が共通の研究上の焦点になっているが，転倒予防の成果を全国レベルで達成するためには，転倒ハイリスク者の早期発見のための標準的評価方法を作成する必要があることが指摘され，合同討論で一致した見解をみた．行政の観点からも，転倒ハイリスク者の早期発見のための標準的評価方法の作成は，老人健診や介護予防検診の改訂に資するためには，早期に行う必要性が指摘され，合同会議で班員が選定され，班長は鳥羽がつとめることとなった．内外のレビュー[1)3)]から，筋力低下，バランス欠如，歩行障害，視力障害，移動障害，認知機能障害，ADL障害，起立性低血，加齢，転倒の既往，慢性疾患，薬剤，段差が必須項目として挙げられた．これらの項目を具体的に質問表のみで被験者が内容を理解し，かつ因子のもつ意味が変容しないよう議論を重ね，問診表を完成した（表1）．

調査対象

全国7地域（北海道浦臼町，宮城県仙台市，長野県塩尻市，東京都多摩地区，高知県香北町，熊本県相良村）の住民2,439名（男性932名，女性1,507名：76.3±7.4歳）．年齢分布は，65歳未満59名，65～69歳373名，70～74歳541名，75～79歳586名，80～84歳477名，85歳以上191名，不明，未記入60名．問診表の意味を説明し調査の同意を得たのち，自記式にて回答，自記不可能な場合は調査員が聞き取り調査を行った．

表1　転倒ハイリスク者の発見のための問診表

1) 過去一年の転んだことがありますか（はい，いいえ）
　はい　の場合転倒回数（　　回／年）
2) つまづくことがありますか（はい，いいえ）
3) 手摺につかまらず，階段の昇り降りを出来ますか（はい，いいえ）
4) 歩く速度が遅くなってきましたか（はい，いいえ）
5) 横断歩道を青のうちにわたりきれますか（はい，いいえ）
6) 1キロメートルぐらい続けてあるけますか（はい，いいえ）
7) 片足で5秒くらい立っていられますか（はい，いいえ）
8) 杖をつかっていますか（はい，いいえ）
9) タオルを固く絞れますか（はい，いいえ）
10) めまい，ふらつきがありますか（はい，いいえ）
11) 背中が丸くなってきましたか（はい，いいえ）
12) 膝が痛みますか（はい，いいえ）
13) 目がみにくいですか（はい，いいえ）
14) 耳が聞こえにくいですか（はい，いいえ）
15) 物忘れが気になりますか（はい，いいえ）
16) 転ばないかと不安になりますか（はい，いいえ）
17) 毎日お薬を5種類以上飲んでいますか（はい，いいえ）
18) 家の中で歩くとき暗く感じますか（はい，いいえ）
19) 廊下，居間，玄関によけてとおるものがおいてありますか（はい，いいえ）
20) 家の中に段差がありますか（はい，いいえ）
21) 階段を使わなくてはなりませんか（はい，いいえ）
22) 生活上家の近くの急な坂道を歩きますか（はい，いいえ）

解　析

1) 再現性：21例において，1カ月後に再調査を行い，単相関にて再現性を検討した．

2) 浦臼町89名において，夏季と冬期の再現性を単相関にて検討した．

3) 項目の陽性頻度は単純集計し%表示で比較した．

4) 転倒の有無によって，各因子の頻度に有意差があるかどうか，対応のないT検定を行った．

5) 過去の転倒歴を従属変数として，調査票2)～22)の21項目のうち4)によって得られた有意な因子，を独立変数として，重回帰分析を行った．有意な因子はロジスティック回帰分析により，危険率（オッズ比）を算定した．

相関検定にてはr値，2群間のT検定，重回帰分析ではp値が0.05未満を統計学的に有意とした．

6) 重回帰分析によって抽出された項目で，転倒予測をROC曲線で解析した．

結　果

1) 繰り返し再現性：1カ月後の再現性は$r=0.74$，$p<0.01$で良好であった．

2）季節変動：r＝0.675，p＜0.001と6カ月後の再現性も良好であった．

表2　質問項目の陽性頻度

1）転倒：回答数2,439名で708例	29.0%
転倒例の平均転倒数：4.7 ± 1.0回／年（Mean ± SE）	
2）つまづくことがある	56.5%
3）手摺につかまらず，階段の昇り降りを出来ない	50.6%
4）歩く速度が遅くなってきた	65.2%
5）横断歩道を青のうちにわたりきれない	17.0%
6）1キロメートルくらい続けてあるけない	35.8%
7）片足で5秒くらい立てない	38.6%
8）杖をつかっている	28.3%
9）タオルを固く絞れない	16.8%
10）めまい，ふらつきがある	32.4%
11）背中が丸くなってきた	44.9%
12）膝が痛む	47.3%
13）目がみにくい	53.1%
14）耳が聞こえにく	42.5%
15）物忘れが気になる	63.7%
16）転ばないかと不安になる	45.8%
17）毎日お薬を5種類以上飲んでいる	31.2%
18）家の中で歩くとき暗く感ずる	11.4%
19）廊下，居間，玄関に障害物	20.8%
20）家の中に段差がある	69.1%
21）階段を使わなくてはならない	27.7%
22）生活上家の近くの急な坂道を歩く	33.3%

3）各項目の出現頻度

過去1年の転倒歴は708名（男性229名，女性479名，平均年齢77.5±7.4歳），転倒率は29.0%であった．

問診表における出現頻度で，50%以上であったものは，身体関係では，「歩く速度が遅くなってきた」が65.2%，「つまずく」56.5%，「階段昇降にてすりが必要」50.6%で，情報関連機能では，「物忘れの自覚」63.7%，「視力低下」53.1%，環境要因では「段差」69.1%であった．逆に20%未満の頻度の低い要因は，身体関係では，「横断歩道を青のうちに渡れない」17.0%，「タオルを堅く絞れない」16.8%で，環境要因では「照明が暗い」11.4%であった（表2）．

4）転倒の有無（有効回答数2,389）による各因子の頻度の有意差を検定すると，段差，階段，坂道以外のすべての項目が，転倒者は非転倒者に比べ，有意に「はい」と答えた率が高かった（表3）．以上の成績から20）段差，21）階段，22）坂道以外の項目を重回帰分析の項目に採用した．

5）過去の転倒歴を従属変数として，調査票2～19）の18項目を独立変数として，重回帰分析を行った．

年齢，性別は強制注入した．有効回答数は2,287例であった．

独立した有意な危険因子として，つまずく（p＜

表3　転倒者と非転倒者の各項目のはい」と答えた率（%）

	非転倒 (n = 1,687)	転倒 (n = 708)	有意差 (p)
2）つまづくことがある	45.3	83.3	＜0.0001
3）手摺りにつかまらず，階段の昇り降りを出来ない	45.0	63.8	＜0.0001
4）歩く速度が遅くなってきた	59.2	79.6	＜0.0001
5）横断歩道を青のうちにわたりきれない	12.7	27.5	＜0.0001
6）1キロメートルくらい続けてあるけない	30.5	48.5	＜0.0001
7）片足で5秒くらい立てない	32.5	53.2	＜0.0001
8）杖をつかっている	22.0	43.7	＜0.0001
9）タオルを固く絞れない	12.2	28.2	＜0.0001
10）めまい，ふらつきがある	24.7	50.6	＜0.0001
11）背中が丸くなってきた	40.3	55.8	＜0.0001
12）膝が痛む	41.1	62.3	＜0.0001
13）目がみにくい	48.4	64.3	＜0.0001
14）耳が聞こえにくい	39.1	50.7	＜0.0001
15）物忘れが気になる	59.4	74.0	＜0.0001
16）転ばないかと不安になる	37.9	64.8	＜0.0001
17）毎日お薬を5種類以上飲んでいる	27.2	40.8	＜0.0001
18）家の中で歩くとき暗く感ずる	8.5	18.3	＜0.0001
19）廊下，居間，玄関に障害物	17.1	29.6	＜0.0001
20）家の中に段差がある	68.9	69.5	0.79 ns
21）階段を使わなくてはならない	27.5	28.2	0.74 ns
22）生活上家の近くの急な坂道を歩く	33.6	32.5	0.60 ns