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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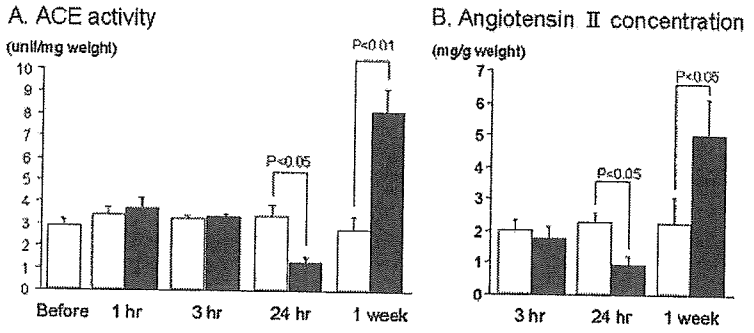

Figure 1. ACE activity and Ang II concentration in rat carotid artery after H_2O_2 treatment. Treated (closed bars) and contralateral (open bar) carotid arteries were harvested at the indicated time points after H_2O_2 treatment. ACE activity and Ang II concentration in tissue homogenates were measured 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).

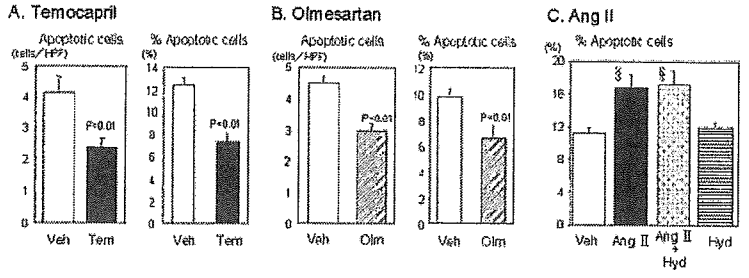

Figure 2. Effects of temocapril (A), olmesartan (B), and Ang II (C) on EC apoptosis after H_2O_2 treatment in rat carotid artery. The number of apoptotic ECs was counted per high power field (HPF; ×200), and the ratio of the apoptotic cell number to the intact cell number was calculated using *en face* 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.

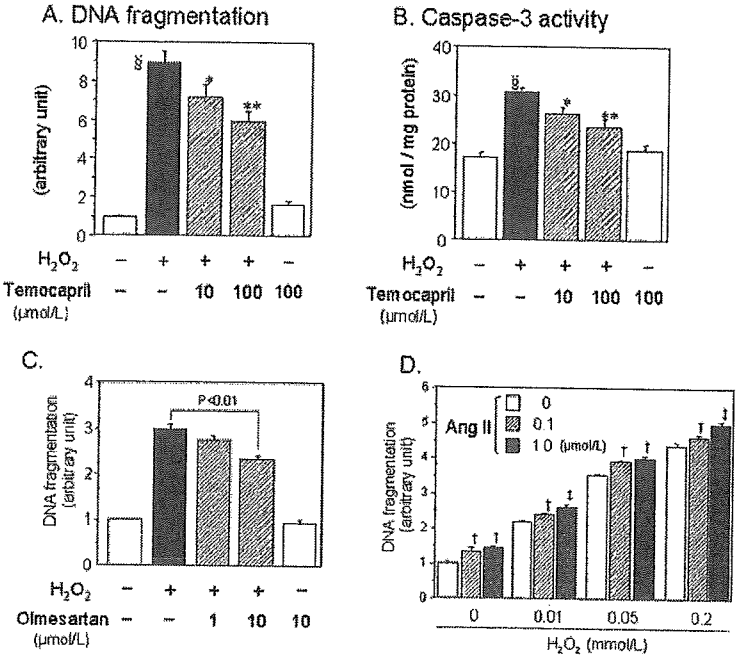

Figure 3. Effects of temocapril (A and B), olmesartan (C), and Ang II (D) on H_2O_2-induced EC apoptosis in culture. A through D, Temocapril, olmesartan, Ang II, or their vehicle was added to the culture medium 24 hours before H_2O_2 treatment until assay. EC apoptosis was evaluated 24 hours after H_2O_2 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

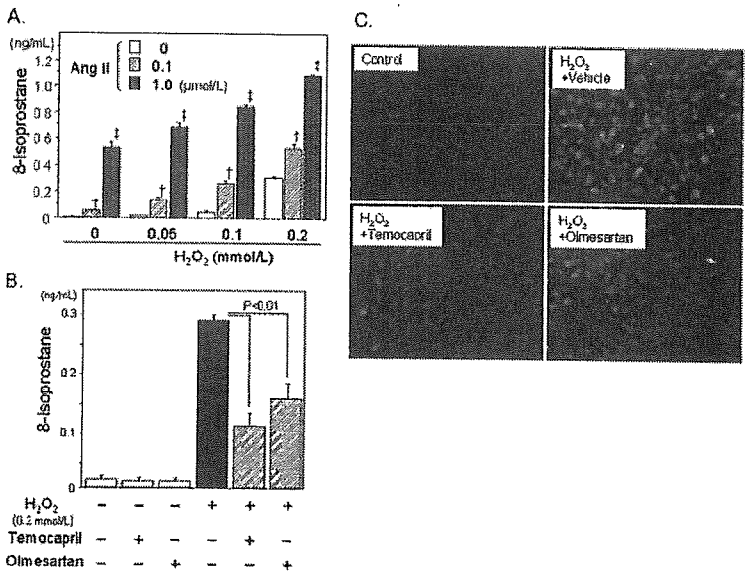

Figure 4. Effects of Ang II (A), temocapril, and olmesartan (B and C) on 8-isoprostane and DCF formation in cultured ECs. Ang II, temocapril (100 μmol/L), olmesartan (10 μmol/L), or their vehicle was added to the culture medium 24 hours before H_2O_2 treatment until assay. Then 8-isoprostane concentration 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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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).

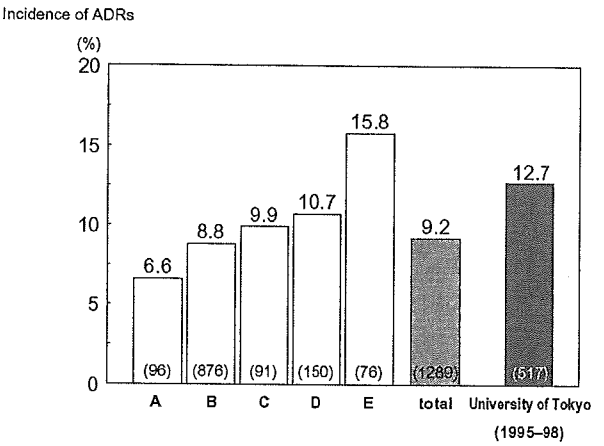

Figure 1 Incidence of ADR in inpatients of geriatric units of five university hospitals. The incidence of ADR in the geriatric unit of University of Tokyo Hospital in 1995–98 is shown as a reference.[9] The numbers of patients surveyed are shown in parentheses.

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

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Abstract

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

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

1. Introduction

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

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

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

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2. Materials and methods

2.1. Induction of EC apoptosis

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

2.2. Cell viability

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

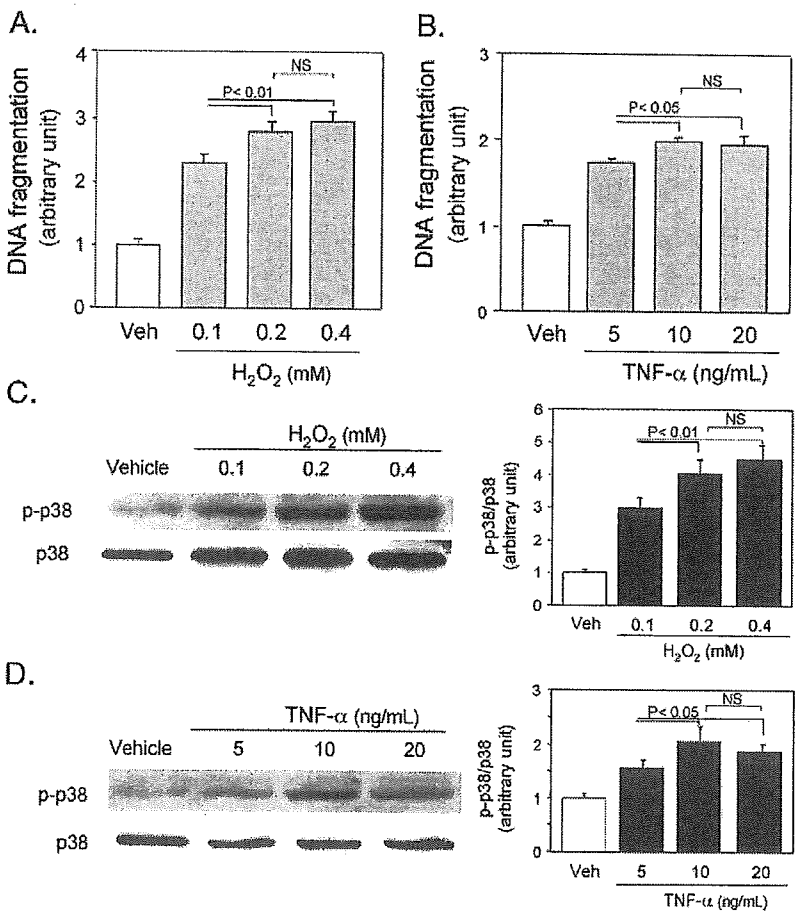

Fig. 1. Dose-dependent effects of H_2O_2 (A, C) and TNF-α (B, D) on EC apoptosis (A, B) and p38 MAP kinase activity (C, D). A and B, apoptosis was evaluated 24 h after H_2O_2 treatment (for 1.5 h) or 72 h after addition of TNF-α by means of DNA fragmentation ($n=3$). C and D, the activity of p38 MAP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

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

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

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

2.4. Immunoblotting

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

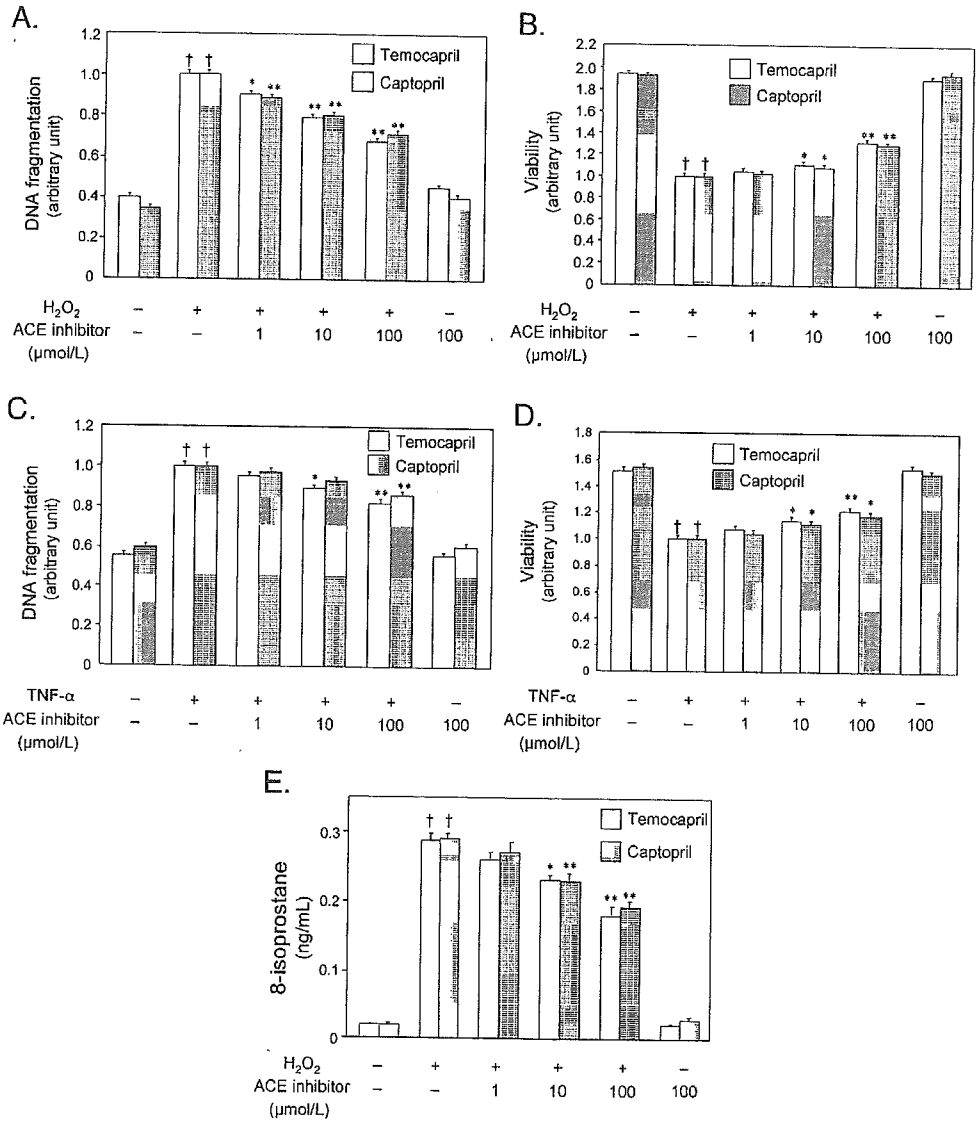

Fig. 2. Effects of ACE inhibitors on H_2O_2-induced (A, B) and TNF-α-induced (C, D) EC apoptosis and the effects of ACE inhibitors on H_2O_2-induced 8-isoprostne formation (E). Temocapril, captopril or their vehicle was added to the culture medium 24 h before H_2O_2 or TNF-α treatment until assay. Apoptosis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

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

2.5. Data analysis

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

3. Results

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

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

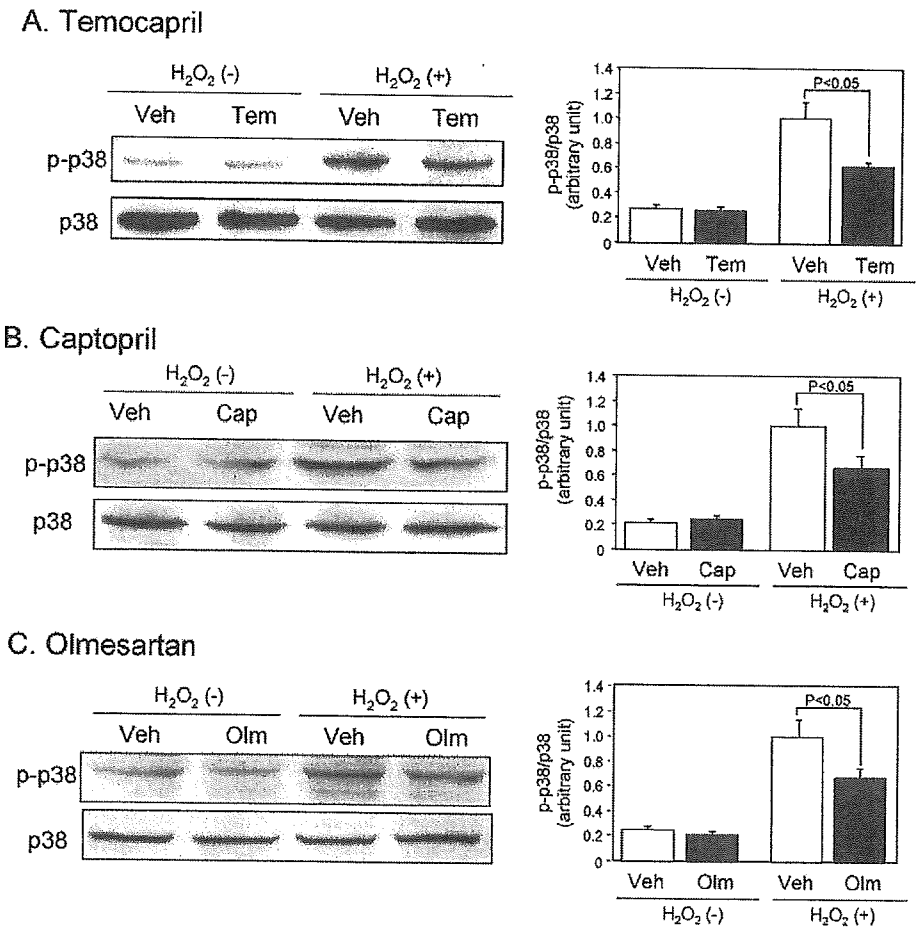

Fig. 3. Effects of temocapril (A), captopril (B) and olmesartan (C) on p38 MAP kinase activity at 30 min after exposure to H_2O_2. Temocapril (100 μmol/l), captopril (100 μmol/l), olmesartan (10 μmol/l) or its vehicle was added to the culture medium 24 h before H_2O_2 treatment until assay. Right panels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

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

3.2. Effect of ACE inhibitors on EC apoptosis

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

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

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

3.3. Effect of ACE inhibitor on p38 MAP kinase activity

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

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

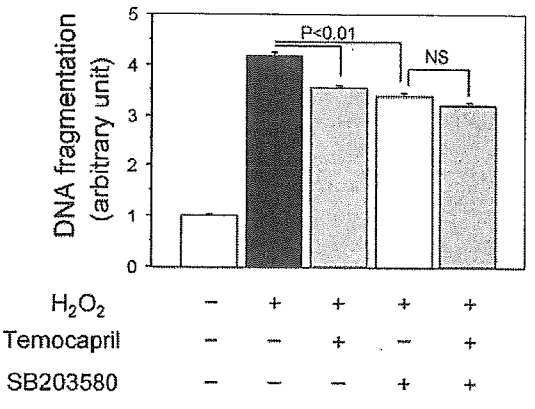

Fig. 4. Effects of temocapril and SB203580 on H_2O_2-induced EC apoptosis. Temocapril (100 μmol/l) or its vehicle was added to the culture medium 24 h before H_2O_2 treatment until assay. SB203580 (10 μmol/l) or its vehicle was added to the culture medium for 1 h before H_2O_2 treatment. EC apoptosis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

4. Discussion

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

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

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

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

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

Acknowledgements

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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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小林　義雄　　町田　綾子　　秋下　雅弘　　佐々木英忠
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

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〈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

鳥羽　研二[1)]　大河内二郎[2)]　高橋　泰[3)]　松林　公蔵[4)]
西永　正典[5)]　山田　思鶴[6)]　高橋龍太郎[7)]　西島　令子[1)]
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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，

1）K. Toba, R. Nishijima, Y. Kobayashi, A. Machida, M. Akishita：杏林大学高齢医学
2）J. Okochi：産業医科大学医学部公衆衛生学
3）T. Takahashi：国際医療福祉大学医療福祉
4）K. Matsubayashi：京都大学東南アジア研究センター
5）M. Nishinaga：高知大学老年病科
6）S. Yamada：老人保健施設まほろばの郷
7）R. Takahashi：東京都老人総合研究所
8）H. Sasaki：東北大学老年・呼吸器内科
受付日：2004. 9. 17，採用日：2004. 10. 15