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Clinica Chimica Acta 364 (2006) 328 – 334
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Angiotensin converting enzyme inhibitor attenuates oxidative stress-induced endothelial cell apoptosis via p38 MAP kinase inhibition

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Received 11 May 2005; received in revised form 22 July 2005; accepted 29 July 2005
Available online 8 September 2005

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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doi:10.1016/j.cca.2005.07.021

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.

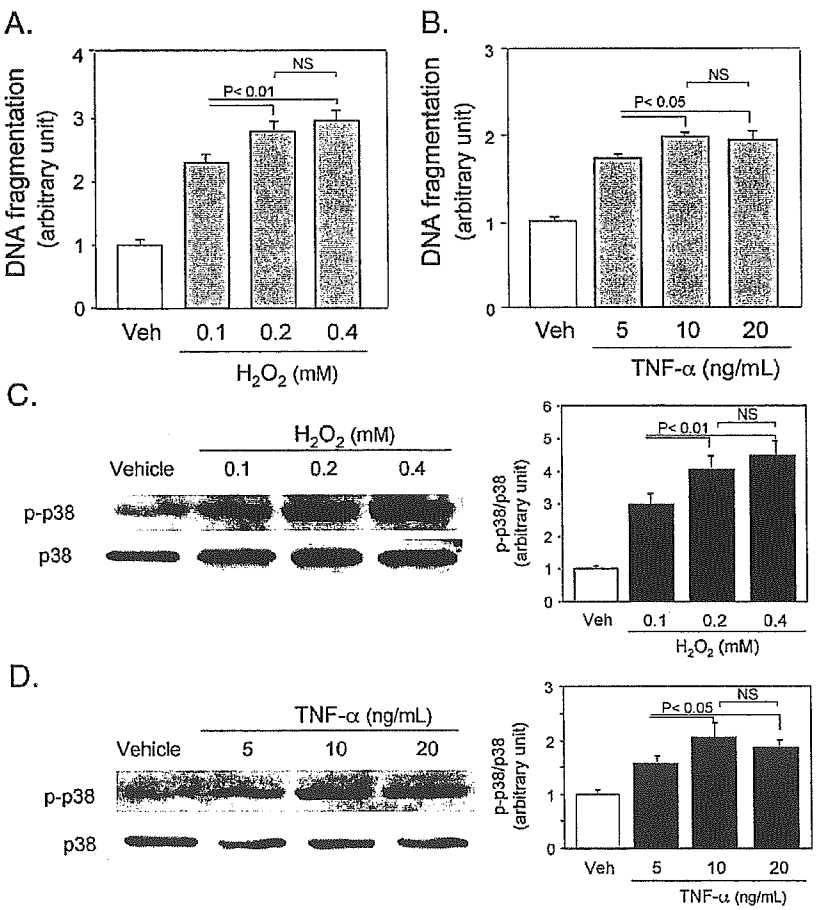

Fig. 1. Dose-dependent effects of H_2O_2 (A, C) and TNF-α (B, D) on EC apoptosis (A, B) and p38 MAP kinase activity (C, D). A and B, apoptosis was evaluated 24 h after H_2O_2 treatment (for 1.5 h) or 72 h after addition of TNF-α by means of DNA fragmentation ($n=3$). C and D, the activity of p38 MAP 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

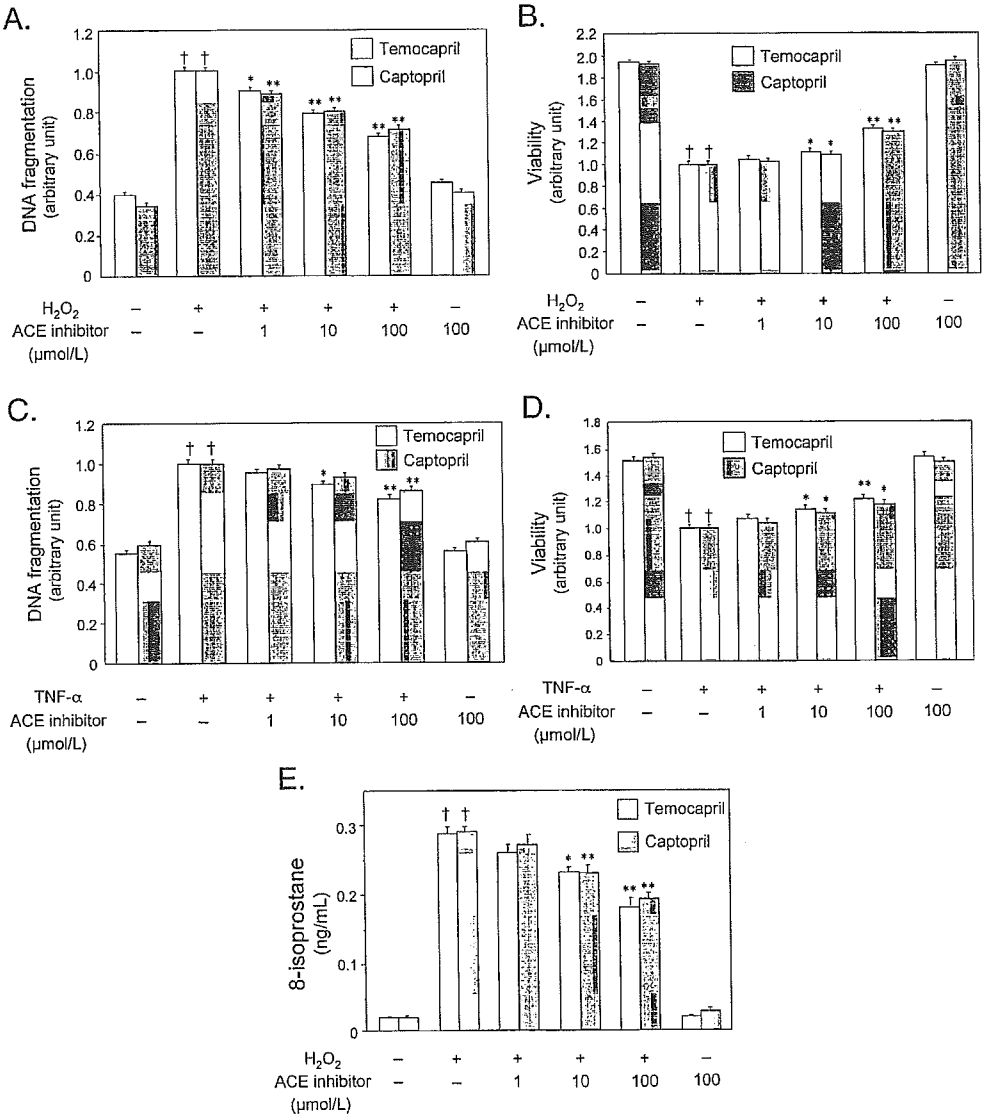

Fig. 2. Effects of ACE inhibitors on H_2O_2-induced (A, B) and TNF-α-induced (C, D) EC apoptosis and the effects of ACE inhibitors on H_2O_2-induced 8-isoprostne formation (E). Temocapril, captopril or their vehicle was added to the culture medium 24 h before H_2O_2 or TNF-α treatment until assay. Apoptosis (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

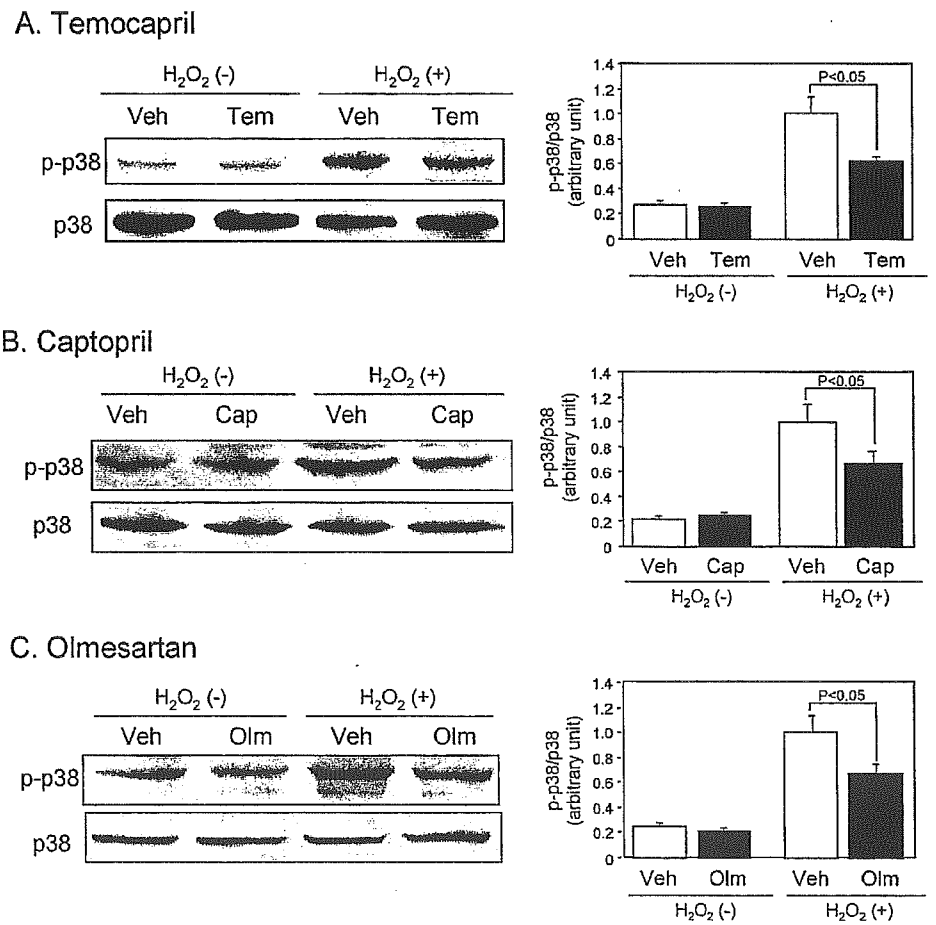

Fig. 3. Effects of temocapril (A), captopril (B) and olmesartan (C) on p38 MAP kinase activity at 30 min after exposure to H_2O_2. Temocapril (100 μmol/l), captopril (100 μmol/l), olmesartan (10 μmol/l) or its vehicle was added to the culture medium 24 h before H_2O_2 treatment until assay. Right panels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

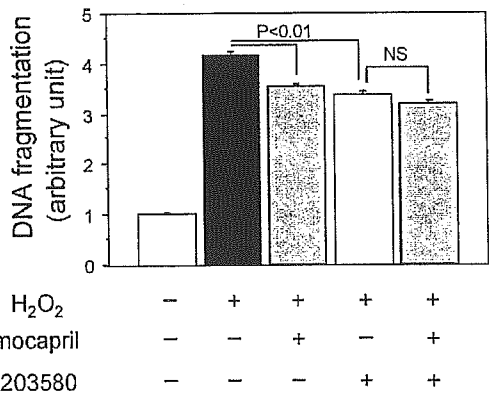

Fig. 4. Effects of temocapril and SB203580 on H_2O_2-induced EC apoptosis. Temocapril (100 μmol/l) or its vehicle was added to the culture medium 24 h before H_2O_2 treatment until assay. SB203580 (10 μmol/l) or its vehicle was added to the culture medium for 1 h before H_2O_2 treatment. EC apoptosis 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

We thank Ms. Mariko Sawano for her excellent technical assistance. This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Culture and Sports of Japan (13670741), and by Health and Labour Sciences Research Grants (H16-Choju-013 and H16-Choju-015) from the Ministry of Health, Labour and Welfare of Japan.

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Microbiol. Immunol., 49(12), 1075–1081, 2005

Editor-Communicated Paper

Analysis of the Distribution of Neuropathogenic Retroviral Antigens Following PVC211 or A8-V Infection

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Communicated by Dr. Hiroshi Kida: Received September 14, 2005. Accepted October 6, 2005

Abstract: **A8-V and PVC211 are neuropathogenic strains of the Friend murine leukemia virus (Fr-MLV) that cause spongiosis in the rat brain after infection at birth. PVC211 exhibited stronger neuropathogenicity than A8-V, and induced more severe neurological symptoms such as hind-leg paralysis. These symptoms correlated with the neuropathological spread and intensity, which were more severe in the spinal cord of rats infected with PVC211 than in those infected with A8-V, without exhibiting neuropathological differences in other areas of the CNS. Interestingly, virus titers recovered from infected spinal cords were similar in PVC211 and A8-V infected animals. However, in the spinal cord infected with PVC211, glial cells attained higher immunohistochemical expression scores for the viral surface antigen, gp70 (Env) than in the A8-V infected spinal cord, although expression levels of viral antigens in blood vessel walls were similar in A8-V and PVC211 infections. Furthermore, many of those glial cells which carried viral antigens were found, by double immunostaining, to be microglia. The results suggested that the spread of viral antigen positive microglia plays an important role in forming the different neuro-pathogenicity observed in A8-V and PVC211 infections.**

Key words: **Ecotropic, Histochemistry, CNS**

The FrC6-V, from which A8-V was molecularly cloned (15), and the PVC211 (5) were separately isolated by different methods (20) from the same source of the Fr-MLV complex which had been maintained by mouse-to-mouse passages for over 30 times (10). The A8-V shares a high degree of homology with PVC211 in the R (98.5%), U5 (100%), 5′ leader (99.6%), *gag* (99.6%), pol (99.5%), and env (99.7%) regions (15). The lowest degree of homology between the A8 virus and PVC211 is in the U3 region of the LTR (81.4%). This low homology was attributed to differences in enhancer elements in the U3 region (15). But our recent study using recombinant viruses revealed that the enhancer elements of the neuropathogenic viruses do not contribute to their neuropathogenicity (19), although A8-V enhancer elements appeared to be responsible for its ability to proliferate at high rates in the CNS and to induce tumorigenesis in the thymus and spleen (18).

One common feature of A8-V and PVC211 is that the primary determinant for the induction of neurodegenerative disease is the env gene, but other viral genes also have effects on neuropathogenicity (15). The 0.3-kb fragment containing 0.04-kb of R, U5, and the 5′ half of the 5′ leader of A8 are essential for the induction of spongiform neurodegeneration (19). In the case of PVC211, the sequences within the 5′ leader sequence, the *gag* gene, and the 5′ quarter of the pol gene also influence neurovirulence (9). However, our serial studies using chimerae from A8-V and 57 virus (57-V), the

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Abbreviations: A8-V, A8 virus; CNS, central nervous system; DAB, 3,3′-diaminobenzidine tetrahydrochloride; EcoR, receptor proteins for ecotropic retroviruses; Env, envelope protein (gp70); 57-V, 57 virus; FrC6-V, FrC6 virus; Fr-MLV, Friend murine leukemia virus; HE, haematoxylin and eosin; MEM, minimum essential medium; NRK, normal rat kidney; PAP, peroxidase-anti-peroxidase; PBS, phosphate-buffered saline; PFU, plaque forming unit.

latter of which is a non-neuropathogenic strain of Fr-MLV proved that the *gag* gene does not need to originate in a neuropathogenic virus in order for a virus to cause neurological disease (16). As part of these studies, we developed recombinant viruses R7c and R7f which lack the enhancer element found in A8-V after the replacement of the fragment with the enhancer element derived from the 57-V gene. These recombinant viruses induced spongiosis with a high incidence, although the isolated viral titers of the infected brain were very low. The mechanism by which viruses with low titers following CNS infection can induce distinct neuropathological lesions was partially revealed by immuno-histochemical studies (21) in which the expression levels of viral antigens following infection with A8-V, R7c, R7f or non-neuropathogenic recombinant virus Rec5 were studied. We employed specific antibodies raised against Env and Gag proteins to evaluate viral antigen expression in blood vessel walls and glia cells in infected brains using the criteria provided in our scoring system. Our data showed that the expression scores of the R7c and R7f viruses were comparable to those of A8-V but distinctly higher than those of Rec5, the latter of which was only minimally expressed in the infected CNS (21).

In this paper, we have compared viral antigen expression levels and neuropathological severity following infection with the A8-V and the PVC211. The main target of A8-V and PVC211 is CNS vascular walls (4, 16), namely endothelial cells (7, 8). The viral antigens in the endothelial cells are distributed not only in or near to the spongiform lesions but also in the areas that appear normal (2, 21). In addition, the viral antigens are not expressed in the neurons of the infected animals although the vacuoles forming spongiosis are located in neuropils (15). The expression levels of Gag and Env proteins assessed after infection with A8-V are identical *in vivo* (21) and *in vitro* (19).

Materials and Methods

Viruses and inoculation. PVC211-producing normal rat kidney cells (NRK) were kindly provided by Dr. Kai (Yamaguchi University, Yamaguchi, Japan). Friend murine leukemia virus (Fr-MLV) clone A8-V was obtained from the virus FrC6-V as described previously (15). Viral titers were determined by the XC cell plaque assay using C182 cells (14) in the presence of 10 μg/ml of Polybrene (14). C182 cells were grown on minimum essential medium (MEM) supplemented with 10% calf serum.

The ability of the viruses to cause disease was assessed using newborn Lewis rats purchased from a commercial breeder. Newborn rats were inoculated by intra-peritoneal (0.1 ml) and intra-cerebral (0.0005 ml) routes with viral supernatant ($1–30\times10^4$ XC-PFU/head). Infected animals were then exsanguinated under deep anesthesia and the dissected organs were homogenized in cold phosphate-buffered saline (PBS) containing 1 mM $MgCl_2$ and 1 mM $CaCl_2$. Infectious virus titers were then determined by the XC cell plaque assay (14).

Histology. Following exsanguination of the animals 6–7 weeks after infection, the collected organs were immersed and fixed in 4% paraformaldehyde buffered with 0.12 M phosphate (pH 7.3). Tissues were then embedded in paraffin for histological staining with haematoxylin and eosin (HE).

The degree of spongiform neurodegeneration was scored as follows: 0—no lesions; 1—less than 20 vacuoles in the total area; 2—20 to 100 vacuoles counted in the light microscopical field at 10× magnification (field (×10)); 3—clusters consisting of over 100 vacuoles spread within one field (×10); 4—more than two clusters consisting of over 100 vacuoles in the area, or clusters of vacuoles occupying over 30% of the total area. Intermediate scoring in between each of the seven established scores was permitted by adding a value of 0.5 to the lower score. To score CNS pathology, five areas were selected: cerebral cortex, thalamus, cerebellum, pons and spinal cord.

For detection of the viral antigen proteins Env and Gag, we performed immunohistochemistry using goat anti-Rauscher MLV gp70 (antiEnv) (Quality Biotech Incorporated Resource Laboratory) and anti-AKR $p30^{Gag}$ (antiGag, Quality Biotech Incorporated Resource Laboratory) on thin paraffin sections (2). Non-specific antibody binding was blocked by incubation of sections and cells with 50% normal mixed serum (fetal calf, calf, pig, and horse) diluted in PBS. Biotinylated rabbit anti-goat IgG (ZYMED Laboratories, Inc.) was used as a secondary antibody followed by treatment with an avidin-peroxidase complex (ZYMED Laboratories, Inc.). Washes in PBS were carried out between each step. After primary antibody incubation, the non-specific activity of endogenous peroxidase activity was blocked by incubating cells with 0.3% H_2O_2 in methanol. For the peroxidase reaction, 0.2 mg/ml 3,3'-diaminobenzidine tetrahydrochloride (DAB) (DOTIDE) in 0.1 M Tris buffer (pH 7.6) was used. In order to confirm that most of the glial cells carrying viral antigens are microglia, double immunostaining was carried. ED-1 monoclonal antibody was used to detect microglia as primary antibody (22). The antiGag reaction was developed first using the PAP method and donkey anti-goat IgG (Bethyl Lab., Inc.), peroxidase labeled goat IgG (DACO), and DAB, to yield a brown

reaction product. ED-1 was visualized using 20 mg/ml of 4-chloro-1-naphtol (WAKO, Japan), which gave a purple reaction product after sequential application of biotinylated rabbit anti-mouse antibody (ZYMED Laboratories, Inc.) and an avidin-peroxidase complex (ZYMED Laboratories, Inc.). Nuclei were counterstained with methyl green.

The intensities of antigen expression were scored separately either in blood vessels or in glial cells of the CNS. Scoring was performed in the same areas used for pathological evaluation. The degree of antigen expression in the blood vessels was scored as follows: 0—no antigens; 1—less than 10% of antigen-positive blood vessels in the sample area; 2—10–20% antigen-positive blood vessels in the sample area; 3—20–50% antigen-positive blood vessels in the sample area; 4—more than 70% antigen-positive blood vessels in the sample area. The degree of antigen expression in glial cells was scored as follows: 1—a few antigen-positive glial cells in total area; 2—more than 10 antigen-positive glial cells in the field at 10× magnification (field (×10)); 3—more than three fields (×10) that contain more than 10 antigen-positive glial cells; 4—fields with more than 10 antigen-positive glial cells occupy more than 70% of the area.

Results

Clinical Sign

None of the 10 rats that were infected with A8-V developed hind-leg paralysis or weakness within 6 weeks after inoculation and three out of ten infected rats manifested hind-leg paralysis afterwards. Three rats infected with A8-V did not show any neurological signs over the course of 8 weeks post inoculation. In contrast, half of the PVC211 infected rats developed hind-leg weakness within 6 weeks of inoculation and most (11/12 infected rats) manifested hind-leg paralysis by 8 weeks post inoculation.

Virus Growth

The virus titers recovered from the brains and spinal cords 6–7 weeks after infection with the A8-V ranged $3–50\times10^4$ and $9–60\times10^4$, respectively. The averages obtained from five infected rats were 1.8×10^5 and 3.4×10^5 respectively (Fig. 1). The infection of the PVC211 induced similar level of viral recovery from the brain and the spinal cord at 6–7 weeks post inoculation ranging from $1.3–3.7\times10^4$ and $4.8–7.2\times10^4$, respectively. The averages obtained from four rats infected with PVC211 were 3.0×10^4 and 6.1×10^4 respectively.

Histopathology

The degree of spongiotic change induced by the infection was assessed by a scoring system (see "Materials and Methods") after evaluation of 5 areas (cerebral cortex, thalamus, cerebellum, pons, and spinal cord) of each infected animal. A typical spongiosis scored as 3 is shown in Fig. 2. There, the HE staining illustrates many vacuoles with diameters of 5–100 μm compose a fairly well demarcated area of spongiosis (Fig. 2A). The average scores obtained from the spinal cords of 7 rats infected with the A8-V and 3 rats infected with PVC211 were 1.4 and 2.8, respectively (Fig. 1), suggesting that PVC211 induced more severe neuropathology in the spinal cord compared to A8-V ($P<0.002$ by Student's test). The average neuropathological scores in the cerebral cortex, thalamus, pons, and cerebellum (designated cumulatively as Brain in Fig. 1) were similar in PVC211 and A8-V infected rats ($P>0.7$).

Histochemistry

Viral antigens were detected predominantly in the vascular walls of infected brains regardless of the virus used (Fig. 2E), and with the exception of the vascular wall, were detected at lower frequencies in glial cell structures (Fig. 2, C and F). The glial structures associated with viral antigens were regularly encountered close to and inside spongiotic lesions and were often attached to the wall of the vacuole (Fig. 2C). On the other hand, virus-expressing blood vessels exhibited a much wider distribution, and were often found in the regions where no spongiosis was observed (Fig. 2E).

This closer correlation of antigen expression to the lesions in glial cells than in vascular walls was statistically analyzed. The expression levels of the Env protein were evaluated according to our evaluation score (see "Materials and Methods"), examined in selected five areas within the CNS as described earlier for a pathological scoring system. When the expression levels of the viral antigen in the glial cells (env/glia in Fig. 1) activated in the spinal cords were compared between PVC211 and A8-V infection, PVC211 infection induced more than twice the levels of antigen expression in glial cells compared to A8-V ($P<0.04$). In contrast, no significant difference was observed in the expression of Env protein in the vascular walls following infection by these viruses (env/vessel in Fig. 1) in the spinal cord ($P>0.3$). Furthermore, there also was no significant difference in the env/glia immunohistochemical scores obtained from the brain (cerebral cortex, thalamus, pons, and cerebellum, designated as Brain in Fig. 1) between the PVC211 and A8-V infected groups ($P>0.4$).

In order to confirm the previous report that spongi-

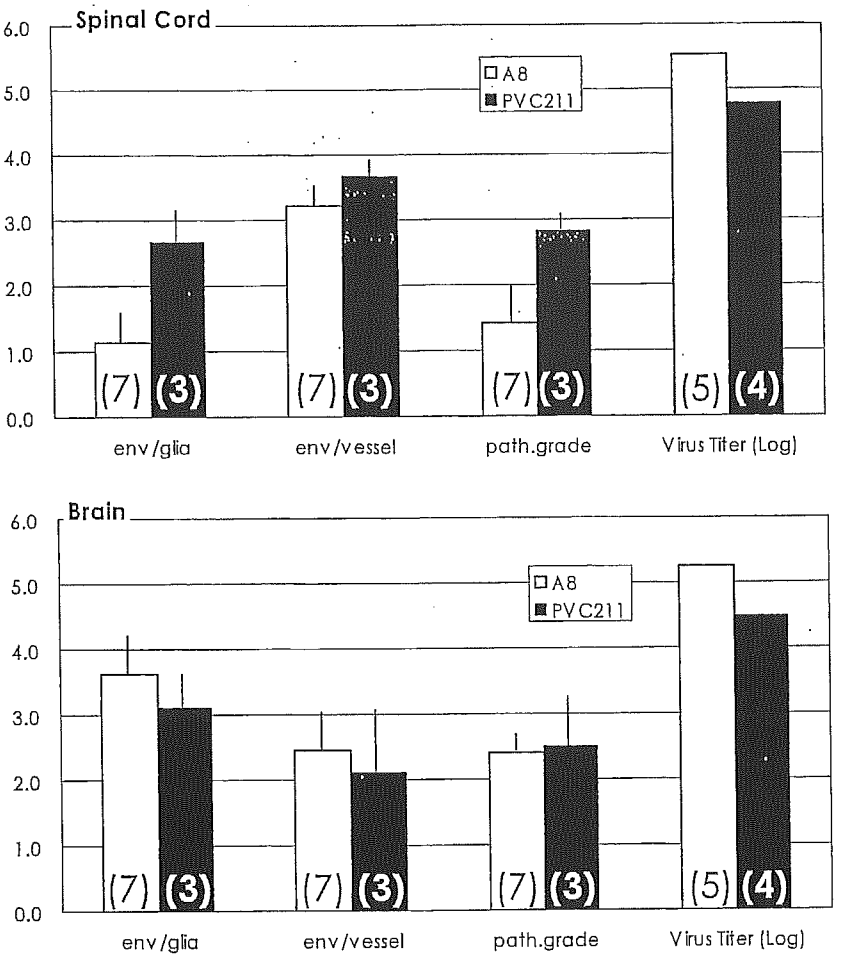

Fig. 1. Histological scores and viral growth. Numbers in the parentheses described in the bars indicate the numbers of rats examined. Env expression estimated by immuno-histochemistry either in the blood vessel wall or glia cells is described as Env/vessel or glia, respectively, and the pathological grade as path. grade. The immunohistochemical and pathological scores of each animal was evaluated by the averaging the data obtained from spinal cord or brain which is investigated in 4 different areas (cerebral cortex, thalamus, cerebellum, and pons).

form degenerations induced by several neuropathogenic murine retroviruses are associated primarily with infection of microglial cells (13), we performed double immunostaining. Our data showed that many of the glial cells that bore viral antigen (Fig. 2F) also expressed a microglial marker protein that was identified by the monoclonal antibody ED-1 (Fig. 2F).

Discussion

Our data showed that PVC211 was more neuropathogenic in neonatal rats than A8-V, as manifested by the higher incidence and more rapid onset of hind-leg paralysis in these animals. This neurological sign was due to the presence of pathological lesions in the spinal cord, which have also been investigated in other neurological diseases which are experimentally induced in rats, such as experimental allergic encephalomyelitis (2). Our neuropathological data confirmed the presence of viral lesions in the spinal cord of rats that developed paralysis and, as expected based on our neurological findings, rats that were infected with PVC211 showed more of such lesions.

The above data notwithstanding, viral titers recovered from the spinal cords of PVC211 and A8-V infected rats were similar (Fig. 1). From a virological view point, it has been considered that the level of viral titer obtained from a target organ is correlated to pathological severity in the relevant organs (11, 13, 14). However, our recent studies using chimeric viruses derived from the genes of A8-V and a non-neuropathogenic Fr-MLV, 57-V revealed that neuropathology induced by A8-V infection is not dependent upon the viral proliferation rate but rather the level of viral antigen expression (19). Nevertheless, this comparative study using chimerae resulted in a comparison between neuropathogenic and

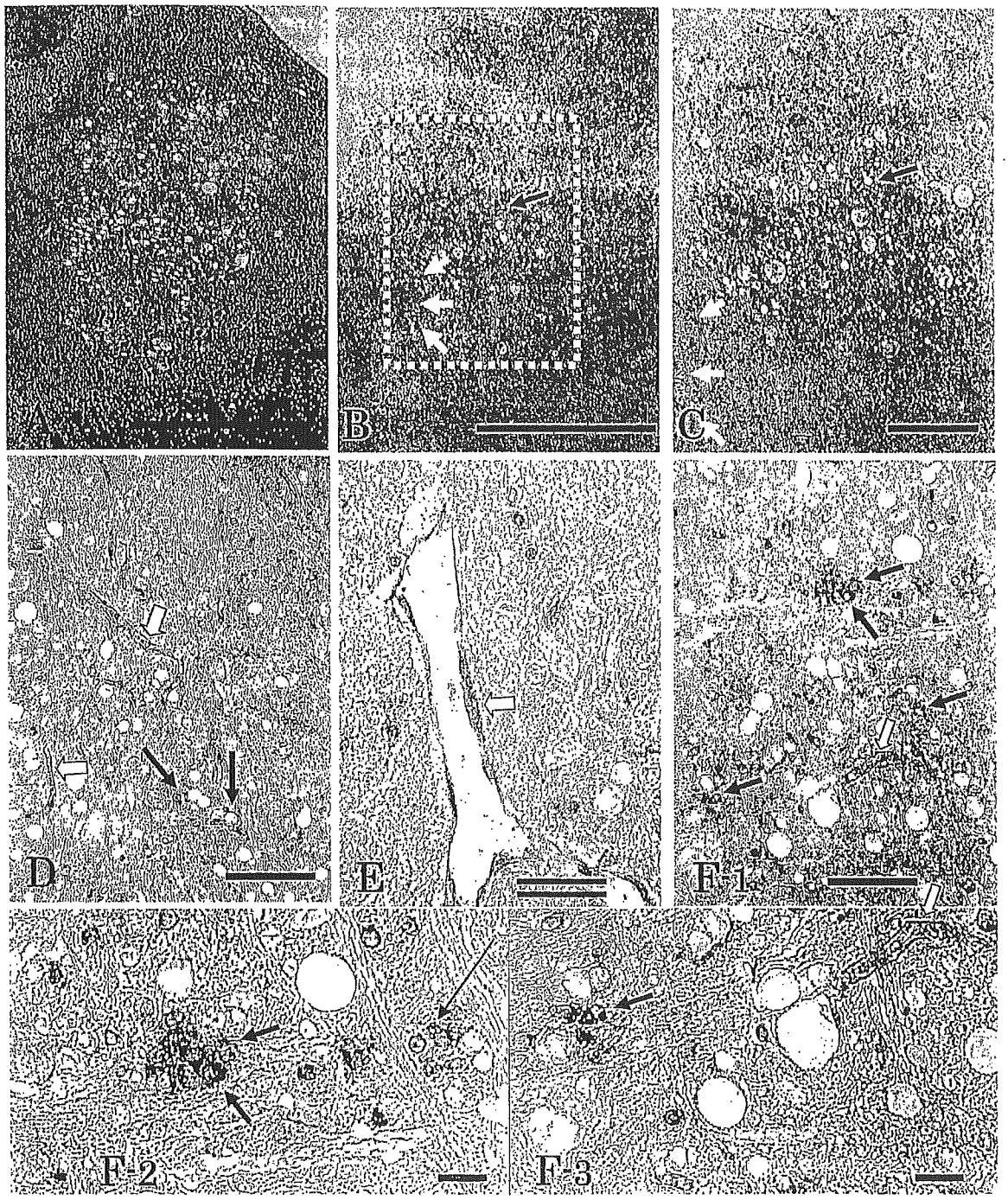

Fig. 2. Histology after infection. HE staining (A) and immuno-histochemistry (B–E) using anti-Gag or anti-Env protein antibodies (designated as Gag or Env in each photogragh respectively) 8 weeks after infection with A8-V or PVC211. The inoculated virus is indicated in each photograph. A–D: Areas around the lateral cerebellar nucleus stained for serial sections. The black arrows indicate glial cells bearing viral antigen which attach to the vacuolar wall while the white arrows indicate the antigen-positive blood vessels. The rectangular region in figure B is shown at higher magnification in C. The expression levels of Env (C) and Gag proteins (D) were similar as we previously reported (in press). E: Note the antigen-positive blood vessel indicated by the white arrow in the area of normal appearance. F: Double immunostaining for Gag protein (brown color) and a microglial marker protein (purple color). Pictures taken at higher magnification of F-1 are shown in F-2 and F-3. Note the viral antigen-positive blood vessel (brown staining) at the white arrow and the double labeled microglial cells staining brown and purple at the bolded black arrows. The long black arrow indicates a positively stained (purple) uninfected microglial cell. The single bars and double lines indicate 500 μm and 125 μm, respectively.

non-neuropathogenic chimerae, and the viral antigen expression in blood vessel walls of the CNS infected with non-neuropathogenic chimerae was also suppressed as well as in glial cells (19). Therefore, the significance of viral antigen positive glial cells in forming spongiotic lesions could not be evaluated in the comparative study mentioned above.

We have proposed that glial cells carrying viral antigens might contribute to developing neuropathological lesions induced by A8-V infection, because we often observe the antigen positive glial cells attaching to the walls of vacuoles which compose spongiotic lesions (17, 21). In addition, numerous reports have suggested that spongiform degeneration induced by neuropathogenic murine retroviruses is primarily associated with infection of microglial cells (1, 3, 6, 12). The results of our double labeling study confirmed that A8-V infection also induces many microglial cells which carry viral antigens (Fig. 2F). However, the importance of the suggested association of infected microglia with neuropathological lesions remains unknown, because viral antigen positive blood vessel walls are encountered in much higher frequency than viral antigen positive microglia in infected CNS (4, 7, 17, 20), and no statistical analysis had been done until now. In this paper, we clarified that viral antigen positive microglial cells were really associated with lesional distribution after a statistical analysis. Furthermore, distribution of antigen positive blood vessels was proved not to be statistically correlated with that of neuropathological lesions. Neuropathological changes induced by infections of A8-V and PVC211, which share a common genetical background, were almost identical except for the intensity of lesions in spinal cords, where similar levels of viral antigen expression in blood vessel walls were obtained from those viral infections. It is not clear how PVC211 induced a higher population of infected microglial cells in infected spinal cords than A8-V, in spite of the fact that these two viruses exhibit similar viral titers in brains and in spinal cords as well. The difference in the infectivity of the two viruses to glial cells in spinal cords might be due to different proliferation rates of these viruses during an earlier period of infection than the time when we compared the outcome at 6–8 weeks after infection, because the viral titers recovered from the CNS infected with A8-V at the newborn stage reach a plateau at 3–4 weeks after infection (15). And the expression levels of the receptor proteins for ecotropic retroviruses (EcoR) in rat brains change at the age of 4–5 weeks (17). We molecularly cloned the EcoR gene from F10 cells (F10-EcoR) (16), which are derived from rat glial cells. Application of a specific antibody for F10-EcoR protein visualized, by an immunohistochemical procedure, the F10-EcoR protein expression both in blood vessel walls and in glial cells of the CNS (16). The expression level of the F10-EcoR protein in rat glial cells reduced dramatically at the age of 4 weeks old, while that in the blood vessel walls of the rat CNS was well maintained afterwards (17). Although a close association of viral antigen positive microglial cell with lesional distribution was indicated, we still do not have any evidence or information as to whether those antigen positive microglial cells really create the vacuoles in the neuropils.

The authors thank Katsumi Goto for performing the histological studies. This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science and "High-Tech Research Center" Project for Private Universities: matching fund subsidy from MEXT, 2004–2008.

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Geriatr Gerontol Int 2005; 5: 293–297

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

Accepted for publication 4 July 2005.

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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).

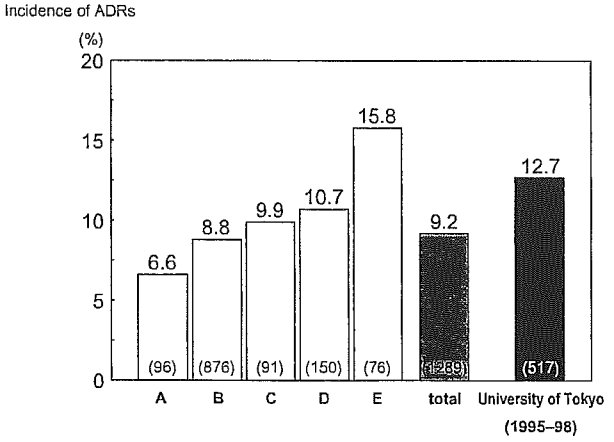

Figure 1 Incidence of ADR in inpatients of geriatric units of five university hospitals. The incidence of ADR in the geriatric unit of University of Tokyo Hospital in 1995–98 is shown as a reference.[9] The numbers of patients surveyed are shown in parentheses.

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.

Acknowledgments

This study was supported by a Research Grant for Longevity Sciences from the Ministry of Health, Labor, and Welfare, Japan.

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Original Article

Impact of Blood Pressure Variability on Cardiovascular Events in Elderly Patients with Hypertension

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Blood pressure variability is one of the characteristic features of hypertension in the elderly. However, its clinical significance remains to be determined. We therefore examined the impact of blood pressure variability on the development of cardiovascular events in elderly hypertensive patients. A total of 106 consecutive hypertensive patients aged more than 60 years old (mean age, 73.9±8.1 years old; male, 54%), all of whom underwent 24-h ambulatory blood pressure monitoring, were followed up (median, 34 months; range, 3–60 months). During the follow-up period, 39 cardiovascular events were observed, including 14 cases of cerebral infarction and 7 cases of acute myocardial infarction. The coefficient of variation (CV) of 24-h systolic blood pressure (SBP) values was used as an index of blood pressure variability. The patients showed a mean CV value of 10.6%, and were divided into two groups according to this mean value as a cut-off point: a high CV group (n=46) and a low CV group (n=60). Although baseline clinical characteristics were similar in the two groups, Kaplan-Meier plots for event-free survival revealed that the rate of cardiovascular events was significantly higher in high CV group than in low CV group (p<0.05). Cox's proportional hazards analysis showed that increased blood pressure variability (a high CV value of 24-h SBP) was an independent predictive variable for cardiovascular events. The CV value of daytime SBP and the SD value of both 24-h SBP and daytime SBP also had positive correlations with the onset of cardiovascular events. These results suggest that increased blood pressure variability may be an independent risk factor for cardiovascular events in elderly hypertensive patients. (*Hypertens Res* 2005; 28: 1–7)

***Key Words*: elderly hypertension, blood pressure variability, cardiovascular events, ambulatory blood pressure monitoring**

Introduction

Hypertension has been well established as a major predisposing factor for cardiovascular disease (*1*). The goal of treatment for hypertensive patients is not only to reduce blood pressure, but also to prevent cardiovascular events. The prevalence of hypertension increases with age (*2*), and elderly hypertensive patients are known to have some specific clinical features, such as isolated systolic hypertension (*3*), blood pressure variability (*4*, *5*), orthostatic hypotension (*6*, *7*) and postprandial hypotension (*8*).

Blood pressure variability is a characteristic feature of hypertension in the elderly (*4*, *5*). The arterial baroreflex

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This work was supported in part by a Grant-in-Aid (No. 08670768) for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.
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Received January 19, 2004; Accepted in revised form August 20, 2004.