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Reduced cell replication and induction of apoptosis by advanced glycation end products in rat Schwann cells

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Abstract

We investigated the effects of advanced glycation end products (AGEs) derived from glucose, glyceraldehyde, and glycolaldehyde (designated as AGE-1, -2, and -3, respectively) on the viability, replication rate, and cytokine production of cultured Schwann cells. AGE-2 and -3, but not AGE-1, induced apoptosis, and significantly decreased the viability measured by MTT assay. The decrease was prevented completely by antioxidant α-lipoic acid and was prevented partially by p38 mitogen-activated protein kinase inhibitor SB202190. The decrease in mitochondrial membrane potential by AGE-2 and -3 was also observed. In addition, AGE-2 and -3 significantly suppressed the replication rate as shown by reduced bromodeoxyuridine uptake, whereas they enhanced the release of TNF-α and IL-1β into the medium and activated nuclear factor-κB. The effects of AGE-1 on these measures were equivocal. The series of events elicited by AGE-2 and -3 may be responsible for some of the aspects of pathogenetic mechanisms in patients with diabetic neuropathy.

Keywords: Diabetic neuropathy; Advanced glycation end products; Schwann cell; Apoptosis

Excessive production of advanced glycation end products (AGEs) is implicated in the pathogenesis of diabetic neuropathy. AGEs accumulate in the peripheral nerve of diabetic patients [1], and the inhibition of AGE formation by anti-glycation agents improved neuropathic changes in experimental diabetic rats [2–4]. In the vascular wall, AGEs activate intracellular signal transduction pathways, thereby augmenting the expression of growth factors and adhesion molecules as well as cytokines, and inhibiting cell replication and/or inducing apoptosis [5–9]. However, the mechanisms of action of AGEs in diabetic neuropathy are poorly understood. To date, it remains unknown whether AGEs themselves directly injure the nerve tissues in diabetic patients, or if they are simply the waste products of hyperglycemia and/or oxidative stress [10,11].

AGEs are formed not only from glucose but also from other short-chain sugars. Various types of AGEs present in human serum are derived from glucose, glyceraldehyde, and glycolaldehyde; levels of these AGEs are elevated in diabetic patients [12] and are currently designated as AGE-1 to -3, respectively, according to the original sugar. These endogenous AGEs have been reported to exert various biological effects on neuronal cells [13], vascular cells [8], and renal cells [9,14].

In this study, we investigated whether AGEs exert any influence on Schwann cells in vitro, by preparing three immunochemically different AGEs from AGE-1 to -3. In particular, effects on cell viability, replication rate, and the production of proinflammatory cytokines were explored using cultured Schwann cells.

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Materials and methods

Isolation of Schwann cells. Schwann cells were isolated from the sciatic nerve of adult male Sprague–Dawley rats (11–13 weeks old), as described previously by Suzuki et al. [15]. Isolated cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Asahi Techno Glass, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS) (Gibco-BRL, Rockville, MD), 2 μM forskolin (Sigma, St. Louis, MO), and 10 μg/ml bovine pituitary extracts (Becton–Dickinson) (SC medium) in poly-lysine coated culture plates. The cells were round-shaped during the proliferating stage and became spindle-shaped when they reached confluence. Treatment with AGEs was carried out in SC medium with the FBS concentration reduced to 5%.

Preparation of AGE proteins. AGE proteins were prepared as described previously by Takeuchi et al. [12]. Briefly, bovine serum albumin (BSA) was incubated under sterile conditions with D-glucose (AGE-1) for 8 weeks or with D-glyceraldehyde (AGE-2) or glycolaldehyde (AGE-3) for 7 days at 37 °C in 0.2 M phosphate buffer (pH 7.4) containing 5 mM diethylenetriamine-pentaacetic acid (DTPA).

Low-molecular-weight reactants and aldehydes were removed by chromatography on a PD-10 desalting column (Amersham–Pharmacia Biotech AB, Uppsala, Sweden) followed by dialysis against phosphate-buffered saline (PBS). These three types of AGEs are reported to be immunochemically distinct and are also detectable in the serum of diabetic patients [12]. For the control experiment, BSA was purified through the same procedures as those described above, except that the AGE-forming compounds were not included. All preparations were passed through a Zeta-Pore filter (Cuno, Tokyo, Japan) to remove any endotoxin present in the solution. Protein concentrations were determined with a Bradford-based assay (Bio-Rad Laboratories, Richmond, CA).

Detection of apoptotic cell death. Schwann cells grown to confluence were incubated with AGE-1, -2, -3 or control BSA at a concentration of 500 μg/ml. After 24 h, the cells were harvested, washed with PBS, and fixed with 3.7% formaldehyde on ice for 15 min. The fixed cells were washed and then suspended in PBS. Two microliters of 100 μg/ml Hoechst 33342 (Calbiochem, La Jolla, CA) was added to 5 μl of the cell suspension, and the cells were observed under a microscope using a U filter (Olympus, Tokyo, Japan). Under randomly selected fields (at least three fields per sample), cells with fragmented and condensed nuclei identified as apoptotic cells among a total of at least 500 cells were counted and expressed as a percentage. Triplicate samples were counted per experimental group.

Quantification of viable cells. Schwann cells grown to confluence in 24-well plates were incubated with AGE-1, -2, -3 or with control BSA for 24 h at concentrations of 10, 500, and 1000 μg/ml, and then the viable cells were quantified by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [16]. The medium was removed, and 0.4 ml of DMEM supplemented with 5% FBS and 40 μl of 5 mg/ml MTT were added to each well. Following incubation for 2 h, 0.4 ml of acid-isopropyl alcohol (0.04 N HCl in isopropyl alcohol) was added, and the cells were stored under dark conditions. The absorbance was read at 570 nm with the background subtraction at 630 nm.

To observe the effects of antioxidant on cell viability, the cells were incubated with 500 μg/ml AGE-3 or control BSA along with 1 mM α-lipoic acid for 24 h. To observe the effects of mitogen-activated protein kinase (MAPK) inhibitors, the cells were pretreated with 10 μM SB202190 (p38 MAPK inhibitor) (Calbiochem) or UO126 (MEK inhibitor, the inhibitor of upstream kinase of ERK) (Promega, Madison, WI) for 1 h, washed with DMEM once, and incubated with 500 μg/ml AGE-3 or control BSA for 24 h. After treatment with the AGEs, the viable cells were quantified by MTT assay.

Detection of 8-hydroxydeoxyguanosine. To observe the oxidative stress introduced by AGEs, immunocytochemistry was used to evaluate 8-hydroxy-deoxyguanosine (8-OHdG) levels in Schwann cells exposed to AGE-2, -3. Schwann cells grown to confluence in poly-lysine coated coverslips (Becton–Dickinson, Braintree, MA) were incubated with 500 μg/ml AGE-2, -3 or with control BSA for 24 h. After incubation, cells were washed twice with Tris-buffered saline (TBS), fixed with Bouin's solution for 15 min, and washed three times with 70% ethanol and TBS, respectively, at room temperature. 8-OHdG was detected using a monoclonal-antibody N45.1 (Nikken Seil, Shizuoka, Japan) [17]. Samples were sequentially treated with 3% H_2O_2 in methanol for 10 min, 0.01 M NaOH in 40% ethanol for 12 min, and 250 μg/ml RNases (Wako Pure Chemical Industry, Osaka, Japan) for 1 h at 37 °C, and then washed with TBS. They were first incubated with primary antibody (1:100 dilution of N45.1) for 1 h at room temperature and washed with TBS. Then they were sequentially exposed to biotinylated anti-mouse IgG (1:200 dilution) and avidin-peroxidase conjugate. The binding sites of peroxidase were demonstrated with 3-amino-9-ethyl carbazole (AEC). Staining of 8-OHdG was visualized under microscope (BX 50, Olympus, Japan) connected to a 3CCD camera unit (XC-003, Sony, Japan), and the intensity of staining was quantified using a software analysis system (IPAP-WIN, Sumika Techno Service, Japan). In approximately 230 Schwann cells from randomly selected 10 fields, the staining intensity in nucleus was measured. In addition, the intensity in cytosol was measured as a background. The relative staining intensity was expressed as the percentage of the control BSA-treated cells.

Mitochondrial membrane potential. Schwann cells grown to confluence were incubated with AGE-2, -3, or control BSA for 24 h. Cells were harvested and suspended in DMEM supplemented with 5% FBS. Following the addition of rhodamine 123 (Molecular Probes, Eugene, OR) to the medium to a final concentration of 10 μM, cells were incubated for 30 min at 37 °C, washed with PBS, and then resuspended in PBS containing 10 μM propidium iodide (PI) (Molecular Probes). Cell fluorescence was quantified using an EPICS XL flow cytometer (Beckman Coulter, Fullerton, CA), and the data were analyzed using EXPO ver. 2 software (Beckman Coulter). To quantify rhodamine 123 fluorescence only in viable cells, a PI-negative (i.e., viable) gate was established using PI and forward light scatter dot plots. Throughout the experiments, the population of PI-positive cells was consistently less than 20%.

Measurement of cell replication rate. Schwann cells were seeded at 5000 cells/well in DMEM supplemented with 10% FBS onto 24-well plates. On the next day, the cultures were switched to SC medium in order to stimulate cell replication, and cells became round-shaped, indicating that the cells were in the proliferating stage. Ten micrograms per milliliter of AGE-1, -2, -3, or control BSA was further added to the cultures on the following day. The cultures were then incubated for five additional days. At days 0, 3, and 5 after the start of the incubation with the AGEs, the viable cells were quantified by MTT assay.

Uptake of bromodeoxyuridine. Schwann cells in the proliferating stage were incubated with AGE-2, -3, or control BSA for 24 h at a concentration of 10 μg/ml, and then incorporation of bromodeoxyuridine (BrdU) was determined using the Cell Proliferation ELISA, BrdU (colorimetric) (Roche Diagnostics, Mannheim, Germany), according to the manufacturer's instructions. Since decreased cell number resulting from AGEs-induced cell death would interfere with determination of replication, the values of BrdU uptake were normalized by amounts of protein that were lysed from the cells in each well of the culture plates. The protein concentration was determined using a Bradford-based assay, and the levels of BrdU uptake per milligram protein were expressed as the percentage of the control BSA.

Measurement of cytokine production. Schwann cells grown to confluence were incubated with 100 μg/ml AGE-1, -2, -3, or control BSA for 4 days, or with 500 μg/ml AGE-3 or control BSA in the presence or absence of 5 μg/ml cyclohexamide (CHX; Wako Pure Chemical Industry) for 24 h following a 12-h pretreatment with or without CHX. After incubation, concentrations of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in the medium were determined with an ELISA kit (BioSource International, Camarillo, CA). The protein concentration in each well was determined with a Bradford-based assay.

Measurement of nuclear factor-κB activation. Schwann cells grown to confluence were incubated with AGE-1, -2, -3, or control BSA at a concentration of 100 μg/ml. After incubation for 24 h, cells were washed twice with ice-cold PBS, harvested, and the activated p65 nuclear factor-κB (NF-κB) levels in the nuclear extracts were measured using a TransAM NF-κB Assay Kit (Active Motif, Carlsbad, CA), according to the manufacturer's instructions. Briefly, the samples were loaded to a 96-well NF-κB assay plate coated with oligonucleotide containing a NF-κB-consensus binding site (5′-GGGACTTTCC-3′), and the activated p65 NF-κB bound to the assay plate was detected using an antibody against p65 subunit.

Statistical analysis. The data were analyzed by EXSAS ver. 5.10 (Arm, Osaka, Japan). The results are shown as means ± SEM. The statistical significance of differences between experimental groups in all experiments, except for the experiment involving the effect of antioxidant and MAPK inhibitors, was analyzed using Dunnett's test. In the experiment involving the effect of antioxidant and MAPK inhibitors, the statistical significance of differences was analyzed using Tukey's test. P values of less than 0.05 were considered to indicate significance.

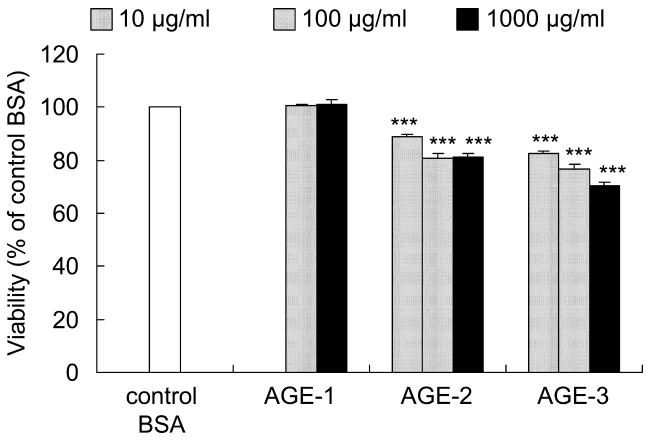

Fig. 2. Effects of AGEs on cell viability in Schwann cells. Schwann cells grown to confluence were incubated with AGEs or control BSA at a concentration of 10, 500, or 1000 μg/ml. After incubation for 24 h, viable cells were quantified by MTT assay. Data are expressed as means ± SEM ($n = 4$). ***$P < 0.001$ vs. control BSA.

Results

Effects of AGEs on cell viability

When cells were treated with 500 μg/ml AGE-2 or -3 for 24 h, apoptotic cells with nuclear condensation and fragmentation were detected (Fig. 1A). Counting the number of cells under microscopy showed increased percentage of apoptotic cells by AGE-2 and -3 compared to control BSA (Fig. 1B). In contrast, there was no significant increase in the number of apoptotic cells in AGE-1-treated cells. To determine the cytotoxic effect of AGEs at various concentrations, we carried out MTT assay, a quantitative method to assess cell viability. As shown in Fig. 2, AGE-2 and -3 induced cell death in a dose-dependent manner (Fig. 2). Of note, these AGEs were effective at low concentration of 10 μg/ml. In contrast, there was no significant effect of AGE-1 on cell viability, even at high concentrations of 500 and 1000 μg/ml. At the same concentrations, control BSA had no effect on the viability.

Involvement of oxidative stress, MAPK, and changes in the mitochondrial membrane potential in AGEs-induced cell death

To investigate a possibility of involvement of oxidative stress and MAPK in the AGEs-induced cell death, we examined the effect of an antioxidant and MAPK inhibitors on cell viability. In this experiment, we used

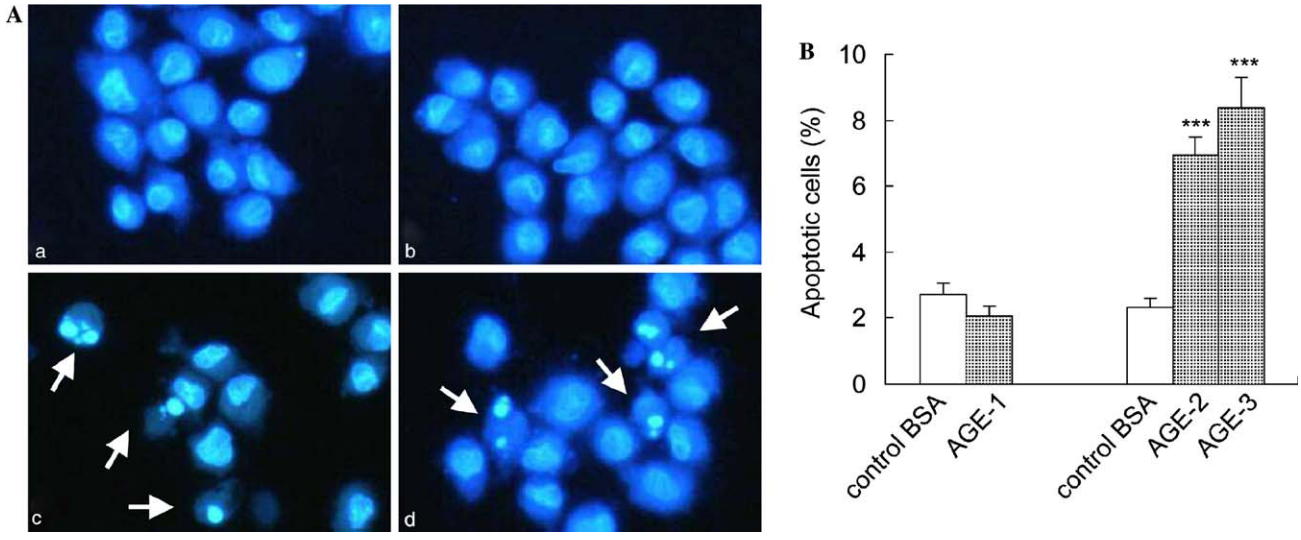

Fig. 1. Induction of apoptosis in cultured Schwann cells after exposure to AGEs. (A) Exposure to 500 μg/ml AGE-2 (c) or AGE-3 (d) induced apoptotic nuclear alterations of Schwann cells (arrows), while there was no apoptosis when the cells were exposed to AGE-1 (b) or control BSA (a). (B) The percentage of apoptotic Schwann cells exposed to AGEs. Data are expressed as means ± SEM ($n = 3$). ***$P < 0.001$ vs. control BSA.

high concentration of AGE-3 (500 μg/ml) to observe preventive effects of inhibitors clearly. Addition of 1 mM α-lipoic acid, an antioxidant, completely prevented AGE-3-induced cell death (Fig. 3A). Ten micromolar SB202190, a p38 MAPK inhibitor, also partially but significantly prevented the cell death (Fig. 3B), but UO126, a MEK (an upstream kinase of ERK) inhibitor, had no effect (data not shown). In addition, treatment with AGE-3 or -2 at 500 μg/ml for 24 h significantly increased the immunostaining intensity against 8-OHdG-antibody in nucleus (Fig. 4). By contrast, the staining intensity in cytosol as a background was less than 25% of the intensity in nucleus and there was no change among experimental groups (control BSA, 100.0 ± 5.4; AGE-2, 102.2 ± 6.0; and AGE-3, 109.5 ± 5.8, % of control BSA, $n = 50$).

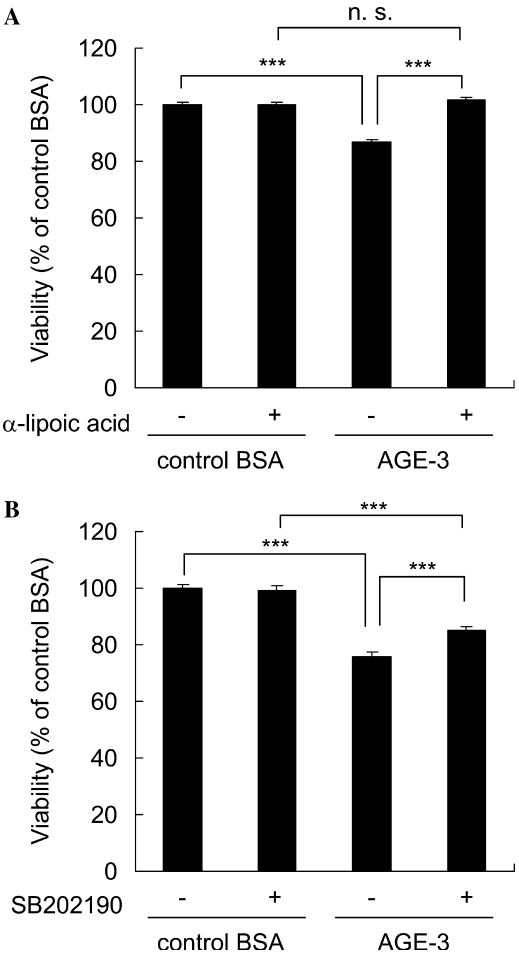

Fig. 3. Effects of the antioxidant α-lipoic acid and the p38 MAPK inhibitor SB202190 on AGE-3 induced decrease in cell viability. (A) Schwann cells grown to confluence were incubated with AGE-3 or control BSA (500 μg/ml) with or without α-lipoic acid (1 mM). After incubation for 24 h, viable cells were quantified by MTT assay. Data are expressed as means ± SEM ($n = 4$). ***$P < 0.001$. n.s., not significant. (B) Schwann cells pretreated with 10 μM SB202190 for 1 h were incubated with AGE-3 or control BSA (500 μg/ml). After incubation for 24 h, viable cells were quantified by MTT assay. Data are expressed as means ± SEM ($n = 6$). ***$P < 0.001$.

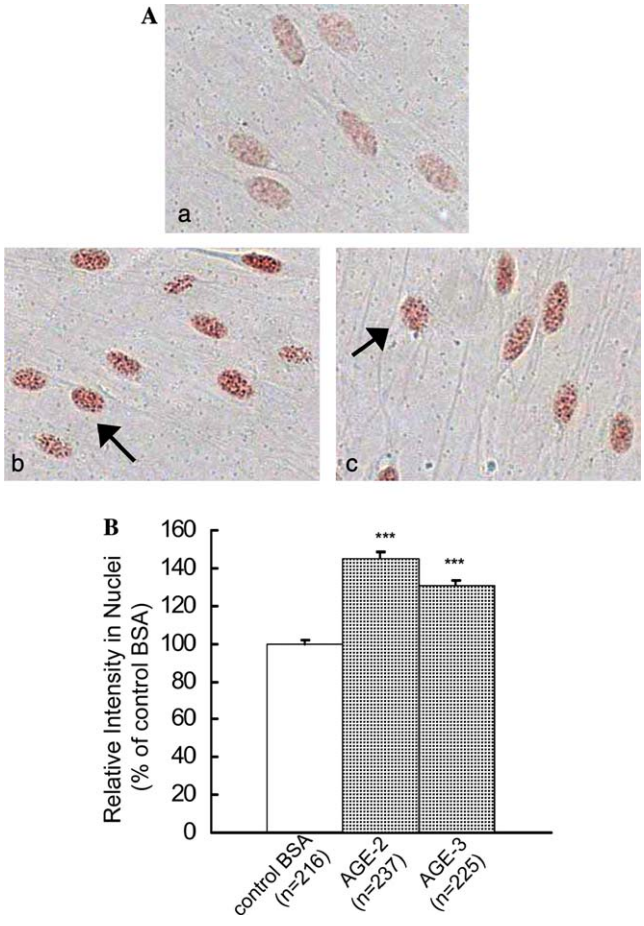

Fig. 4. Increase in 8-OHdG levels in cultured Schwann cells after exposure to AGEs. (A) Exposure to 500 μg/ml AGE-2 (b), -3 (c) induced the increase in the immunostaining intensity against 8-OHdG-antibody in nucleus (arrows) compared with control BSA-treated cells (a). (B) The relative staining intensity in nucleus was expressed as the percentage of the control BSA-treated cells. Data are expressed as means ± SEM. ***$P < 0.001$ vs. control BSA.

We further investigated the changes in mitochondria, which play an important role in the cascade of apoptotic cell death in various types of cells. As shown in Fig. 5, the mitochondrial membrane potential was reduced in cells exposed to AGE-2 and -3.

Effects of AGEs on cell replication

To determine effects of AGEs on growth curve, Schwann cells in proliferating stage were incubated with AGEs, and viable cells were quantified by MTT assay before the cells reached confluence (at days 0, 3, and 5 after AGEs addition). The growth curve was found to be shifted downwards by the treatment of 10 μg/ml AGE-2 and -3 (Fig. 6A). AGE-1 exerted no influence on growth curve.

We further investigated whether AGE-2 and -3 exert influence on DNA synthesis. To this end, BrdU uptake was measured after proliferating Schwann cells were

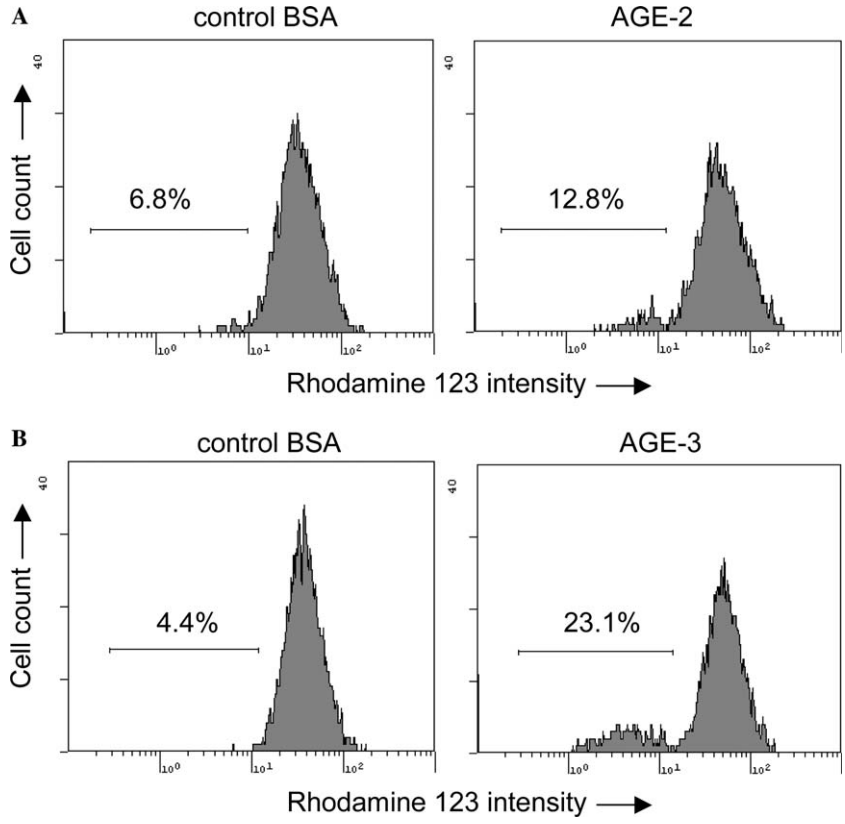

Fig. 5. Effects of AGE-3 on mitochondrial membrane potential in Schwann cells. Schwann cells grown to confluence were incubated with 500 μg/ml AGE-2 or AGE-3 or control BSA. After incubation for 24 h, cells were harvested and incubated with Rhodamine 123 at 37 °C for 30 min. The stained cells were analyzed by flow cytometry.

incubated with AGEs for 24 h. As shown in Fig. 5B, BrdU uptake was markedly decreased by 10 μg/ml AGE-2 and -3.

Effects of AGEs of cytokine production and NF-κB activation

When cells were exposed to 100 μg/ml AGE-2 and -3 for 4 days, both TNF-α and IL-1β levels in the medium were significantly increased (Fig. 7). In contrast, AGE-1 exerted no influence on the production of these cytokines. To address whether AGE generates these cytokines in transcriptional levels, we assessed the effect of CHX, a protein synthesis inhibitor, on the production of these cytokines by AGE-3. Treatment with CHX significantly inhibited the increase in both TNF-α and IL-1β production in the cells exposed to 500 μg/ml AGE-3 for 24 h. In contrast, treatment with CHX had no effect on control-BSA-treated cells (Fig. 8).

Furthermore, since NF-κB is known to control the transcription of proinflammatory cytokine genes, we investigated whether AGEs induce activation of NF-κB. As shown in Fig. 9, treatment with AGE-2 and -3 at 100 μg/ml for 24 h significantly increased the activation of NF-κB, whereas AGE-1 had no effect.

Discussion

In this study, we demonstrated for the first time that AGEs directly exert biological effects on Schwann cells. Inhibitory effects of AGE-2 and -3, glyceraldehyde and glycolaldehyde derived AGEs, respectively, on cell viability were dose-dependent and related to an increased rate of apoptotic cells. In contrast, AGE-1 and control BSA had no effect on cell viability. Cell replication and BrdU uptake in Schwann cells were also inhibited by AGE-2 and -3. Exposure of Schwann cells to these types of AGEs further promoted the activation of NF-κB, as well as secretion of TNF-α and IL-1β in the medium. Based on these results, it is likely that elevated levels of tissue or serum AGEs, in particular AGE-2 and -3, observed in patients with long-standing diabetes [12,18] contribute to the functional and structural abnormalities observed in nerves of those patients.

Excessive oxidative stress, the induction of cell signaling pathways, as well as protein kinase C-MAP-kinase or NF-κB activation have all been proposed as major downstream cascades towards the cytopathic effects of AGEs in vascular cells [19–23]. In the present study, the decreased viability of Schwann cells by AGEs

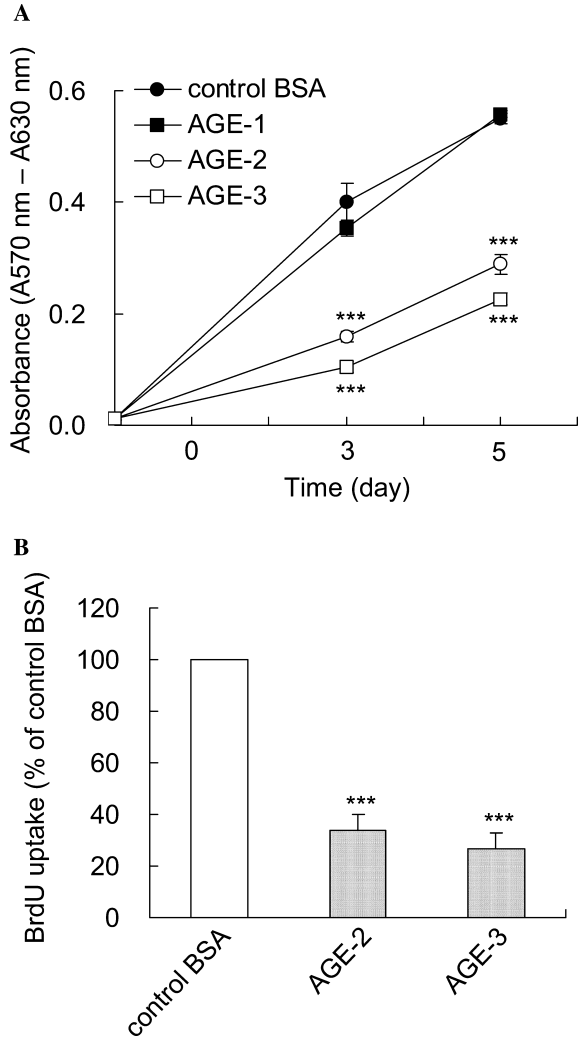

Fig. 6. Effects of AGEs on cell replication in Schwann cells. (A) Schwann cells in proliferating stage were incubated with AGEs or control BSA at 10 μg/ml, and viable cells were quantified by MTT assay at days 0, 3, and 5 after AGEs addition. Data are expressed as means ± SEM ($n = 4$). ***$P < 0.001$ vs. control BSA. (B) Schwann cells in proliferating stage were incubated with AGEs or control BSA at 10 μg/ml. After incubation for 24 h, BrdU uptake into the cells was assayed. The levels of BrdU uptake per mg protein were expressed as the percentage of the control BSA. Data are expressed as means ± SEM ($n = 4$). ***$P < 0.001$ vs. control BSA.

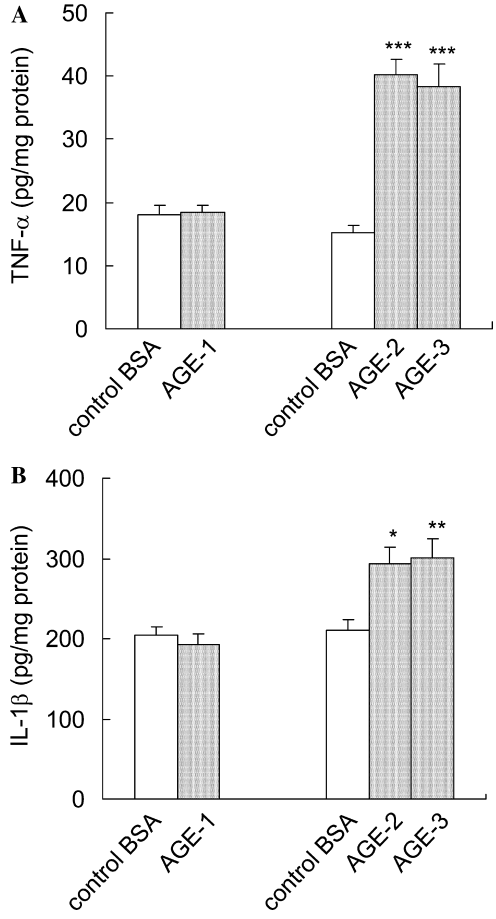

Fig. 7. Effects of AGEs on production of proinflammatory cytokines in Schwann cells. Schwann cells grown to confluence were incubated with AGEs or control BSA at 100 μg/ml. After incubation for 4 days, concentrations of TNF-α (A) and IL-1β (B) in the medium were determined with ELISA. Data are expressed as means ± SEM ($n = 4$). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs. control BSA.

was completely prevented by a potent antioxidant, α-lipoic acid. In addition, AGEs produced an increase in levels of 8-OHdG, a marker of intracellular oxidative stress generation. A specific inhibitor of p38 MAPK (SB202190) also inhibited the effects of AGEs on cell viability, but this was not the case with the MEK inhibitor UO206. These results indicate that oxidative stress is involved in the pathway of AGE-induced cytotoxic effects, which agree with other research showing that AGEs induce the generation of reactive oxygen species [19,23]. Furthermore, we found that p38 MAPK, which responds to cellular stress inducers such as oxidative stress, was also involved in this pathway, although only partially, whereas the ERK pathway was not so. The decrease in the mitochondrial membrane potential after exposure to AGEs detected in this study may reflect the impairment of mitochondrial function leading to the release of pro-apoptotic factors such as caspase and/or cytochrome *c*.

NF-κB is a key regulatory molecule in oxidative stress-induced cell injury, and it controls the transcription of genes encoding the mediators of inflammatory responses [24]. In the present study, AGEs induced the activation of NF-κB, as well as the excessive production of the proinflammatory cytokines TNF-α and IL-1β. These results suggest that the activation of NF-κB due to excessive oxidative stress is possibly involved in the elevated expression of TNF-α and IL-1β induced by AGEs.

In human diabetic nerves, the major pathology involves fiber loss, axonal degeneration, and demyelination, as well as microangiopathic changes [25,26]. Various degenerated features of Schwann cells are ob-

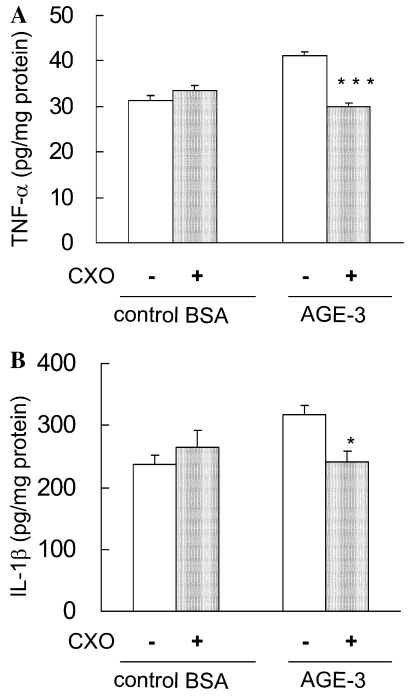

Fig. 8. Effects of cyclohexamide (CXO) on production of proinflammatory cytokines in Schwann cells exposed to AGEs. Schwann cells grown to confluence were treated with or without 5 μg/ml CXO for 12 h, and then incubated with 500 μg/ml AGE-3 or control BSA in the presence or absence of continuous treatment of CXO for 24 h. After incubation, concentrations of TNF-α (A) and IL-1β (B) in the medium were determined with ELISA. Data are expressed as means ± SEM ($n = 6$). *$P < 0.05$, ***$P < 0.001$ vs. not CXO-treated cells (CXO−).

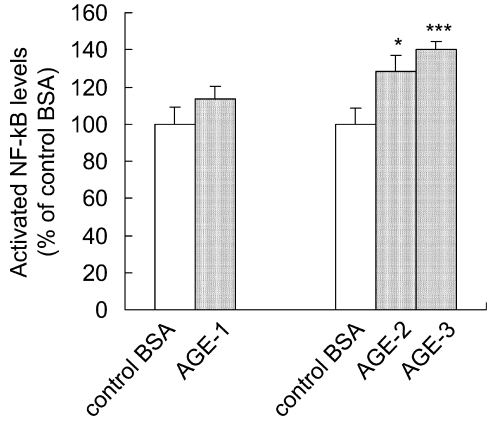

Fig. 9. Effects of AGEs on the activation of NF-κB. Schwann cells grown to confluence were incubated with AGEs or control BSA at 100 μg/ml. After incubation for 24 h, activated p65 NF-κB levels in the nuclear extracts were measured. Data are expressed as means ± SEM ($n = 4$). *$P < 0.05$, ***$P < 0.001$ vs. control BSA.

served in human and animal diabetic peripheral nerves [27,28]. The accumulation of AGEs detected in the nerves of diabetics [1] and the AGEs-induced apoptosis of Schwann cells shown in this study may be related to the repeated cell death of Schwann cells leading to demyelination and abnormal structure formation around the degenerated nerve fibers [29,30]. When nerves are injured, Schwann cells replicate to form continuous cell columns and to secrete neurotrophins, serving as a guide for axonal sprouts. As demonstrated in this study, AGEs-induced reduced cell replication in Schwann cells may be involved in the impairment of regeneration in damaged nerves of diabetic patients. The cytokines exerted by AGEs in Schwann cells may also enhance nerve fiber damage, since these cytokines themselves are suggested to be deleterious to peripheral nerves [31,32].

The current study demonstrated that the effects of AGEs on Schwann cells were not uniform but instead differed among several AGEs. Cell viability, replication, and the production of proinflammatory cytokines were significantly affected by AGE-2 and -3, whereas AGE-1 had no effect on Schwann cells, even at high concentrations. The findings of toxicity associated with aldehyde-derived AGEs and the non-toxicity of AGE-1 in Schwann cells are consistent with previous report concerned with neuronal cells. In cortical neuronal cells isolated from the rat brain, AGE-2 caused more severe cytotoxic effects than did AGE-1 [13]. Additionally, in retinal pericytes, overexpression of the receptor for AGEs (RAGE) significantly enhanced the effects of aldehyde-derived AGEs, but not those of AGE-1, implicating the role of RAGE in AGEs biological effects [8].

In summary, our study demonstrated that AGEs derived from glyceraldehyde and glycolaldehyde directly perturbed the function of Schwann cells; such impairment of function may in turn be responsible for impaired survival and regeneration of peripheral nerves in diabetic patients. These findings support the hypothesis that AGEs play a causative role in the pathogenesis of diabetic neuropathy. The inhibition of the deleterious effects of these AGEs should therefore be studied carefully for the future treatment of this intractable disorder.

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