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Glycolaldehyde induces endoplasmic reticulum stress and apoptosis in Schwann cells

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ABSTRACT

Schwann cell injury is caused by diabetic neuropathy. The apoptosis of Schwann cells plays a pivotal role in diabetic nerve dysfunction. Glycolaldehyde is a precursor of advanced glycation end products that contribute to the pathogenesis of diabetic neuropathy. In this study, we examined whether glycolaldehyde induces endoplasmic reticulum (ER) stress and apoptosis in rat Schwann cells. Schwann cells treated with 500 μM glycolaldehyde showed morphological changes characteristic of apoptosis. Glycolaldehyde activated apoptotic signals, such as caspase-3 and caspase-8. Furthermore, it induced ER stress response involving RNA-dependent protein kinase-like ER kinase (PERK), inositol-requiring ER-to-nucleus signal kinase 1α (IRE1α), and eukaryotic initiation factor 2α (eIF2α). In addition, glycolaldehyde activated CCAAT/enhancer-binding homologous protein (CHOP), an ER stress response factor crucial to executing apoptosis. Knockdown of nuclear factor E2-related factor 2 (Nrf2), which is involved in the promotion of cell survival following ER stress, enhanced glycolaldehyde-induced cytotoxicity, indicating that Nrf2 plays a protective role in the cytotoxicity caused by glycolaldehyde. Taken together, these findings indicate that glycolaldehyde is capable of inducing apoptosis and ER stress in Schwann cells. The ER stress induced by glycolaldehyde may trigger the glycolaldehyde-induced apoptosis in Schwann cells. This study demonstrated for the first time that glycolaldehyde induced ER stress.

1. Introduction

Glycolaldehyde (GA), a highly reactive α-hydroxyaldehyde, is formed from glycated proteins during hyperglycemia and plays an important role in the production of advanced glycation end products (AGEs) [1]. During the glycation of proteins leading to AGE formation, dicarbonyl compounds, such as methylglyoxal (MG), glyoxal, and 3-deoxyglucosone, are produced as intermediates, as well as GA [2]. It is reported that AGEs are associated with diabetic complications, including neuropathy, retinopathy, and nephropathy [3]. However, it is unknown whether GA is implicated in the pathogenesis of diabetic complications. Diabetic neuropathy is one of the most common long-term complications in diabetes mellitus patients [4–6]. The apoptosis of Schwann cells plays a pivotal role in diabetic nerve dysfunction [7,8]; it leads to demyelination and abnormal structure formation around degraded nerve fibers [9,10]. It is reported that AGEs and MG induce apoptosis in Schwann cells [7,8,11]. In our previous report, we demonstrated that GA decreased the viability of rat Schwann cells [12]. Moreover, GA had a greater effect on Schwann cells than dicarbonyl compounds, such as MG. However, it is unknown how GA causes the cytotoxicity in Schwann cells.

Apoptosis plays a substantial role in development, homeostasis, and disease processes, including diabetes mellitus and diabetic complications [13,14]. Oxidative stress is a well-known mediator of apoptosis [15]. There are several reports indicating that AGEs and MG induce apoptosis via oxidative stress [7,8,11]. However, almost nothing is known about whether GA causes apoptosis or not. There is only one report showing that GA induces apoptosis via oxidative stress in breast cancer cells [16]. On the other hand, we have reported that oxidative stress is not a major contributor to the GA-induced cytotoxicity in Schwann cells [12]. Endoplasmic reticulum (ER) stress was found to be involved in the induction of apoptosis that occurs in a wide range of metabolic diseases, including diabetes mellitus [17]. The protein involved in the ER-stress-mediated apoptosis pathway is CCAAT/enhancer-binding homologous protein (CHOP), also termed growth-arrest and DNA-damage inducible

Abbreviations: GA, glycolaldehyde; MG, methylglyoxal; AGEs, advanced glycation end products; ER, endoplasmic reticulum; PERK, RNA-dependent protein kinase-like ER kinase; eIF2, eukaryotic initiation factor 2; IRE1, inositol-requiring ER-to-nucleus signal kinase 1; ATF6, activating transcription factor 6; CHOP, CCAAT/enhancer-binding homologous protein; Nrf2, nuclear factor E2-related factor 2; HO-1, heme oxygenase-1.

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gene 153 (GADD153) [18,19]. However, the effect of GA on ER stress is unknown.

ER is the key organelle where newly synthesized proteins form correct tertiary structures by posttranslational modification, folding, and oligomerization [20]. Such abnormalities as unfolded protein accumulation in the ER are collectively called ER stress [21]. There are three major transducers for sensing ER stress in the ER: RNA-dependent protein kinase-like ER kinase (PERK), inositol-requiring ER-to-nucleus signal kinase 1 (IRE1), and activating transcription factor 6 (ATF6). The proapoptotic pathway involving the CHOP transcription factor is induced via PERK and IRE1 [22]. Moreover, PERK activates the transcription factor nuclear factor E2-related factor 2 (Nrf2) [23,24], a protein that contributes to cellular redox homeostasis by regulating antioxidant response. Nrf2 activation is implicated in the promotion of cell survival following ER stress [25]. We previously reported that GA induced Nrf2 activation in Schwann cells [12]. It is possible that GA affects ER stress sensors, including PERK.

This study aims to investigate whether GA causes apoptosis and induces ER stress in Schwann cells.

2. Materials and methods

2.1. Materials

GA dimer (G6805; crystalline, mixture of stereoisomers) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), Dulbecco's phosphate-buffered saline (DPBS), and phosphate-buffered saline (PBS) at pH 7.4 were from Life Technologies (Carlsbad, CA, USA). All other chemicals used were of reagent grade.

2.2. Cell culture and treatment with GA

Rat Schwann cells were purchased from Dainippon Sumitomo Pharma (Osaka, Japan). Cell culture was performed according to a previous report [12]. The cells were grown to 80–90% confluence in DMEM containing 10% fetal bovine serum (FBS), L-glutamine (4 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Then, the cells were passaged by trypsinization and those between passages 3 and 20 were used.

Before treating Schwann cells with GA, the culture medium was replaced with DMEM containing 2% FBS because serum might include antioxidants that would cause chelation of transition metal ions and high-density lipoproteins [26]. After GA (100–500 μM) was added to the medium, the cells were incubated for 0–24 h.

2.3. Annexin-V propidium iodide (PI) assay

An Annexin-V-FLUOS Staining Kit (Roche, Penzberg, Germany) was used. Schwann cells treated with GA were collected by trypsinization and centrifugation. The cells were stained with annexin-V-fluorescein and PI, and analyzed under a laser scanning microscope (Carl Zeiss, Oberkochen, Germany) and a flow cytometer (Beckman Coulter, Fullerton, CA, USA). Fluorescence was detected with FL1 and FL2.

2.4. Flow cytometric detection of activated caspase-3 and caspase-8

The activation of caspase-3 and caspase-8 was determined by flow cytometry. Schwann cells were treated with GA. The cells were fixed by adding 4% paraformaldehyde. After the cells were washed with PBS, they were permeabilized for 30 min at 4 °C with ice-chilled methanol. The cells were washed with PBS again and treated with PBS containing 0.5% blocking reagent (GE Healthcare, Buckinghamshire, UK). The cells were incubated with primary antibodies, washed, and stained with secondary antibodies. The primary antibodies used were anti-mouse caspase-3 monoclonal antibody and anti-mouse caspase-8 monoclonal antibody (Cell Signaling Technology, Danvers, MA, USA). The secondary antibody was Alexa Fluor 488-conjugated goat anti-mouse IgG (Cell Signaling Technology). The stained cells were washed with DPBS and analyzed by flow cytometry (Beckman Coulter). Fluorescence was detected with FL1.

2.5. Measurement of mRNA

Real-time RT-PCR was performed to measure PERK, IRE1α, and heme oxygenase-1 (HO-1) mRNA levels. Total RNA from GA-treated cells was extracted with a PureLink® RNA Mini Kit (Life Technologies) according to the manufacturer's protocol. mRNAs were reverse-transcribed into cDNA with a High-Capacity cDNA Reverse Transcription Kit (Life Technologies). Quantitative RT-PCR was performed with a 7500 Fast Real-Time PCR System (Life Technologies). Primers for rat PERK, rat IRE1α, and rat HO-1 were purchased from Life Technologies. mRNA levels were obtained from the threshold cycle (Ct) values of PERK, IRE1α, and HO-1, normalized to that of GAPDH. Relative mRNA levels were expressed as percentage relative to control levels.

Semi-quantitative RT-PCR was performed to measure CHOP mRNA levels. Total RNA from GA-treated cells was extracted with a PureLink® RNA Mini Kit. Total RNA was reverse-transcribed into cDNA with a High-Capacity cDNA Reverse Transcription Kit. Primer sequences and their respective PCR product lengths were as follows: CHOP Fw, 5′-ACCACACCTGAAAGCAGAAAC-3′; CHOP Rv, 5′-TCAGTCAGCCAAGCTAGGGA-3′.

2.6. Measurement of protein

IRE1α, eukaryotic initiation factor 2α (eIF2α), and caspase-3 protein levels were analyzed by western blotting. Schwann cells were treated with 500 μM GA for 24 h. The cells were washed with DPBS and lysed in radioimmunoprecipitation assay (RIPA) buffer (Pierce, Rockford, IL, USA) containing protease inhibitors. The lysate was centrifuged at 10,000 × *g* for 15 min, and 15 μg of protein in the supernatant was resolved by 6% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The separated proteins were blotted onto a PVDF membrane. The membrane was incubated with primary antibodies for IRE1α, eIF2α, caspase-3, and β-actin and with horseradish-peroxidase-conjugated secondary antibodies. Chemiluminescence was detected with an ECL Prime Western blot Detection Kit (GE Healthcare).

PERK, CHOP, and HO-1 protein levels were analyzed by fluorescence microscopy studies. GA-treated Schwann cells were fixed with 4% paraformaldehyde. The cells were incubated with primary antibodies, washed, and stained with secondary antibodies. The primary antibodies used were anti-rabbit PERK monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-mouse CHOP monoclonal antibody (Santa Cruz Biotechnology), and anti-rabbit HO-1 monoclonal antibody (Cell Signaling Technology), respectively. The secondary antibody was Alexa Fluor 488-conjugated goat anti-mouse IgG (Life Technologies) or Alexa Fluor 488-conjugated goat anti-rabbit IgG (Life Technologies). The stained cells were washed with DPBS and analyzed under a laser scanning microscope.

2.7. Hoechst stain

After the Schwann cells were treated with 500 μM GA for 24 h, they were washed with DPBS and fixed with 4% paraformaldehyde. Hoechst 33258 was added to the fixed cells, and cells with frag-

mented and condensed nuclei, identified as apoptotic cells, were observed under a laser scanning microscope.

2.8. Knockdown of Nrf2 with small interfering RNA (siRNA)

The knockdown of Nrf2 was performed according to a previous report [12]. Oligonucleotides directed against rat Nrf2 and control siRNA (Life Technologies) were transfected into Schwann cells by Lipofectamine RNAiMAX (Life Technologies) according to the manufacturer's protocol. Both Nrf2 siRNA and control siRNA were diluted with Opti-MEM medium and then, diluted Lipofectamine RNAiMAX was added. The transfection mixture was incubated at room temperature for 20 min. When the cells reached 30–50% confluence, the culture medium was replaced with DMEM (FBS-free) and the transfection mixture was added to each well. The final concentration of siRNA was 20 nM.

2.9. Cell viability

Cell viability assay was carried out according to a previous report [12]. CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS assay) from Promega (Madison, WI, USA) was used. Briefly, Schwann cells on 96-well plates were treated with 500 μM GA for 24 h. After the medium was removed, the cells remaining on the 96-well plates were washed with DMEM (FBS-free) and incubated with fresh DMEM (100 μL) and MTS assay solution (10 μL) at 37 °C for 60 min. Produced MTS formazan was measured at 490 nm with a Bio-Rad Model 680 microplate reader (Hercules, CA, USA).

2.10. Statistical analysis

All experiments were performed independently at least three times. Data were combined and expressed as means ± SD. Statistical significance between two groups was evaluated using the Student's *t*-test. A value of $p < 0.05$ was considered significant.

3. Results

3.1. GA induces apoptosis in Schwann cells

We first examined whether GA induces apoptosis in Schwann cells. Noticeable features of apoptosis included externalization of phosphatidylserine and chromatin condensation. We examined the binding of fluorescein-labeled annexin-V to externalized phosphatidylserine by confocal laser microscopy to detect early apoptotic cells, and measured PI uptake to assess cells in the late stages of apoptosis or cells that sustained direct plasma membrane damage. Schwann cells were treated with 100–500 μM GA for 24 h. Early apoptotic cells (annexin-V+, PI−) and late apoptotic cells (annexin-V+, PI+) were observed in the cells treated with 500 μM GA (Fig. 1A). The fluorescence intensities of annexin-V and PI in the GA-treated cells were increased by approximately 3.0-fold and 3.5-fold, respectively, compared with that of control cells. We also performed flow cytometric analyses and defined stained cell populations as follows: A, viable or undamaged cells (annexin-V−, PI−); B, early apoptotic cells (annexin-V+, PI−); and C, late apoptotic cells (annexin-V+, PI+). Compared with control, GA increased the numbers of early apoptotic cells (B) and late apoptotic cells (C) in a time-dependent manner (Fig. 1B).

Next, we examined the effect of GA on caspase-8 and -3 activities. Fig. 2A and B shows the results of flow cytometric analyses with anti-caspase-8 antibody. The fluorescence intensities of Schwann cells treated with 500 μM GA for 24 h were shifted to the right side of the panel compared with that of control. The fluorescence intensities of the GA-treated Schwann cells incubated with anti-caspase-8 showed an approximately 3.5-fold increase compared with that of control cells. Caspase-3 is a critical executioner of apoptosis. When Schwann cells were treated with 500 μM GA for 24 h, the fluorescence intensities were shifted to the right side of the panel compared with that of control (Fig. 2C). The fluorescence intensities of the GA-treated Schwann cells incubated with anti-caspase-3 were increased by approximately 2.8-fold compared with that of control cells (Fig. 2D). In addition, western blot analysis revealed a marked increase in cleaved caspase-3 protein levels in Schwann cells treated with GA (Fig. 2E and F). These results suggest that GA induces apoptosis in Schwann cells.

3.2. Effect of GA on ER stress sensors

ER stress response is mediated by such membrane receptors as PERK and IRE1α. PERK and IRE1α, both ER stress sensors, are activated by phosphorylation. We examined the effect of GA on the ER stress sensors in Schwann cells. When Schwann cells were treated with 500 μM GA for 24 h, PERK was phosphorylated (Fig. 3A). However, GA had no effect on PERK mRNA levels (Fig. 3B). In contrast, GA induced the phosphorylation of IRE1α and increased IRE1α mRNA levels (Fig. 3C and D). These results indicate that GA activates ER stress sensors in Schwann cells. The majority of PERK signaling is mediated by the phosphorylation of eIF2 [27]. Fig. 3E demonstrates that GA induced the phosphorylation of eIF2α. CHOP is one of the components of the ER-stress-mediated apoptosis pathway. CHOP expression is induced via IRE1 and PERK signaling [28]. GA increased CHOP protein levels and the translocation of CHOP to the nucleus, resulting in the activation of CHOP (Fig. 3F). In addition, GA increased CHOP mRNA levels (Fig. 3G).

3.3. Effect of Nrf2 on GA-induced cytotoxicity

Nrf2 activity has been implicated in the promotion of cell survival following ER stress [29]. We previously reported that GA induced Nrf2 activation in Schwann cells [12]. In this study, GA at 500 μM predominantly increased HO-1 protein and mRNA expression levels (Fig. 4A and B). HO-1 is a typical Nrf2 target gene that has been shown to protect cells from a variety of pathologies [30]. As shown in Fig. 4C, the knockdown of Nrf2 by siRNA enhanced GA-induced cytotoxicity, indicating that the Nrf2 system plays a protective role in the cytotoxicity caused by GA.

4. Discussion

GA levels are increased during inflammation and hyperglycemia [31]. In addition, during inflammation, GA is the major product of L-serine oxidation by myeloperoxidase [32]. Although plasma GA level has not been quantified, it is considered that its physiological concentration is of mM order [33] based on several *in vitro* studies demonstrating GA-induced cytotoxicity at that concentration [33–35]. The present study revealed that GA at 500 μM caused apoptosis in Schwann cells. Furthermore, treatment of Schwann cells with GA resulted in the activation of PERK, eIF2α, IRE1α, and CHOP.

Apoptosis, or programmed cell death, is one of the results of stress. In this study, Schwann cells treated with 500 μM GA exhibited morphological changes indicative of apoptosis in a time-dependent manner (Fig. 1B). In the same condition, GA induced caspase-8 and caspase-3 activation (Fig. 2). In addition, Hoechst staining revealed nuclear condensation in GA-induced apoptotic cells (Fig. 3F). These results indicate that GA causes apoptosis in Schwann cells. Similar to GA, MG is a potent precursor of AGEs. Studies have proved that MG induces apoptosis in some cell lines, including HL-60 [36] and U937 [37]. MG-induced apoptosis is caused by oxidative stress due to mitochondrial injury [7,11]. We also reported that MG induced ROS production and caused apoptosis in bovine aortic endothelial cells [38,39]. In breast cancer

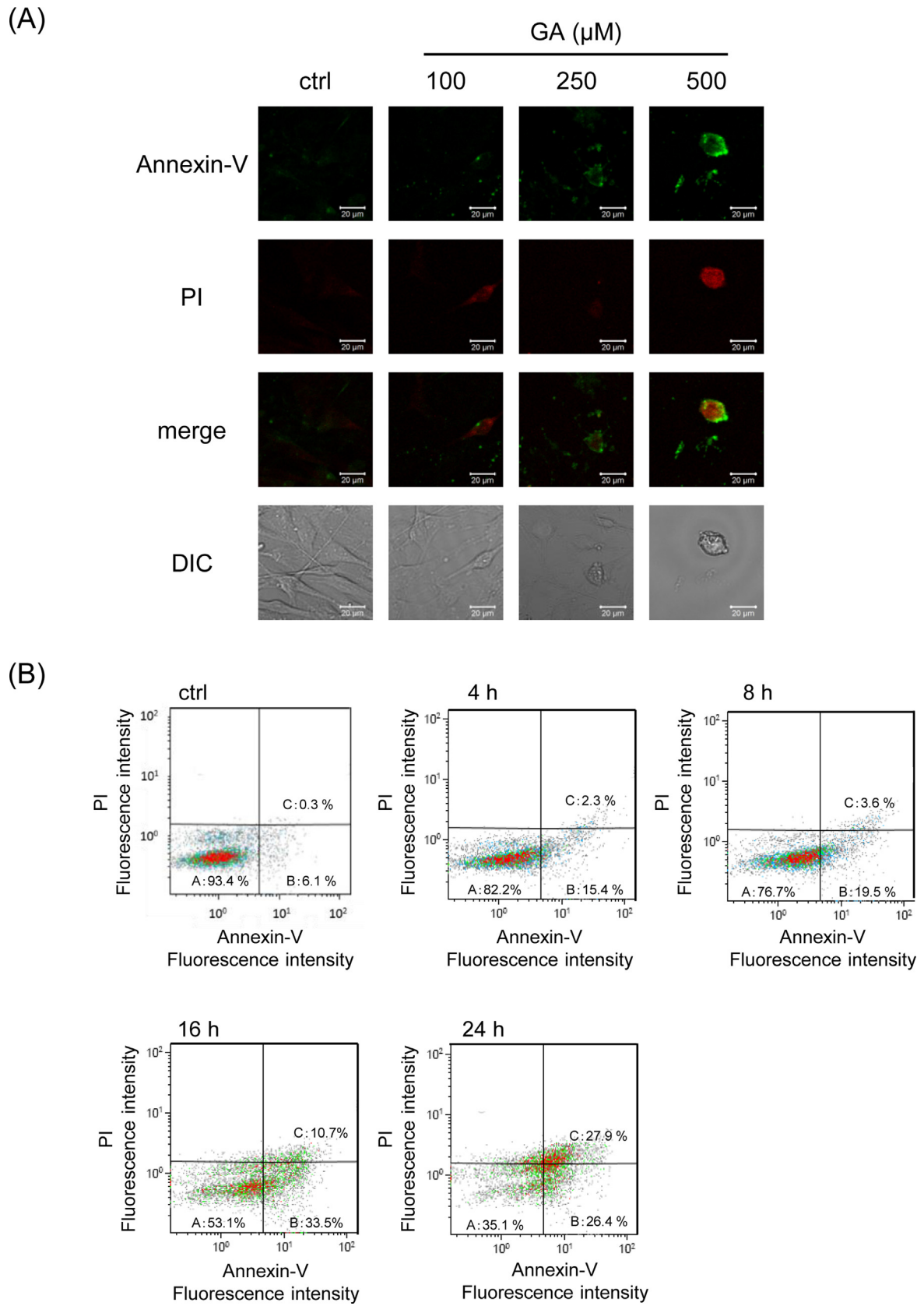

Fig. 1. Effect of GA on morphology of apoptotic cells.
(A) Schwann cells were treated with GA (100, 250 or 500 μM) for 24 h. Cells were analyzed under a laser scanning microscope using the annexin-V/PI double staining method. Magnification, 40×. Scale bar, 20 μm. (B) Schwann cells were treated with 500 μM GA (0–24 h). Cells were analyzed by flow cytometry using the annexin-V/PI double staining method. A, viable or undamaged cells (annexin-V−, PI−); B, early apoptotic cells (annexin-V+, PI−); and C, late apoptotic cells (annexin-V+, PI+).

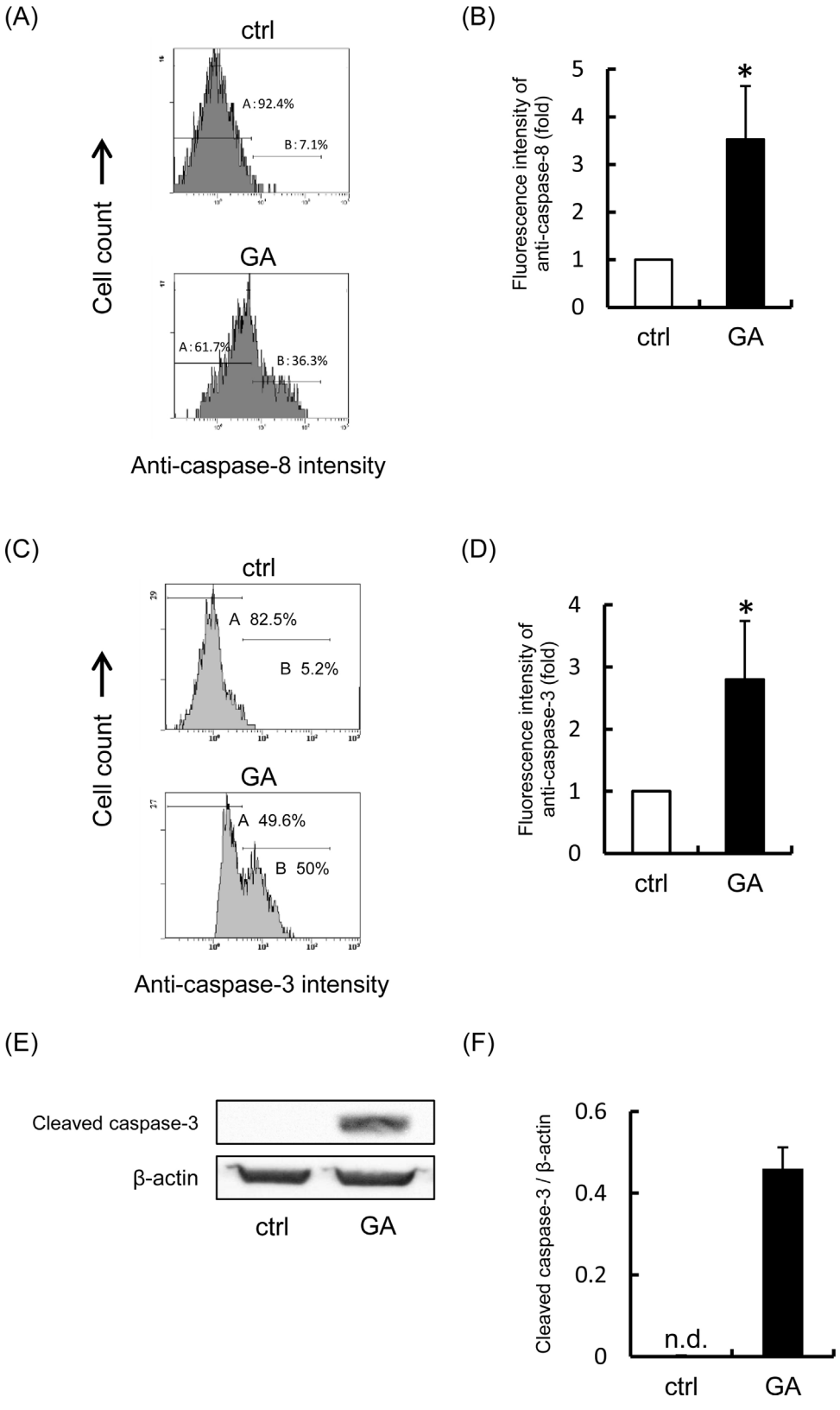

Fig. 2. Effect of GA on caspase-8 and -3 activities.
Schwann cells were treated with 500 μM GA for 24 h. Subsequently, caspase-8 (A) and caspase-3 (C and E) activation was analyzed by flow cytometry and by western blot. (B) and (D) Relative fluorescence of untreated cells was set to 1.0. (F) Relative band intensity was normalized for β-actin. Data are means ± S.D. of three independent experiments. *Significant difference from the value of control ($p < 0.05$).

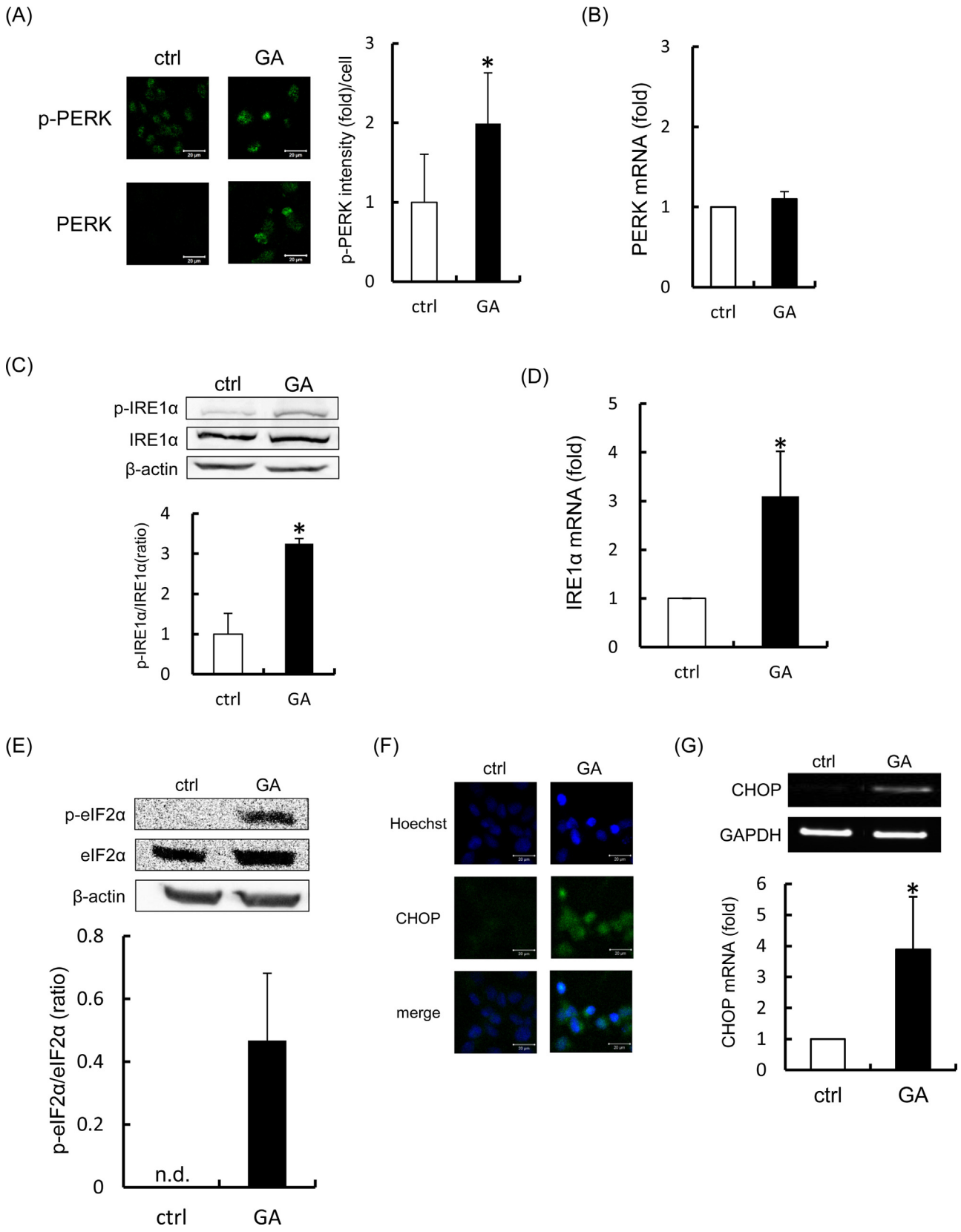

Fig. 3. Effect of GA on ER stress sensors.
Schwann cells were treated with 500 μM GA for 24 h. (A) PERK phosphorylation was analyzed under a laser scanning microscope. Scale bar, 20 μm. (B) PERK mRNA expression was analyzed by real-time RT-PCR. (C) IRE1α phosphorylation was analyzed by Western blot. (D) IRE1α mRNA expression was analyzed by real-time RT-PCR. (E) eIF2α phosphorylation was analyzed by Western blot. (F) CHOP protein expression was analyzed under a laser scanning microscope. Scale bar, 20 μm. (G) CHOP mRNA expression was analyzed by RT-PCR. Values are means ± S.D. of three experiments. *Significant difference from the value of control ($p < 0.05$).

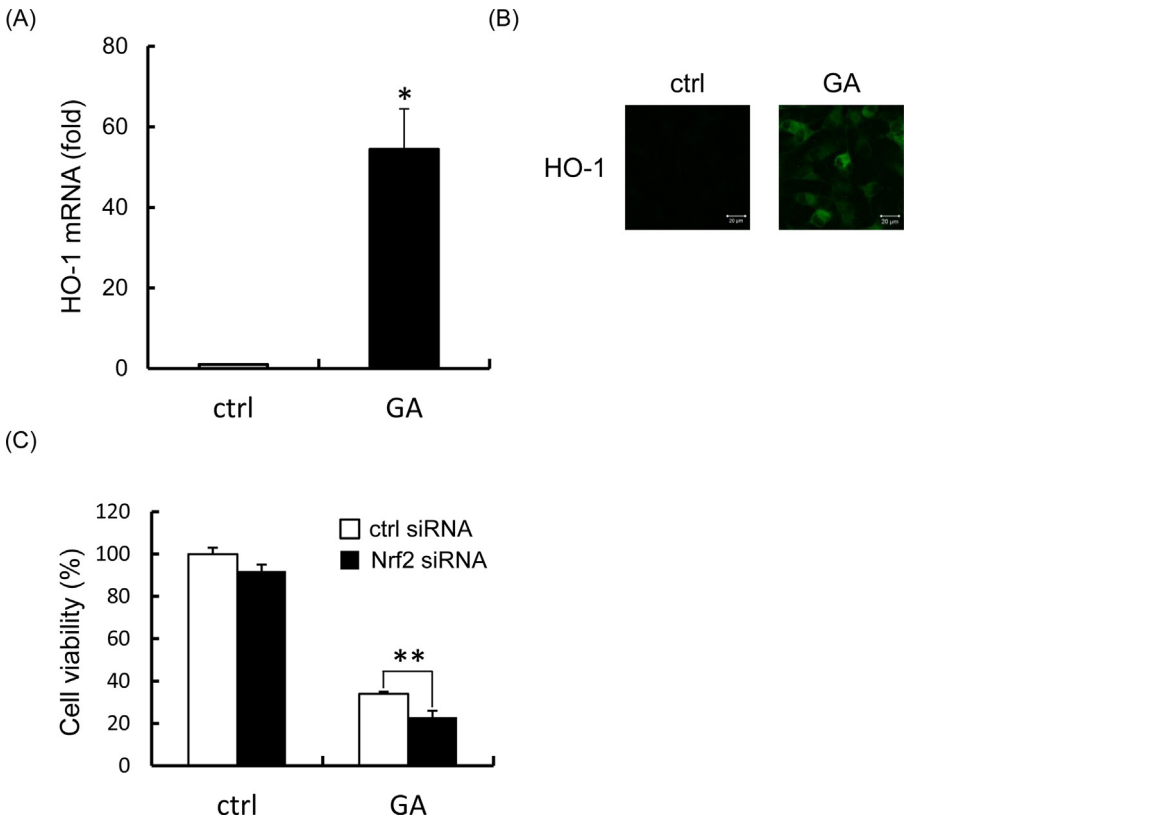

Fig. 4. Effect of Nrf2 on GA-induced cytotoxicity.
Schwann cells were treated with 500 μM GA for 24 h. (A) HO-1 mRNA expression was analyzed by real-time RT-PCR. (B) HO-1 protein expression was analyzed under a laser scanning microscope. Scale bar, 20 μm. (C) Schwann cells were transfected with control siRNA (ctrl siRNA) or Nrf2 siRNA and were treated or not treated with GA. Data are means ± S.D. of three independent experiments. *Significant difference from the value of control ($p < 0.05$). **Significant difference from the value of control treated with GA ($p < 0.05$).

cells, GA induced apoptosis via oxidative stress [16,40]. However, we found that oxidative stress was not a major contributor to the GA-induced loss of viability in Schwann cells. GA did not increase the fluorescence of ROS probes [12]. In addition, GA did not change mitochondrial membrane potential (data not shown), although mitochondria are a significant source of ROS. It was found in a recent study that heme degradation by heme oxygenase protected mitochondria but induced ER stress [41]. This finding supports our results that GA induces ER stress and HO-1. Therefore, oxidative stress is likely not implicated in the GA-induced apoptosis in Schwann cells. These results suggest that the GA-induced apoptosis in Schwann cells has a different mechanism from that in breast cancer cells.

It was demonstrated that AGE3, a GA-derived AGE, induced apoptosis in chondrocytes [42]. We were concerned about whether GA directly induced apoptosis in Schwann cells. The intracellular accumulation of AGEs in chondrocytes requires incubation of the cells with GA for more than 24 h [42]. An increase of annexin-V fluorescence in Schwann cells was observed upon incubation with GA for 8 h (Fig. 1B). Moreover, in our previous work, we found that GA decreased the viability of Schwann cells after incubation for 8 h [12]. These results suggest that not AGE3 but GA directly induces apoptosis in Schwann cells. On the other hand, glyoxal was found to be the major metabolite of GA [34,43]. Glyoxal did not affect Schwann cells even if it was formed under our experimental conditions. We demonstrated that 500 μM glyoxal had no effect on Schwann cell viability [12].

ER stress is characterized by the activation of ER stress sensors (PERK, IRE1, and ATF6), thereby resulting in the unfolded protein response and the subsequent induction of apoptosis [44]. It is known that ER stress is induced by AGEs and not by the intermediates of AGEs. There is only one report indicating that MG induces ER stress in human lens epithelial cells [45]. In this study, we revealed that GA induced the phosphorylation of PERK, eIF2α, and IRE1α, and activated CHOP (Fig. 3). These results indicate that GA is capable of inducing ER stress in Schwann cells. ER stress is caused by the accumulation of unfolded proteins. GA likely contributes to the accumulation of unfolded proteins without ROS generation.

CHOP, an ER stress response factor crucial to executing apoptosis, is located downstream of PERK and IRE1 [22]. PERK/eIF2 mainly induces the CHOP pathway [20]. Moreover, the DR5/caspase-8 pathway is one of the CHOP-induced apoptosis pathways [46,47]. The caspase-8 pathway is one of the major routes in ER-stress-induced apoptosis [48,49]. We demonstrated that GA induced CHOP activation (Fig. 3F and G). In addition, we found that GA induced caspase-8 and caspase-3, the key executioners of apoptosis (Fig. 2A–F). These results suggest that ER stress induced by GA participates in the GA-induced apoptosis in Schwann cells. Apoptosis induced by ER stress is involved in the activation of the cJUN NH2-terminal kinase (JNK) pathway, which is mediated by the formation of the IRE1-TNF receptor-associated factor 2-apoptosis signal-regulating kinase 1 complex [50,51]. These reports support the possibility that GA-induced ER stress is involved in the apoptosis, because GA induced the activation of IRE1α (Fig. 3C and D).

ER stress is one of the contributors to Nrf2 activation. Nrf2, a bZIP transcription factor, dissociates from its cytoplasmic inhibitor Kelch-like ECH-associated protein 1 and binds to a cis-element antioxidant response element in its target gene promoter [52–54]. Nrf2 plays a significant role in protecting cells from endogenous

and exogenous stress [55–57]. HO-1 is representative enzyme that is regulated by Nrf2 [30,57]. We confirmed that HO-1 mRNA and protein levels were markedly increased by GA (Fig. 4A and B). We also reported that GA induced an increase in Nrf2 mRNA and nuclear levels in Schwann cells [12]. Moreover, the GA-induced cytotoxicity was enhanced in Nrf2 knockdown cells (Fig. 4C). These results suggest that Nrf2 activated by GA plays a protective role in the cytotoxicity. It was reported that Nrf2 was activated by the phosphorylation of PERK [24]. Moreover, one study revealed that PERK-dependent Nrf2 activation was implicated in cell survival [23]. Because GA caused the phosphorylation of PERK, as shown in Fig. 3A, this phosphorylation of PERK might be participating in not only the apoptosis regulated by CHOP but also the cytoprotection promoted by Nrf2. A model has been proposed in which PERK senses the accumulation of unfolded proteins in the ER and activates both Nrf2 and eIF2 [29]. That report supports our findings, because GA caused the phosphorylation of eIF2α (Fig. 3E).

We previously reported that GA at near-physiological concentration caused cytotoxicity in Schwann cells. In the present study, we demonstrated for the first time that GA at the same concentration induced ER stress and caused apoptosis in rat Schwann cells. At present, it is not clear whether these findings are observed in other cell lines. Further investigations are needed to examine ER stress and apoptosis caused by GA in other cell lines, including human. ER stress is a novel therapeutic target because it is implicated in the pathogenesis of various diseases, including neurodegenerative diseases, metabolic diseases, and cancers [58–60]. GA may be participating in the development of these diseases associated with ER stress.

Transparency document

The Transparency document associated with this article can be found in the online version.

References

[1] M.A. Glomb, V.M. Monnier, Mechanism of protein modification by glyoxal and glycolaldehyde, reactive intermediates of the Maillard reaction, J. Biol. Chem. 270 (1995) 10017–10026.

[2] P.J. Thornalley, A. Langborg, H.S. Minhas, Formation of glyoxal, methylglyoxal and 3-deoxyglucosone in the glycation of proteins by glucose, Biochem. J. 344 (1999) 109–116.

[3] M. Jack, D. Wright, Role of advanced glycation endproducts and glyoxalase I in diabetic peripheral sensory neuropathy, Transl. Res. 159 (2012) 355–365, http://dx.doi.org/10.1016/j.trsl.2011.12.004.

[4] G. Said, Diabetic neuropathy–a review, Nat. Clin. Pract. Neurol. 3 (2007) 331–340.

[5] A.I. Vinik, T.S. Park, K.B. Stansberry, G.L. Pittenger, Diabetic neuropathies, Diabetologia 43 (2000) 957–973.

[6] J.L. Edwards, A.M. Vincent, H.T. Cheng, E.L. Feldman, Diabetic neuropathy: mechanisms to management, Pharmacol. Ther. 120 (2008) 1–34, http://dx.doi.org/10.1016/j.pharmthera.2008.05.005.

[7] K. Ota, J. Nakamura, W. Li, M. Kozakae, A. Watarai, N. Nakamura, Y. Yasuda, E. Nakashima, K. Naruse, K. Watabe, K. Kato, Y. Oiso, Y. Hamada, Metformin prevents methylglyoxal-induced apoptosis of mouse Schwann cells, Biochem. Biophys. Res. Commun. 357 (2007) 270–275.

[8] H. Sekido, T. Suzuki, T. Jomori, M. Takeuchi, C. Yabe-Nishimura, S. Yagihashi, Reduced cell replication and induction of apoptosis by advanced glycation end products in rat Schwann cells, Biochem. Biophys. Res. Commun. 320 (2004) 241–248.

[9] P.C. Johnson, K. Brendel, E. Meezan, Human diabetic perineurial cell basement membrane thickening, Lab. Invest. 44 (1981) 265–270.

[10] C. Giannini, P.J. Dyck, The fate of Schwann cell basement membranes in permanently transected nerves, J. Neuropathol. Exp. Neurol. 49 (1990) 550–563.

[11] M. Fukunaga, S. Miyata, B.F. Liu, H. Miyazaki, Y. Hirota, S. Higo, Y. Hamada, S. Ueyama, M. Kasuga, Methylglyoxal induces apoptosis through activation of p38 MAPK in rat Schwann cells, Biochem. Biophys. Res. Commun. 320 (2004) 689–695.

[12] K. Sato, R. Tatsunami, K. Yama, Y. Tampo, Glycolaldehyde induces cytotoxicity and increases glutathione and multidrug-resistance-associated protein levels in Schwann cells, Biol. Pharm. Bull. 36 (2013) 1111–1117.

[13] S. Elmore, Apoptosis: a review of programmed cell death, Toxicol. Pathol. 35 (2007) 495–516.

[14] D.A. Allen, M.M. Yaqoob, S.M. Harwood, Mechanisms of high glucose-induced apoptosis and its relationship to diabetic complications, J. Nutr. Biochem. 16 (2005) 705–713.

[15] L. Annunziato, S. Amoroso, A. Pannaccione, M. Cataldi, G. Pignataro, A. D'Alessio, R. Sirabella, A. Secondo, L. Sibaud, G.F. Di Renzo, Apoptosis induced in neuronal cells by oxidative stress: role played by caspases and intracellular calcium ions, Toxicol. Lett. 139 (2003) 125–133.

[16] M.A. Al-Maghrebi, F. Al-Mulla, L.T. Benov, Glycolaldehyde induces apoptosis in a human breast cancer cell line, Arch. Biochem. Biophys. 417 (2003) 123–127.

[17] M. Yokouchi, N. Hiramatsu, K. Hayakawa, A. Kasai, Y. Takano, J. Yao, M. Kitamura, Atypical, bidirectional regulation of cadmium-induced apoptosis via distinct signaling of unfolded protein response, Cell Death Differ. 14 (2007) 1467–1474.

[18] S. Oyadomari, E. Araki, M. Mori, Endoplasmic reticulum stress-mediated apoptosis in pancreatic beta-cells, Apoptosis 7 (2002) 335–345.

[19] K.D. McCullough, J.L. Martindale, L.O. Klotz, T.Y. Aw, N.J. Holbrook, Gadd153 sensitizes cells to endoplasmic reticulum stress by down-regulating Bcl2 and perturbing the cellular redox state, Mol. Cell. Biol. 21 (2001) 1249–1259.

[20] H. Kadowaki, H. Nishitoh, H. Ichijo, Survival and apoptosis signals in ER stress: the role of protein kinases, J. Chem. Neuroanat. 28 (2004) 93–100.

[21] H. Matsumoto, S. Miyazaki, S. Matsuyama, M. Takeda, M. Kawano, H. Nakagawa, K. Nishimura, S. Matsuo, Selection of autophagy or apoptosis in cells exposed to ER-stress depends on ATF4 expression pattern with or without CHOP expression, Biol. Open 2 (2013) 1084–1090, http://dx.doi.org/10.1242/bio.20135033.

[22] R. Sano, J.C. Reed, ER stress-induced cell death mechanisms, Biochim. Biophys. Acta 1833 (2013) 3460–3470, http://dx.doi.org/10.1016/j.bbamcr.2013.06.028.

[23] H.P. Kim, H.O. Pae, S.H. Back, S.W. Chung, J.M. Woo, Y. Son, H.T. Chung, Hem oxygenase-1 comes back to endoplasmic reticulum, Biochem. Biophys. Res. Commun. 404 (2011) 1–5, http://dx.doi.org/10.1016/j.bbrc.2010.11.067.

[24] K. Itoh, K.I. Tong, M. Yamamoto, Molecular mechanism activating Nrf2-Keap1 pathway in regulation of adaptive response to electrophiles, Free Radic. Biol. Med. 36 (2004) 1208–1213.

[25] S.B. Cullinan, J.A. Diehl, PERK-dependent activation of Nrf2 contributes to redox homeostasis and cell survival following endoplasmic reticulum stress, J. Biol. Chem. 279 (2004) 20108–20117.

[26] S. Parthasarathy, J. Barnett, L.G. Fong, High-density lipoprotein inhibits the oxidative modification of low-density lipoprotein, Biochim. Biophys. Acta 1044 (1990) 275–283.

[27] J.D. Malhotra, R.J. Kaufman, Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? Antioxid. Redox Signal. 9 (2007) 2277–2294, http://dx.doi.org/10.1089/ars.2007.1782.

[28] K. Hayakawa, S. Nakajima, N. Hiramatsu, M. Okamura, T. Huang, Y. Saito, Y. Tagawa, M. Tamai, S. Takahashi, J. Yao, M. Kitamura, ER stress depresses NF-kappaB activation in mesangial cells through preferential induction of C/EBP beta, J. Am. Soc. Nephrol. 21 (2010) 73–81.

[29] S.B. Cullinan, D. Zhang, M. Hannink, E. Arvisais, R.J. Kaufman, J.A. Diehl, Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival, Mol. Cell. Biol. 23 (2003) 7198–7209, http://dx.doi.org/10.1128/MCB.23.20.7198-7209.2003.

[30] T. Jarmi, A. Agarwal, Heme oxygenase and renal disease, Curr. Hypertens. Rep. 11 (2009) 56–62.

[31] Y.J. Xu, M. Qiang, J.L. Zhang, Y. Liu, R.Q. He, Reactive carbonyl compounds (RCCs) cause aggregation and dysfunction of fibrinogen, Protein Cell 3 (2012) 627–640.

[32] M.M. Anderson, S.L. Hazen, F.F. Hsu, J.W. Heinecke, Human neutrophils employ the myeloperoxidase-hydrogen peroxide-chloride system to convert hydroxy-amino acids into glycolaldehyde, 2-hydroxypropanal, and acrolein. A mechanism for the generation of highly reactive alpha-hydroxy and alpha,beta-unsaturated aldehydes by phagocytes at sites of inflammation, J. Clin. Invest. 99 (1997) 424–432.

[33] H. Ukeda, Y. Hasegawa, Y. Harada, M. Sawamura, Effect of carnosine and related compounds on the inactivation of human Cu,Zn-superoxide dismutase by modification of fructose and glycolaldehyde, Biosci. Biotechnol. Biochem. 66 (2002) 36–43.

[34] K. Yang, C. Feng, H. Lip, W.R. Bruce, P.J. O'Brien, Cytotoxic molecular mechanisms and cytoprotection by enzymic metabolism or autoxidation for glyceraldehyde, hydroxypyruvate and glycolaldehyde, Chem. Biol. Interact. 191 (2011) 315–321, http://dx.doi.org/10.1016/j.cbi.2011.02.027.

[35] H. Ukeda, Y. Hasegawa, T. Ishi, M. Sawamura, Inactivation of Cu,Zn-superoxide dismutase by intermediates of Maillard reaction and glycolytic pathway and some sugars, Biosci. Biotechnol. Biochem. 61 (1997) 2039–2042, http://dx.doi.org/10.1271/bbb.61.2039.

[36] Y. Kang, L.G. Edwards, P.J. Thornalley, Effect of methylglyoxal on human leukaemia 60 cell growth: modification of DNA G1 growth arrest and induction of apoptosis, Leuk. Res. 20 (1996) 397–405.

[37] A. Okado, Y. Kawasaki, Y. Hasuike, M. Takahashi, T. Teshima, J. Fujii, N. Taniguchi, Induction of apoptotic cell death by methylglyoxal and 3-deoxyglucosone in macrophage-derived cell lines, Biochem. Biophys. Res. Commun. 225 (1996) 219–224.

[38] K. Takahashi, R. Tatsunami, Y. Tampo, Methylglyoxal-induced apoptosis of endothelial cells, Yakugaku Zasshi 128 (2008) 1443–1448.

[39] R. Tatsunami, K. Takahashi, T. Oba, Y. Tampo, Methylglyoxal-induced superoxide anion production in endothelial cells, Yakugaku Zasshi 129 (2009) 147–153.
[40] K.S. Al-Enezi, M. Alkhalaf, L.T. Benov, Glycolaldehyde induces growth inhibition and oxidative stress in human breast cancer cells, Free Radic. Biol. Med. 40 (2006) 1144–1151.
[41] A. Müllebner, R. Moldzio, H. Redl, A.V. Kozlov, J.C. Duvigneau, Heme degradation by heme oxygenase protects mitochondria but induces ER stress via formed bilirubin, Biomolecules 5 (2015) 679–701, http://dx.doi.org/10.3390/biom5020679.
[42] S. Yamabe, J. Hirose, Y. Uehara, T. Okada, N. Okamoto, K. Oka, T. Taniwaki, H. Mizuta, Intracellular accumulation of advanced glycation end products induces apoptosis via endoplasmic reticulum stress in chondrocytes, FEBS J. 280 (2013) 1617–1629, http://dx.doi.org/10.1111/febs.12170.
[43] A. Okado-Matsumoto, I. Fridovich, The role of alpha,beta-dicarbonyl compounds in the toxicity of short chain sugars, J. Biol. Chem. 275 (2000) 34853–34857, http://dx.doi.org/10.1074/jbc.M005536200.
[44] R. Kim, M. Emi, K. Tanabe, S. Murakami, Role of the unfolded protein response in cell death, Apoptosis 11 (2006) 5–13.
[45] P. Palsamy, K.R. Bidasee, M. Ayaki, R.C. Augusteyn, J.Y. Chan, T. Shinohara, Methylglyoxal induces endoplasmic reticulum stress and DNA demethylation in the Keap1 promoter of human lens epithelial cells and age-related cataracts, Free Radic. Biol. Med. 72 (2014) 134–148, http://dx.doi.org/10.1016/j.freeradbiomed.2014.04.010.
[46] M. Lu, D.A. Lawrence, S. Marsters, D. Acosta-Alvear, P. Kimmig, A.S. Mendez, A.W. Paton, J.C. Paton, P. Walter, A. Ashkenazi, Cell death. Opposing unfolded-protein-response signals converge on death receptor 5 to control apoptosis, Science 345 (2014) 98–101, http://dx.doi.org/10.1126/science.1254312.
[47] N. Liu, V.L. Scofield, W. Qiang, M. Yan, X. Kuang, P.K. Wong, Interaction between endoplasmic reticulum stress and caspase 8 activation in retrovirus MoMuLV-ts1-infected astrocytes, Virology 348 (2006) 398–405.
[48] T. Iizaka, M. Tsuji, H. Oyamada, Y. Morio, K. Oguchi, Interaction between caspase-8 activation and endoplasmic reticulum stress in glycochenodeoxycholic acid-induced apoptotic HepG2 cells, Toxicology 241 (2007) 146–156.
[49] R. Mishra, A.A. Karande, Endoplasmic reticulum stress-mediated activation of p38 MAPK, caspase-2 and caspase-8 leads to abrin-induced apoptosis, PLoS One 9 (2014) e92586, http://dx.doi.org/10.1371/journal.pone.0092586.
[50] F. Urano, X. Wang, A. Bertolotti, Y. Zhang, P. Chung, H.P. Harding, D. Ron, Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1, Science 287 (2000) 664–666.
[51] H. Nishitoh, A. Matsuzawa, K. Tobiume, K. Saegusa, K. Takeda, K. Inoue, S. Hori, A. Kakizuka, H. Ichijo, ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats, Genes Dev. 16 (2002) 1345–1355.
[52] T.W. Kensler, N. Wakabayashi, S. Biswal, Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway, Annu. Rev. Pharmacol. Toxicol. 47 (2007) 89–116.
[53] R.H. Hübner, J.D. Schwartz, P. De Bishnu, B. Ferris, L. Omberg, J.G. Mezey, N.R. Hackett, R.G. Crystal, Coordinate control of expression of Nrf2-modulated genes in the human small airway epithelium is highly responsive to cigarette smoking, Mol. Med. 15 (2009) 203–219, http://dx.doi.org/10.2119/molmed.2008.00130.
[54] S. Thangapandiyan, S. Miltonprabu, Epigallocatechin gallate supplementation protects against renal injury induced by fluoride intoxication in rats: role of Nrf2/HO-1 signaling, Toxicol. Rep. 1 (2014) 12–30, http://dx.doi.org/10.1016/j.toxrep.2014.01.002.
[55] T.W. Kensler, N. Wakabayashi, S. Biswal, Cell survival responses to environmental stresses via the Keap1-Nrf2-ARE pathway, Annu. Rev. Pharmacol. Toxicol. 47 (2007) 89–116.
[56] K.M. Kim, H.O. Pae, M. Zheng, R. Park, Y.M. Kim, H.T. Chung, Carbon monoxide induces heme oxygenase-1 via activation of protein kinase R-like endoplasmic reticulum kinase and inhibits endothelial cell apoptosis triggered by endoplasmic reticulum stress, Circ. Res. 101 (2007) 919–927.
[57] E. Kansanen, S.M. Kuosmanen, H. Leinonen, A.L. Levonen, The Keap1-Nrf2 pathway: mechanisms of activation and dysregulation in cancer, Redox Biol. 1 (2013) 45–49, http://dx.doi.org/10.1016/j.redox.2012.10.001.
[58] T. Minamino, I. Komuro, M. Kitakaze, Endoplasmic reticulum stress as a therapeutic target in cardiovascular disease, Circ. Res. 107 (2010) 1071–1082, http://dx.doi.org/10.1161/circresaha.110.227819.
[59] P.D. O'Brien, L.M. Hinder, S.A. Sakowski, E.L. Feldman, ER stress in diabetic peripheral neuropathy: a new therapeutic target, Antioxid. Redox Signal. 21 (2014) 621–633, http://dx.doi.org/10.1089/ars.2013.5807.
[60] L. Ozcan, I. Tabas, Role of endoplasmic reticulum stress in metabolic disease and other disorders, Annu. Rev. Med. 63 (2012) 317–328, http://dx.doi.org/10.1146/annurev-med-043010-144749.