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Astaxanthin Inhibits H_2O_2-Mediated Apoptotic Cell Death in Mouse Neural Progenitor Cells *via* Modulation of P38 and MEK Signaling Pathways

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In the present study, the neuroprotective effects of astaxanthin on H_2O_2-mediated apoptotic cell death, using cultured mouse neural progenitor cells (mNPCs), were investigated. To cause apoptotic cell death, mNPCs were pretreated with astaxanthin for 8 h and followed by treatment of 0.3 mM H_2O_2. Pretreatment of mNPCs with astaxanthin significantly inhibited H_2O_2-mediated apoptosis and induced cell growth in a dose-dependent manner. In Western blot analysis, astaxanthin-pretreated cells showed the activation of p-Akt, p-MEK, p-ERK, and Bcl-2, and the reduction of p-P38, p-SAPK/JNK, Bax, p-GSK3b, cytochrome *c*, caspase-3, and PARP. Because H_2O_2 triggers caspases activation, this study examined whether astaxanthin can inhibit caspases activation in H_2O_2-treated mNPCs. After H_2O_2 treatment, caspases activities were prominently increased, but astaxanthin pretreatment significantly inhibited H_2O_2-mediated caspases activation. Astaxanthin pretreatment also significantly recovered the ATP production ability of H_2O_2-treated cells. These findings indicate that astaxanthin inhibits H_2O_2-mediated apoptotic features in mNPCs. Inhibition assays with SB203580 (10 μM, a specific inhibitor of p38) and PD98059 (10 μM, a specific inhibitor of MEK) clearly showed that astaxanthin can inhibit H_2O_2-mediated apoptotic death *via* modulation of p38 and MEK signaling pathways.

Keywords: Antioxidant, apoptotic cell death, astaxanthin, H_2O_2, mouse neural progenitor cells

Oxidative damage is mediated by reactive oxygen species (ROS). Generally, ROS can be generated following cell lysis and oxidative burst as part of the immune response [2, 9] or the presence of an excess of free transition metals, which can act catalytically to generate free radicals [10]. ROS can also be generated as a byproduct of normal cellular respiration, primarily associated with mitochondrial electron transport [28]. ROS-mediated oxidative damage can be induced by imbalances in generation of ROS and the activity of protection mechanisms, which can lead to excessive exposure of cells to ROS, and subsequent free radical-mediated damage. These ROS-mediated damaging events are normally kept under control by endogenous antioxidant systems including ascorbic acid, glutathione, and ROS scavenging-related enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase. However, the functional disorder of antioxidant systems by ROS induces various oxidative damages, which can lead to cell death. Specifically, cultured neural progenitor cells (NPCs) are very sensitive to increases of ROS and result in apoptotic cell death.

Various cellular defense mechanisms against ROS-mediated oxidative damage, such as enzymatic conversion of ROS to less reactive species, chelation of transition metal catalysts, and detoxification of ROS by antioxidants, have been reported [35]. Oxidative damage to mitochondria is a critical event in oxidative cell damage, and mitochondrial ROS should be a primary target for drug development [22]. In the mitochondrial membrane, various proapoptotic and antiapoptotic proteins are present. Cytochrome *c*, a component of the respiratory chain, has been identified as one of the proapoptotic molecules [20]. The release of cytochrome *c* from the injured mitochondria was recently shown to activate caspase-3 [15]. Bcl-2, an antiapoptotic protein, is predominantly present in mitochondria and inhibits various agents-mediated apoptosis [11]. Moreover, Bcl-2 inhibits cell death by suppressing oxyradical-mediated

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membrane damage, stabilizing mitochondrial membrane potential, and preventing cytochrome *c* release [4, 6].

Astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione), a potent antioxidant, is widely distributed in nature, and it is the principal pigment in crustaceans, salmonoids, and many other organisms. However, the biosynthesis of astaxanthin is limited to a few species of microorganisms such as *Xanthophyllomyces dendrorhous* and *Haematococcus pluvialis* [1, 12]. Astaxanthin has important metabolic functions in animals, including conversion to vitamin A [3], enhancement of immune response [14, 25], and protection against diseases such as cancer by scavenging oxygen radicals [13, 32–34]. The antioxidant activity of astaxanthin has been reported to be approximately 10 times stronger than that of other carotenoids tested, including zeaxanthin, lutein, canthaxanthin, and β-carotene, and 100 times greater than that of α-tocopherol [16, 17, 21, 23]. These effects are considered to be defense mechanisms against the attack of ROS. Astaxanthin also shows strong activity as an inhibitor of oxygen radical-mediated lipid peroxidation [19, 26] and protects retinal cells against oxidative stress *in vitro* and in mice *in vivo* [24].

In the present study, the neuroprotective effects of astaxanthin on hydrogen peroxide (H_2O_2)-mediated apoptotic cell death using cultured mNPCs were investigated. Since the protective effects of astaxanthin were related with redox modulation evidenced by decreased lipid peroxidation, we therefore hypothesized that such protective effects might be directly involving its antioxidant properties, most probably related with optimum H_2O_2 removal, and a consequent inhibition of proapoptotic events including caspase-3 and caspase-9 activation. To assess this hypothesis, this work explored astaxanthin's ability to, and signal mechanisms that, inhibit H_2O_2-mediated apoptotic cell death.

Materials and Methods

Astaxanthin Reagent

Astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione) was purchased from Sigma Chemicals (St. Louis, MO, U.S.A.) and used in all the cell culture experiments (Fig. 1). The stock solution of astaxanthin was made with dimethyl sulfoxide (DMSO) and stored at 4°C. The stock solution was diluted to working concentrations before use.

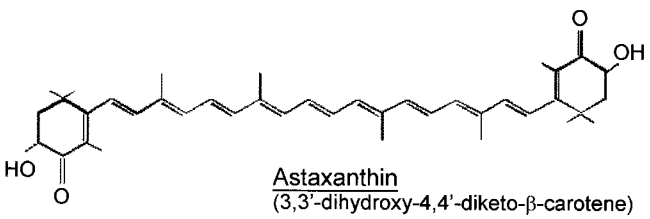

Fig. 1. Chemical structure of astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione).

Mouse Neural Progenitor Cells (mNPCs) Culture

For preparation of mNPCs, mice were anesthetized deeply using a pentobarbital in 0.9% sterile saline solution and killed by decapitation. To obtain mNPCs, the region of the complete cervical enlargement (spinal cord level C3 through T1) was dissected out. The tissue was minced, washed in sterile Dulbecco's phosphate-buffered saline (DPBS), and digested in a solution of 0.125% trypsin, DNase (0.01%, Sigma) in Hank's balanced salt solution (HBSS) for 30 min at 37°C. The cells were transferred to culture dishes containing serum-free growth medium, which consisted of neurobasal (NB) medium with B27 supplement, basic fibroblast growth factor (bFGF, 20 ng/ml), and epidermal growth factor (EGF, 20 ng/ml).

Chemicals Treatment

The optimum concentrations of astaxanthin and H_2O_2 were selected according to the preliminary experiment related to the cytotoxicity and survival effects of a broad range of each reagent. A 0.3 mM concentration of H_2O_2 in mNPCs was determined to be optimal for this study (data not shown). Moreover, 1, 5, and 10 ng/ml concentrations of astaxanthin were tested, and 10 ng/ml was optimized through the experiment (Fig. 2). The mNPCs were seeded in NB media and cultured at 37°C in a CO_2 incubator. To investigate the neuroprotective effects of astaxanthin on H_2O_2-mediated apoptosis, 80% density of mNPCs was pretreated with astaxanthin (1, 5, and 10 ng/ml) for 8 h and followed by treatment with 0.3 mM H_2O_2 for a further 72 h. Dilutions of H_2O_2 were made fresh from a 30% stock solution into Dulbecco's modified Eagle's medium (DMEM) just prior to each experiment. Exposures to H_2O_2 were performed by simple addition of a small volume of H_2O_2 diluted in DMEM at ×100 directly to each well, followed by light agitation. Cultures were incubated for the times noted and then toxicity or biochemical measurements were performed. We observed that the toxicity of H_2O_2 was reduced if the H_2O_2 exposures were not performed within 5 min of dilution. This is likely due to the highly reactive nature of H_2O_2 and its short half-life in dilute solutions.

To confirm the effects of astaxanthin on cell growth, cultured mNPCs were seeded in 10-cm dishes at a density of 5×10^5 cells per dish and cultured in NB media at 37°C in a CO_2 incubator. The cells were then treated with astaxanthin at various concentrations (1, 5, and 10 ng/ml) for 72 h.

Analysis of Cell Viability and TUNEL

Cell viability was assessed by visual cell counts in conjunction with tryphan blue exclusion. In all viability assays, triplicate wells were used for each condition, and each experiment was repeated at least three times. The apoptotic cell death of mNPCs was estimated using TUNEL staining. Briefly, 4% paraformaldehyde-fixed cells were incubated at 37°C for 90 min in the terminal deoxynucleotidyl transferase biotin-dUTP nick (TUNEL) reaction mixture containing TdT, biotinylated dUTP, and the TdT reaction buffer. After washing with PBS, samples were incubated at room temperature for 30 min with an anti-horseradish peroxidase antibody conjugated to FITS and then visualized by green fluorescence using a Leica fluorescence microscope (Leica Microsystems).

Measurement of ATPase Activity

The amount of protein was determined using a protein assay kit (Bio-Rad, Hercules, CA, U.S.A.) following the manufacturer's instructions. Cells were resuspended in buffer containing 150 mM/l

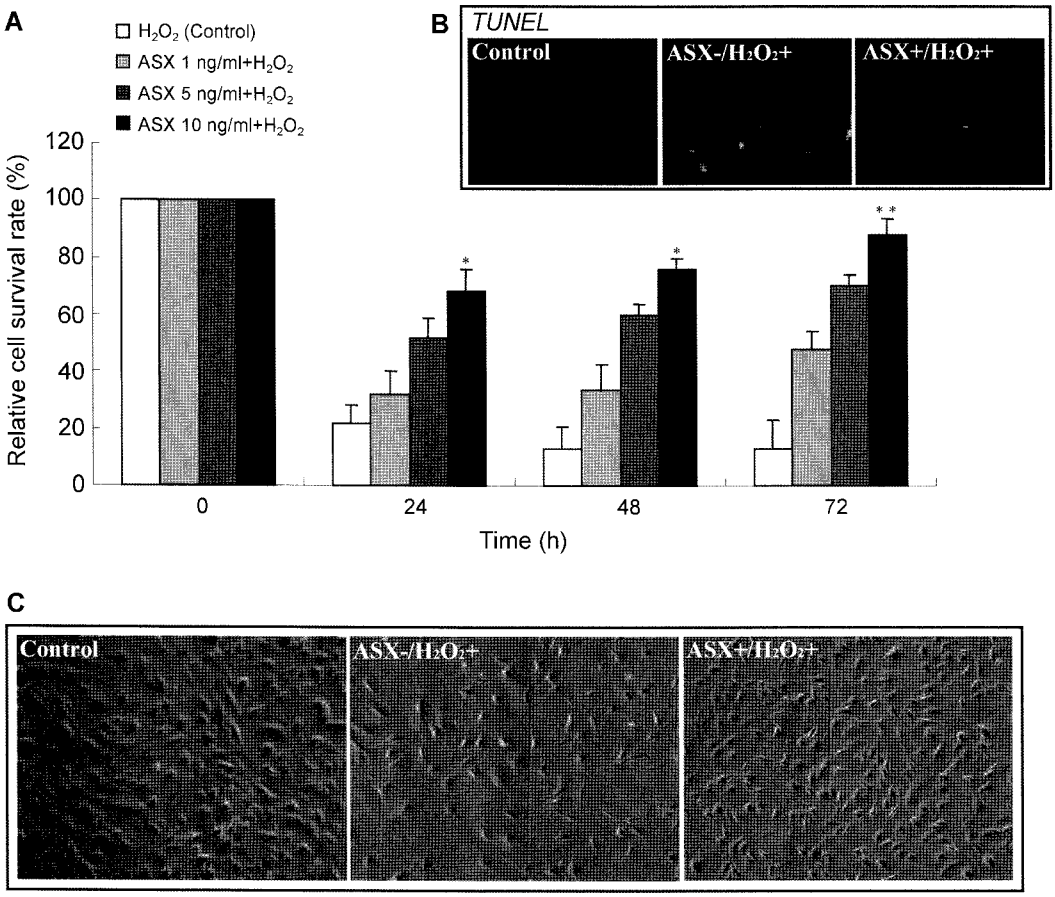

Fig. 2. Astaxanthin protects mNPCs from H_2O_2-mediated apoptotic cell death and induces cell growth.
Cultured mNPCs were pretreated with astaxanthin (1, 5, and 10 ng/ml) for 8 h and followed by treatment of 0.3 mM H_2O_2 for 72 h. **A**. In all cell viability assays, triplicate wells were used for each condition, and each experiment was repeated at least three times. Data are plotted as the mean±SEM (*P<0.05; **P<0.01). **B**. The apoptosis of mNPCs was assessed by TUNEL staining. TUNEL-positive apoptotic cells were quantified *via* the counting of the positively stained cells. **C**. Microphotographs clearly showed that 10 ng/ml astaxanthin can inhibit H_2O_2-mediated apoptotic death.

KCl, 25 mM/l Tris-HCl pH 7.6, 2 mM/l EDTA pH 7.4, 10 mM/l KPO_4 pH 7.4, 0.1 mM/l $MgCl_2$, and 0.1% (w/v) BSA at a concentration of 1 mg protein per milliliter of buffer. ATP synthesis was initiated by the addition of 250 ml of the cell suspension to 750 μl of substrate buffer (10 mM/l malate, 10 mM/l pyruvate, 1 mM/l ADP, 40 μg/ml digitonin, and 0.15 mM/l adenosine pentaphosphate). Cells were incubated at 37°C for 10 min. At 0 and 10 min, 50-μl aliquots of the reaction mixture were withdrawn, quenched in 450 μl of boiling 100 mM/l Tris-HCl and 4 mM/l EDTA, pH 7.75, for 2 min and further diluted 1/10 in the quenching buffer. The quantity of ATP was measured in a luminometer (Berthold, Detection Systems, Pforzheim, Germany) with the ATP Bioluminescence Assay Kit (Roche Diagnostics, Basel, Switzerland) following the manufacturer's instructions. Raw data from each experiment were analyzed using analysis of variance with Fisher's exact test or *t*-test.

Caspases Assays and Quantification

For caspase-3 and caspase-9 activity assay, 10 μg of protein in a 50-μl total volume was mixed with 50 μl of equilibrated caspase-Glo 3 or 9 reagents (Promega). After incubating at room temperature for 2 h, luminescence was measured using a TD 20/20 Luminometer (Turner Designs, Sunnyvale, CA, U.S.A.). Blank values were subtracted and the fold-increase in activity was calculated based on activity measured from untreated cells. Each sample was measured in triplicate.

Western Blot Analysis

For confirmation of differentially expressed proteins after astaxanthin treatment in cultured mNPCs, astaxanthin-pretreated cells lysed in 500 μl of lysis buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM EGTA, 1 mM glycerophosphate, 1 mM Na_3VO_4, and 1 mM PMSF). Lysates were clarified by centrifugation at 15,000 ×*g* for 10 min and the total protein content was determined by a Bio-Rad (Millan, Italy) protein assay kit. Proteins were fractionated on 12% gels and then electrophoretically transferred to a nitrocellulose membrane followed by blocking in 5% milk protein for 1 h prior to incubating with appropriate primary and secondary antibodies. Anti-PARP (1:1,000, Cell Signaling), anti-p-SAPK/JNK (1:1,000, Cell Signaling), anti-p-MEK (1:1,000, Cell Signaling), anti-p-ERK (1:1,000, Cell Signaling), anti-Bcl-2 (1:1,000, Cell Signaling), anti-Bax (1:1,000, Cell Signaling), anti-p-P38 (1:1,000, Cell Signaling), anti-p-Akt (1:1,000, Cell Signaling), anti-cytochrome *c* (1:1,000, Cell Signaling), anti-p-GSK3b (1:1,000, Cell Signaling), anti-caspase-3 (1:1,000, Cell Signaling), and anti-β-actin (1:500, Sigma) antibodies were incubated with membranes.

Relative band intensities were determined by Quality-one 1-D analysis software (Bio-Rad, U.S.A.).

Statistical Analysis

All data were presented as the mean±SEM. from five or more independent experiments. The statistical significance of difference between groups was calculated by using the Student's two tailed *t*-test.

RESULTS

Astaxanthin Protects mNPCs from H_2O_2-Mediated Apoptosis and Induces Cell Growth

To estimate the neuroprotective effects of astaxanthin on H_2O_2-mediated apoptosis, 80% density of mNPCs was pretreated with astaxanthin (1, 5, and 10 ng/ml) for 8 h and followed by treatment of 0.3 mM H_2O_2 for a further 72 h. After H_2O_2 treatment, the relative survival rate of mNPCs untreated and pretreated with different concentrations (1, 5, and 10 ng/ml) of astaxanthin for 72 h was assessed by trypan blue exclusion and TUNEL staining. As shown in Fig. 2, H_2O_2-mediated apoptosis was clearly observed in the 24 h culture of mNPCs but was prevented in astaxanthin-pretreated cultures. Moreover, pretreatment of mNPCs with astaxanthin inhibited H_2O_2-mediated apoptosis in a dose-dependent manner. Specifically, about 70% of mNPCs was shown to have survived in the 10 ng/ml astaxanthin-pretreated culture after treatment with 0.3 mM H_2O_2. Microphotographs clearly showed that 10 ng/ml astaxanthin can inhibit H_2O_2-mediated apoptotic death. Therefore, 10 ng/ml of astaxanthin was determined to be optimal for this study.

In Fig. 2, H_2O_2-treated mNPCs grew (*P<0.05; **P<0.01) for 72 h in astaxanthin-containing medium. This result suggested that astaxanthin can induce the proliferation of mNPCs. To confirm the effects of astaxanthin on cell growth, the proliferation of mNPCs pretreated with different concentrations (1, 5, and 10 ng/ml) of astaxanthin for 72 h was evaluated. As shown in Fig. 3, astaxanthin pretreatment for 72 h significantly increased the proliferation of mNPCs in a dose-dependent manner. In particular, mNPCs grew well (*P<0.05; **P<0.01) for 72 h with 10 ng/ml astaxanthin pretreatment. These results indicated that astaxanthin can protect mNPCs from H_2O_2-mediated apoptosis and induce cell growth.

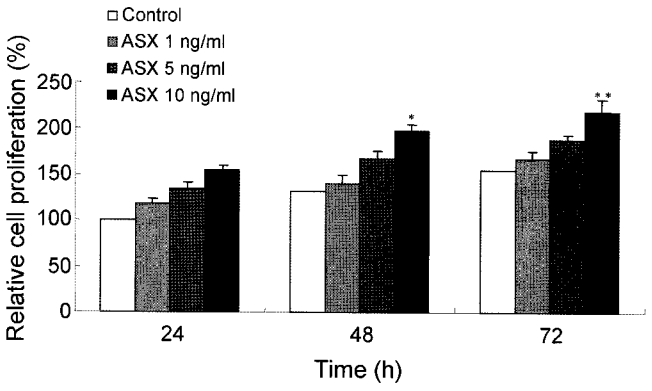

Fig. 3. Astaxanthin induces cell proliferation in mNPCs.
The proliferation of mNPCs pretreated with different concentrations of astaxanthin (1, 5, and 10 ng/ml) for 72 h was evaluated. Astaxanthin pretreatment for 72 h significantly increased the proliferation of mNPCs in a dose-dependent manner. Each experiment was repeated at least three times. Data are plotted as the mean±SEM (*P<0.05; **P<0.01).

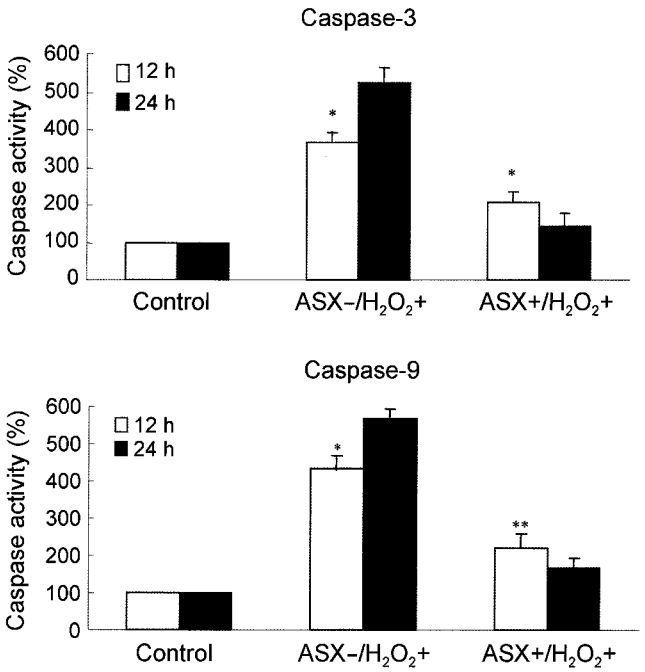

Fig. 4. Astaxanthin pretreatment significantly inhibits H_2O_2-mediated apoptotic features such as caspases activation in mNPCs.
After H_2O_2 treatment for 12 h and 24 h, respectively, the activities of caspase-3 and caspase-9 were prominently increased but astaxanthin pretreatment significantly (*P<0.05; **P<0.001) inhibited H_2O_2-mediated caspases activation. Each sample was measured in triplicate. Data are plotted as the mean±SEM (*P<0.05; **P<0.001).

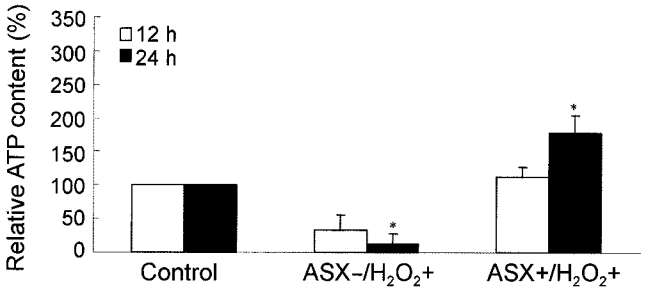

Fig. 5. Astaxanthin pretreatment significantly recovers the ATP production ability of H_2O_2-treated mNPCs.
In response to H_2O_2 exposure for 12 h and 24 h, respectively, ATP production decreased prominently, but astaxanthin pretreatment significantly recovered the ATP production ability of H_2O_2-treated cells. Data are plotted as the mean±SEM (*P<0.05).

Astaxanthin Inhibits H_2O_2-Mediated Apoptotic Features H_2O_2 effectively induces cellular oxidative stress and apoptosis, which triggers caspase activation and cytochrome *c* release. The present study investigated whether astaxanthin can inhibit caspase-3 and caspase-9 activation in H_2O_2-treated mNPCs. After H_2O_2 treatment for 12 h and 24 h, respectively, the activities of caspase-3 and caspase-9 were prominently increased, but astaxanthin pretreatment significantly (*P<0.05; **P<0.001) inhibited H_2O_2-mediated caspases activation (Fig. 4). This study also examined the effect of astaxanthin on ATP production after treatment of H_2O_2 in mNPCs. In response to H_2O_2 exposure for 12 h and 24 h, respectively, ATP production decreased prominently, but astaxanthin pretreatment significantly (*P<0.05) recovered the ATP production ability of cells (Fig. 5). In addition, we tested whether astaxanthin could inhibit cytochrome *c*

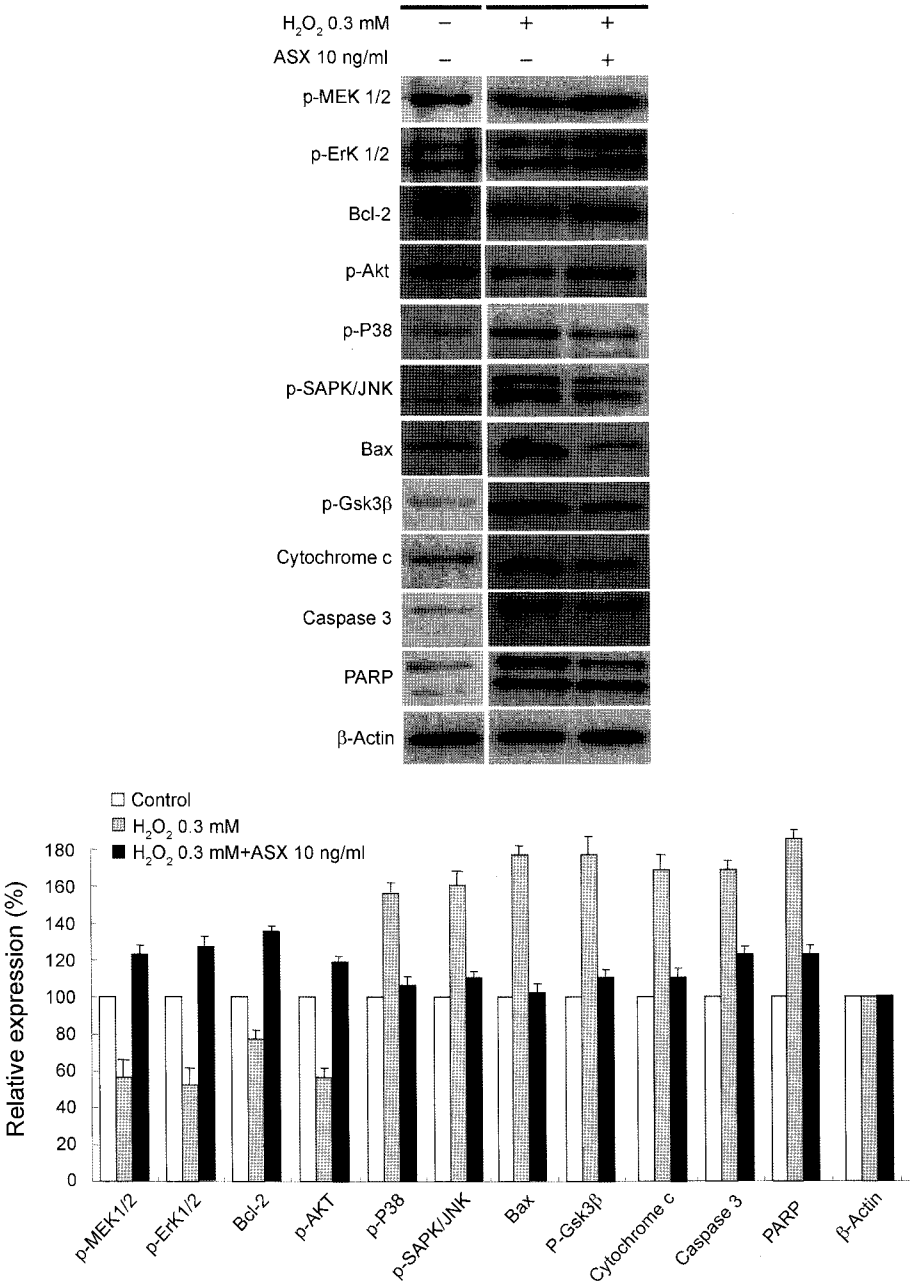

Fig. 6. Astaxanthin inhibits the expression of H_2O_2-mediated apoptotic-related proteins in mNPCs.
For confirmation of the differential expression of the apoptotic signal proteins in the cultured mNPCs after astaxanthin pretreatment, mNPCs were analyzed by Western blotting. In Western blot analysis, astaxanthin-pretreated cells showed the activation of survival and proliferation related factors such as p-Akt, p-MEK, p-ERK, and Bcl-2, and the reduction of apoptotic marker proteins such as p-P38, p-SAPK/JNK, Bax, p-GSK3b, cytochrome *c*, caspase-3, and PARP. Relative band intensities were determined by Quality-one 1-D analysis software. Data are plotted as the mean±SEM (*P<0.001).

release from the mitochondria in astaxanthin-pretreated cells. The results of Western blot analysis showed that H_2O_2-treated cells released a lot of cytochrome *c* into the cytosol, whereas astaxanthin-pretreated cells reduced the release of cytochrome *c* into the cytosol in mNPCs (Fig. 6). These results indicated that astaxanthin inhibits H_2O_2-mediated apoptotic cell death by maintaining mitochondria integrity.

Astaxanthin Induces Prevention of H_2O_2-Mediated Apoptotic Cell Death *via* Modulation of p38 and MEK Signaling Pathways

Cell survival requires the active inhibition of apoptosis. To confirm the astaxanthin ability and signal mechanisms for inhibition of H_2O_2-mediated apoptotic cell death, expression levels of survival and apoptotic marker proteins were

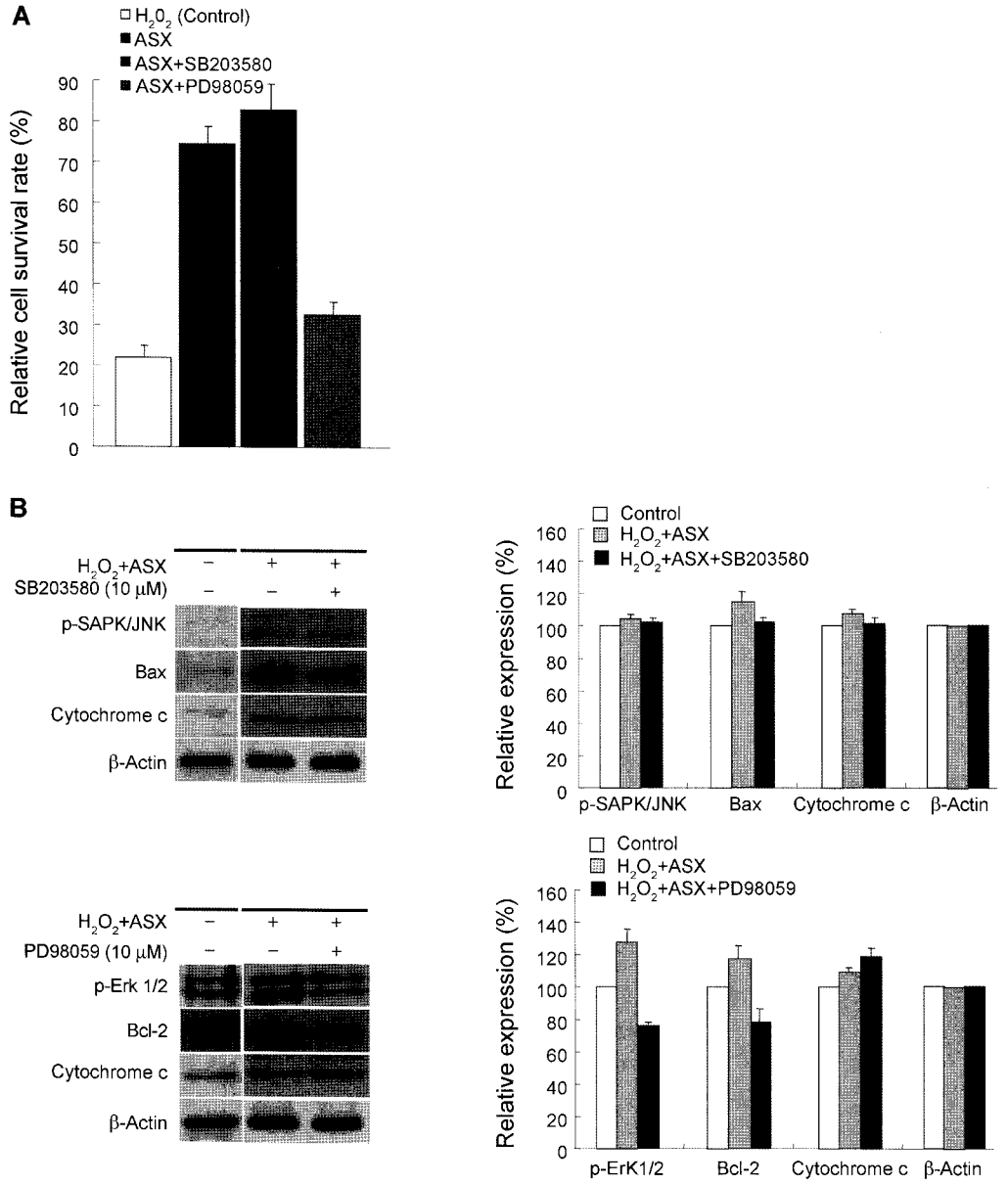

Fig. 7. Astaxanthin induces mNPCs protection against H_2O_2-mediated apoptotic cell death *via* modulation of p38 and MEK signaling pathways.

To examine the relevance of the p38 and MEK signaling pathways in controlling the apoptotic cell death in astaxanthin-pretreated mNPCs, inhibition assays were performed with SB203580 (10 mM, a specific inhibitor of p38) and PD98059 (10 mM, a specific inhibitor of MEK). **A**. After SB203580 and PD98059 treatment, the relative survival rate of mNPCs pretreated with 10 ng/ml astaxanthin was assessed by trypan blue exclusion. SB203580 treatment increased the protective effect of astaxanthin, whereas PD98059 treatment caused significant increase of H_2O_2-mediated apoptosis. **B**. Western blot analysis revealed that SB203580 treatment induced downregulation of p-SAPK/JNK, Bax, and cytochrome *c* compared with astaxanthin alone pretreatment. Moreover, PD98059 treatment induced downregulation of p-ERK and Bcl-2 with upregulation of cytochrome *c* compared with astaxanthin alone pretreatment. Inhibition assays speculated that astaxanthin causes inhibition of p38 and activation of MEK signaling pathways for prevention of H_2O_2-mediated apoptotic cell death.

estimated by Western blot analysis. In Western blot analysis, astaxanthin-pretreated cells showed the activation of survival and proliferation related factors such as p-Akt, p-MEK, p-ERK, and Bcl-2, and the reduction of apoptotic marker proteins such as p-P38, p-SAPK/JNK, Bax, p-GSK3b, cytochrome *c*, caspase-3, and PARP (Fig. 6). Specifically, astaxanthin attenuated activation of mitochondria-mediated cell-death-related protein *via* modulating caspases activities and cytochrome *c* release (Fig. 6).

To identify the relevance of the p38 and MEK signaling pathways in controlling the apoptotic cell death by astaxanthinin, inhibition assays were performed with SB203580 (10 μM, a specific inhibitor of p38) and PD98059 (10 μM, a specific inhibitor of MEK). After SB203580 and PD98059 treatment, respectively, the relative survival rate of mNPCs pretreated with 10 ng/ml astaxanthin was assessed by trypan blue exclusion and results are shown in Fig. 7A. SB203580 treatment increased the protective effect of astaxanthin, whereas PD98059 treatment caused significant increase of H_2O_2-mediated apoptosis. Western blot analysis revealed that SB203580 treatment induced downregulation of p-SAPK/JNK, Bax, and cytochrome *c* compared with astaxanthin alone pretreatment (Fig. 7B). Moreover, PD98059 treatment induced downregulation of p-ERK and Bcl-2 with upregulation of cytochrome *c* compared with astaxanthin alone pretreatment (Fig. 7B). Therefore, SB203580 increased the cell survival ability through enhancement of astaxanthin-mediated p38 signaling pathway inactivation. On the other hand, PD98059 caused attenuation of the cell survival ability through inhibition of astaxanthin-mediated MEK signaling pathway activation. Inhibition assays speculated that astaxanthin causes inhibition of p38 and activation of MEK signaling pathways for prevention of H_2O_2-mediated apoptotic cell death (Fig. 8). These data clearly suggested that astaxanthin can induce prevention of apoptotic cell death *via* modulation of the p38 and MEK signaling pathways.

DISCUSSION

Generation of ROS by oxidative injury is tightly involved in various neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, amyotropic lateral sclerosis, spinal cord injury, and conditions such as ischemia and excitotoxicity [5, 7, 8, 27, 29–31]. Various cellular defense mechanisms such as enzymatic conversion of ROS to less reactive species, chelation of transition metal catalysts, and detoxification of ROS by antioxidants have been reported to work against ROS-mediated oxidative damage [24, 35]. Many antioxidants are particularly known to provide protection from ROS-mediated cellular damage. This effect is considered to be defense mechanisms against the attack of ROS. In addition, antioxidants have been linked to regulatory functions in cell growth, survival, cytotoxicity, and transformation possibly involving redox regulation and chemical toxicity, as well as the transcription regulation of various genes [18]. However, the correct biochemical function of antioxidants is not yet fully resolved, especially concerning about astaxanthin. Therefore, the present study demonstrated that astaxanthin has a key role in neuroprotection and anti-oxidation responses.

An important intracellular signaling pathway that leads to ROS-mediated apoptosis is involved in activation of the caspases. Astaxanthin, a potent antioxidant, can reduce

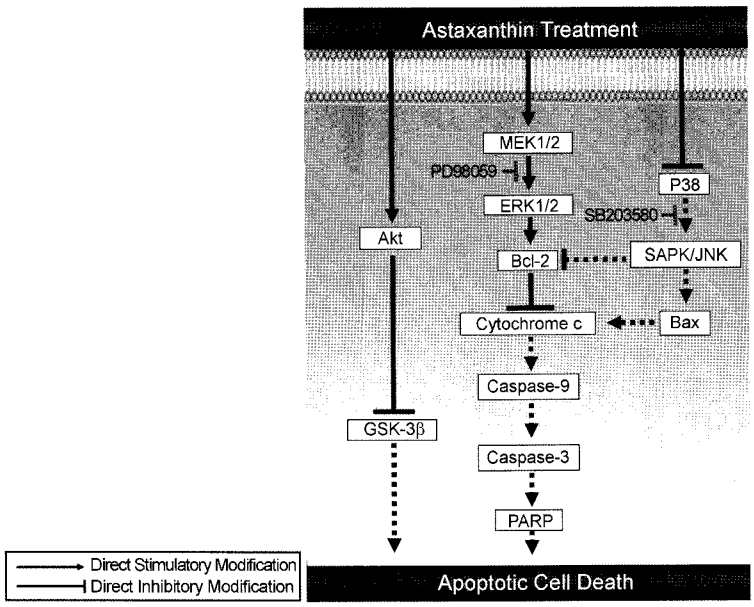

Fig. 8. Putative inhibition mechanism of astaxanthin on H_2O_2-mediated apoptotic cell death.

caspases activities by its antioxidant function and by modulating the downstream pathway. The short-term effect of astaxanthin is mainly antiapoptosis. ROS-mediated cellular damage was greatly reduced by astaxanthin pretreatment. Astaxanthin pretreatment resulted in the inhibition of caspase-3 and caspase-9 activities. Moreover, activation of apoptotic marker proteins such as p-P38, p-SAPK/JNK, and Bax was effectively inhibited, and the ATP production ability of cells was significantly recovered. Based on our present data, we suggest a model, shown in Fig. 8, for explanation of the inhibition mechanism of astaxanthin on H_2O_2-mediated apoptotic cell death. Inhibition assays with SB203580 and PD98059 clearly showed that astaxanthin can induce prevention of apoptotic cell death *via* modulation of p38 and MEK signaling pathways.

The neuroprotective effect of astaxanthin to attenuate apoptotic cell death requires further study in order to elucidate a mechanism. Astaxanthin might be a novel and safe approach for the prevention of neurodegenerative diseases.

Acknowledgment

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Abbreviations

ASX, astaxanthin; bFGF, basic fibroblast growth factor; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; DPBS, Dulbecco's phosphate-buffered saline; EGF, epidermal growth factor; ERK1/2, extracellular regulated kinases 1/2; GSK3β, glycogen synthase kinase-3b; HBSS, Hank's balanced salt solution; H_2O_2, hydrogen peroxide; MEK, Mitogen-activated protein kinase kinase; mNPCs, mouse neural progenitor cells; NB, neurobasal; PARP, polyADP-ribose polymerase; P38 MAPK, P38 mitogen-activated protein kinase; SAPK/JNK, stress-activated protein kinase/c-Jun N-terminal protein kinase.

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