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Alginate protects NT2 neurons against H_2O_2-induced neurotoxicity

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ABSTRACT

Increased oxidative stress is a widely accepted factor in the development and progression of Alzheimer's disease. Here we introduced alginate – an antioxidant oligosaccharide – as a protective agent on NT2 neural cell line against $H_2O_2/FeSO_4$-induced cell death. Our results demonstrate that alginate not only protects the neurons against cell death, as measured by MTT test and caspase-3 activity determination, but also decreases amyloid β formation. In the present study we could induce Aβ formation through oxidative stress in NT2 neurons, one of the most appropriate cell line models in Alzheimer's disease and provided the first documentation that alginate can be neuroprotective by suppressing Aβ formation. We further showed that alginate exerts its protective effect by up regulation of HO-1, γ-GCS, Hsp-70, Nrf2 and inhibiting caspase-3 and NF-κB. This study raises the possibility of developing alginate as a potential neuroprotective agent.

1. Introduction

Oxidative stress-induced cell damage has long been implicated both in the physiological process of aging and in a variety of neurodegenerative diseases such as Alzheimer's disease (AD) (Butterfield, Drake, Pocernich, & Castegna, 2001; Gotz, Kunig, Riederer, & Youdim, 1994). Oxidative damage, mediated by reactive oxygen species which can be generated following cell lysis, oxidative burst or the presence of an excess of free transition metals can attack proteins, deoxynucleic acid and lipid membranes, thereby disrupting cellular function and integrity. Hydrogen peroxide (H_2O_2) one of the main reactive oxygen species, is produced during the redox process and is recently considered as a messenger in intracellular signaling cascades (Behl, Davis, Lesley, & Schubert, 1994). In addition, it is well known that H_2O_2 could cause lipid peroxidation and DNA damage, thus inducing apoptosis in many different cell types (Halliwell & Aruoma, 1991; Yoshikawa, Saito, & Maruyama, 2006). Therefore, therapeutic strategies aimed at preventing or delaying reactive oxygen species-induced apoptosis might be a reasonable choice for the treatment of these diseases. Among various therapeutic strategies, one of the plausible ways is to augment or fortify endogenous defense against oxidative stress through dietary or pharmacological intake of antioxidants. Many synthetic chemicals such as phenolic compounds have been proven to be strong radical scavenger, but they usually have some severe adverse effects (Heilmann, Merfort, & Weiss, 1995). Recently, attention has been focused on searching for natural substances with neuroprotective potential that can scavenger free radicals and protect cells from oxidative damage.

Alginate is regarded as a biocompatible, non-toxic, non-immunogenic and biodegradable polymer, making it an attractive candidate for biomedical application (Kumar, 2000). It is a natural linear polysaccharide comprised of β-D-mannuronic acid (M block) and α-L-guluronic acid (G block) units arranged in blocks rich in G units or M units. The antioxidant property of alginate has attracted increasing attention (Anraku et al., 2008; Bylund, Burgess, Cescutti, Ernst, & Speert, 2006; Jeon et al., 2003; Kamil, Jeon, & Shahidi, 2002). This oligosaccharide possess additional characteristics such as anti-aggregatory effects (Khodagholi, Eftekharzadeh, & Yazdanparast, 2008; Rezaii & Khodagholi, 2009), anti-inflammatory effects (Mo, Son, Rhee, & Pyo, 2003), enhancing protection against infection with some pathogens (An et al., 2009) and advanced glycation end-products (AGEs) inhibitory effect (Sattarahmady, Khodagholi, Moosavi-Movahedi, Heli, & Hakimelahi, 2007). Formation of AGEs is the end-products of millard reactions that are induced by reactive oxygen species and are associated with various age-related pathologies such as AD (Reddy & Beyaz, 2006).

In the present investigation we wished to examine whether this oligosaccharide can protect NT2 neurons from cell death and whether its presence could inhibit Aβ formation, a major cause of AD pathology. In addition we provide some insight into its mechanism.

2. Materials and methods

2.1. Materials

Antibodies directed against caspase-3, NF-κB, heat shock protein 70 (HSP-70) and β-actin were obtained from Cell Signaling

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Company. Glutamylcysteine synthetase (γ-GCS) and hemeoxigenase-1 (HO-1) were from ABCAM. The polyclonal Nrf2 (C-20) was purchased from Santa Cruz Biotechnology. All the other reagents, unless otherwise stated, were from Sigma Aldrich (St. Louis, MO).

2.2. Cell culture and NT2 differentiation

NT2 cells from ATCC (Manassas, USA), were maintained in Dulbecco's modified Eagle's medium (DMEM) (Sigma Aldrich), supplemented with 5% fetal bovine serum and 1% antibiotic mixture comprising penicillin–streptomycin, in a humidified atmosphere at 37 °C with 5% CO_2. For differentiation 2×10^6 cells were plated in 75 cm^2 culture flasks and exposed to 10 μM all-trans retinoic acid (RA) for 5 weeks. Growth medium was changed three times a week. Cells were then replated and, after 48 h, the mitotic inhibitors cytosine arabinoside (1 μM), fluorodeoxyuridine (10 μM) and uridine (10 μM) were added for 2 weeks to inhibit the division of non-neuronal cells. Experiments were performed 4–5 weeks after cessation of RA treatment (Pleasure, Page, & Lee, 1992; Tamango et al., 2002).

2.3. Preparation of alginate

Alginate solutions were prepared by dissolving different amounts of sodium alginate in 100 ml of distilled water containing alginase (30 μg/ml) heating with constant agitation for 1 h.

2.4. Treatment conditions

NT2 neuronal cells, plated in 75 cm^2 culture flasks, were incubated with alginate for 1 h prior to our experiments, then the cells were treated with H_2O_2 (10 μM) and $FeSO_4$ (100 μM) for 2 h.

2.5. Measurement of cell viability

Cell viability was determined by the conventional MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) reduction assay. The dark blue formazan crystals formed in intact cells were solubilized with lysis buffer (10% sodium dodecylsulfate, 0.01 M HCl) and the absorbance at 550 nm was measured. Results were expressed as the percentages of reduced MTT, assuming the absorbance of control cells as 100%.

2.6. Western blot analysis

For Western blot analysis, the cells were lysed in buffer containing complete protease inhibitor cocktail. Then the total proteins were electrophoresed in 12% SDS–PAGE gels, transferred to polyvinylidene fluoride membranes and probed with specific antibodies. Immunoreactive polypeptides were detected by chemiluminescence using enhanced ElectroChemiLuminescence (ECL) reagents (Amersham Bioscience, USA) and subsequent autoradiography. Quantification of the results was performed by densitometric scan of films. Data analysis was done by Image.J. Protein concentrations were determined according to Bradford's method (Bradford, 1976). A standard plot was generated using bovine serum albumin. Nuclear and cytoplasmic proteins were isolated as described by Kutuk and Basaga (2003).

2.7. Measurement of glutathione levels

The concentration of glutathione (GSH) was determined in whole cell lysate using dithionitrobenzoic acid (DTNB) method at 412 nm (Ellman, 1959) and GSH concentrations were expressed as % control.

2.8. ELISA analysis of Aβ

For determination of Aβ 1–42 level, a specific sandwich enzyme-linked immunosorbent (ELISA) employing monoclonal antibody was used. The ELISA was performed according to the instructions given in the Aβ ELISA kit by Wako chemicals (USA).

2.9. Data analysis

All data are represented as the mean ± SD. Comparison between groups was made by one-way analysis of variance (ANOVA) followed by a specific post hoc test to analyze the difference. The statistical significances were achieved when $P < 0.05$.

3. Results

3.1. Alginate suppresses oxidative stress-induced cell death in NT2 neurons

The present investigation was aimed to determine the protective role of alginate in oxidative-stress-induced cell death and Aβ formation. Therefore we chose NT2 cells as an in vitro model and H_2O_2 as the oxidative marker (Tamango et al., 2002). NT2 neurons are potentially a reliable experimental model for evaluating the relationship between oxidative stress and Aβ formation as a very credible in vitro model for AD.

To assess if alginate can protect NT2 neurons, cells were plated in 6-well plates, treated with different doses of alginate, and exposed to $H_2O_2/FeSO_4$ 1 h later. These cells then were assayed for cell viability. As the results show in Fig. 1, pretreatment with alginate dose-dependently protected neurons against H_2O_2 caused cell death compared to the control. Thus treatment of cells with alginate oligosaccharide protects NT2 neurons from oxidative stress-induced death.

3.2. Alginate inhibits caspase-3 activation in NT2 neurons

Caspases play a major role in effecting cell death in cells undergoing apoptosis (Miller, 1997) and previous studies demonstrated efficacy of caspase inhibitors in preventing Aβ-induced apoptosis in a cultured tumor cell line (Guo et al., 1999; Keller et al., 1998). As shown in Fig. 2, H_2O_2 induced the appearance of cleaved active caspase-3, arguing for involvement of caspase-3 in H_2O_2-induced cell death in NT2 neurons. In those cells pretreated with alginate, band of cleaved (active) caspase-3 was weaker, demonstrating the ability of this biopolymer to suppress oxidative stress-induced activation of caspase-3 in NT2 neurons.

3.3. Alginate treatment results in induction of HO-1 in NT2 neurons

Perhaps the first line of defense against oxidative injury comes from the molecular switches that recognize redox changes within the intracellular environment. In eukaryotes HO-1 is a ubiquitous and redox-sensitive enzyme that catabolizes heme into carbon monoxide (CO), iron and biliverdin (Maines, 1997; Ponka, 1999; Tenhunen, Marver, & Schmid, 1969). HO-1 is usually expressed at low levels under basal conditions, but is highly inducible in response to various agents causing oxidative stress. Because CO and bilirubin may act as adaptors to provide cytoprotection against various environmental stresses, induction of HO-1 by chemopreventive agents has been postulated to be part of the chemoprotective mechanisms (McNally, Harrison, Ross, Garden, & Wigmore, 2007; Ogborne, Rushworth, Charalambos, & O'Connell, 2004). To determine whether alginate induces HO-1, NT2 neurons were pretreated with two different concentrations (0.5% w/v and 1% w/v) of

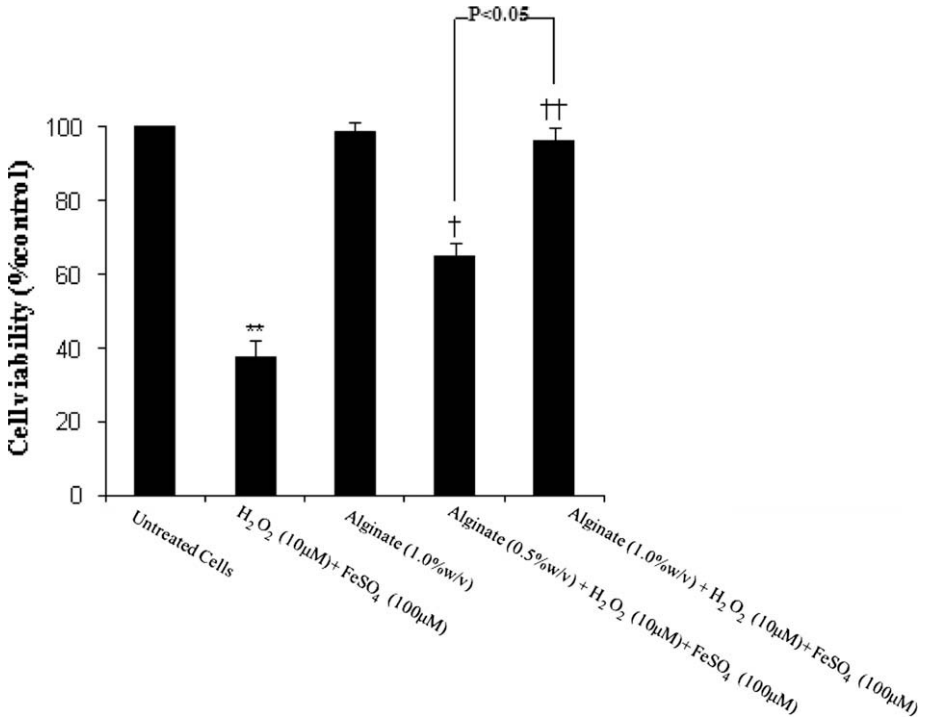

Fig. 1. Effect of alginate on cell viability. NT2 neurons were pretreated with alginate at indicated concentrations for 1 h and then exposed to $H_2O_2/FeSO_4$ for 2 h. Cell viability was determined by MTT test. Viability was calculated as the percentage of living cells in treated cultures compared to those in control cultures. Each value represents the mean ± SD ($n = 3$). *Significantly different from untreated cells. †Significantly different from $H_2O_2/FeSO_4$-treated cells.

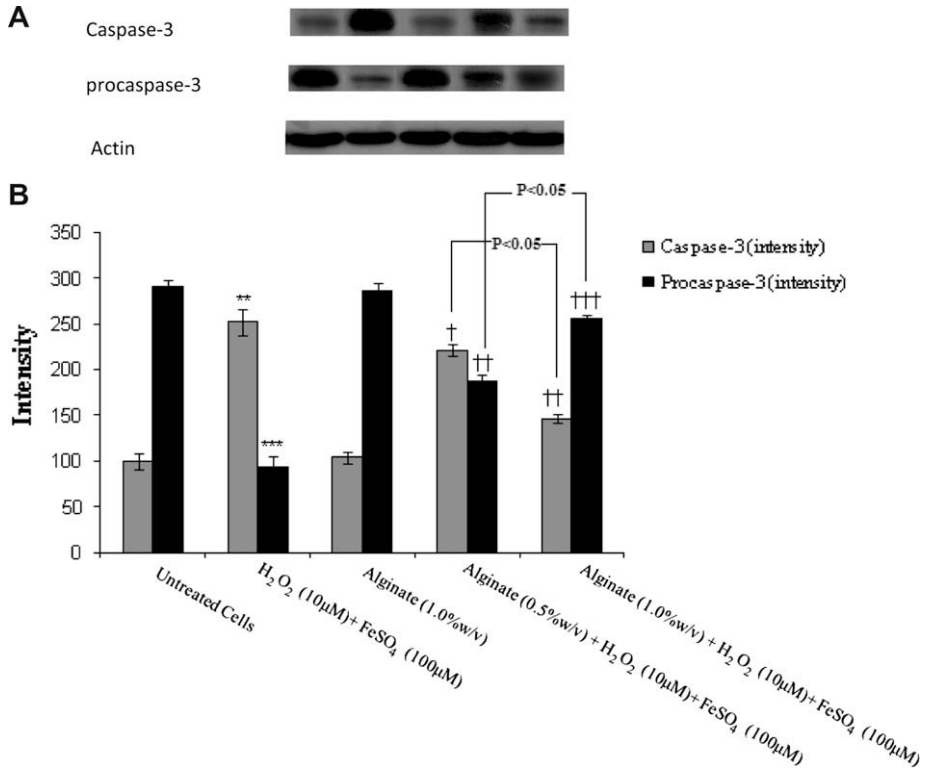

Fig. 2. Decrease of caspase-3 activity in NT2 neurons pretreated with alginate. (A) Procaspase-3 response to alginate on NT2 cells, pretreated with different concentrations of alginate for 1 h and then exposed to $H_2O_2/FeSO_4$ for 2 h. Twenty micrograms of proteins were separated on SDS–PAGE, Western blotted, probed with anti-caspase antibody and reprobed with anti-β-actin antibody. (One representative Western blot was shown; $n = 3$). (B) The densities of corresponding bands were measured and the ratio was calculated. The median of three independent experiments is shown. *Significantly different from untreated cells. †Significantly different from $H_2O_2/FeSO_4$-treated cells.

alginate for 1 h and then exposed to $H_2O_2/FeSO_4$ for 2 h. HO-1 protein level in the cell lysates was detected by Western blot. As seen in Fig. 3, alginate pretreatment could increase HO-1 protein, compared to the control cells.

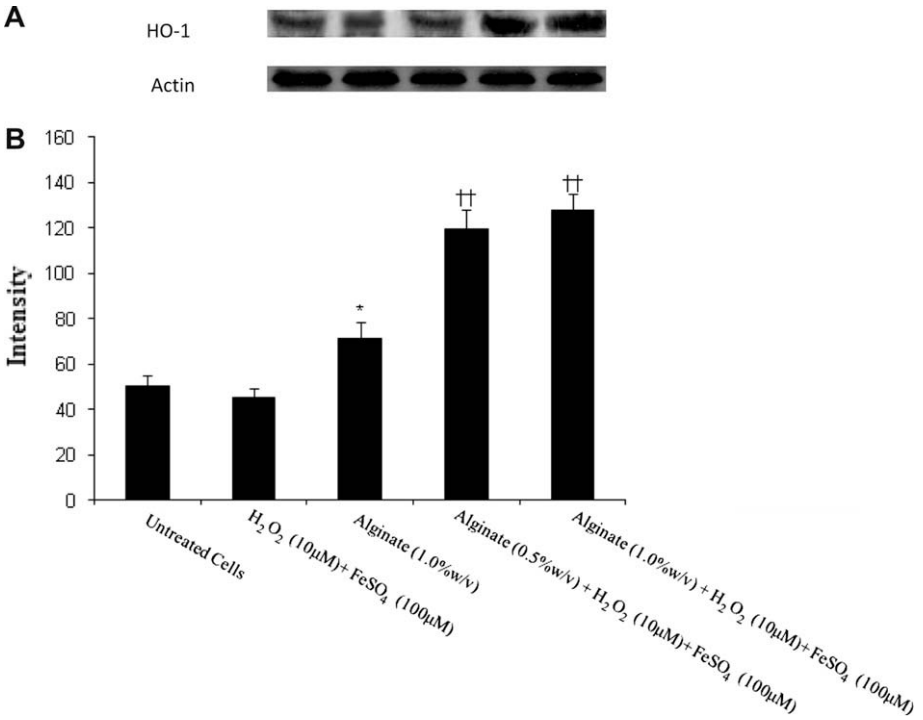

Fig. 3. Western blot analysis to measure the effects of alginate on HO-1 in NT2 neurons. (A) HO-1 response to alginate on NT2 cells pretreated with different concentrations of alginate for 1 h and then exposed to $H_2O_2/FeSO_4$ for 2 h. Twenty micrograms of proteins were separated on SDS–PAGE, Western blotted, probed with anti-HO-1 antibody and reprobed with anti-β-actin antibody. (One representative Western blot was shown; $n = 3$). (B) The densities of corresponding bands were measured and the ratio was calculated. The median of three independent experiments is shown. *Significantly different from untreated cells. †Significantly different from $H_2O_2/FeSO_4$-treated cells.

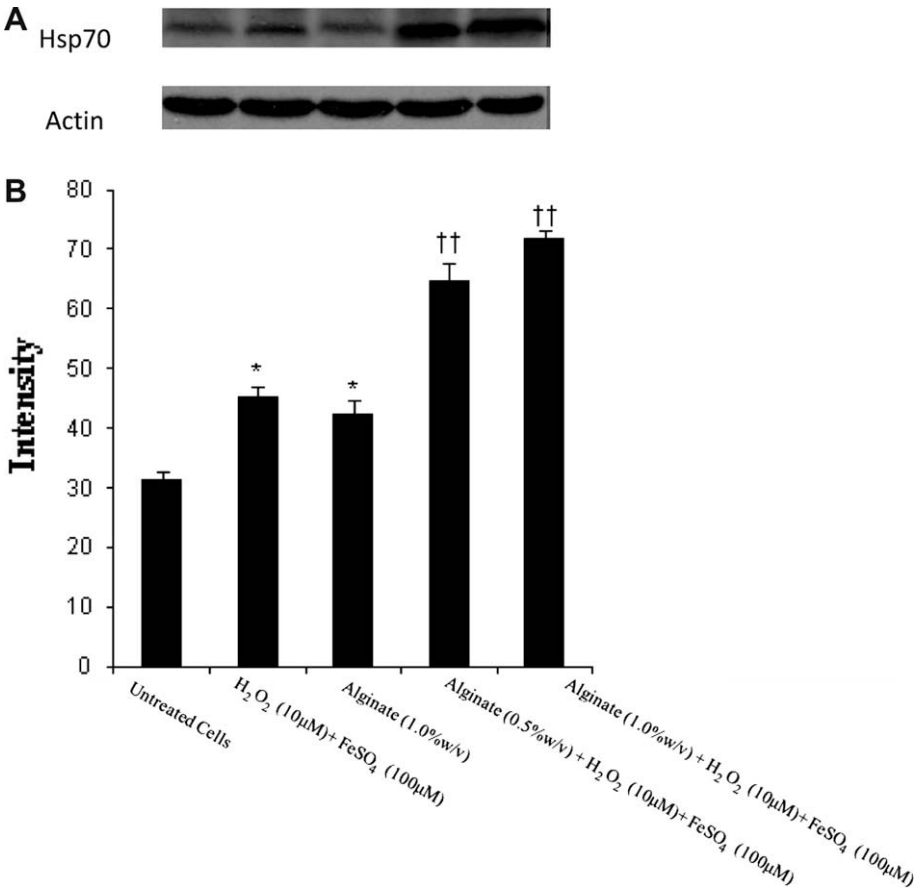

Fig. 4. Western blot analysis to measure the effects of alginate on Hsp-70 in NT2 neurons. (A) Hsp-70 response to alginate on NT2 cells pretreated with different concentrations of alginate for 1 h and then exposed to $H_2O_2/FeSO_4$ for 2 h. (B) The densities of corresponding bands were measured and the ratio was calculated. The median of three independent experiments is shown. *Significantly different from untreated cells. †Significantly different from $H_2O_2/FeSO_4$-treated cells.

3.4. Alginate treatment results in induction of Hsp-70 in NT2 neurons

A number of genes are upregulated following exposure to a stress conditioning, among them a set of Hsps, mainly Hsp-70 (Chiu et al., 2003; Currie et al., 2000; Kalmar & Greensmith, 2009; Kelly, 2005; Rordorf, Koroshetz, & Bonventre, 1991; Sato, Saito, & Matsuki, 1996).This Hsp has cytoprotective effects. Indeed, following exposure to cell stress, the chances of neuronal survival are related to the ability of the cell to elevate Hsp-70 synthesis. Therefore, we sought to determine the amount of Hsp-70 in the presence of alginate. Although alginate itself caused an upregulation of Hsp-70, the combination of alginate and oxidant upregulated Hsp-70 significantly. (Fig. 4). For the combinations, the increase was concomitant with decrease of caspase-3 activity (Fig. 2).

3.5. Alginate increases the glutathione level in NT2 neurons through γ-GCS

As is indicated in Fig. 5, pretreatment of NT2 cells by alginate 1% (w/v) resulted in more than 3-fold increase in GSH levels compared with the control cells. This result is consistent with our hypothesis that alginate can activate the antioxidant system, indicating that GSH metabolism can be regulated by this biopolymer. This observation is likely due to mediated expression of γ-glutamylcysteine synthetase (γ-GCS) because the rate limiting step in GSH biosynthesis is mediated by γ-GCS, which is upregulated by Nrf2 (Lee & Johnson, 2004; Sun, Erb, & Murphy, 2005; Wild, Moinova, & Mulcahy, 1999). Here the role of γ-GCS was confirmed by determination of its amount. As is illustrated in Fig. 6, alginate stimulated an increase in the amount of γ-GCS.

3.6. Alginate treatment results in dose-dependent induction of Nrf2 in NT2 neurons

It is generally accepted that Nrf2 plays a key role in the adaptive response to oxidative and electrophilic stress, maintaining the cellular self-defense. To establish whether the Keap1-Nrf2 pathway was involved in HO-1 and γ-GCS induction by alginate in NT2 neurons, we examined protein levels and nuclear localization in response to alginate. The level of Nrf2 protein is kept relatively low under basal conditions due to proteasomal degradation of Nrf2. Activation of the Keap1-Nrf2 pathway leads to stabilization of Nrf2 protein by detaching it from Keap1 repression (Nguyen, Sherratt, & Pickett, 2003). As the results in Fig. 7a show, a low level of Nrf2 was detected in untreated NT2 cells, while pretreatment with alginate for 1 h before exposure to H_2O_2/$FeSO_4$, with doses that induced HO-1, caused a significant increase in Nrf2 nuclear protein levels. The dose-dependent increase in Nrf2 paralleled the increase in HO-1 and γ-GCS in response to alginate, as indicated by Western blot analysis. As shown in Fig. 7b, densitometric analyses revealed about 4.0-fold increases in Nrf2 level in the presence of 1% w/v of alginate compared to control. Time-dependent induction of Nrf2 by Western blot showed the same amounts of Nrf2 after 0.5 and 2 h exposure to H_2O_2 in those cells pretreated by alginate. However, in the absence of alginate a small increase in the amount of Nrf2 was observed after 0.5 h that decrease to the basal level after 2 h (data not shown). It suggests that the cytoprotective effect of alginate is due to its ability to stabilize Nrf2 in the nucleus and upregulate the Nrf2-target genes.

3.7. Alginate treatment results in inhibition of NF-κB in NT2 neurons

To further explore the mechanisms underlying the protective effect of alginate against cell death, we analyzed NF-κB activity be-

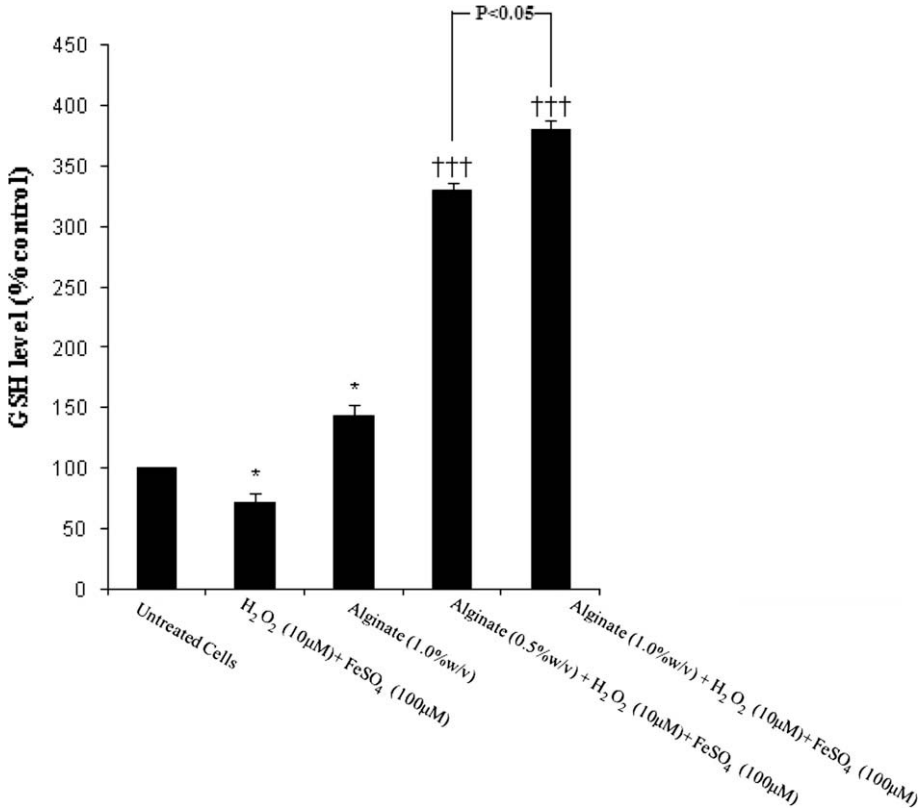

Fig. 5. Effect of alginate on glutathione concentration in NT2 neurons. Neurons pretreated with different concentrations of alginate for 1 h and then exposed to H_2O_2/$FeSO_4$ for 2 h. The glutathione content determined by DTNB test. Each value represents the mean ± SD (n = 3). *Significantly different from untreated cells. †Significantly different from H_2O_2/$FeSO_4$-treated cells.

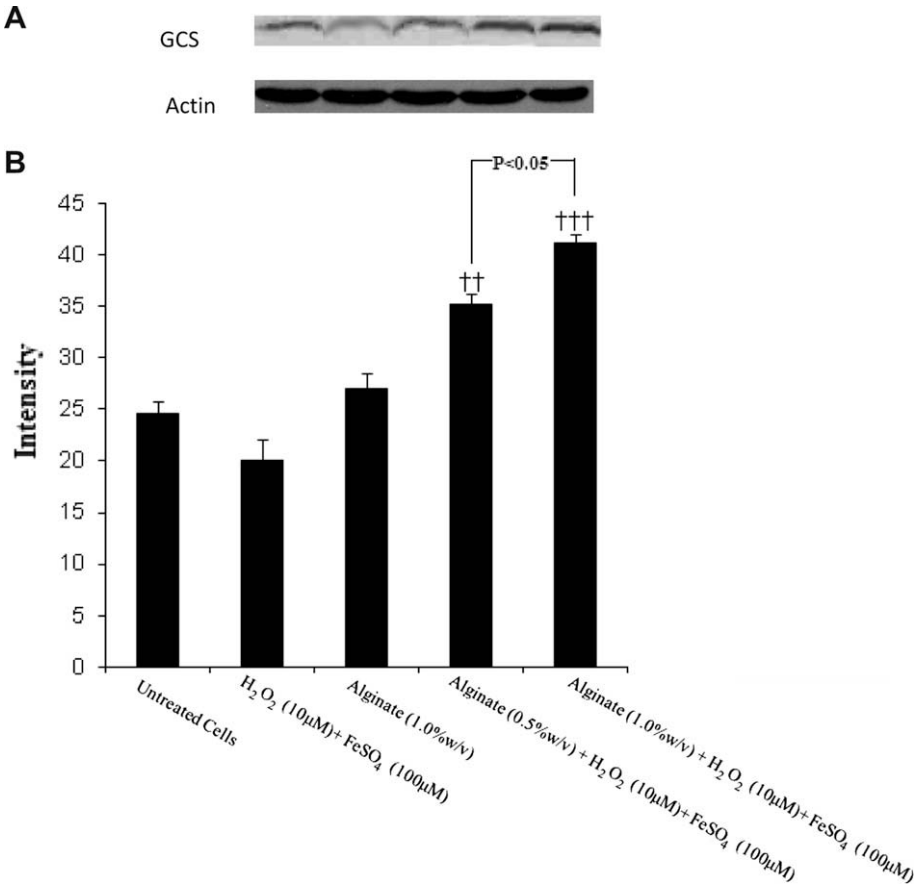

Fig. 6. Determination of alginate effect on γ-GCS in NT2 neurons. (A) Western blot analysis to measure the effects of alginate on γ-GCS in NT2 cells, pretreated with alginate for 1 h and then exposed to H_2O_2/$FeSO_4$ for 2 h. (B) The densities of corresponding bands were measured and the ratio was calculated. The median of three independent experiments is shown. †Significantly different from H_2O_2/$FeSO_4$-treated cells.

cause one of the events that occurs following oxidative damage is the activation of inflammatory pathways. The inflammatory response is mainly regulated by NF-κB (Kalayarasan et al., 2009). NF-κB is normally located in the cytosol, bound and inhibited by I-κB. Following oxidative stress, NF-κB is released from this inhibition, translocates to the nucleus and activates transcription of downstream inflammatory mediators. We found that in those cells exposed to H_2O_2 the level of NF-κB was increased in nucleus as determined by Western blot (Fig. 7a and c). Interestingly, this increase was prevented by pretreating cells directly with alginate. This inhibition is associated with a prevention of cell death induced by H_2O_2. Therefore in oxidative stress conditions, different cell defense mechanisms act synergistically to protect cells against death.

3.8. Alginate reduces the level of Aβ formation in NT2 neurons

We further tested the hypothesis that alginate could suppress Aβ formation and protect the cells from lesions caused by it. The study was based on the hypothesis that increase of an important product of oxidation may induce Aβ formation. Therefore differentiated NT2 neurons were incubated for 1 h with different concentrations of alginate and then exposed to an oxidative reagent which is known to induce Aβ formation (Frederriske, Garland, Zigler, & Piatigorsky, 1996; Misonou, Morishima-Kawashima, & Ihara, 2000; Paola et al., 2000). The Aβ levels were quantified against Aβ standards in the cell lysate and in the culture medium. When NT2 differentiated cells were exposed to H_2O_2, a significant increase of Aβ formation was observed in NT2 neurons. We found that Aβ formation by NT2 neurons pretreated with alginate was significantly lower than that of control cells exposed only to H_2O_2 (Fig. 8). These data indicate that Aβ level can be controlled by treatment with this polysaccharide, suggesting protective effect of alginate in AD. Moreover, the results indicate that oxidative stress fosters Aβ formation in NT2 neurons, creating a vicious neurodegenerative loop.

4. Discussion

Many studies have shown that oxidative stress is a major cause of cellular injuries in a variety of human diseases including neurodegenerative disorders. Reactive oxygen species such as hydrogen peroxide, superoxide anion and hydroxyl radical readily damage biological molecules, which can ultimately lead to apoptotic or necrotic cell death (Halliwell & Aruoma, 1991; Yoshikawa et al., 2006). Thus, removal of excess reactive oxygen species or suppression of their generation by antioxidants may be effective in preventing oxidative cell death. Recently, researchers have made considerable efforts to search for natural antioxidants with neuroprotective potential.

From the other side, the tissue concentrations of the AGEs, the end-products of millard reactions that are induced by reactive oxygen species, are associated with various age-related pathologies such as AD (Reddy & Beyaz, 2006). Consequently, development of AGE inhibitors as therapeutics has been attracting renewed attention in recent years. In view of the adverse side-effects associated with many of the potent AGE inhibitors, it is apparent that naturally occurring antiglycating agents can offer practical approaches for the prevention and/or treatment of AGE-associated diseases,

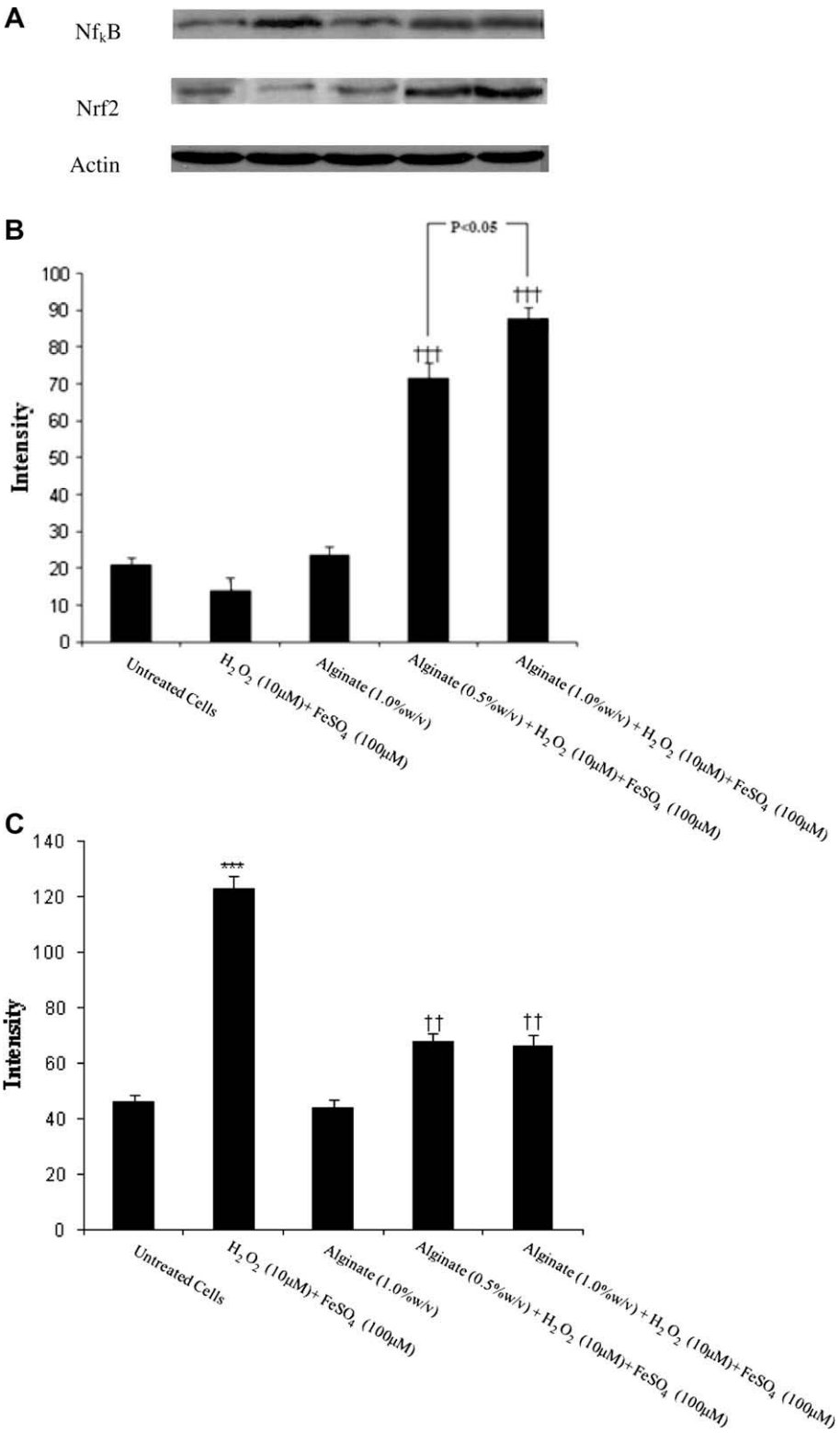

Fig. 7. Western blot analysis to measure the effects of alginate on the nuclear levels of Nrf2 and NF-κB in NT2 neurons. (A) Nrf2 and NF-κB response to alginate on NT2 cells, pretreated with different concentrations of alginate for 1 h and then exposed to $H_2O_2/FeSO_4$ for 2 h. Twenty micrograms of proteins were separated on SDS–PAGE, Western blotted, probed with anti-Nrf2 and/or anti-NF-κB antibody and reprobed with anti-β-actin antibody. (One representative Western blot was shown; $n = 3$). The densities of Nrf2 (B) and NF-κB (C) bands were measured and the ratio was calculated. The median of three independent experiments is shown. *Significantly different from untreated cells. †Significantly different from $H_2O_2/FeSO_4$-treated cells.

like AD (Reddy & Beyaz, 2006). Alginate is a natural oligosaccharide that has been recently reported as an antiglycating agent as well (Sattarahmady et al., 2007). Therefore in this study we wished to examine whether this oligosaccharide has neuroprotective effect.

H_2O_2 has been extensively used as an inducer of oxidative stress in vitro model (Behl et al., 1994). The exposure of cultured cell to H_2O_2 results in an imbalance in energy metabolism and the deleterious effects of hydroxyl and peroxyl radicals on membrane lipids

and proteins. Our data confirmed that treating cells with H_2O_2 resulted in cell death which was greatly decreased in the presence of alginate. This result indicated that alginate did significantly protect NT2 neurons from H_2O_2-induced cytotoxicity.

Although many signals and metabolic events are important in the regulation of cell death, the intracellular redox level, in particular, has been shown to play a critical role. Redox regulation is an interesting and important issue which tightly associates with oxidative stress. In intercellular redox modulation system, HO-1 is one of the antioxidant proteins in response to oxidative stress. HO-1 belongs to a family of cytoprotective and detoxification genes that possess antioxidant responsive elements (AREs) in their regulatory regions. The Nrf family of transcription factors can bind the ARE (Nguyen et al., 2003). Previous studies suggest that Nrf2 plays a key role in antioxidant-induced HO-1 expression. In this study alginate induced Nrf2 nuclear translocation. In addition alginate increased γ-GCS level (the rate limiting step in GSH biosynthesis), one of the other Nrf2-target genes, suggesting that Nrf2 is indeed important for alginate-induced cytoprotective effect in NT2 neurons.

Based on our data, alginate has a high capability to increase GSH levels, suggesting that the increment of intracellular GSH level contributes the ability of alginate to mediate cellular oxidative stress. Besides, in contrast to most other Nrf2-activators like flavonoids, sulforaphane or curcumin (Balogun et al., 2003; Scapagnini et al., 2006) which are already toxic at low concentration, alginate showed no toxicity in our assays in vitro and was able to activate Nrf2 at low concentrations. In addition to Nrf2-target genes, other groups of proteins are regulated by oxidative stress, including Hsps and NF-κB.

It has been shown that Hsp-70 plays a role in sensing oxidative stress, and is also able to inhibit cell death pathways, including the inflammation and apoptotic pathways (Kalmar & Greensmith, 2009).The anti-inflammatory action of Hsp-70 is mediated by binding of Hsp-70 to NF-κB and its subsequent inhibition through prevention of nuclear translocation of NF-κB. The link between oxidative stress and NF-κB was established mainly from the inhibition of NF-κB activation by cellular oxidants (Feinstein, Galea, & Reis, 1997; Guzhova, Darieva, Melo, & Margulis, 1997; Kalmar & Greensmith, 2009; Ran et al., 2004). Our results also clearly indicate that inhibition of NF-κB is concomitant by increase of Hsp-70 and decrease of caspase-3 level, which makes alginate an applicable agent. Because recently it has been shown that induction of Hsps is a promising therapeutic approach for neurodegenerative diseases and as a next step toward developing a therapy, it is ideal to pharmacologically induce endogenous molecular chaperones by administration of small chemical compounds, instead of to express exogenous genes (Fujikake et al., 2008). In our study alginate itself caused an upregulation of Hsp-70, perhaps as a response to osmotic effect and/or viscosity changes. However, it seems that the system can cope with this stress, through inhibition of caspase-3 (as a result of Hsp-70 induction) and therefore does not cause cell death.

In addition this study demonstrates that alginate is able to inhibit Aβ formation and protect NT2 neurons from cell death. To date, most investigations try to protect neural cells against the cytotoxicity of Aβ. In the present study we induced Aβ formation through oxidative stress in NT2 neurons, one of the most appropriate cell line models in AD. NT2 neurons are potentially a reliable experimental model for evaluating the relationship between oxidative stress and Aβ formation as a very credible in vitro model for AD, since they show a phenotype similar to primary neurons and express high level of $A\beta PP_{696}$, the major AβPP isoform expressed in brain (Wertkin et al., 1993), generate intracellular Aβ peptide (Turner, Suzuki, Chuyung, Younkin, & Lee, 1996), and are highly susceptible to oxidative stress (Tamango et al., 2000). Here we provide the first documentation of the neuroprotective effect of alginate through suppression of Aβ formation. The present study confirms and extends previous observations on the relationship between oxidative stress and Aβ production.

In summary we have determined that alginate not only effectively prevents the oxidative stress-induced neurotoxicity but also can inhibit Aβ formation in our in vitro model of AD. It seems that in this cytoprotection, HO-1 and γ-GCS upregulation through Nrf2 pathway play the key roles. In addition in this system, induction of Hsp-70 and inhibition of NF-κB are the other main factors that protects neurons against oxidative stress-induced caspase-3 activity and Aβ formation. It has been shown that alginate has antioxidant properties, exhibit direct scavenging on ROS in cell free system and these radical scavenging properties are expanded into oxidation-induced system (Bylund et al., 2006; Pedersen, Kharazmi, Espersen, & Hoiby, 1990). Therefore, the inhibitory action of alginate on H_2O_2-induced Aβ formation is likely associated with ROS-scavenging abilities of alginate. Similarly, many reports discuss the possibility that ROS promote apoptosis by targeting caspase-3 activity

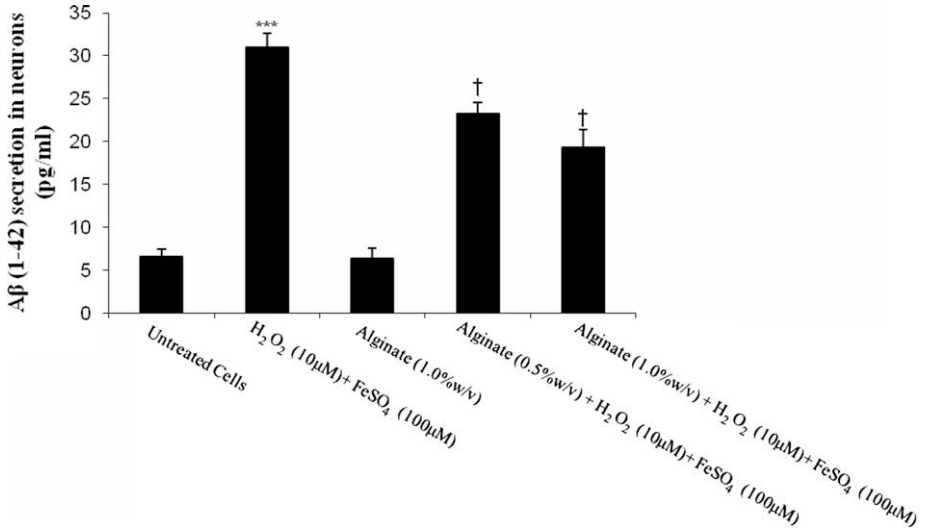

Fig. 8. Effect of alginate on Aβ secretion by NT2 neurons in oxidative stress condition. Cells were plated at equal density in 6-well plates, pretreated with different concentrations of alginate for 1 h and then exposed to $H_2O_2/FeSO_4$ for 2 h. The amount of Aβ was determined by ELISA kit. Each value represents the mean ± SD ($n = 3$). *Significantly different from untreated cells. †Significantly different from $H_2O_2/FeSO_4$-treated cells.

(Perskvist, Long, Stendahl, & Zheng, 2002). The interdependent relationship between caspase-3 activation and ROS generation inspires us to assume that the inhibition of alginate on caspase-3 activation might also be in relation to ROS-scavenging abilities of alginate. However, we can not rule out the possibility of involvement of other mechanisms. In fact, the mechanisms underlying the neuroprotective effects of alginate remain to be further explored. A further study of the detailed mechanisms is now in progress in our laboratory.

Acknowledgments

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