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Neuroprotective effects of α-tocopherol on oxidative stress in rat striatal cultures

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Abstract

Oxidative stress caused by an increase in free radicals plays an important role in neuronal death. We investigated the effects of α-tocopherol on oxidative stress-induced cytotoxicity using primary cultures of rat striatal neurons. α-Tocopherol at concentrations of 1–10 μM significantly prevented cytotoxicity induced by superoxide radical ($O_2^{-}\cdot$) donor, 1,1′-dimethyl-4,4′-bipyridium dichloride (paraquat). In contrast, α-tocopherol did not affect the cytotoxicity of hydrogen peroxide (H_2O_2), which enhances hydroxyl radical (·OH) formation by metal-catalyzed Fenton reactions. α-Tocopherol significantly inhibited the cytotoxicity of nitric oxide (NO) donors, *S*-nitrosocysteine and 3-morpholinosydnonimine (SIN-1). α-Tocopherol showed potent protection against cytotoxicity induced by L-buthionine-[*S*,*R*]-sulfoximine (BSO), which causes depletion of intracellular glutathione. Moreover, α-tocopherol afforded a moderate but significant inhibition of cytotoxicity induced by a non-specific protein kinase inhibitor, staurosporine, which is known to induce apoptosis in many types of cells including neurons. These results suggest that α-tocopherol protects striatal neurons by the reduction of oxidative stress, presumably by decreasing intracellular $O_2^{-}\cdot$ levels, and at least partly by the inhibition of apoptosis.

Keywords: α-Tocopherol; Neuroprotection; Oxidative stress; Apoptosis; Striatal neuron; (In vitro); (Rat)

1. Introduction

Neurons in the central nervous system are vulnerable to oxidative stress caused by reactive oxygen species and nitric oxide (NO). Those radicals may play crucial roles in neuronal death during the process of neurodegenerative diseases. Increased formation of free radicals is responsible for a variety of neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis and for pathological conditions such as ischemia and excitotoxicity (Coyle and Puttfarcken, 1993; Olanow, 1993). Free radicals are able to initiate injurious processes of neurons such as lipid peroxidation, oxidative alteration of proteins and DNA damage. Furthermore, it has been suggested that apoptotic neuronal death is involved in neurodegenerative diseases (Yuan and Yankner, 2000). Accordingly, substances which can reduce oxidative stress and inhibit apoptosis are expected to be potential tools in the therapy of various neurodegenerative diseases.

There has been considerable interest in the potential role of vitamin E in the treatment of age-associated neurodegenerative diseases such as Alzheimer's disease. Epidemiologic studies have suggested that intake of antioxidant nutrients such as vitamin E or vitamin C is associated with a reduced risk of developing Alzheimer's disease (Morris et al., 1998). A clinical trial suggested that administration of vitamin E to patients with Alzheimer's disease significantly slows the progression of the disease (Sano et al., 1997). These studies prompted us to investigate the effects of vitamin E on neurotoxicity induced by oxidative stress in vitro.

Vitamin E is the term for a group of tocopherols and tocotrienols. Tocopherols and tocotrienols have the same

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basic chemical structure characterized by a long phytyl tail attached to chromane ring. Tocopherols have a saturated, whereas tocotrienols have an unsaturated phytyl tail, and individual isoforms of tocopherols and tocotrienols differ in the number and the position of the methyl groups attached to the aromatic ring (Ricciarelli et al., 2001). Each of these forms of vitamin E has a different biopotency. In the vitamin E group, α-tocopherol is suggested to have the highest biological activity (Hosomi et al., 1997; Weimann and Weiser, 1991). In the present study, we demonstrated the neuroprotective action of α-tocopherol on oxidative stress by using primary cultures of rat striatal neurons.

2. Materials and methods

2.1. Materials

Eagle's minimum essential medium (Eagle's MEM) was purchased from Nissui Pharmaceutical (Tokyo, Japan). Fetal bovine serum was obtained from JRH Biosciences (Lenexa, KS, USA.). L-Cysteine, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), glucose, glutamine, HEPES, hydrochloric acid (HCl), $NaHCO_3$, sodium nitrite and α-tocopherol were from Nacalai Tesque (Kyoto, Japan). L-Buthionine-[*S*,*R*]-sulfoximine (BSO) and 1,1′-dimethyl-4,4′-bipyridium dichloride (paraquat) were obtained from Sigma (St. Louis, MO, USA). Hydrogen peroxide (H_2O_2) was purchased from Wako (Osaka, Japan). 3-Morpholino-sydnonimine (SIN-1) was from Dojindo Laboratory (Kumamoto, Japan). 2-[2-(4-Hydroxyphenyl)-6-benzimidazoyl]-6-(1-methyl-4-piperazyl)-benzimidazole, 3 HCl (Hoechst 33258) and staurosporine were purchased from Calbiochem (San Diego, CA, USA). Mn(III)tetrakis(4-benzoic acid)-porphyrin chloride (Mn-TBAP), a cell permeable superoxide dismutase mimetic was obtained from Cayman Chemical (Ann Arbor, MI, USA). Cytotoxicity Detection lactate dehydrogenase (LDH) kit was purchased from Kyokuto Pharmaceutical Industrial (Tokyo, Japan).

2.2. Cell culture

Primary cultures were prepared from the anterior striatum of fetal Wistar rats (Nihon SLC, Shizuoka, Japan) on the 17–19th days of gestation, according to the procedures described previously (Amano et al., 1994) with slight modifications. Briefly, the anterior striatum of fetal rats was removed bilaterally, mechanically dissociated using scalpel blades, and then filtered through a stainless steel mesh. Single-cell suspensions were plated on 0.1% polyethylenimine-coated 24-well plates or 2-well chamber slides at a density of $3.5–4.0 \times 10^5$ cell/cm^2. Cultures were incubated in Eagle's MEM supplemented with 10% heat-inactivated fetal bovine serum, glutamine (2 mM), glucose (total 11 mM), $NaHCO_3$ (24 mM), and HEPES (10 mM). Cultures were maintained at 37 °C in a humidified 5% CO_2 atmosphere. The animals were treated in accordance with the guidelines of Kyoto University animal experimentation committee, and the guidelines of the Japanese Pharmacological Society.

2.3. Drug treatment

At 3 days in vitro, cultures were incubated in a medium containing various concentrations of paraquat, H_2O_2, *S*-nitrosocysteine, SIN-1, or staurosporine for 24 h, or BSO for 48 h. *S*-Nitrosocysteine was prepared according to the method described previously (Kume et al., 1997). In brief, L-cysteine and sodium nitrite were combined in equimolar amounts and dissolved in double-distilled water. To this solution, 10 N HCl was added to give a final normality of 0.5 N and a final *S*-nitrosocysteine concentration of 100 mM. α-Tocopherol or Mn-TBAP was simultaneously added to a toxin-containing medium. After exposure of cultures to drugs, cell viability was determined by LDH release assay or MTT assay. In each experiment, cells on 4 wells were used to obtain means ± S.E.M. of the cell viability. Reproducibility of the results was confirmed by at least three different sets of experiments. Data shown in figures are from a representative set of experiments. In a pilot study, exposure to α-tocopherol alone at up to 100 μM did not influence cell viability (data not shown).

2.4. LDH release assay

Cytotoxicity was quantified by measurement of LDH released in the medium during the exposure to drugs. LDH release was determined using a Cytotoxicity Detection LDH kit according to the manufacturers' instructions. In this colorimetric assay, β-nicotinamide adenine dinucleotide (NAD) is reduced to NADH through the conversion of lactate to pyruvate by LDH, and then NADH reduces tetrazolium dyes to formazan dyes in the presence of diaphorase. Briefly, 25 μl of culture supernatants were mixed with 75 μl of the LDH substrate mixture in a 96-well plate. After incubation for 1 h at room temperature, the reaction was stopped by adding 100 μl of 1 N HCl and the absorbance was measured at 570 nm. The background absorbance obtained from the culture medium was subtracted. Cell viability of the cultures was evaluated as relative to the total LDH, which can be released by exposure to 1 mM H_2O_2 for 24 h or 1 mM BSO for 48 h.

2.5. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

MTT assay measures dehydrogenase activity by the reduction of MTT tetrazolium salt to MTT formazan. The culture medium was replaced by serum-free medium containing 0.5 mg/ml MTT tetrazolium salt, and then incubation was continued for 3 h at 37 °C. The medium was aspirated, and the cells were solubilized by adding isopro-

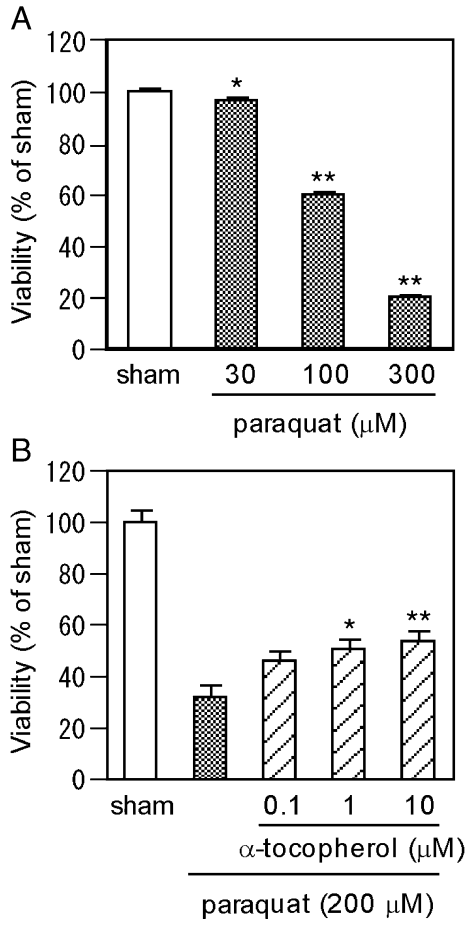

Fig. 1. Effect of α-tocopherol on paraquat-induced cytotoxicity in rat striatal cultures. Cytotoxicity was quantified by LDH release assay. (A) Concentration-dependent reduction of cell viability by paraquat. Cultures were exposed to paraquat (30–300 μM) for 24 h. $*P<0.05$, $**P<0.01$, compared with sham treatment. (B) Protective effect of α-tocopherol on paraquat-induced cytotoxicity. α-Tocopherol (0.1–10 μM) was simultaneously added with paraquat (200 μM) for 24 h. $*P<0.05$, $**P<0.01$, compared with paraquat alone. Paraquat: 1,1′-dimethyl-4,4′-bipyridium dichloride.

panol. Aliquots were transferred to a 96-well plate and the absorbance was measured photometrically at 595 nm. The viability of the cultures was expressed as a percentage of the absorbance measured in non-treated cells.

2.6. Hoechst staining

To reveal the nuclear morphological changes in cultured neurons, cells were stained with a nuclear dye Hoechst 33258. After staurosporine treatment, cultures were fixed with neural formaldehyde for 30 min, rinsed three times with phosphate-buffered saline, and then incubated with 1 mg/ml Hoechst 33258 for 30 min at room temperature. After washing three times with phosphate-buffered saline, specimens were dehydrated through a graded series of ethanol (70%, 90% and 100%) and mounted with glycerol. Cells were visualized and photographed under ultraviolet illumination using fluorescent microscopy (Leica, Wetzlar, Germany).

2.7. Statistics

Values were expressed as means ± S.E.M. The statistical significance of difference between groups was determined by one-way analysis of variance (ANOVA) followed by Dunnett's test. Probability values less than 5% were considered to be significant.

3. Results

3.1. Effects of α-tocopherol on paraquat- and H_2O_2-induced cytotoxicity

Superoxide radical ($O_2^{-}\cdot$), which has limited toxic effects itself, can dismutate into hydrogen peroxide (H_2O_2). In turn, H_2O_2 exerts its toxic effects mainly through the ferrous iron-

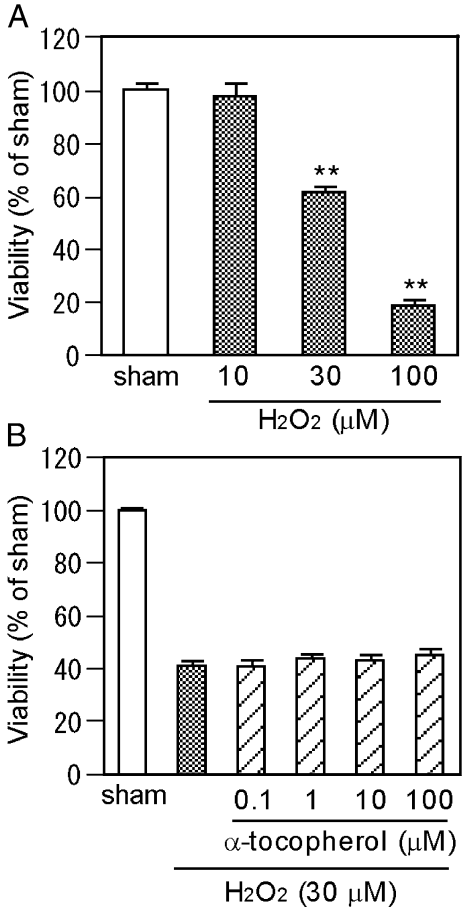

Fig. 2. Effect of α-tocopherol on H_2O_2-induced cytotoxicity in rat striatal cultures. Cytotoxicity was quantified by LDH release assay. (A) Concentration-dependent reduction of cell viability by H_2O_2. Cultures were exposed to H_2O_2 (10–100 μM) for 24 h. $**P<0.01$, compared with sham treatment. (B) Effect of α-tocopherol on H_2O_2-induced cytotoxicity. α-Tocopherol (0.1–100 μM) was simultaneously added with H_2O_2 (30 μM) for 24 h. H_2O_2: hydrogen peroxide.

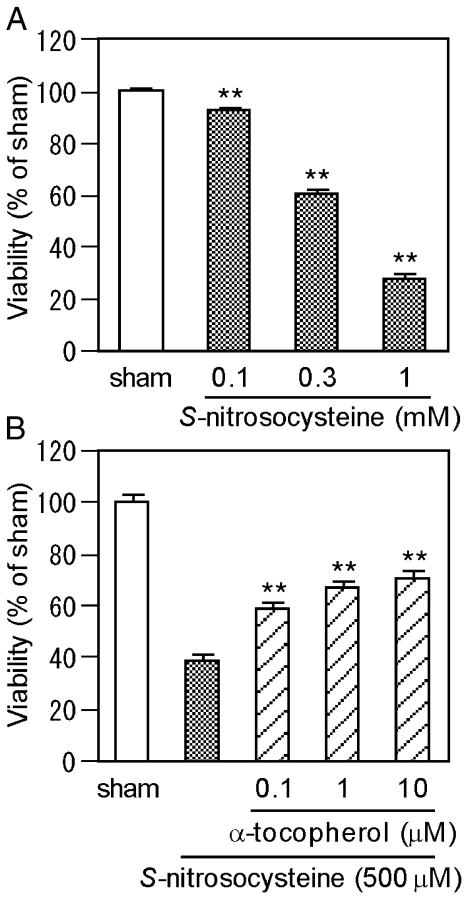

Fig. 3. Effect of α-tocopherol on *S*-nitrosocysteine-induced cytotoxicity in rat striatal cultures. Cytotoxicity was quantified by LDH release assay. (A) Concentration-dependent reduction of cell viability by *S*-nitrosocysteine. Striatal cultures were exposed to *S*-nitrosocysteine (0.1–1 μM) for 24 h. $^{**}P<0.01$, compared with sham treatment. (B) Protective effect of α-tocopherol on *S*-nitrosocysteine-induced cytotoxicity. α-Tocopherol (0.1–10 μM) was co-administered with *S*-nitrosocysteine (500 μM) for 24 h. $^{**}P<0.01$, compared with *S*-nitrosocysteine alone.

dependent formation of the highly reactive hydroxyl radical (·OH). In the first set of experiments, we examined the effect of α-tocopherol on paraquat-induced cytotoxicity by LDH release assay. Paraquat reacts with NADPH to generate paraquat radical, and it reacts with O_2 to generate $O_2^{-}\cdot$ (Day et al., 1995; Patel et al., 1996). Exposure of the striatal cultures to paraquat (30–300 μM) for 24 h reduced cell viability in a concentration-dependent manner (Fig. 1A). Coapplication of α-tocopherol (0.1–10 μM) with paraquat for 24 h significantly attenuated paraquat-induced cytotoxicity in a concentration-dependent manner (Fig. 1B). To reveal the neuroprotective mechanism of α-tocopherol against $O_2^{-}\cdot$ donor-induced neuronal death, we investigated whether α-tocopherol exhibits protection against H_2O_2-induced neuronal death. The viability of striatal neurons was reduced by treatment with H_2O_2 (10–100 μM) for 24 h (Fig. 2A). Coapplication of α-tocopherol (0.1–100 μM) with H_2O_2 for 24 h did not prevent H_2O_2-induced cytotoxicity (Fig. 2B). These results suggest that α-tocopherol suppresses cytotoxicity induced by $O_2^{-}\cdot$ but not by H_2O_2 or its product ·OH.

3.2. Effects of α-tocopherol against NO donor-induced cytotoxicity

$O_2^{-}\cdot$ can react with NO to yield peroxynitrite anion ($ONOO^{-}$), and rapid degradation of $ONOO^{-}$ produces ·OH (Beckman et al., 1990; Bonfoco et al., 1995; Hogg et al., 1992; Kume et al., 1997; Lipton et al., 1993). Therefore, we examined the effects of α-tocopherol on cytotoxicity induced by an NO donor, *S*-nitrosocysteine by LDH release assay. *S*-Nitrosocysteine spontaneously releases NO which reacts with endogenous $O_2^{-}\cdot$ to produce $ONOO^{-}$ (Bonfoco et al., 1995; Kume et al., 1997; Lipton et al., 1993). Application of *S*-nitrosocysteine (0.1–1 μM) to the striatal cultures for 24 h caused a concentration-dependent reduction in cell viability (Fig. 3A). Simultaneous administration of α-tocopherol (0.1–10 μM) with *S*-nitrosocysteine for 24 h mark-

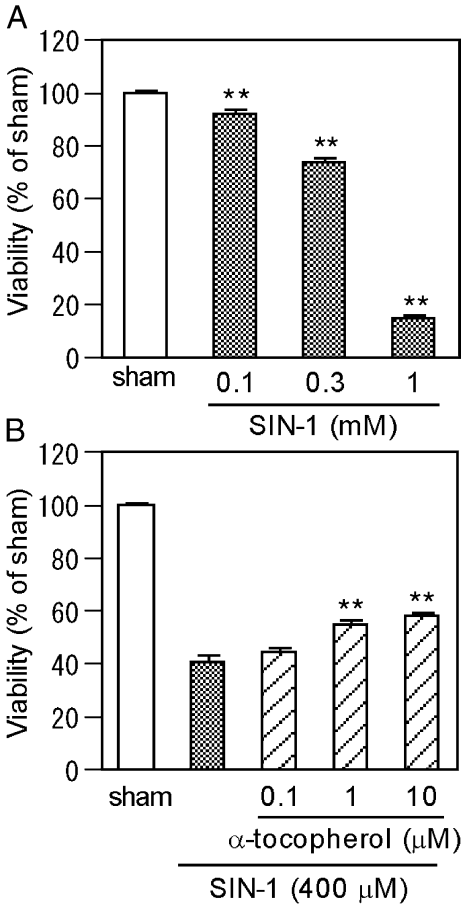

Fig. 4. Effect of α-tocopherol on SIN-1-induced cytotoxicity in rat striatal cultures. Cytotoxicity was quantified by LDH release assay. (A) Concentration-dependent reduction of cell viability by SIN-1. Cultures were exposed to SIN-1 (0.1–1 mM) for 24 h. $^{**}P<0.01$, compared with sham treatment. (B) Protective effect of α-tocopherol on SIN-1-induced cytotoxicity. α-Tocopherol (0.1–10 μM) was simultaneously applied with SIN-1 (400 μM) for 24 h. $^{**}P<0.01$, compared with SIN-1 alone. SIN-1: 3-morpholinosydnonimine.

edly inhibited *S*-nitrosocysteine-induced neuronal death (Fig. 3B). Thus, we further investigated the effects of α-tocopherol on a different type of NO donor, SIN-1. SIN-1 simultaneously releases NO and O_2^-· to generate $ONOO^-$, thereby resulting in the formation of ·OH (Beckman et al., 1990; Bonfoco et al., 1995; Hogg et al., 1992; Kume et al., 1997; Lipton et al., 1993). Exposure of the striatal cultures to SIN-1 (0.1–1 mM) for 24 h caused cell death in a concentration-dependent manner (Fig. 4A). Coapplication of α-tocopherol (0.1–10 μM) with SIN-1 exerted concentration-dependent suppression of SIN-1-induced neuronal death (Fig. 4B). Cytotoxicity induced by *S*-nitrosocysteine and SIN-1 was also blocked by co-administration of Mn-TBAP responsible for scavenging intracellular O_2^-· (data not shown), suggesting that the suppression of intracellular O_2^-· levels and reduced formation of $ONOO^-$ contributes to neuroprotection against NO cytotoxicity.

3.3. Effect of α-tocopherol on BSO-induced cytotoxicity

BSO is an irreversible inhibitor of γ-glutamylcysteine synthase, which is the rate-limiting step in glutathione (GSH) synthesis, and causes intracellular GSH depletion and oxidative stress (Ibi et al., 1999; Merad-Boudia et al., 1998). Since the above results suggest that α-tocopherol reduced oxidative stress by suppressing O_2^-· levels, we decided to investigate whether α-tocopherol ameliorates BSO-induced neurotoxicity in rat striatal cultures. Exposure of cultures to BSO (100 μM) for 48 h reduced the survival of striatal neurons, as determined by LDH release assay. Simultaneous application of α-tocopherol (0.01–10 μM) with BSO for 48 h markedly inhibited BSO-induced cytotoxicity (Fig. 5). α-Tocopherol at a concentration as low as 0.1 μM virtually abolished BSO-induced neurotoxicity.

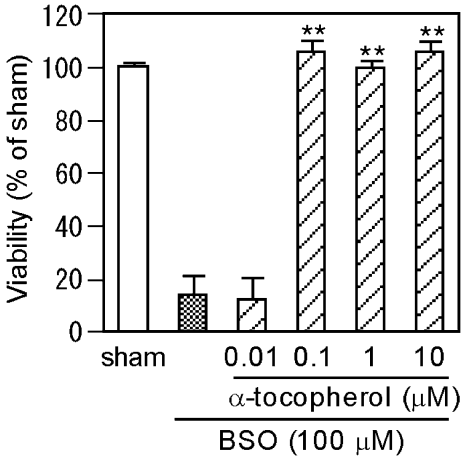

Fig. 5. Protective effect of α-tocopherol on BSO-induced cytotoxicity in rat striatal cultures. Cytotoxicity was quantified by LDH release assay. Striatal cultures were treated with BSO (100 μM) for 48 h. α-Tocopherol (0.01–10 μM) was simultaneously added to BSO-containing medium. $^{**}P<0.01$, compared with BSO alone. BSO: L-buthionine-[*S*,*R*]-sulfoximine.

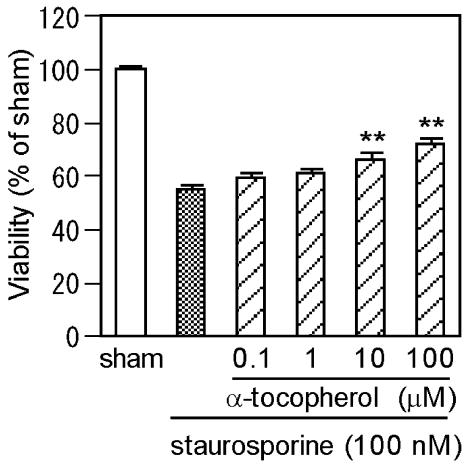

Fig. 6. Effect of α-tocopherol on staurosporine-induced cytotoxicity in rat striatal cultures. Cytotoxicity was quantified by MTT assay. Striatal cultures were exposed to staurosporine (100 nM) for 24 h. α-Tocopherol (0.1–100 μM) was simultaneously added to staurosporine-containing medium. $^{**}P<0.01$, compared with staurosporine alone.

3.4. Effect of α-tocopherol on staurosporine-induced cytotoxicity

In the final set of experiments, we employed staurosporine to examine the anti-apoptotic effect of α-tocopherol in

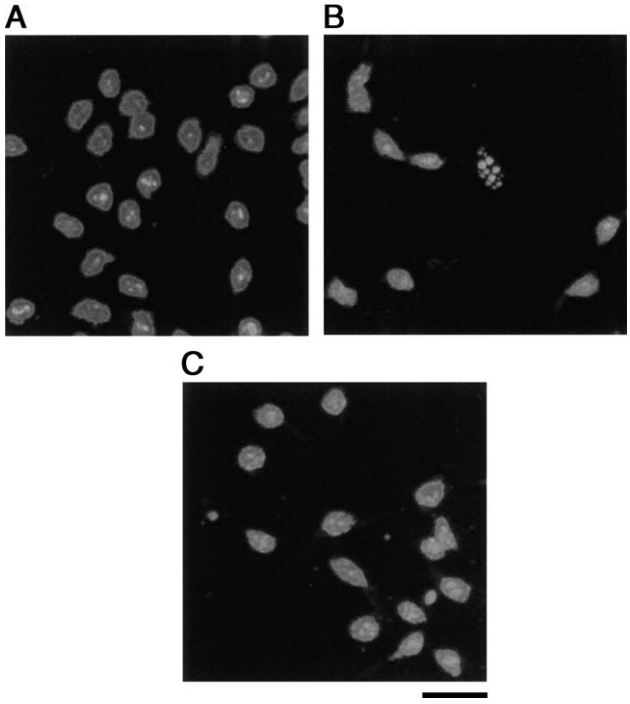

Fig. 7. Effect of α-tocopherol on nuclear changes induced by staurosporine in rat striatal cultures. Cells were stained with Hoechst 33258. Photomicrographs (A, B and C) show sham treatment, staurosporine treatment, and staurosporine + α-tocopherol treatment respectively. Cultures were exposed to staurosporine (100 nM) for 24 h. α-Tocopherol (100 μM) was simultaneously added to staurosporine-containing medium. Calibration bar = 20 μm.

cultured striatal neurons. Staurosporine, a broad-spectrum protein kinase inhibitor, has been reported to induce apoptosis, which is accompanied by cell body shrinkage, chromatin condensation and DNA laddering (Honda et al., 2001; Koh et al., 1995; Yuste et al., 2002). Consistent with these reports, Hoechst 33258 staining revealed that treatment with staurosporine (100 nM) for 24 h caused cell death with apoptotic features such as nuclear fragmentation and chromatin condensation in rat striatal cultures (Fig. 7A,B). LDH release assay measures the activity of the cytoplasmic enzyme LDH in the culture medium. However, one of the hallmarks of apoptotic death is the integrity of the plasma membrane. Thus, internal and external membranes may be preserved, and the cellular content may not be released in the neighborhood. Therefore, staurosporine-induced apoptosis was quantified by MTT assay, which measures the mitochondrial reduction capacity. Application of staurosporine (100 nM) to the striatal cultures for 24 h reduced the cell viability. α-Tocopherol simultaneously added with staurosporine showed significant protection against staurosporine-induced cytotoxicity at concentrations of 10 and 100 μM (Fig. 6). Moreover, concurrent treatment of striatal cultures with α-tocopherol (100 μM) and staurosporine inhibited nuclear fragmentation and chromatin condensation, as evaluated by Hoechst 33258 staining (Fig. 7C).

4. Discussion

The present study demonstrated that α-tocopherol showed neuroprotective effects in rat striatal cultures. α-Tocopherol has been reported to reduce oxidative stress. For example, α-tocopherol prevented free radical formation caused by the membrane-permeant oxidant *tert*-butylhydroperoxide in human neuroblastoma SH-SY5Y cells, as determined by the fluorescent probe 2′,7′-dichlorodihydrofluoresein diacetate (Amoroso et al., 1999). α-Tocopherol provided a concentration-dependent protection against paraquat-induced cytotoxicity but did not affect H_2O_2-induced cytotoxicity. $O_2^{-}\cdot$ dismutates into H_2O_2 and then generates ·OH through the breakdown of H_2O_2 in the presence of metal ions. Thus, the present results suggest that α-tocopherol reacts with $O_2^{-}\cdot$ before the formation of ·OH. α-Tocopherol may effectively scavenge $O_2^{-}\cdot$ rather than ·OH in the striatal cultures. The present study suggests that neuroprotective effect of α-tocopherol is attributed to its ability to decrease levels of $O_2^{-}\cdot$, although the ability of α-tocopherol to scavenge both $O_2^{-}\cdot$ and ·OH has been demonstrated by electron spin resonance spectrometry (Wang and Jiao, 2000) and photometric assay (Yamaguchi et al., 2000). In addition to direct scavenging activity, α-tocopherol may up-regulate anti-oxidative molecules such as superoxide dismutase, catalase and GSH, to reduce effectively $O_2^{-}\cdot$-induced cytotoxicity in rat striatal cultures (Lopez-Torres et al., 1998).

Pretreatment with superoxide dismutase and catalase attenuates the apoptosis caused by NO donors *S*-nitrosocysteine and SIN-1 in cultured cortical neurons (Bonfoco et al., 1995). A cell-permeable superoxide dismutase mimetic also prevents NO-dependent cell death in rat motor neurons (Estevez et al., 1998). These findings suggest decreased levels of $O_2^{-}\cdot$ inhibit NO cytotoxicity by attenuating $ONOO^{-}$ formation. Accordingly, we tested whether α-tocopherol prevented NO donor-induced cytotoxicity in rat striatal cultures. α-Tocopherol significantly protected striatal cultures against cytotoxicity of *S*-nitrosocysteine and SIN-1. Together with the above results, it is suggested that the neuroprotective mechanisms of α-tocopherol against NO donor-induced cytotoxicity also involve its ability to reduce $O_2^{-}\cdot$ levels. Overall, suppression of intracellular $O_2^{-}\cdot$, not ·OH, may constitute an important mechanism of neuroprotective action of α-tocopherol in rat striatal cultures.

GSH is abundant in neurons and acts as a major cellular antioxidant. Reduction of the intracellular GSH content enhances oxidative stress and eventually results in cell death (Ibi et al., 1999; Merad-Boudia et al., 1998). BSO inhibits GSH synthesis and causes GSH depletion and neuronal death. In the present study, α-tocopherol potently inhibited BSO-induced cytotoxicity in rat cultured striatal neurons. This suggests that α-tocopherol is very effective in preventing oxidative stress triggered by deterioration of cellular functions to reduce reactive oxygen species levels. The potent effect of α-tocopherol on BSO-induced neuronal death led us to estimate that α-tocopherol possesses anti-apoptotic action, because recent studies showed that BSO caused apoptosis in rat mesencephalic cultures (Sawada et al., 2000) and a neuronal cell line (Merad-Boudia et al., 1998). Staurosporine is known to induce apoptosis in cultured rat cortical neurons (Honda et al., 2001; Koh et al., 1995) and human neuroblastoma cells (Yuste et al., 2002). Therefore, we investigated the effect of α-tocopherol on staurosporine-induced cytotoxicity in rat striatal cultures, and found that α-tocopherol showed a moderate but significant protective action on staurosporine-induced apoptosis. These results suggest that α-tocopherol at high concentrations possesses a neuroprotective action that is independent of its antioxidant/radical scavenging ability.

Recent studies revealed that α-tocopherol inhibited smooth muscle cell proliferation (Boscoboinik et al., 1991a), decreased protein kinase C (PKC) activity (Boscoboinik et al., 1991b), and increased phosphoprotein phosphatase 2A activity (Ricciarelli et al., 1998). α-Tocopherol effects on PKC inhibition have also been reported in different cell types, including platelets (Freedman et al., 1996), neutrophils (Kanno et al., 1995), and monocytes (Devaraj et al., 1996). The effect on PKC may be related to the interference on intracellular signaling of apoptosis. Moreover, α-tocopherol prevented oxidized low-density lipoprotein-mediated apoptosis by decreasing IκB degradation and nuclear factor-κB activation (Li et al., 2000), and by reducing the mitogen-activated protein kinase and Jun

kinase cascade activation and apoptotic protein of the Bcl-2 family in addition to nuclear activities of several transcription factors (De Nigris et al., 2000). Those findings suggest that a non-antioxidant property of α-tocopherol is involved in the anti-apoptotic action. The concentrations of α-tocopherol eliciting these non-antioxidant actions are approximately 10–100 μM (Boscoboinik et al., 1991a,b; de Nigris et al., 2000; Ricciarelli et al., 1998). Consistent with these studies, α-tocopherol at 10–100 μM ameliorated staurosporine-induced apoptosis in the present study. Therefore, anti-apoptotic neuroprotection by α-tocopherol may be attributable, at least partly, to a non-antioxidant property in rat striatal cultures.

α-Tocopherol was reported to prevent β-amyloid-induced toxicity in cultured rat hippocampal neurons (Goodman and Mattson, 1994) and PC12 cells (Behl et al., 1992), and glutamate-induced cytotoxicity in rat cerebellar granule cultures (Ciani et al., 1996) and a neuronal cell line (Schubert et al., 1992). In addition, vitamin E protected against impaired water maze performance resulting from treatment with AF64A, a cholinotoxin ethylcholine mustard aziridinium ion that induced oxidative stress (Wortwein et al., 1994). The present findings provide evidence that vitamin E may have a therapeutic significance for neurodegenerative disease such as Alzheimer's disease. Since vitamin E was demonstrated to have far fewer adverse effects, it would become a noteworthy nutrient or drug for treatment of neurodegenerative diseases.

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