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Protective Effects of Potassium Ion on Rotenone-Induced Apoptosis in Neuronal (Neuro 2A) Cells

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Objective : The authors investigated whether rotenone induces cellular death also in non-dopaminergic neurons and high concentration of potassium ion can show protective effect for non-dopaminergic neuron in case of rotenone-induced cytotoxicity.

Methods : Neuro 2A cells was treated with rotenone, and their survival as well as cell death mechanism was estimated using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium(MTT) assay, Lactate dehydrogenase(LDH) release assay, fluorescence microscopy, and agarose gel electrophoresis. The changes in rotenone-treated cells was also studied after co-treatment of 50mM KCl. And the protective effect of KCl was evaluated by mitochondrial membrane potential assay and compared with the effects of various antioxidants.

Results : Neuro 2A cells treated with rotenone underwent apoptotic death showing chromosome condensation and fragmentation as well as DNA laddering. Co-incubation of neuro 2A cells with 50mM KCl prevented it from the cytotoxicity induced by rotenone. Intracellular accumulation of reactive oxygen species(ROS) resulting by rotenone were significantly reduced by 50mM KCl. Potassium exhibited significantly similar potency compared to the antioxidants.

Conclusion : The present findings showed that potassium attenuated rotenone-induced cytotoxicity, intracellular accumulation of ROS, and fragmentation of DNA in Neuro 2A cells. These findings suggest the therapeutic potential of potassium ion in neuronal apoptosis, but the practical application of high concentration of potassium ion remains to be settled.

KEY WORDS : Potassium ion · Rotenone · Non-dopaminergic neuron · Apoptosis.

Introduction

Rotenone is a classical, high affinity inhibitor of mitochondrial complex I, and typically used to define the specific activity of the complex[5]. It is also used commonly as naturally occurring organic pesticide in lakes and reservoirs to kill nuisance fish. Because it is extremely lipophilic, it easily crosses biological membranes easily and independent of transporters, and it gets into the brain very rapidly[24].

Mitochondria are considered as mediator of cell death in neuro-degenerative disorders. Moreover, mitochondria are the primary mediators of cell death caused by oxidative stress and apoptosis in metabolic failure[10]. The mitochondrial-derived ROS are vital not only because mitochondrial respiratory chain components are present in almost all eukaryotic cells, but also because ROS produced in mitochondria can readily influence mitochondrial function without having to cope with long diffusion times from the cytosol. Mitochondrial-derived ROS could be modified when the mitochondrial respiratory chain was interrupted under pathological conditions or by respiratory chain inhibitors[27,28].

Inhibition of the mitochondrial respiratory chain by rotenone has been widely used to study the role of the mitochondrial respiratory chain in apoptosis[1,4]. Also, early reports showed that rotenone can induce mitochondrial ROS production and that rotenone-induced mitochondrial ROS production is closely related to rotenone-induced apoptosis[18].

High potassium ion is directly or indirectly relevant to survival of some specific neuronal cells. The definite evidences from many reports suggest that apoptotic cells actually become hypotonic when compared with their non-dying counterparts. In these reports, the nucleases and the activity of caspase-1

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are effectively inhibited at physiological K^+ concentration[3,17,29]. Thus, the transition of an apoptotic cell from a state of high ionic strength to low ionic strength permits both the loss in cell volume and the activation of enzymes that mediate apoptosis[3]. Secondary, high potassium-induced depolarization induces the protective effects against the damage of neuronal cells by various toxins. Potassium-induced depolarization increases intracellular Ca^{2+} concentration to a modest degree, which provides protection against apoptosis[8]. This action of potassium ion-induced depolarization for neuroprotection contains signaling sequences such as the activation of MAP kinase and molecule cyclic AMP-response element binding protein(CREB)[13]. And also high potassium-induced depolarization protects beta-amyloid-mediated neurotoxicity in cultured rat hippocampal neurons[22]. Cultured cerebellar granule neurons(CGC) increase survival in a medium containing 25mM KCl (25K), and they die apoptotically when cultures are transferred to a 5mM KCl containing medium (5K)[6,9]. Thirdly, cell death induces by these conditions was partially prevented when cultures were maintained under alkaline conditions. The acidification of the medium is a critical factor of cell death. Cultures transferred to 5mM K^+ suffered an immediate intracellular alkalinization and remained constant during the time 5mM K^+ was present[7]. Both 150mM KCl and alkaline pH inhibit cytochrome c-mediated activation of procaspase-3 by blocking acidification during the induction of apoptosis.

Many papers suggest that rotenone induced apoptosis in dopaminergic neuron cells. However, no report is published about any correlation of rotenone and non-dopaminergic cells. Additionally, the intracellular signaling pathway by which K^+-induced depolarization protects neuronal cells from rotenone-induced apoptosis is unclear. So, the authors performed the study to demonstrate whether rotenone induces apoptosis also in non-dopaminergic neurons. And whether potassium ion display the protective effect in non-dopaminergic neuronal (Neuro 2A) cells treated with rotenone and its mechanism were also studied.

Materials and Methods

Cell culture

Neuro-2A cells, a subclone of the C1300 murine neuroblastoma derived spontaneously in the A/J mouse(American Type Culture Collection, Manassas, VA, USA) were maintained in a humidified 37°C, 5% carbon dioxide incubator. Cells underwent serial passage as monolayers in Dulbecco's Modified Eagle Medium(DMEM)(Gibco BRL, Grand Island, NY, USA), which was supplemented with nonessential amino acids, 100IU/ml penicillin, 100㎍-ml streptomycin, 2mmol/L L-glutamine, and 10% fetal calf serum(Gibco BRL, Grand Island, NY, USA).

MTT assay

MTT(Sigma Chemical Company, St Louis, MO, USA) is a yellow-colored tetrazolium that is taken up and cleaved only by metabolically active cells, reducing it to a colored, water-insoluble formazan salt. Once the formazan has been solubilized, it can be easily and rapidly read in a conventional enzyme-linked immunosorbent assay plate reader at 595nm. The absorbance directly correlates with cell number. MTT reagent (7.5mg/ml) in phosphate-buffered saline was added (10㎕/well), and the cultures were incubated at 37°C for 30 min. The reaction was stopped by the addition of acidified triton buffer (0.1M HCl, 10%(v/v) triton X-100; 50㎕/well), and the tetrazolium crystals were dissolved with dimethyl sulfoxide(DMSO)(Sigma Chemical Company) by mixing on shaker at room temperature for 20min. The samples were measured on microplate reader(Molecular Devides Corp., Sunnyvale, CA, USA) at a test wavelength of 595nm and a reference wavelength of 650nm. Results represent the mean ± S.E. of 5 wells from one experiment that is representative of experiments repeated at least three times. The results are optical density readings expressed as a percentage of controls.

LDH release assay

To estimate cell viability, the amount of LDH released into the culture medium was measured using a Cytotoxicity Detection LDH kit(Sigma Chemical Company, St Louis, MO, USA). In this assay, 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㎕ of culture supernatant was mixed with 75㎕ of the LDH substrate mixture in a 96-well plate. After incubation for 1 h at room temperature, the reaction was stopped by addition of 100㎕ of 1 N HCl, and the absorbance was measured at 570nm using a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). The reading of background absorbance, consisting of the culture medium and each drug in the absence of cells, was subtracted from each value. The cell viability was evaluated relative to the total LDH from whole cell lysate for 48 hours.

Nuclear Morphology

Morphological study was performed by phase-contrast microscopy. After rotenone treatment, the cells were collected, washed with phosphate-buffered saline(PBS), fixed with 3.7% formaldehyde for 20 min, and incubated in 2g/ml 4',6-diamidine-2-phenylindole(DAPI)(Sigma Chemical Company,

St Louis, MO, USA) for 5 minutes. Nuclear morphology was examined using fluorescence microscopy.

Analysis of DNA Fragmentation by Agarose Gel Electrophoresis

The detection of DNA fragmentation was performed by using agarose gel electrophoresis. Cells were collected by centrifugation and washed with PBS. The washed cells were lysed in a solution of 10mM Tris-HCl, pH 8.0, 10mM EDTA, 0.5%(w/v) sodium dodecyl sulfate(SDS), and 0.1%(w/v) RNase A, with incubation for 60 minutes at 50°C The lysate was incubated for an additional 60 minutes at 50°C with 1mg/ml proteinase K and then subjected to electrophoresis for 60 minutes at 50 V in a 1% (w/v) agarose gel in 40mM Tris acetate, pH 7.5, which contained 1mM EDTA. After electrophoresis, DNA was visualized by staining with ethidium bromide.

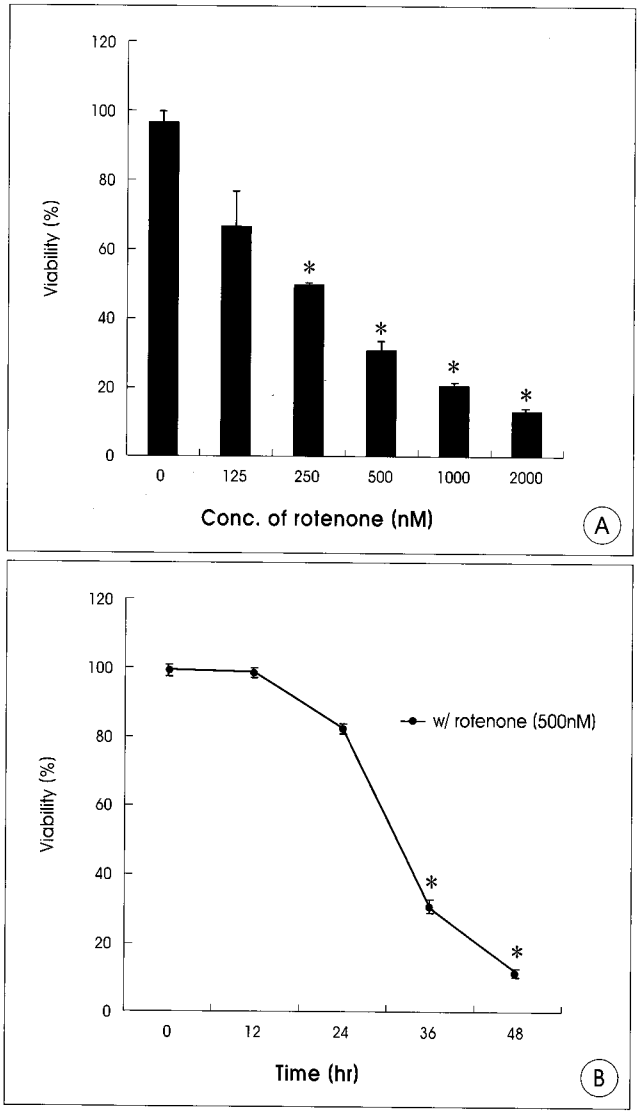

Fig. 1. Rotenone decreased the viability of Neuro 2A in dose and time-dependant manner. (A), cells are treated with various doses of rotenone for 36 h. (B), cells are treated with 500 nM rotenone for the indicated periods. Cell viability was measured by MTT assay. Results represent as the mean ± S.D. of three independent experiments ($p < 0.01$).

Fluorescent Measurement of Intracellular ROS

ROS production was assayed with 2',7'-dichloroflourescein-diacetate (DCF-DA, Alexsis, Grünberg, Germany), which is transformed by oxygen radicals and can be visualized at 488nm. Cells were grown to 80% confluence on culture slides and incubated for 1-3 h. Cells were fixed with 4% parafor-maldehyde for 20 minutes, washed in 0.1 MPBS three times for 10 minutes at 4~8°C and kept dark. The relative amount of ROS was determined by confocal laser scanning microscopy using a 256 value gray scale.

Mitochondrial membrane potential assay

The mitochondrial membrane potential of cells was measured with use of the probe 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolyl carbocyanine iodide(JC-1, Molecular Probes, Eugene, OR, USA). JC-1 is able to enter mitochondria selectively, which appears green at low concentrations or at low membrane potential as a monomer. However, at high concentrations, mitochondria shows as red fluorescent aggregates. JC-1 is sensitive to mitochondrial membrane potential and the changes in the ratio between green and red fluorescence can provide information regarding the mitochondrial membrane potential. After the treated cells were loaded with JC-1 1μ mol/L for 10min at 37°C, the fluorescent dye was excited at 490nm, and the fluorescence intensities of both monomer and aggregated molecules were recorded at 590nm under a confocal scanning laser microscopy.

Statistical analysis

Results were expressed as mean ± SD of triplicate values for each experiment. Statistical evaluation was performed by Student's t-test. $P<0.05$ was considered to be statistically significant.

Results

Rotenone induced apoptosis in mouse neuronal (neuro 2A) cells

To define the proper conditions of cell death induced by rotenone, we treated mouse neuronal (Neuro 2A) cells with various concentration (125~2000nM) for 36 hours. The loss of cell viability in culture is generally measured by the reduction of MTT activity and the release of LDH into the media. Treatment of Neuro 2A cells with rotenone caused cell death in a concentration- and time-dependent manner (Fig. 1A, B). The viability of Nuero 2A cells treated with rotenone were decreased dose-dependently. Exposure of Neuro 2A cells to

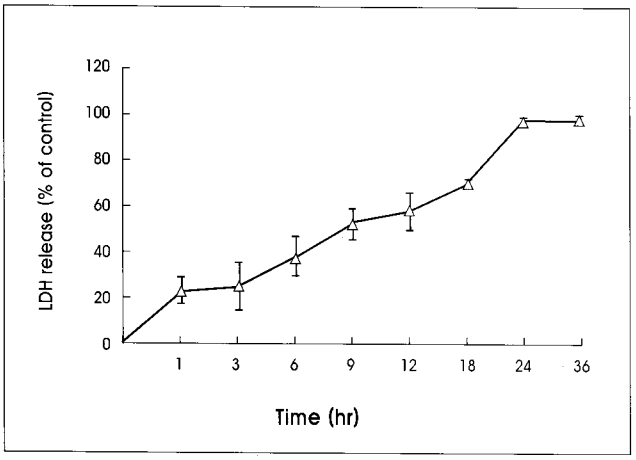

Fig. 2. Rotenone increased lactate dehydrogenase(LDH) release from Neuro 2A in a time-dependant manner. Cells are treated with 500nM rotenone for the indicated periods. Treatment with rotenone resulted in significant increase in LDH release to 96% in a time-dependent manner. Results represent as the mean ± S.D. of three independent experiments ($p < 0.01$).

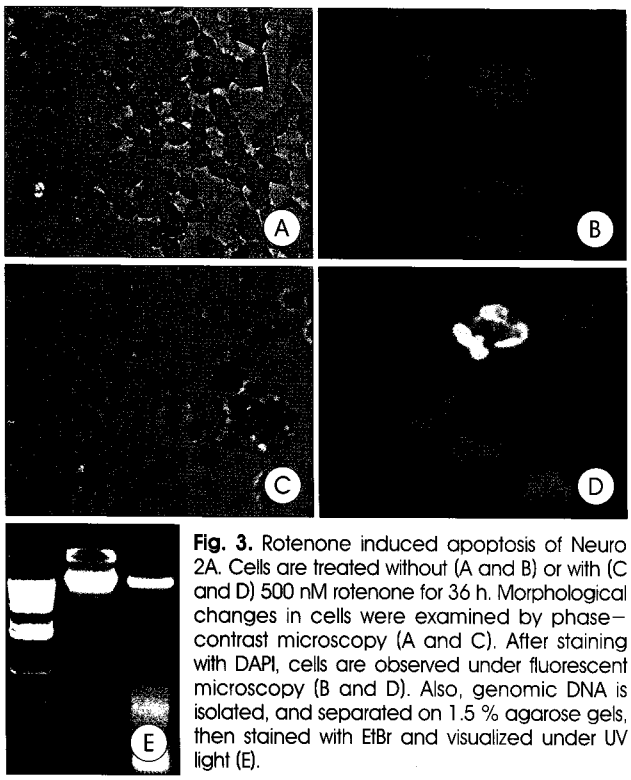

Fig. 3. Rotenone induced apoptosis of Neuro 2A. Cells are treated without (A and B) or with (C and D) 500 nM rotenone for 36 h. Morphological changes in cells were examined by phase-contrast microscopy (A and C). After staining with DAPI, cells are observed under fluorescent microscopy (B and D). Also, genomic DNA is isolated, and separated on 1.5 % agarose gels, then stained with EtBr and visualized under UV light (E).

500nM rotenone for 3 hours or less did not induce the release of LDH from the cells so much, but a pronounced LDH release occurred in following 24 hours of treatment with rotenone (Fig. 2). These data showed that rotenone also induced cell death in non-dopaminergic neuronal cells.

To define the mode of cell death induced by rotenone, we treated neuro 2A cells with 500nM rotenone and examined its effect on nuclear morphology. Cells were treated without (Fig. 3A, B) or with (Fig. 3C, D) 500nM rotenone for 36

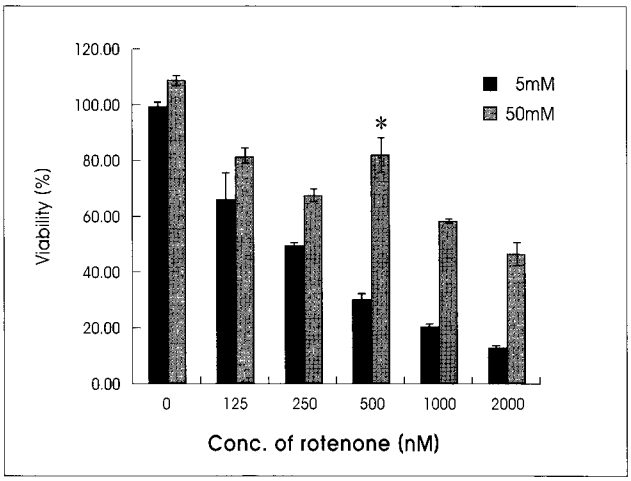

Fig. 4. Potassium ion protected rotenone-induced cytotoxicity of Neuro 2A. Cells are co-treated with 50 mM potassium ion in the presence or absence of various concentration of rotenone for 36 h. Cell viability is measured by MTT assay. Results represent as the mean ± S.D. of three independent experiments ($p < 0.01$).

hours. At 500nM rotenone, the condensation of cell bodies, nuclear fragmentation, and condensation into discrete dense chromatin clumps were occurred (Fig. 3C, D). Also, we examined rotenone induced-DNA fragmentation in mouse non-dopaminergic Neuro 2A cells. The histone-associated DNA fragmentation caused by 500nM rotenone was detected (Fig. 3E). Therefore, these are hallmarks of morphological changes associated with apoptosis. These results confirm that rotenone induces apoptosis in Neuro 2A cells.

Potassium ion protected rotenone-induced apoptosis

To define whether potassium ion protects rotenone-induced apoptosis, we treated mouse neuronal (Neuro 2A) cells with 500nM rotenone for 36 hours. The loss of cell viability in culture is generally measured by the reduction of MTT activity and the release of LDH into the media. The exposure of Neuro 2A to 500nM rotenone for 36 h resulted in a significant decrease in cell viability (Fig. 4), to 30.71% and a significant increase in LDH release to 96.57% (Fig. 5). But Nuero 2A cells co-treated with 50mM potassium ion and 500nM rotenone were increased the viability to 82.16% (Fig. 4) and decreased LDH release to 36.79% (Fig. 5).

So, in co-treatments with potassium and rotenone for 36 hours, it was observed that dose of 50mM of potassium ion was enough to protect rotenone induced apoptosis in Neuro 2A cells. Cells were treated without (Fig. 6A, B) or with (Fig. 6C, D) 500nM rotenone for 36 hours. In nuclear morphological study, cells were caused condensation of the cell body, nuclear fragmentation, and condensation into discrete dense chromatin clumps at 500nM rotenone (Fig. 6C, D), but co-treatment of cells with 50mM KCl inhibited these apoptotic hallmarks (Fig. 6E, F). And in electrophoresis, potassium ion

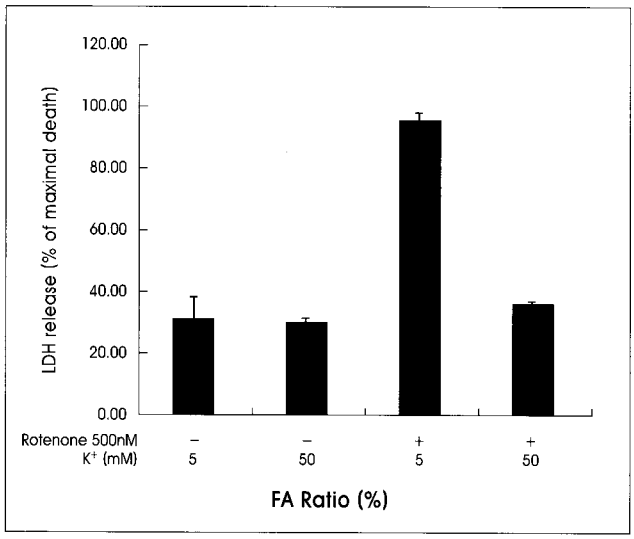

Fig. 5. Potassium ion induced the reduction of Lactate dehydrogenase (LDH) release of Neuro 2A. Cells are treated with 500nM rotenone for the indicate periods. The exposure of Neuro 2A cells to 500nM rotenone resulted in a significant increase in LDH release 96.57%, but Neuro 2A cells co-treated with 50mM potassium ion were decreased LDH release to 36.79%. Results represent as the mean ± S.D. of three independent experiments ($p < 0.01$).

blocked the histone-associated DNA fragmentation caused by 500nM rotenone (Fig. 6G). Therefore, potassium ion protects rotenone-induced apoptosis in Neuro 2A, non-dopaminergic cells.

Potassium ion inhibited rotenone-induced intracelluar ROS product of Neuro 2A cells

Neuro 2A cells were co-treated with 50mM potassium ion in the presence or absence of 500nM rotenone for 36h. After DCF-DA staining, cells were observed under fluorescent microscopy. Compared with controls (Fig. 7A, B), intracellular ROS product generated in rotenone-treated Neuro 2A cells increased markedly (Fig. 7C), and the decrease of Rotenone-induced ROS production by 50mM KCl was also noticed (Fig. 7D). To confirm the protective effect of potassium ion against rotenone-induced apoptosis by inhibiting ROS production, three antioxidants, N-acetylcysteine(NAC), glutathion(GSH), and trolox, were pretreated for 1 h in the presence or absence of 500nM rotenone. NAC is a thiol antioxidant, cysteine source for GSH synthesis[3], and trolox is a vitamin E analogue. The viability of Neuro 2A cells pretreated with antioxidants or co-treated with potassium ion were significantly higher than the rotenone untreated cells (Fig. 8). Therefore, potassium ion protected rotenoneinduced apoptosis through inhibition of ROS production such as antioxidants.

Potassium ion inhibited rotenone-induced a loss of membrane potential in mouse Neuro 2A cells

To define whether potassium ion inhibits rotenone-induced transition in mitochondria permeability and significant loss of membrane potential, Neuro 2A cells were co-treated with potassium ion in the presence or absence of 500nM rotenone for 36 hours. After JC-1 staining, cell were observed under fluorescent microscopy. Untreated control cells exhibited numerous, brightly stained mitochondria that emitted red-orange fluorescence, which was indicative of normal high membrane potential (Fig. 9A). Cells were co-treated 50mM potassium ion in the absence of 500nM rotenone (Fig. 9B). But treatment with rotenone induced a transition in mitochondrial permeability and a significant loss of membrane potential noticed by color change (Fig. 9C). However, cotr-

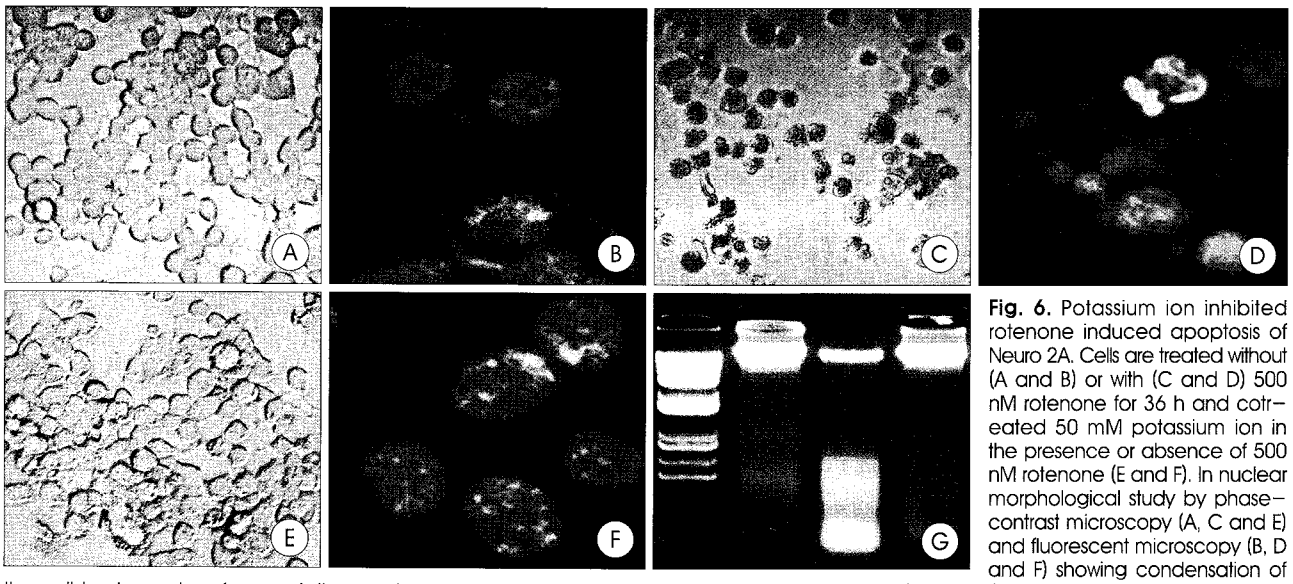

Fig. 6. Potassium ion inhibited rotenone induced apoptosis of Neuro 2A. Cells are treated without (A and B) or with (C and D) 500 nM rotenone for 36 h and cotr-eated 50 mM potassium ion in the presence or absence of 500 nM rotenone (E and F). In nuclear morphological study by phase-contrast microscopy (A, C and E) and fluorescent microscopy (B, D and F) showing condensation of the cell body, nuclear fragmentation, and condensation into discrete dense chromatin clumps (C and D) and inhibition these apoptotic hallmarks (E and F). And in electrophoresis, potassium ion blocked the histone-associated DNA fragmentation caused by 500nM rotenone (G).

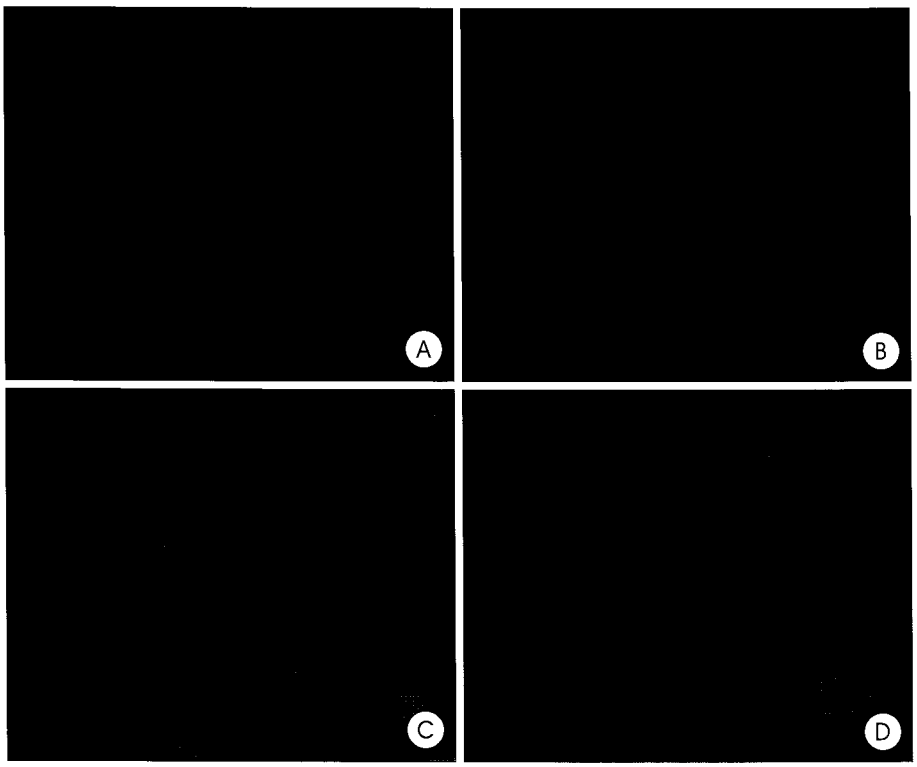

Fig. 7. Potassium ion inhibited rotenone induced intracellular ROS production of Neuro 2A. Cells are treated without (A) or with (C) 500nM rotenone for 36 h and co-treated 50mM potassium ion in the absence (B) or presence (D) of 500nM rotenone. After staining with 4',6-diamidine-2-phenylindole (DAPI) and 2',7'-dichloro fluorescein diacetate(DCF-DA), compared with controls (A and B), intracellular ROS product generated in rotenone-treated Neuro 2A cells increasing markedly (C), and decreasing by 50 mM KCl (D).

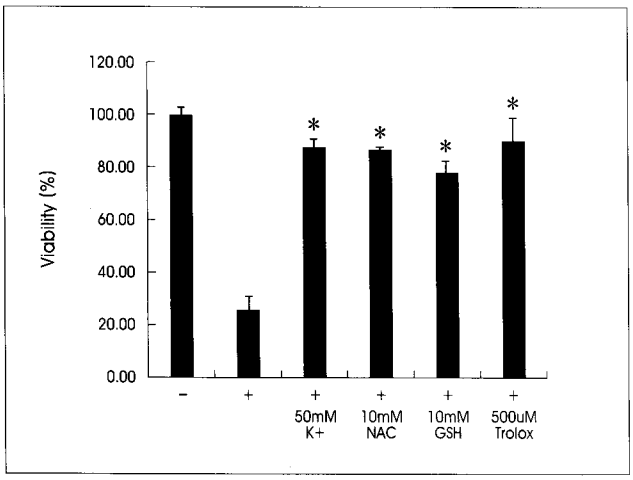

Fig. 8. Antioxidants protected rotenone-induced cytotoxicity of Neuro 2A. The viability of Neuro 2A cells pretreated with antioxidants or co-treated with potassium ion are significantly higher than the rotenone untreated cells. Results represent as the mean ± S.D. of three experiments ($p<0.01$).

eatment of 50mM potassium ion prevented the collapse of mitochondrial membrane potential induced by rotenone in Neuro 2A cells (Fig. 9D). Potassium ion gradually resumed the mitochondrial membrane potential at 50mM concentrations as indicated via reappearance of red mitochondrial staining.

Discussion

In this study, it was demonstrated for the first time that rotenone, known to inhibit mitochondrial complex I, induces apoptosis in non-dopaminergic neuroblastoma, Neuro 2A cells. Previously, many studies reported that rotenone induced α-synuclein[12], Bad dephosphorylation[31], caspase-9 activation, and ultimately apoptosis in human dopaminergic SH-SY5Y cells[15,25]. Some papers showed rotenone-induced apoptosis in non-dopaminergic cells except Neuro 2A cell[14,15,19]. However, the application of rotenone to induce in non-dopaminergic cells such as Neuro 2A still is not reported.

Here, new information were presented about rotenone-induced in Neuro 2A cells, which is different from a model of nigral degeneration in Parkinson's disease. In this study, it was demonstrated that rotenone-induced cell death with condensation of the cell body and nuclear fragmentation in N-euro 2A cells (Fig. 3). ROS production was detected as one of apoptotic factors in rotenone-treated cells (Fig. 7C). Some reports also suggested that the mechanism of rotenone-induced apoptosis is mainly connected with intracellular ROS production[1,4,11]. In addition, rotenone induced a transition in mitochondria permeability and a significant loss of membrane potential in Neuro 2A cells (Fig. 9C). Although the mechanism of rotenone-induced apoptosis is still elusive, severe concomitant events, which induces shutdown of the electron transfer through respiratory chain complex I, decreasing cellular ATP level, increasing mitochondrial ROS production, and decreasing mitochondrial membrane potential, appeared in various cells. The decrease of mitochondrial membrane potential and the opening of the mitochondria permeability transition pore, but not ATP reduction, have been shown to be involved in apoptosis[1,4,11]. In current study, the role of rotenone-induced mitochondrial ROS production has not been fully investigated, therefore it has to be still proven that intracellular ROS may be a crucial factor of apoptotic pathway in Neuro 2A cells. Also, other studies obtained similar results in cortical neuronal cells following exposure with rotenone[21].

It was also demonstrated that high potassium ion has sig-

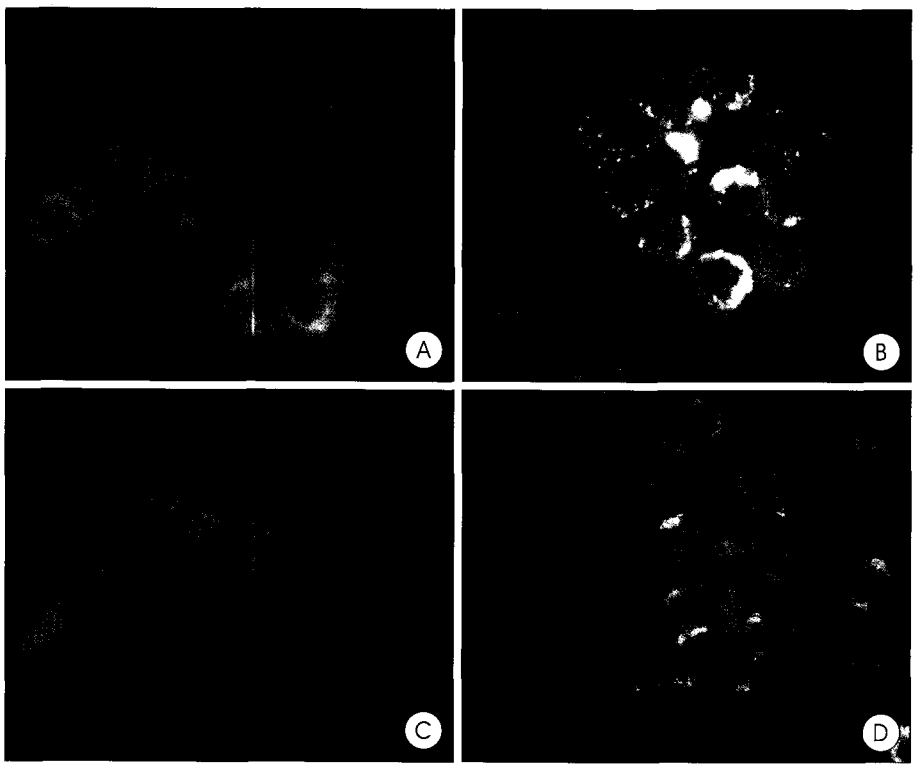

Fig. 9. Potassium ion inhibited rotenone-induced a loss of membrane potential in mouse neuronal (neuro 2A) cells. Cells are treated without (A) or with (C) 500 nM rotenone for 36 h and co-treated 50 mM potassium ion in the absence (B) or presence (D) of 500 nM rotenone. Compared with normal high membrane potential(A and B), treatment with rotenone inducing a transition in mitochondrial permeability and a significant loss of membrane potential noticed by color change (C). However, co-treatment of 50 mM potassium ion preventing the collapse of mitochondrial membrane potential induced by rotenone (D) under fluorescent microscopy after staining with JC-1.

nificant neuroprotective effects against apoptosis induced by rotenone in Neuro 2A cells. Potassium ion at 50mM showed protective effect against rotenone-induced cytotoxicity in non-dopaminergic Nuero 2A cells (Fig. 4, 5, 6). Moreover, rotenone-induced intracellular ROS production was inhibited by the addition with high potassium ion (Fig. 7D). Potassium ion at 50mM protects on rotenone-induced apoptosis via the decease of LDH release and the inhibition of condensation of the cell body and nuclear fragmentation and intracellular ROS production in Neuro 2A cells. Potassium ion also inhibited the collapse of mitochondrial membrane potential induced by rotenone in Neuro 2A cells and gradually resumed the mitochondrial membrane potential with 50mM concentrations as indicated via the reappearance of red mitochondrial staining (Fig. 9D). The observation that antioxidants cause to inhibit rotenone-induced dopaminergic[20] and non-dopaminergic neuronal death in our experiment (Fig. 8) suggests that intracellular ROS production plays a crucial role in the fate of neuronal cells[16,26]. Though it has still to be elucidated the exact protective mechanism of potassium ion against rotenone-cytotoxicity of Neuro 2A cells, it is speculated that high potassium ion may interact with the prosurvival signaling by high ionic strength[3,17] or deporlarization[22] or control the alkaline condition of culture media pH[6,9]. It is also speculated that high potassium ion stimuli may initiate a synthesis of protein in defensive system against oxidative stress and affect on activation of voltage gated potassium channel or ATP-sensitive potassium channels in mitochondria (mitoK(ATP) channels)[20,23].

Taken together, these results have provided new information to be taken into consideration in the development of a good therapeutic target. Nevertheless, the defensive mechanism of potassium in rotenone-mediated cell death and the side effects from treatment of high potassium are still to be determined in further experiments.

Conclusion

Considerable evidences of apoptosis of non-dopaminergic Neuro 2A cells by rotenone was showed. In this study, we also demonstrated that high potassium ion inhibits rotenone-induced apoptosis in mouse neuronal (neuro 2A) cells. High potassium ion induced cellular survival signs, such as the decease of LDH release and the inhibition of condensation of the cell body, nuclear fragmentation, and intracellular ROS production in Neuro 2A cells. Also, potassium ion inhibited rotenone-induced loss of membrane potential in neuro 2A cells. These results suggest that in Neuro 2A cells, potassium ion protects rotenone-induced apoptosis via inhibition of intracellular ROS prodution and mitochondrial permeability transition.

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Commentary

The authors examined the neuroprotective effects of potassium ion in a in vitro model of rotenone-induced apoptosis using mouse neuronal cells. To define the cell viability, they used the 3-(4,5-Dimethythiazol-2-yl)-2,5-diphenyltetrazolium assay and the lactate dehydrogenase release assay. Cell viability was markedly reduced by exposure to rotenone in a concentration and time-dependent manner. Morphological changes of apoptosis in mouse neuronal cells by the exposure to 500nM rotenone for 36 hours, were clearly demonstrated. Co-treatment with 50mM KCl and 500nM rotenone inhibited the apoptotic hallmarks such as condensation of cell body, nuclear fragmentation, and condensation into discrete dense chromatin clumps. To evaluate the protective action of potassium ion, the cells were observed under fluorescent micrroscopy using DCF-DA or JC-1staining. Reactive oxygen species(ROS) product increased by exposure to rotenone was significantly reduced by the treatment with 50mM KCl. Potassium ion prevented the collapse of mitochondrial membrane potential induced by retenone and gradually resumed the motochondrial membrane potential.

In rotenone-induced apoptotic cells, there have been observed various events such as shut-down of electron transfer through respiratory chain complex 1, cellular ATP decrease, increase of mitochondrial ROS production, decrease of mitochondrial membrane potential, and opening of mitochondrial permeability transition pore[1-3]. Alterations of ionic fluxes through ion transporters either could be directly responsible for the early phase of apoptosis or lead to other alterations such as changes in cell volume or intracellular Ca^{++} and pH. Apoptotic death of cortical neurons is associated with an enhancement of K^+ current that lead to a total loss of intracellular K^+[4]. Also, the blockade of the K^+ efflux with elevated extracellular K^+ markedly reduces neuronal apoptosis[4]. Although the exact role of K^+ is unknown, it is clear that K^+ may play a critical role in apoptosis. This study well demonstrated the neurop-

rotective effects of potassium ion in rotenone-induced apoptosis using mouse neuronal cells. In vitro study cited by the authors suggest that neuroprotection can be provided by application of high concentration of potassium ion in view of ROS production and mitochondrial membrane potential.

Even though this study can provide new information for the development of experimental model for a good therapeutic target, it is still limited to know the further important infor-mation for the crucial factor of rotenone-induced apoptotic pathway and the protective mechanism of high potassium ion. In addition, in vivo situation is always more complex. Multiphasic dose-responsiveness of KCl on apoptotic cell should be further investigated to evaluate the therapeutic potential of potassium ion suitable to neuroprotection.

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