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Protective effect of geranylgeranylacetone against hydrogen peroxide-induced oxidative stress in human neuroblastoma cells

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ABSTRACT

Aims: Heat shock protein 70 (HSP70), one of the major HSPs, has been reported to suppress apoptosis and formation of pathogenic proteins in neurodegenerative disorders. Geranylgeranylacetone (GGA), an anti-ulcer drug, induces HSP70 and thereby protects against cellular damage in various diseases. We investigated the effect of GGA on hydrogen peroxide (H_2O_2)-induced neurotoxicity in human neuroblastoma SH-SY5Y cells.
Main methods: H_2O_2-induced neuronal toxicity was measured by a CCK-8 assay and Hoechst 33342 staining. We also assessed oxidative stress and apoptosis by measuring reactive oxygen species (ROS) generation with 2′,7′-dichlorofluorescein diacetate (DCFH-DA), caspase-3 activity, and mitogen-activated protein kinase (MAPK) pathway.
Key findings: GGA showed a concentration-dependent inhibition on H_2O_2-induced apoptotic cell death. H_2O_2-induced induction of HSP70 was enhanced by GGA pretreatment. GGA effectively suppressed the up-regulation of Bax and down-regulation of Bcl-2. GGA also blocked the H_2O_2-induced phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2). In addition, GGA attenuated H_2O_2-induced ROS generation and caspase-3 activity.
Significance: These results demonstrate that GGA protects SH-SY5Y cells from H_2O_2-induced apoptosis, at least in part by enhancing HSP70 production. Neuroprotective properties of GGA indicate that this compound may be a potential therapeutic agent for the treatment and prevention of neurodegenerative diseases.
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1. Introduction

Reactive oxygen species (ROS) are mitochondrial by-products of normal cell respiration and highly reactive molecules that contain one or more unpaired oxygen atoms [1]. Oxidative stress occurs as a result of an imbalance between ROS formation and the capacity of antioxidant defenses [2] and is widely implicated in neuronal cell death which is associated with a variety of chronic neurodegenerative disorders such as Parkinson's disease [3]. Oxidative stress-induced neuronal cell death is involved in necrosis and apoptosis, the form of death being dependent upon the severity of oxidative insult [4].

Heat shock proteins (HSPs) compose a part of tightly regulated systems for maintenance of cellular homeostasis during normal cell growth and for survival after detrimental environmental stresses [5]. Previous studies have shown that HSPs not only protect cells from stress but also suppress apoptosis [6]. These proteins have been classified in four families according to molecular sizes such as HSP90, HSP70, HSP60, and small HSPs. Among these, HSP70 is the best characterized endogenous factor involved in protecting cells, tissues, and organs from injury under various pathological conditions in both in vivo and in vitro experimental models [7]. Furthermore, HSP70 has been suggested to be a promising molecule for controlling apoptosis. Oxidative stress down-regulates HSP70 expression, and overexpression of HSP70 can inhibit oxidative stress-induced release of second mitochondria-derived activator of caspases (SMAC) from mitochondria, activation of caspase-9 and -3, and apoptosis [8].

Geranylgeranylacetone (GGA), also known as teprenone, has been used as an anti-ulcer drug in Japan for more than 20 years with no major adverse effects. Previous studies have shown that GGA can exert cytoprotective action against various stresses on a variety of cells and tissue [9], and additionally suppress hydrogen peroxide (H_2O_2)-induced apoptosis in osteoarthritic chondrocytes [10] and gastric mucosal cells [11]. The authors of these investigations reported that the protective action of GGA is associated with induction of HSP70.

In this study, the protective effect of GGA against H_2O_2-induced apoptotic cell death was investigated in SH-SY5Y cells.

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2. Materials and methods

2.1. Cell culture and treatments

SH-SY5Y cell was purchased from American Type Culture Collection (Manassas, VA, USA) and grown in a 1:1 mixture of Dulbecco's Modified Eagle Medium and Nutrient F-12 medium (DMEM/F-12; WELGENE, Daegu, Korea) with 10% fetal bovine serum (Hyclone, Logan, UT, USA) and 1% antibiotic–antimycotic (GIBCO, Grand Island, NE, USA) at 37 °C in a humidified atmosphere with 5% CO_2. Cells were pretreated with GGA (Sigma, St. Louis, MO, USA) for 1 h, and then exposed to H_2O_2.

2.2. Cell viability assay

Cell viability was assessed by Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan). Cells were grown on 96-well plates and treated with chemicals. After 24 h, 5% CCK-8 was added to each well and incubated at 37 °C for 3 h. Absorbance at 450 nm was measured by a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

2.3. Measurement of apoptotic cell death

Cells were fixed using 4% paraformaldehyde at room temperature for 30 min. After washing with PBS, the cells were stained in the dark with Hoechst 33342 dyes (Sigma, 1 μg/ml in PBS) for 20 min. The morphological change was examined under UV illumination under a fluorescence microscope. The dye was excited at 340 nm, and emission was observed through a 510 nm barrier filter. To quantify the apoptotic process, cells with fragmented or condensed DNA and normal DNA were counted. Data were shown as apoptotic cells as a percentage of the total cells.

2.4. Western blot analysis

Cells were lysed in RIPA buffer containing a protease inhibitor. Protein content was measured by Lowry's method [12] with bovine serum albumin as the standard, and separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred electrophoretically to a poly-vinyl difluoride (PVDF) membrane (Thermo Scientific, Rockford, IL, USA). The membranes were blocked with 5% skim milk, and then probed with primary antibodies [HSP70, Bax, P38 (Santa Cruz, CA, USA), Bcl-2, Beta-actin (Abcam, Cambridge, MA, USA), extracellular signaling-regulating kinases (ERK1/2), phospho-ERK1/2, SAPK/JNK, phospho-SAPK/JNK, phospho-P38 MAPK (Cell Signaling, Danvers, MA, USA)] overnight at 4 °C. After being rinsed with PBS-T, the membranes were incubated for 1 h with secondary antibodies. The protein bands were visualized using Chemi-Doc (Micro Chemi 4.2; DNR Bio-imaging System, Jerusalem, Israel) with a chemiluminescence reaction (Santa Cruz).

2.5. Detection of ROS generation

Intracellular ROS production was evaluated with 2′,7′-dichlorofluorescin diacetate (DCFH-DA; Sigma). Cells wer seeded in a black 96-well plate for 24 h, and then incubated with GGA for 1 h. DCF-DA (20 μM) in medium without serum was added directly to each well, and the plate was incubated at 37 °C for 30 min. After washing using PBS, 100 μM H_2O_2 was added to each well. The formation of fluorescent dichlorofluorescein (DCF) due to oxidation of DCFH in the presence of ROS was read after every 10 min at an excitation wavelength of 480 nm and an emission wavelength of 520 nm using a fluorescence microplate reader (Molecular Devices).

2.6. Caspase-3 activity

Caspase-3 activity was measured using a caspase-3 colorimetric assay (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's

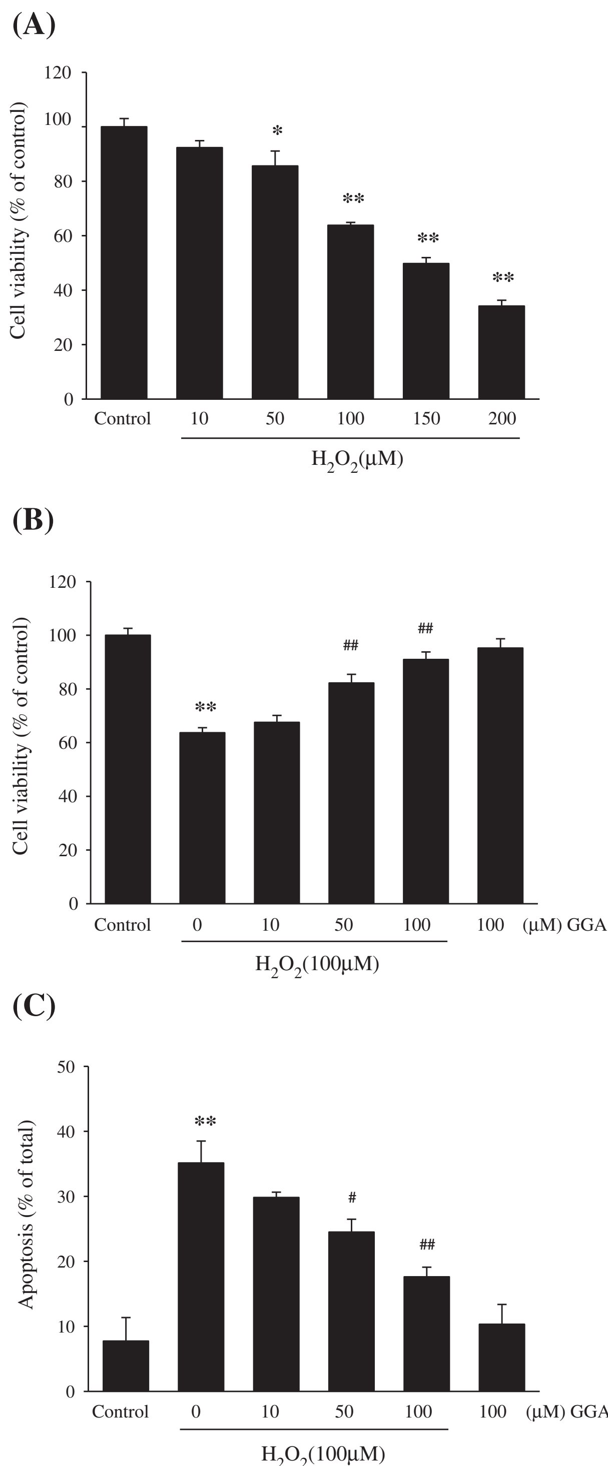

Fig. 1. Effects of GGA on cell viability in H_2O_2-treated SH-SY5Y cells. (A) H_2O_2 dose-dependently decreased cell viability. Cell viability was determined by MTT assay. The absorbance of non-treated cells was regarded as 100%. (B) GGA dose-dependently reversed the H_2O_2-induced decrease in cell viability. (C) Apoptotic cells measured by Hoechst 33342 staining were counted from five to six fields per well. The apoptosis rate was assessed by the ratio of apoptotic nuclei/total nuclei. Values are expressed as mean ± S.E.M. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to control cells. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ compared to H_2O_2-treated cells.

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