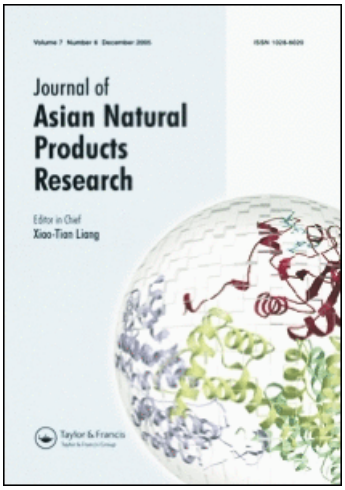

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Daidzin protects PC12 cells from serum deprivation-induced apoptosis

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DAIDZIN PROTECTS PC12 CELLS FROM SERUM DEPRIVATION-INDUCED APOPTOSIS

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This article examines the effect of daidzin on PC12 cell apoptosis induced by serum-free medium. PC12 cell survival was measured by MTT assay. The DNA content and percentage of apoptosis were monitored by flow cytometry and DNA fragmentation was analyzed by agarose gel electrophoresis. The results showed that serum-free (12 h) medium induced apoptosis in PC12 cells. When the cells had been treated with daidzin (0.1, 1 μM) for 12 h, the percentage of PC12 cell apoptosis was significantly decreased to 12.21 and 4.24% from 91.94% in the group with serum deprivation, and DNA fragmentation was prevented. Daidzin (0.01–10 μM) attenuated the cytotoxic effect of sodium cyanide (20 mM), glutamate (0.5 mM) and sodium nitroprusside (0.5 mM) in a manner dependent on concentration. The results suggested that daidzin prevented PC12 cell from serum free-induced apoptosis.

INTRODUCTION

Pueraria lobata is a Chinese traditional medicine used for the treatment of coronary heart disease in clinics [1]. Daidzin is one of the pueraria isoflavonoids, which are known to have antioxidant properties and scavenge lipid peroxidation [2]. However, basic information on the action of daidzin in neuroprotective effects is scarce.

The use of PC12 cells to investigate neuronal apoptosis holds much promise for future studies, as with these cells, it is possible to obtain large homogeneous cell populations to facilitate molecular and biochemical analysis [3]. In the present study, we observed the effect of daidzin on PC12 cell apoptosis induced by serum-free medium. Moreover, the effects of daidzin on sodium cyanide (NaCN)-, glutamate (Glu)- and sodium nitroprusside (SNP)-induced cytotoxicity were also examined.

RESULTS AND DISCUSSION

Our study revealed that daidzin prevented PC12 cell apoptosis when these cells are treated *in vitro* with serum-free medium using flow cytometry and DNA agarose gel electrophoresis. It was demonstrated that NaCN-, Glu- or SNP-induced PC12 cell damage was attenuated by daidzin pretreatment in a dose-dependent manner.

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Effects of Daidzin on NaCN-, Glu- and SNP-induced Cytotoxicity in PC12 Cells

The exposure of PC12 cells to NaCN (20 mM), Glu (0.5 mM) or SNP (0.5 mM) for 20 min and subsequent incubation with serum-free medium for 24 h can produce an obvious decrease in the cell viability, as assayed by MTT. When PC12 cells pretreated with daidzin (0.01 – 10 μM) for 30 min, and then exposed to NaCN, Glu or SNP, the cell damage was greatly attenuated (Table I).

Flow Cytometry Assay

When treated with serum-free medium, the percentage of apoptosis cells with fractional DNA content was 91.94% and a large amount of debris was observed, indicating a pronounced degree of cell disruption. When daidzin was added to serum-free treated cells, at concentrations of 0.1 and 1 μM, the percentage of apoptosis cells was significantly reduced (12.21 and 4.24%, respectively, Fig. 1).

DNA Fragmentation

Detection of internucleosomal DNA fragmentation provides a sensitive means to monitor early events of apoptosis. PC12 cell cultures were deprived of serum and different concentrations of daidzin (0.1 and 1 μM) were added. After 12 h incubation, DNA fragmentation was observed to be prevented. In untreated cells, spontaneous DNA fragmentation was observed, as evidence of the characteristic "DNA ladder" profile that was considered the hallmark of apoptosis (Fig. 2).

TABLE I Effect of daidzin on NaCN-, Glu- or SNP-induced cytotoxicity in PC12 cells. Cells in serum-free medium were pretreated with or without different concentration (0.01 – 10 μM) of daidzin for 30 min and then exposed to NaCN (20 mM), Glu (0.5 mM) or SNP (0.5 mM) for 20 min

Drug (μM)	A_{570}	*Inhibitory rate* (%)
Control	1.34 ± 0.13	
NaCN	0.79 ± 0.079*	
Daidzin 0.01 + NaCN	0.83 ± 0.089†	7.27
Daidzin 0.1 + NaCN	0.94 ± 0.091†	27.27
Daidzin 1 + NaCN	1.03 ± 0.11‡	43.64
Daidzin 10 + NaCN	1.11 ± 0.10¶	58.18
Control	1.32 ± 0.12	
Glu	0.83 ± 0.090*	
Daidzin 0.01 + Glu	0.87 ± 0.080†	8.16
Daidzin 0.1 + Glu	0.98 ± 0.11†	30.61
Daidzin 1 + Glu	1.09 ± 0.13‡	44.89
Daidzin 10 + Glu	1.19 ± 0.12¶	61.22
Control	1.36 ± 0.12	
SNP	0.87 ± 0.080*	
Daidzin 0.01 + SNP	0.91 ± 0.071†	8.16
Daidzin 0.1 + SNP	0.99 ± 0.10†	24.49
Daidzin 1 + SNP	1.08 ± 0.068‡	42.85
Daidzin 10 + SNP	1.14 ± 0.072¶	55.10

$n = 4$ (4 wells in each experiments). Means ± standard deviation.
* $P < 0.01$ vs. control group.
† $P > 0.05$ vs. model group.
‡ $P < 0.05$ vs. model group.
¶ $P < 0.01$ vs. model group.

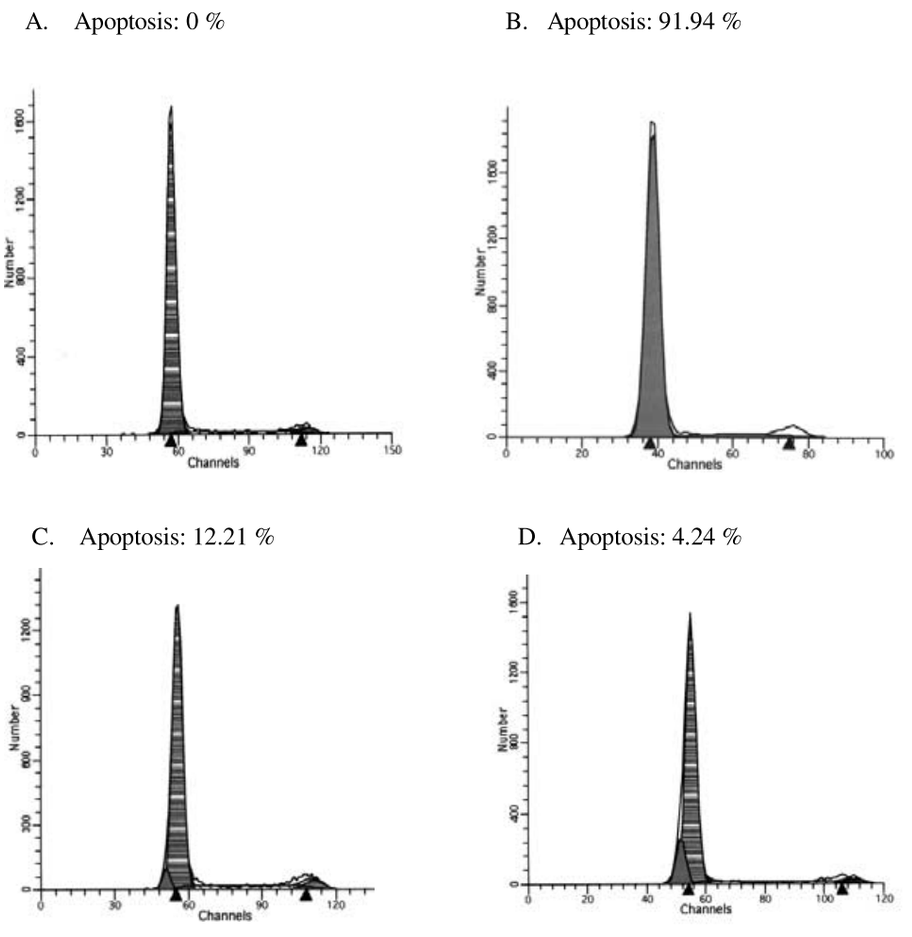

FIGURE 1 Flow cytometric assay of PC12 cells: (A) control; (B) serum-free medium; (C) serum-free medium plus daidzin 0.1 μM; (D) serum-free medium plus daidzin 1 μM.

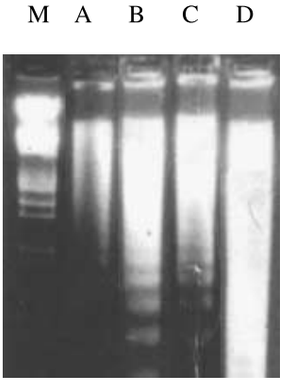

FIGURE 2 Effect of daidzin on prevention of serum-free induced DNA internucleosomal cleavage in PC12 cells. Lane M, molecular weight; Lane A, control; Lane B, cells incubated serum-free medium for 12 h; Lane C, cells incubated serum-free medium and daidzin 0.1 μM; Lane D, cells incubated serum-free medium and daidzin 1 μM.

Apoptotic neuronal cell death occurs normally during development and may also occur in a variety of neurological disorders [4]. The development of strategies to arrest apoptotic death of neurons may, therefore, be used to ameliorate a number of neurological afflictions [5]. Flavonoids, which are widely distributed in the plant, have been reported to have strong antioxidant and free radical scavenging effects. Puerariae isoflavone (500, 1000 mg/kg × 7 d, po) significantly antagonized the increase of water, Ca^{2+} and DNA contents and the attenuation of Ca^{2+}-ATPase and SOD activity in the brain of rats treated with repeated brief ischemia–reperfusion, and markedly prolonged respiratory duration after decapitation in mice [2]. Our results are consistent with these studies.

In conclusion, daidzin inhibited the NaCN-, Glu- or SNP-induced decrease in cell viability, and prevented apoptosis of PC12 cells. Although the exact mechanism by which daidzin acts remains unknown, it is suggested that the antioxidant and free radical scavenging effect may be involved in its neuroprotective action. Future experiments conducted using primary cultured neurons and animal models would be worthwhile and might provide new insights.

EXPERIMENTAL SECTION

Material

Daidzin was prepared and furnished by Prof. Zi-Da Min (Department of Phytochemistry, China Pharmaceutical University), then dissolved in dimethyl sulfoxide (Me_2SO). The concentration of Me_2SO in the final culture media was $\leqslant 0.1\%$ and had no toxic effect on PC12 cells. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Fluka, and Dulbecoo's modified Eagle's medium (DMEM) was the product of Gibco. All other chemicals were of analytical grade.

Cell Culture

The PC12 cell line was obtained from the Shanghai Institute of Cell Biology. The cells were cultured at 37°C in a humidified CO_2 (5%) incubator in DMEM containing 10% heat-inactivated fetal bovine serum, 100,000 U/l penicillin and 100 mg/l streptomycin. Cells were used when in exponential growth. For the experiments in serum-free medium, cells were washed three to four times with serum-free DMEM. Cells (5×10^4 cells in 100 μl medium each well) in a 96-well plate were used for MTT assay. For DNA fragmentation and flow cytometry, cells (5×10^6 cells in 2 ml medium each well) were grown in 6-well plates.

Viability Assay

Cell viability was measured using MTT assay [6]. PC12 cells were treated with different concentrations of daidzin (0.01–10 mM) for 30 min, then NaCN (20 mM), Glu (0.5 mM) or SNP (0.5 mM) was added to each well. After 10–20 min, the medium was separated from the wells and washed three times. The fresh serum-free medium was appended and incubated at 37°C for 20 h then MTT solution (0.5 g/l) was added. After incubation for an additional 4 h, the formazan crystals were dissolved by 150 μl of Me_2SO. The optical density at 570 nm was then measured using an ELISA plate reader (Hua Dong Electronic Company, Nanjing, China).

Flow Cytometry

At 12 h, following withdrawal of the serum support the PC12 cells were harvested for flow cytometry [7]. Briefly, the cells were fixed in 70% ethanol at 4°C overnight. Subsequently, cells were treated with Tris–HCl buffer (pH 7.4) containing 1% RNase A and were stained with propidium iodide (PI) (5 mg/l). The distribution of cells with different DNA contents was determined by flow cytometry (Facscalibur, Becton Dickinson, USA) and the data were analyzed by multicycle DNA content and cell cycle analysis software (Modfit LT 2.0).

DNA Fragmentation

Experiments were performed as described by Troy and Shelanski [8]. In brief, PC12 cells were washed and plated in serum-free DMEM medium with or without indicated additives. After incubation for 12 h at 37°C, fragmented DNA was analyzed by electrophoresis. The cellular DNA was extracted, dialyzed, electrophoresed in 1.2% agarose gel, and visualized under UV light after staining with ethidium bromide.

Statistics

Data were expressed as means ± standard deviation of n experiments. A statistical difference was analyzed using Student's t-test.

References

[1] Fan, L.L., Keefe, D.D. and Powell, W.J. (1984), *Acta Pharm. Sin.* **19**, 801–807.
[2] Yu, Z.L., Zhang, G.Q. and Zhao, H.Q. (1997), *J. Chin. Pharm. Univ.* **28**, 310–312.
[3] Mesner, P.W., Winters, T.R. and Green, S.H. (1992), *J. Cell. Biol.* **119**, 1669–1680.
[4] Sastry, P.S. and Rao, K.S. (2000), *Neurochemistry* **74**, 1–20.
[5] Stefanis, L., Park, S.D., Yan, C.Y.I., Farinelli, S.E., Troy, C.M., Shelanski, M.L., *et al.* (1996), *J. Biol. Chem.* **271**, 30663–30671.
[6] Ding, Q.L., Zhang, G., Liu, G.Q. and Huang, W.L. (1999), *Chin. Pharm. J.* **34**, 742–745.
[7] Darzynkiewicz, Z., Bino, S.B.G. and Gorczyca, W. (1992), *Cytometry* **13**, 795–799.
[8] Troy, C.M. and Shelanski, M.L. (1994), *Proc. Natl Acad. Sci. USA* **91**, 6384–6387.