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# Desipramine antagonized corticosterone-induced apoptosis in cultured PC12 cells

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**KEY WORDS** desipramine; antidepressive agents; corticosterone; PC12 cells; apoptosis

## ABSTRACT

**AIM**: To study possible action mechanism of a tricyclic antidepressant, desipramine (DIM). **METHODS**: Cultured PC12 cells were exposed to corticosterone in the absence or presence of DIM for 5 d. Agarose gel electrophoresis, flow cytometry, and electron microscopy were used to detect the apoptosis of PC12 cells. **RESULTS**: Corticosterone 10 μmol/L treatment for 5 d elicited typical apoptotic biochemical and morphological changes including condensed chromatin shaped like crescent moon, nuclear fragmentation, and DNA degradation. The highest percentage of apoptotic cells accumulated to 28 % ± 9 %. Agarose gel electrophoresis showed typical DNA ladders pattern. While in the presence of DIM 1 or 5 μmol/L, apoptosis percentage was markedly decreased with lightened DNA ladder and ultrastructure of the cells was improved. **CONCLUSION**: DIM could antagonize the apoptosis in PC12 cells induced by corticosterone, which may be one of the cellular mechanisms of its antidepressant effect.

## INTRODUCTION

Tricyclic antidepressants (TCA) were widely used for treatment of depressive disorders and the curative effects were satisfying, but their biochemical mechanism was not clear.

Clinical study found that hypothalamic-pituitary-adrenal (HPA) axis of the patients in depression was hyperactive and the serum glucocorticoids (GC) were increased[1], which caused hypercorticism. There were receptors that mediated the effects of GC in the brain and the GC receptors expression in hippocampus were the highest[2]. As a result of high concentration of GC in serum, the GC receptors in hippocampus would be down-regulated, subsequently, the feed-back of hippocampus on HPA axis was defected and the high concentration of GC in serum would be maintained[3]. It was well known that hippocampus was the key brain region which regulated emotion, behavior, learning, and memory, *etc*. Many studies indicated that the occurance of depression was related to the selective lesion of hippocampus induced by GC[4], but the lesion process and mechanism in details were not clear. TCA not only up-regulated the GC receptors and normalized the feed-back of HPA axis, but also increased the expressions of neurotrophic factors such as nerve growth factor (NGF)[3,5], so it could finally improve the depression syndrome.

*Differentiated* PC12 cell line, a clonal cell line of the rat adrenal pheochromocytoma, processed typical characters of neurons, furthermore, the expression of GC receptors in PC12 cells membrane was abundent[6]. So, we simulated chronic lesion condition of brain neurons with high concentration of corticosterone and detected the protective effect of desipramine (DIM) on the apoptosis of PC12 cells induced by corticosterone.

## MATERIALS AND METHODS

**Drugs and reagents** DIM (Sigma, USA) was dissolved in distilled water at 0.01 mol/L; corticosterone (Sigma, USA) was dissolved in 95 % ethanol at 0.01 mol/L; DMEM was from GIBCO, USA.

**PC12 cells culture and treatment** The pheochromocytoma cells (PC12) was kindly presented by Dr WAN You in Peking University. Cells were planted in 24 well plates or 50 mL plastic culture flask (Costar, USA) at a density of $2 \times 10^8$/L in the growth medium consisted of 90 % DMEM, 5 % heat-inactivated horse serum, 5 % fetal calf serum, benzylpenicillin 200 kU/

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L, and streptomycin 100 mg/L. Incubation was carried out at 37 ℃ in atmosphere with 5 % $CO_2$ (humidified incubator, Napco, USA) for 4 – 5 d and the culture medium was renewed every 2 – 3 d. The cells were treated with corticosterone 10 μmol/L and cultured for 3, 4, and 5 d, respectively. In DIM-treated groups the cells were treated with corticosterone 10 μmol/L and DIM 1 or 5 μmol/L and cultured for 5 d.

**DNA extraction** PC12 cells were seeded onto 50 mL plastic culture flask. After 5 d, cells were harvested, washed with PBS buffer, and resuspended in 2.5 mL PBS, then 2.5 mL solution Ⅰ (containing Tris 10 mmol/L, pH 7.6, KCl 10 mmol/L, $MgCl_2$ 10 mmol/L) and 60 μL NP-40 were added. The sample were mixed fully and centrifuged at 250 × *g* for 10 min. The pellets were resuspended in 400 μL solution Ⅱ (Tris 10 mmol/L, pH 7.6, KCl 10 mmol/L, $MgCl_2$ 10 mmol/L, SDS 0.5 %, edetic acid 2 mmol/L, NaCl 0.5 mol/L). Tris-hydroxybenzene 200 μL was added, and the sample were mixed fully and centrifuged at 6400 × *g* for 10 min at 4 ℃, then the upper portion of water solution was collected and blended with 100 μL Tris-hydroxybenzene as well as 100 μL mixture of chloroform:isoamyl alcohol (24:1, v/v). The solution was centrifuged at 6400 × *g* for 10 min at 4 ℃, then the upper portion was collected, mixtured with 350 μL chloroform: isoamyl alcohol (24:1, v/v), and centrifuged again. The supernatant was gathered, then DNA was precipitated by double volume of ethanol and more than 1/20 volume of acetic acid sodium (3 mol/L, pH 5.2) at – 20 ℃ for at least 12 h, and DNA precipitates were recovered by centrifugation at 7500 × *g* for 20 min. After washing with 70 % ethanol, the DNA was dissolved in 20 μL TE buffer (containing Tris 10 mmol/L and edetic acid 1 mmol/L, pH 8.0) and restored at – 20 ℃.

**Gel electrophoresis** DNA sample 20 μL was loaded on 1.5 % horizontal agarose gels with bromophenol blue/cyanol xylene tracking dyes. Gels were run at 70 V for 45 min submerged in TAE buffer (Tris 40 mmol/L, acetic acid 20 mmol/L, and edetic acid 1 mmol/L), stained with ethidium bromide 0.5 mg/L, and photographed under UV light. *Eco*R Ⅰ and *Hind* Ⅲ restriction digest of lambda DNA was used as molecular size marker.

**Flow cytometry** The cells were removed from the 24-well plates and transfered to 2 mL plastic tubes, then centrifuged at 400 × *g* for 5 min and washed with PBS twice. After resuspending in 1 mL cold 70 % ethanol, the cells were incubated at 4 ℃ for 24 – 48 h, then centrifuged at 900 × *g* for 10 min. After washing, the cells were resuspended in RNase A solution containing RNase A 200 mg/L and incubated at 37 ℃ for 30 min. Then 1 mL PBS was added into each tube and the sample was centrifuged once more at 1300 × *g* for 10 min. The cells were protected from light and stained with 300 μL propidium iodide solution 10 mg/L at 4 ℃ for 30 min. Using Epics XL flow cytometry (Coulter, USA), $2 \times 10^4$ cells were counted and the percentage of apoptotic cells was detected.

**Electron microscopy** Cells were planted in 50 mL plastic culture flask. In the absence or presence of DIM 1 and 5 μmol/L, corticosterone was added in the DMEM medium at the final concentration of 10 μmol/L. Incubation were carried out at 37 ℃, 5 % $CO_2$ for 5 d. Cells were collected into 1.5 mL EP tubes and fixed with 4 % paraformaldehyde for 5 min. The sections were prepared and photographed under PHILIPS 400T Electron microscopy (USA).

## RESULTS

***Effects of DIM on DNA fragmentation in corticosterone treated PC12 cells*** Cells treated with corticosterone 10 μmol/L for 3, 4, and 5 d, respectively, showed typical DNA ladders pattern in a time-dependent manner which indicated the occurance of apoptosis (Fig 1). After DIM 1 and 5 μmol/L treatment, DNA ladders were lightened (Fig 2).

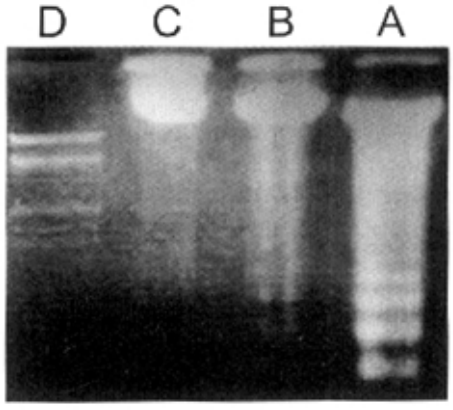


**Fig 1. Agarose gel electrophoresis of DNA fragmentation in corticosterone 10 μmol/L-treated PC12 cells for 5 d (A), 4 d (B), and 3 d (C). λDNA *Eco*R Ⅰ/*Hind* Ⅲ marker (D).**

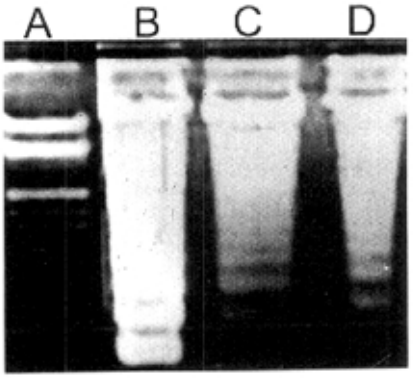


**Fig 2. Effect of DIM on DNA fragmentation in corticosterone treated PC12 cells. (A) DNA marker; (B) Corticosterone 10 μmol/L; (C): Corticosterone + DIM 1 μmol/L; (D) Corticosterone + DIM 5 μmol/L. PC12 cells were treated for 5 d and the DNA fragmentation was detected by agarose gel electrophoresis.**

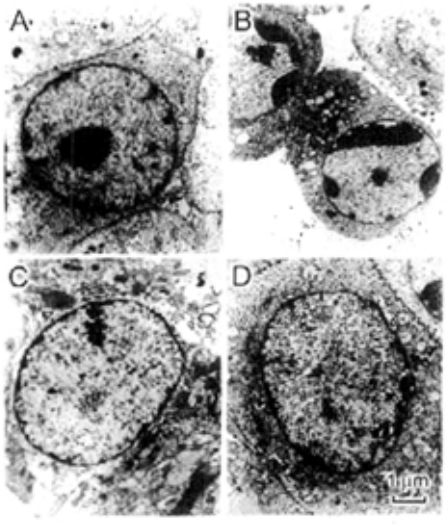


**Fig 3. Effect of DIM on ultrastructure of corticosterone treated PC12 cells. (A) Control; (B) Corticosterone 10 μmol/L; (C) Corticosterone + DIM 1 μmol/L; (D) Corticosterone + DIM 5 μmol/L. PC12 cells were treated for 5 d before examination by electron microscopy. × 10000.**

**Effect of DIM on percentage of apoptotic cells** Cells exposed to corticosterone 1, 3, and 10 μmol/L for 5 d showed apoptosis. DIM 1 and 5 μmol/L decreased the accumulation of the apoptotic cells in sub-G1 peak compared with corticosterone 10 μmol/L treated group (Tab 1).

**Tab 1. Effect of DIM on the percentage of apoptotic cells induced by corticosterone. $n = 3-5$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* corticosterone group.**

| Groups | Concentration/μmol·L$^{-1}$ | Apoptotic cells/% |
|---|---|---|
| Normal | – | 1.3 ± 0.8 |
| Corticosterone | 10 | 28 ± 9 |
| + DIM | 1 | 10.3 ± 1.7$^c$ |
| + DIM | 5 | 11.7 ± 2.6$^c$ |

PC12 cells were exposed to corticosterone 10 μmol/L with or without DIM for 5 d and the percentage of cells in sub-$G_1$ peak was measured by flow cytometry.

**Effect of DIM on ultrastructure of corticosterone-treated PC12 cells** After the exposure to corticosterone 10 μmol/L for 5 d, a great deal of PC12 cells showed typical morphological characteristics of apoptosis: appearance of fragmented chromatin and the apoptotic bodies (Fig 3B). In DIM 1 and 5 μmol/L treated group, cells almost had not any pathologic changes described above (Fig 3C, D), indicating that DIM could antagonize the apoptosis induced by corticosterone.

## DISCUSSION

Former studies used with MTT method indicated that corticosterone in high concentration caused injury of cultured PC12 cells, while traditional antidepressants protected cells from the lesion through increasing the expression of neurotrophic factors. But the biochemical mechanism in detail was still not clear. In fact, necessary suggestions were given by the studies on stress-induced defect of immune system. It was reported that stress, starvation, or low blood sugar could lead to the increase of serum corticosterone which induced apoptosis in thymus lymphocytes in rats[7]. Chronic social stress or corticosterone injection could bring out the apoptosis in neutrophils or spleen lymphocytes, while glucocorticoid receptor (GR) antagonist RU486 could intercept this action which indicated the involvement of GR[8], but the biochemical mechanism was still unknown.

Our studies found that DIM, a tricyclic antidepressant, antagonized corticosterone induced apoptosis in PC12 cells directly. The former research that DIM raised the NGF mRNA in PC12 cells provided evidence of its anti-apoptotic effect. These studies offered a better foreground for deepening the mechanism of research of antidepressants and drug screening.

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## REFERENCES

1 Barden N. Modulation of glucocorticoid receptor gene expression by antidepressant drugs. Pharmacopsychiatry 1996; 29: 12-22.
2 McEwen BS, De Kloet ER, Rostene W. Adrenal steroid receptors and actions in the nervous system. Physiol Rev 1986; 66: 1121-88.
3 Heuser I. The hypothalamic-pituitary-adrenal system in depression. Pharmacopsychiatry 1998; 31: 10-3.
4 Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW. Hippocampal atrophy in recurrent major depression. Proc Natl Acad Sci USA 1996; 93: 3908-13.
5 Nibuya M, Nestler EJ, Duman RS. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. J Neurosci 1996; 16: 2365-72.
6 Anderson DJ, Michelsohn A. Role of glucocorticoids in the chromaffin neuron developmental decision. Int J Dev Neurosci 1989; 7: 475-87.
7 Collier SD, Wu WJ, Pruett SB. Endogenous glucocorticoids induced by a chemicalstressor(ethanol) cause apoptosis in the spleen in B6C3F1 female mice. Toxicol Appl Pharmacol 1998; 148: 176-82.
8 Sendo F, Kato T, Yazawa H. Modulation of neutrophil apoptosis by psychological stress and glucocorticoid. Int J Immunopharmacol 1997; 19: 511-6.

## 地昔帕明对皮质酮诱导培养的 PC12 细胞凋亡的拮抗作用

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**关键词** 地昔帕明；抗抑郁药；皮质酮；PC12 细胞；细胞凋亡

**目的**：探讨三环类抗抑郁剂的作用机制. **方法**：运用 DNA 电泳法、流式细胞仪法、电镜法检测了皮质酮诱导的 PC12 细胞凋亡并观察了去甲丙米嗪(DIM)的效应. **结果**：皮质酮 10 μmol/L 处理 5 d 可显著诱导 PC12 细胞凋亡，凋亡发生率最高达 (28±9) %，DNA 电泳图谱则表现出典型的梯状条带，DIM 的 1 和 5 μmol/L 使凋亡发生率明显降低并使梯状条带变浅、减轻，细胞的超微结构也有明显改善. **结论**：DIM 对皮质酮诱导的 PC12 细胞凋亡有拮抗作用，这可能是其抗抑郁效应的细胞机制之一.

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