Desipramine antagonized corticosterone-induced apoptosis in cultured PC12 cells

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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 increased1, which caused hypercorticism. There were receptors that mediated the effects of GC in the brain and the GC receptors expression in hippocampus were the highest2. 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 maintained3. It was well known that hippocampus was the key brain region which regulated emotion, behavior, learning, and memory, etc. Many studies indicated that the occurrence of depression was related to the selective lesion of hippocampus induced by GC4, but the lesion process and mechanism in details were not clear. TCA not only up-regulated the GC receptors and normalized the feedback of HPA axis, but also increased the expressions of neurotrophic factors such as nerve growth factor (NGF)5, so it could finally improve the depression syndrome.

Differentiated PC12 cell line, a clonal cell line of the rat adrenal pheochromocytoma, possessed typical characters of neurons, furthermore, the expression of GC receptors in PC12 cells membrane was abundant6. 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 x 10^5/L in the growth medium consisted of 90 % DMEM, 5 % heat-inactivated horse serum, 5 % fetal calf serum, benzylpenicillin 200 KU/
L, and streptomycin 100 mg/L. Incubation was carried out at 37 °C in atmosphere with 5% CO₂ (humidified incubator, Napco, USA) for 4 to 5 d and the culture medium was renewed every 2 to 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, 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, MgCl₂ 10 mmol/L) and 60 μL NP-40 were added. The sample was 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, MgCl₂ 10 mmol/L, SDS 0.5%, edetic acid 2 mmol/L, NaCl 0.5 mol/L). Tris-hydroxybenzene was added, and the sample was mixed fully and centrifuged at 6400 × g for 10 min at 4 °C, then the upper portion of water solution was collected and blended with 100 μL Tris-hydroxybenzene as well as 100 μL mixture of chloroform; isomyl alcohol (24:1, v/v). The solution was centrifuged at 6400 × g for 10 min at 4 °C, then the supernatant was collected, mixed with 350 μL chloroform; isomyl 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 °C 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 solution 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 °C.

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 transferred 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 °C 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 °C 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 °C for 30 min. Using Epics XL flow cytometry (Coulter, USA), 2 × 10⁶ 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 was carried out at 37 °C, 5% CO₂ 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 occurrence 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).
Tab 1. Effect of DIM on the percentage of apoptotic cells induced by corticosterone. $n = 3 - 5$. $x \pm s$. $P < 0.01$ vs corticosterone group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Concentration/μmol·L$^{-1}$</th>
<th>Apoptotic cells/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>-</td>
<td>1.3 $\pm$ 0.8</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>10</td>
<td>26 $\pm$ 9</td>
</tr>
<tr>
<td>+ DIM</td>
<td>1</td>
<td>10.3 $\pm$ 1.7$^a$</td>
</tr>
<tr>
<td>+ DIM</td>
<td>5</td>
<td>11.7 $\pm$ 2.6$^c$</td>
</tr>
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</table>

PC12 cells were exposed to corticosterone 10 μmol/L with or without DIM for 5 d and the percentage of cells in sub-G1 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$^{[5]}$. 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.
REFERENCES

1 Barden N. Modulation of glucocorticoid receptor gene expression by antidepressant drugs. Pharmacopsychiatry 1996; 29: 12–22.