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Dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) inhibit the apoptosis in human peripheral blood lymphocytes

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Abstract

Background: Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS) are the major steroid hormones secreted by the adrenal gland. Administration of DHEA has been reported to have beneficial effects on aging, diabetes, and atherosclerosis. Apoptosis is a normal physiologic process that occurs during embryonic development as well as in the maintenance of tissue homeostasis. In this study, we examined the suppressive effect of DHEA(S) on staurosporine-induced apoptosis in human peripheral blood lymphocytes (PBL). *Methods*: Apoptosis was induced in human PBL with staurosporine and measured by flow cytometry utilizing Annexin V and propidium iodide (PI) staining. The quantity of FITC+/PI− cells corresponded to early apoptosis, while that of FITC+/PI+ cells corresponded to late apoptosis or secondary necrosis. *Results*: The fraction of staurosporine-induced early apoptosis but not that of secondary necrosis in PBL was reduced by the treatment with either DHEA or DHEAS. Furthermore, this apoptosis was neither associated with androgen receptor (AR) nor with estrogen receptor (ER). *Conclusions*: This is the first study showing that DHEA(S) inhibits apoptosis in human PBL through a mechanism independent of either ARs or ERs. DHEA(S) may be a promising chemopreventive drug for aging, diabetes, and atherosclerosis.

Keywords: DHEA; Apoptosis; Human peripheral blood lymphocytes; Aging; Atherosclerosis

1. Introduction

Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS) are the major steroid hormones secreted by the adrenal gland. Serum DHEA and DHEAS levels peak in humans during their twenties, after which the levels decrease gradually [1]. Administration of DHEA has been reported to have beneficial effects on aging, diabetes, and atherosclerosis [2–4]. It is not clear whether DHEA exerts effects directly or after conversion to androgens or estrogens because a steroid receptor for DHEA has not been identified and the hormone is generally considered to exert its effects via conversion to steroid metabolites with estrogenic or androgenic activity [5,6]. However, a previous study demonstrated that murine T cells and human T cells may have a specific receptor for DHEA [7,8]. Many hormones, including DHEA and DHEAS, can affect apoptosis. Apoptosis is a normal physiologic process that occurs during embryonic development as well as helps in the maintenance of tissue homeostasis or in the prevention of carcinogenesis. Some authors have reported the protective effect of DHEA against apoptosis in mice or rats [9]; however, the effects of DHEA on chemical-induced apoptosis in human cells have not yet been reported. In this study, we examined the suppressive effect of DHEA(S) on staurosporine-induced apoptosis in human PBL.

2. Materials and methods

2.1. Reagents

DHEA, DHEAS, staurosporine, tamoxifen, flutamide, and foetal bovine serum (FBS) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Sodium phosphate dibasic-anhydrous, sodium phosphate monobasic-anhydrous, and NaCl were purchased from Wako pure Chemical Industries (Tokyo, Japan), Ficoll-Paque was purchased from Amersham Pharmacia Biotech (Uppsala, Sweden), and RPMI 1640 was purchased from Invitrogen Corp. (Carlsbad, CA, USA). Annexin V-FITC and propidium iodide (PI) were purchased from Pharmingen (San Diego, CA, USA).

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2.2. Blood collection and lymphocyte preparation

Informed consent was obtained from eight healthy, non-smoking male volunteers, ranging between 24 and 55 years of age. PBL were isolated from 5 ml of venous whole blood collected in a 15 ml polypropylene conical tube (Becton Dickinson, Franklin Lakes, NJ, USA). The whole blood was diluted 1:1 with 1× phosphate-buffered saline (PBS: 58 mm sodium phosphate dibasic-anhydrous, 17 mm sodium phosphate monobasic-anhydrous, 68 mm NaCl). The mixture was layered over 3 ml Ficoll-Paque, separated by density centrifugation, and washed twice with 1× PBS. Cells were diluted to 1×10^6 cells/ml with RPMI 1640 supplemented with 10% FBS. The samples were placed on culture plates and incubated in a humidified chamber at 37 °C in 5% CO_2. Then the cell cultures were incubated for 24 h with four concentrations (0.01, 0.1, 1, and 10 μM) of DHEA or DHEAS. Tamoxifen (100 nm, an estrogen receptor blocker) or flutamide (100 nm, an androgen receptor blocker) was added 1 h prior to the treatment with 100 nm DHEA or DHEAS. Apoptosis in PBL was induced by staurosporine after 24 h of incubation with DHEA(S) (1 μM, 3 h).

2.3. Flow cytometry

After culturing them for 24 h for the induction of apoptosis, 100 μl aliquots of cell suspension (1×10^5 cells) were labeled fluorescently by adding 5 μl of Annexin V-FITC and 2 μl of PI (50 μg/ml) to each sample for the detection of apoptotic and necrotic cells. Samples were mixed gently and incubated at room temperature in the dark for 15 min. Analysis was carried out using a FACScan (Becton Dickinson, Franklin Lakes, NJ, USA), and a minimum of 10,000 cells within the gated region were analyzed.

2.4. Assessment of apoptosis and necrosis

Phosphatidylserine externalization of apoptosis was determined by two-color flow cytometric analysis of Annexin V-FITC binding and PI uptake using the FACScan flow cytometer as described above. The proportions of FITC+/PI− cells corresponds to early apoptosis and that of FITC+/PI+ cells corresponds to late apoptosis or secondary necrosis (Fig. 1).

2.5. Data analysis

All data are presented as mean ± S.E.M. Statistical analysis were performed using Fisher's PLSD test ; $P = 0.05$ was defined as significant.

3. Results

3.1. DHEA and DHEAS inhibit the early stage apoptosis in PBL

The results of the measurements of apoptosis using flow cytometry are given in Figs. 2 and 3. These include eight sets of data for each experiment. In the samples of PBL with or without staurosporine (1 μM, 3 h), the percentages of the early apoptotic cells (Annexin V-FITC+/PI−) were 9.2 and 22.8%, respectively, and those of secondary necrosis (Annexin V-FITC+/PI+) were 13.3 and 13.1%, respectively. When the cells were treated with DHEA (0.01, 0.1, 1, and 10 μM), the percentages of the early apoptotic cells were 19.7, 19.8, 17.8, and 16.5%, respectively, and those of secondary necrotic cells were 13.5, 13.2, 13.1, and 12.1%, respectively (Fig. 2(a)). When the cells were treated with DHEAS (0.01, 0.1, 1, 10 μM), the percentages of the early apoptotic cells were 18.7, 18.4, 18.4, and 16.8%, respectively, and those of secondary necrotic cells were 13.5, 14.0, 13.1, and 15.7%, respectively (Fig. 3(a)). These results show that with the cells treated either with DHEA or DHEAS, the percentage of the early apoptotic cells was significantly decreased in a dose-dependent manner while the percentage of secondary necrotic cells exhibited no significant change.

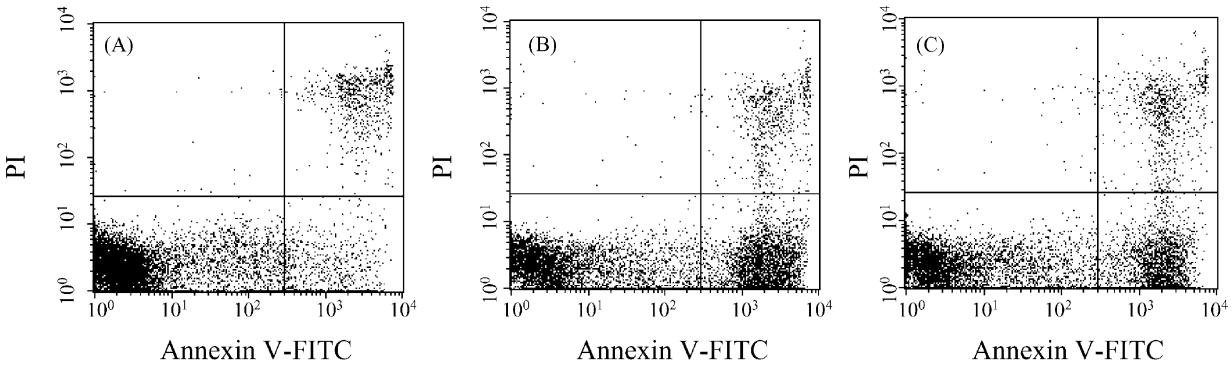

Fig. 1. Representative analysis by two-parameter Annexin-V FITC/PI flow cytometry of PBL. The proportion of FITC+/PI− cells corresponds to early apoptosis, and that of FITC+/PI+ cells corresponds to late apoptosis or secondary necrosis. (A) Control. (B) Incubation with staurosporine (1 μM, 3 h). (C) Pre-incubation with DHEA (10 μM, 24 h).

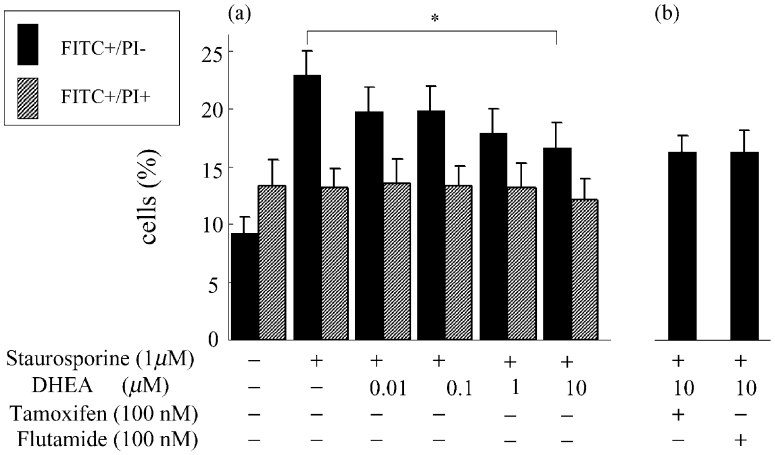

Fig. 2. (a) Effects of DHEA on staurosporine-induced apoptosis in human PBL. After 24 h of incubation with or without DHEA, cells were exposed to staurosporine (1 μM, 3 h). (b) Tamoxifen or flutamide was added 1 h prior to the treatment with 10 μM DHEA. Samples are scored by flow cytometory. Data points are the mean ± S.D. $*P < 0.05$.

3.2. Tamoxifen and flutamide do not block the inhibitory effect of DHEA(S)

There was no significant difference in the percentage of early apoptotic cells among DHEA-treated with or without added tamoxifen or flutamide. When the cells were treated with DHEA (10 μM) alone, with additional tamoxifen (100 nM), or with flutamide (100 nM), the percentages of the early apoptotic cells were 16.5, 16.2, and 16.2%, respectively (Fig. 2(a) and (b)). When the cells were treated with DHEAS alone, with additional tamoxifen (100 nM), or with flutamide (100 nM), the percentages of the early apoptotic cells were 16.8, 16.8, and 16.3%, respectively (Fig. 3(a) and (b)). The effects of DHEA(S) were neither blocked by the steroid hormone antagonist tamoxifen nor by flutamide.

4. Discussion

Flow cytometric evaluation of apoptosis is based on the expression of Annexin V in combination with PI. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet phospholipid-binding protein that has a high affinity for PS, and binds to cells with exposed PS. Externalization of PS occurs in the earlier stages of apoptosis and thus Annexin V-FITC staining can identify apoptosis at an earlier stage. Cells that are in the early stage of apoptosis are Annexin V-FITC positive and PI negative. Cells that are both Annexin V-FITC and PI positive are either in the late stage of apoptosis or secondary necrosis [10–13].

Our results showed that DHEA(S) decreases the number of apoptotic cells at an earlier stage. The protective effects

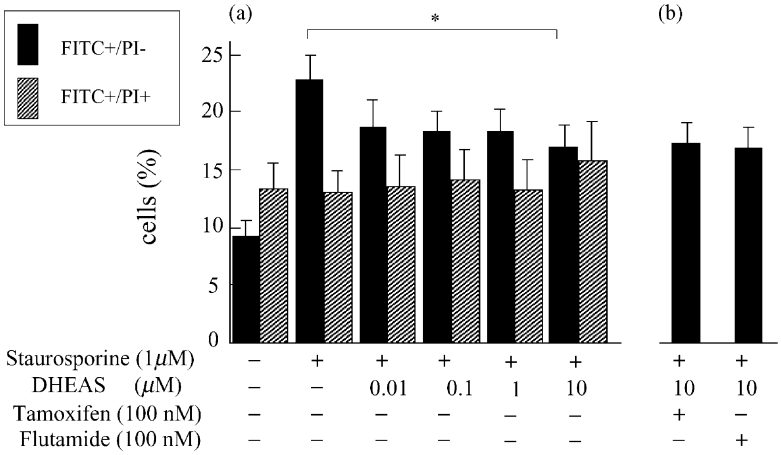

Fig. 3. (a) Effects of DHEAS on staurosporine-induced apoptosis in human PBL. After 24 h of incubation with or without DHEAS, cells were exposed to staurosporine (1 μM, 3 h). (b) Tamoxifen or flutamide was added 1 h prior to the treatment with 10 μM DHEAS. Samples are scored by flow cytometory. Data points are the mean ± S.D. $*P < 0.05$.

of DHEA(S) were dose-dependent. It is well recognized that daily plasma levels of DHEA is 0.001–0.03 μM, and that of DHEAS is 2–6 μM. In this study, we have demonstrated that DHEA(S) decrease the number of apoptotic cells in 10 μM. This is above the daily concentrations. However, it is not shown that daily plasma levels of these steroids have no beneficial effects. The apoptotic cells gradually decrease depending on the concentration; it means the high plasma levels of DHEA(S) have beneficial effect in patients with apoptosis-induced disease. Furthermore, apoptosis in human PBL induced by staurosporine was not associated with ARs or ERs. To our knowledge, this is the first study showing that DHEA(S) inhibits apoptosis of human PBL through a mechanism independent of either ARs or ERs.

Recent data suggest that not only DHEA(S) but also many hormones (growth hormone, IGF-1, estrogen), inhibit apoptosis, and they are considered to act through specific receptors [14–16]. However, the detailed molecular mechanism and signaling pathways of these effects of DHEA(s) have not yet been elucidated. While DHEA is enzymatically metabolized to androgens and estrogens, it is not clear whether DHEA exerts its effects directly or after conversion to these hormones. The results presented here showed that the staurosporine-induced early apoptosis of human PBL, but not that of secondary necrosis, was reduced by treatment with either DHEA or DHEAS.

DHEA is reported to have beneficial effects, those reduces proliferation of human aortic smooth muscle cells, and improves cellular immune function, after inhibiting apoptosis. Furthermore, DHEA may have beneficial effect in patients with atherosclerosis, immunodeficiency disease or inflammatory disease [17–19].

In conclusion, DHEA(S) decrease apoptosis in human PBL through a mechanism independent of ARs or ERs, and may be promising chemopreventive drugs for aging, diabetes, and atherosclerosis.

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