Korean J. of Pharmacology
Vol. 32, No. 1, pp. 113~123, 1996

Inhibitory Role of Polyamines in Dexamethasone-induced Apoptosis of Mouse Thymocytes

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ABSTRACT

It has been well known that polyamines ensure the stability of chromatin structure and the fidelity of DNA transcription. This study was carried out to evaluate the effect of polyamines on the apoptosis of mouse thymocytes induced by dexamethasone and polyamine synthesis inhibitors.

1) In the histological death findings of thymocytes double-stained with acridine orange and ethidium bromide, the apoptotic and the necrotic fractions (AF; NF) in the control group were 9.4±4.2 % and 4.5±5.3 %, respectively. Dexamethasone (3×10^{-8} M: DX) in creased AF upto 52.0± 8.1 % and did not change NF, but A23187 (5×10^{-7} M: A2) increased AF and NF upto 45.0±8.9 % and 20.5±10.6 %, respectively.

2) The thymocyte viability was significantly reduced by DX, DHEA (1×10^{-4} M), A2, DFMO (1×10^{-4} M), and MGBG (1×10^{-4} M), respectively. It was, however, little affected by aminoguanidine (1×10^{-4} M: AG), putrescine (1×10^{-5} M: PT), spermidine (1×10^{-5} M: SD), and spermine (1×10^{-5} M: SM).

3) The genomic DNA of mouse thymocyte was markedly fragmented by DX and A2, respectively, and to a lesser extent, by DHEA, but was little affected by MGBG, DFMO, AG, and each of polyamines.

4) The DX induced reduction of thymocyte viability was moderately attenuated by DHEA, but little affected by DFMO, MGBG, and AG. However, SM significantly attenuated the viability reduction induced by A2 as well as DX.

5) The thymocyte viability reduction by MGBG and DFMO was significantly attenuated by only SM among three polyamines applied in this study.

6) The thymocyte viability redution by combined treatments of DX with DFMO and MGBG, respectively, was significantly attenuated by SM, and moderately by PT. But the viability reduction by combined treatment of DX with AG or DHEA was not affected by polyamines.

These results suggest that polyamines, particularly spermine, might play the inhibitory role in thymocyte apoptosis and the inhibitory effect can be ascribed in part to the increase of polyamine uptake by thymocytes pretreated with DFMO and MGBG.

Key Words: Thymocytes, Apoptosis, Dexamethasone, Polyamine, Spermine, DHEA, A23187, DFMO, MGBG, Aminoguanidine

Abbreviations: DHEA; dehydroepiandrosterone, DFMO; α-DL-difluoromethylornithine, MGBG; methylglyoxal bis(guanylhydrazone) dihydrochloride

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INTRODUCTION

Apoptosis is known to be the active and gene-regulatory programmed death of cells (Kerr and Harmon, 1991; McConkey *et al.*, 1994), which accounts for the maintenance of homeostasis of cells as well as to the morphogenesis during development (Ellis *et al.*, 1991; Schwartz and Osborne, 1993; White *et al.*, 1994). Apoptosis can be found in various physiological processes of selective cell deletion such as embryogenesis, cellular differentiation, aging, metamorphogenesis, atrophy of certain cells due to the deficiency of hormones, and physiologic turnover of normal cells, especially the negative selection in the thymus to remove self-reactive lymphocytes (Goya, 1986; Kerr and Harmon, 1991; Compton and Cidlowski, 1994).

Thymocyte apoptosis can be induced by various external stimuli including glucocorticoids (Wyllie, 1980), Ca^{2+} ionophores (McConkey *et al.*, 1989a), certain toxins including dioxin (McConkey *et al.*, 1988), TCR/CD3 signaling and Fas/Fas-ligand interaction (Smith *et al.*, 1989), low dose radiation (Sellins and Cohen, 1987), TNF (Laster *et al.*, 1988) and lymphotoxins and cytotoxins (Schmid *et al.*, 1986; Liu *et al.*, 1987).

The progenitor cells committed to the T cell lineage are derived from stem cells in the bone marrow, selectively migrate to the thymus, in which some of them proliferates and differentiate into mature T cells in the thymus (von Boehmer, 1988; van Ewijk, 1991). But more than 95% of the immature thymocytes found in the thymus cortex undergo the negative selection process that eliminate autoreactive T-cell clones and preserve the minority of developing T cells that express self major histocompatibility (MHC)-restricted, foreign antigen-specific T cell receptors during the development of cellular immune self-tolerance. (McConkey *et al.*, 1989b; Nagata, 1994).

The apoptosis is known to be associated with the increase of $[Ca^{2+}]_i$ and activation of protein kinase C, the increase of ATP-dependent Ca^{2+} uptake into cell and nucleus (Johnes *et al.*, 1989), the increase of nuclear Ca^{2+}, Mg^{2+}-dependent endonuclease activity (Cohen and Duke, 1984), and the ladder-like fragmentation of chromatin DNA into oligomers of about 180 base pairs on agarose gel electrophoresis. (Wyllie, 1980; Wyllie *et al.*, 1984).

However, there are some evidences that the DNA fragmentation is rather not obligatorily associated with the increase of $[Ca^{2+}]_i$ (Baffy *et al.*, 1993; Barry and Eastman, 1992; Barry *et al.*, 1993). In spite of high $[Ca^{2+}]_i$, apoptosis may be inhibited by phorbol esters and growth factors (McConkey *et al.*, 1989c; 1990). Also cellular Ca^{2+}-influx may be required to resist against an apoptotic cell death (Iwata *et al.*, 1991; Rodriguez-Tarduchy *et al.*, 1992), and glucocorticoids can induce apoptosis in the cells which have no defined endonuclease activity (Alnemri and Litwack, 1989; 1990).

Polyamines are well known to be the pivotal factors of cellular proliferation and differentiation (Williams-Ashman and Carnellakis, 1979; Pegg, 1986) and the regulators of signaling pathway in various cells, and the change of cellular polyamine metabolism affects the DNA-supercoiling status and increases the susceptibility of DNA to DNAse (Snyder, 1989). Lymphocytes actively synthesize and uptake polyamines to maintain relatively higher cellular levels of them (Grillo *et al.*, 1990).

And the quantitative balance of suppressor and helper lymphocytes may be regulated by polyamines (Sharkis *et al.*, 1983). Brüne *et al.* (1991) reported that the glucocorticoid- or Ca^{2+} ionophore-induced apoptosis was inhibited by polyamines, especially spermine, and our recent studies showed that dexamethasone-induced apoptosis of mouse thymocytes was inhibited by DHEA, aminoguanidine and nimodipine, and DFMO, on the other hand, enhanced the apoptotic activity of dexamethasone.

This study was carried out to evaluate the effect of polyamines on the apoptosis of mouse thymocytes induced by dexamethasone, A232187, polyamine synthesis inhibitors (DFMO, MGBG, and aminoguanidine), and combined treatment of dexamethasone and polyamine synthesis inhibitors.

MATERIALS AND METHODS

Primary culture and drug treatment of mouse thymocytes

The thymus removed from an ICR-mouse (male, 3 week old, 12±2 g) was washed in 0.85% saline, immersed in 70% ethanol for 3 sec, and finally washed in PBS. The washed thymus was minced with scissors in DMEM and filtered through stainless steel screen (50 mesh).

The filtered thymocyte suspension, 4 ml, was layered on 2 ml of Ficoll-Hypaque solution and centrifuged at 400×g for 30 min (at room temperature). The monocyte fraction of white band in the intermediate layer, collected by Pasteur pipette, was mixed with 3 volumes of DMEM and centrifuged at 200×g for 10 min. This DMEM washing of monocytes was repeated 2 times more. The cell pellet was obtained and suspended in RPMI1640 medium supplemented with 1% (w/v) BSA.

The RPMI1640 suspension of thymocytes was diluted to 1.0×10^7 cells/ml with RPMI1640 media containing 5% heat-inactivated FBS, 25 μg/ml penicillin-streptomycin, 2 mM L-glutamate and 50 μM 2-mercaptoethanol, and incubated at 37℃. Cell viability was maintained greater than 90%, using exclusion of 0.4% trypan blue. 100 μl of the suspension medium was inoculated in an 96 well microplate, and incubated in humidified 5% CO_2 in air.

Drug treatments: After preconditioning of thymocytes for 30 min, the inhibitors of polyamine synthesis and metabolism (DFMO, MGBG, and aminoguanidine) and DHEA were added to the culture cells, and then, after 30 min interval, polyamines were added, and then, after one hr interval, dexamethasone and A23187 were finally added. And the assays for viability and DNA fragmentation of thymocytes were performed at 18 hr after the last drug treatment.

The drugs used in this study were as the followinga: dexamethasone (3×10^{-9} M), DHEA (1×10^{-4} M), A23187 (5×10^{-7} M), DFMO (1×10^{-4} M), MGBG (1×10^{-4} M), aminoguanidine (1×10^{-4} M), putrescine (1×10^{-3} M), spermidine (1×10^{-5} M), and spermine(1×10^{-5} M), and the concentration levels of them were settled from the data obtained in the preliminary experiments for thymocytes viability.

MTT assay for thymocyte viability

Using a MTT kit (Boehringer Mannheim), the viability of thymocytes was assayed by the method of McGahon *et al.* (1995). 10 μl of stock MTT solution (3-[4,5-dimethylthiazol-2-yl] -2,5-diphenyltetrazolium, 2.5 mg/ml) was added to a microplate well containing 100 μl of culture aliquot, and the microplate was further incubated in humidified 5% CO_2 in air for 4 hr. At the end of the incubation period, the media carefully removed from the well, ensuring that no cells were aspirated. To solubilize the formazan product, 100 μl of 0.04 M HCl in isopropanol was added, and after 5 min incubation, the optical absorbance of each well was read at 600 nm by a microplate reader (Labsystems Uniskan II).

Double staining of thymocytes with acridine orange & ethidium bromide

500 μl aliquots of cultured thymocytes (1×10^7 cells) were washed with PBS and then centrifuged at 200×g for 10 min. The pellet was suspended in 500 μl PBS and the suspension was treated with 20 μl dye mixture (PBS solution of acridine orange, 100 μg/ml and ethidium bromide, 100 μg/ml). The 20 μl of cell suspension was placed onto a microscope slide and observed and photographed on an inverted fluoromicroscope system (Nikon Diaphot and Nikon EM camera) under a 40×60×objetive with a filter (Fig. 1).

A minimum of 200 thymocytes were discriminated into three groups: (L) group-living cells with green chromatin of well organized structure, (A) group-early apoptotic cells with green or yellow-orange chromatin of highly condensed or fragmented structure and late apoptotic cells with green chromatin of highly condensed or fragmented structure, and (N) group-necrotic cells with yellow-orange chromatin of amorphous structure. And the percentages of apoptotic cells and necrotic cells were respectively calculated.

DPA Assay of DNA fragmentation

The cultured thymocytes were harvested by

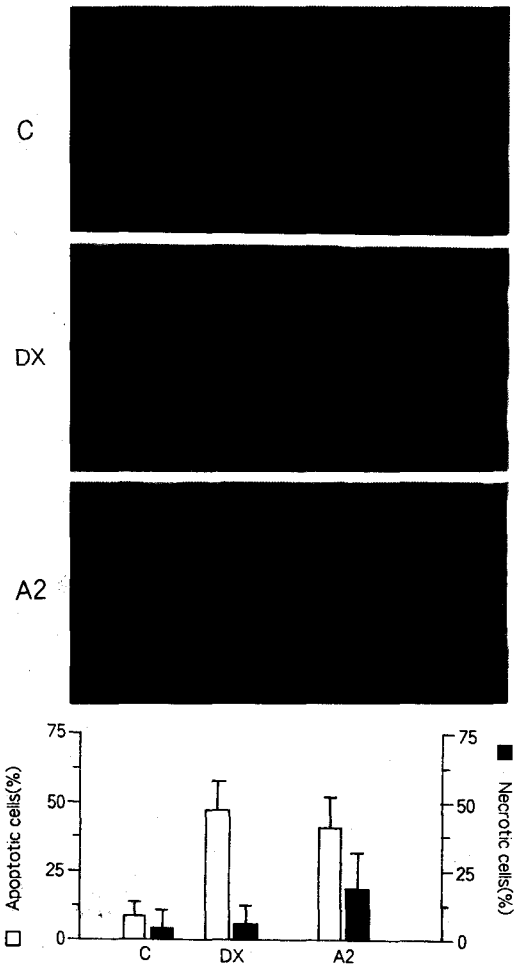

Fig. 1. Effects of dexamethasone and A23187 on the nuclei of the cultured thymocytes stained with acridine orange and ethidium bromide.
C: control, PBS
DX: dexamethasone, 3×10^{-8} M
A2: A23187, 5×10^{-7} M

centrifugation at 200×g for 10 min, and the sediment was lysed by 400 μl of hypotonic buffer (10 mM Tris, 1 mM EDTA, pH 7.5) containing 0.2% Triton X-100. After incubation on ice for 20 min, the lysate was centrifuged at 13000 ×g for 10 min. The supernatant (S) was separated to a separate tube, and both of the remained supernatant and the sediment (T) were mixed with equal volume of 25% trichloroacetic acid. The mixture was leaved at 4℃ for 24 hr

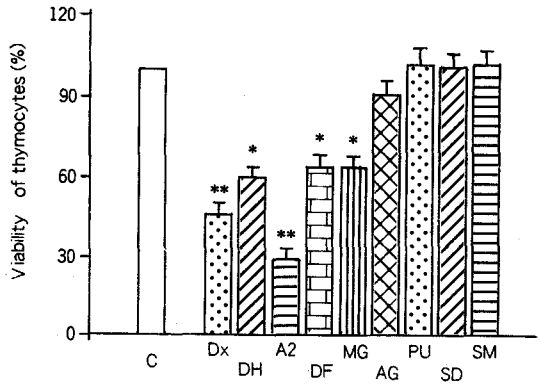

Fig. 2. Effects of dexamethasone, A23187, DHEA, DFMO, MGBG, aminoguanidine, putrescine, spermidine and spermine on the viability of thymocytes. The drugs were treated to the culture cells of conditioning for 30 min after seeding and the assays were performed at 24 hr after the drug treatments.
* and ** represent $p<0.01$ and $p<0.001$, respectively.
C: Control, PBS; DH: DHEA, 1×10^{-4} M
DF: DFMO, 1×10^{-4} M; MG: MGBG, 1×10^{-4} M
AG: aminoguanidine, 1×10^{-4} M
PU: putrescine, 1×10^{-5} M; SD: spermidine, 1×10^{-5} M
SM: spermine, 1×10^{-5} M

and then the precipitate was sedimented at 13000×g for 10 min. The sediment was hydrolyzed by boiling in 80 μl of 5% trichloroacetic acid for 10 min. The fragmented DNAs of both the supernatant and the sediment was added with 0.16 ml of DPA color reagent (diphenylamine, 0.15 g; 0.15 ml of sulfuric acid, and 0.05 ml of 1.6% acetaldehyde in 10 ml of glacial acetic acid) (Burton, 1956) and incubated for 24 hr at room temperature (or for 4 hr at 37℃), and then the optical absorbance of the mixture (200 μl) was measured at 600 nm. 'Percent frament' refers to the ratio of supernatant DNA in 'S' to the total DNA recovered in 'T' (Wyllie, 1980).

RESULTS

Thymocyte viability

After full incubation of thymocyte in primary culture for about 20 hr, the apoptotic fraction

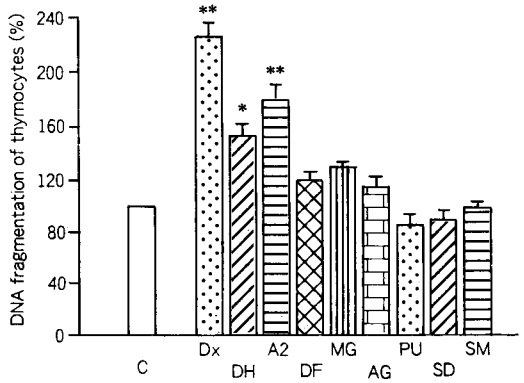

Fig. 3. Effects of dexamethasone, A23187, DHEA, DFMO, MGBG, aminoguanidine, putrescine, spermidine and spermine on DNA fragmentation of mouse thymocytes.

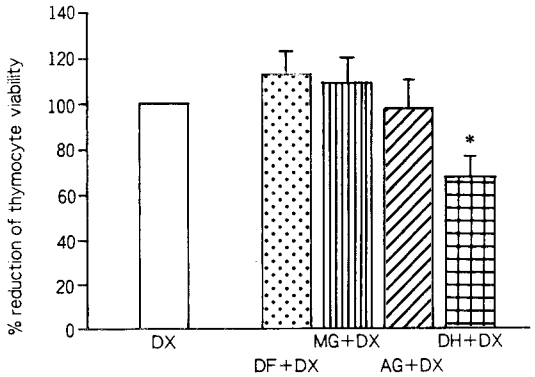

Fig. 4. Effects of DFMO, MGBG, aminoguanidine and DHEA on the dexamethasone-induced decrease of thymocyte viability. The drugs were added to the cells at 1 hr before dexamethasone treatment.

and the necrotic fraction of thymocytes double-stained with acridine orange and ethidium bromide, were 9.4±4.2% and 4.5±5.3%, respectively. Dexamethasone induced the significant increase of the apoptotic fraction to 52.0±8.1% but did not affect the necrotic fraction. However, A23187 produced the significant increases of both apoptotic and necrotic fractions to 45.0±8.9% and 20.5±10.6 %, respectively (Fig. 1).

Dexamethasone and A23187 significantly decreased the viability of thymocytes to 46.2±4.5 % and 29.0±3.8% of the control, respectively. Also the viability was decreased by DHEA (to 60.4±3.9%), DFMO (to 64.0±4.5%), and MGBG (to 64.3±5.7%), respectively. But aminoguanidine and polyamines did not affect thymocyte viability (Fig. 2).

DNA fragmentation of thymocytes

Dexamethasone and A23187 significantly enhanced the DNA fragmentation of thymocytes to 226±10% and 180±11% of the control, respectively, and DHEA also increased the DNA fragmentation to 153±9%. But polyamines, DFMO, MGBG and aminoguanidine showed no effect on the fragmentation of thymocyte DNA (Fig. 3).

Effects of DFMO, MGBG, aminoguanidine, DHEA, and polyamines on dexamethasone-induced cell death

The dexamethasone-induced cell death was significantly inhibited (to 68.1±8.7%) by DHEA, but was not decreased by DFMO, MGBG and aminoguanidine (Fig. 4). Spermine significantly inhibited both the reduction of thymocyte viability by dexamethasone (to 77.0±8.2 %) and by A23187 ⌜(to 75.9±7.8%), (to 75.9±7.8%)⌟, respectively. Putrescine and spermidine did not affect the reduction of thymocyte viability induced by dexamethasone and slightly inhibited the A23187-induced reduction (Fig. 5).

Effects of polyamines on the reduction of thymocyte viability induced by DFMO and MGBG

DFMO and MGBG, respectively, reduced the thymocyte viability to about 64 % of the control. Spermine significantly inhibited the reduction of thymocyte viability induced by DFMO (to 52.6±4.5%) and MGBG (to 74.1±8.0%), respectively. But putrescine and spermidine did not affect the viability reduction by DFMO or MGBG (Fig. 6).

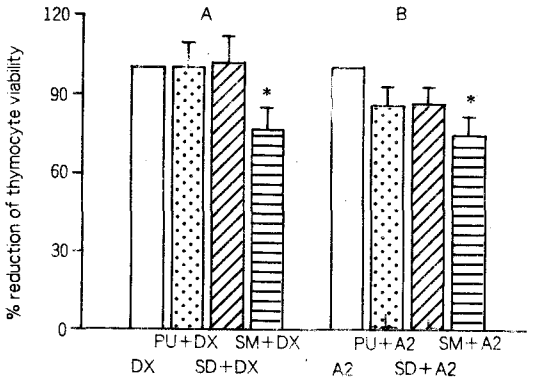

Fig. 5. Effects of putrescine, spermidine and spermine on the dexamethasone-induced and the A23187-induced decrease of thymocyte viability. Polyamines were added to the cells at 1 hr before the treatment of dexamethasone or A23187.

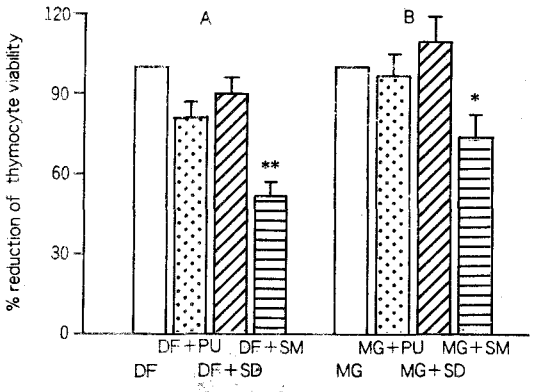

Fig. 6. Effects of putrescine, spermidine and spermine on the DFMO-induced and the MGBG-induced decrease of thymocyte viability. Polyamines were added at 1 hr after the treatment of DFMO or MGBG.

Effects of polyamines on the reduction of thymocyte viability by treatment of dexamethasone and DHEA, DFMO, MGBG, or aminoguanidine

As shown Fig. 2 and Fig. 3, the viability and DNA fragmentation of thymocytes were significantly reduced by DFMO, MGBG and DHEA, but not significantly affected by aminoguanidine. Even so, the dexamethasone-induced reduction of thymocyte viability was little affected by DFMO, MGBG, and aminoguanidine, but significantly inhibited by DHEA (to about 68 %).

By the way, the reduction of thymocyte viability by combined treatment of dexamethasone with DFMO was attenuated by putrescine to about 75% and by spermine to about 80%, but little affected by spermidine; the reduction of thymocyte viability by both dexamethasone and MGBG was attenuated by putrescine to about 80% and by spermine to about 67%, but also little affected by spermidine. However, the reduction of thymocyte viability by combined treatment of dexamethasone with aminoguanidine was moderately attenuated by spermine but not affected by putrescine or spermidine, and furthermore, the reduction by both dexamethasone and DHEA was not affected by all of the polyamines (Fig. 7).

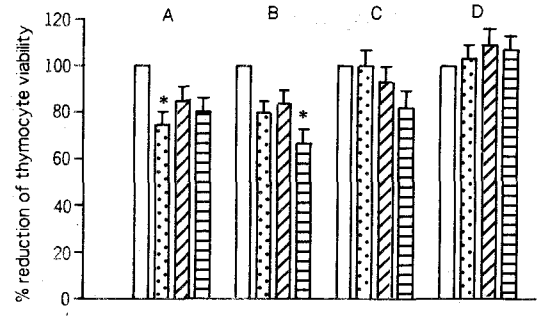

Fig. 7. Effects of putrescine(⊡), spermidine(▨) and spermine(▤) on the reduction of thymocyte viability by combined treatment of dexamethasone with DFMO (A), MGBG (B), aminoguanidine (C) and DHEA (D), respectively.

DISCUSSION

Apoptosis has been known to be associated

with the activation of phospholipase C, protein kinase C, and protease; the increases of $[Ca^{2+}]_i$ and calmodulin-dependent Ca^{2+} uptake into nucleus; the activation of Ca^{2+},Mg^{2+}-dependent endonuclease; the activation of certain gene expression; and the changes of chromatin structure (Cohen and Duke, 1984; Johnes *et al.*, 1989; McConkey *et al.*, 1994).

The apoptosis processes can be activated through the multiple signaling pathways, which may be mediated by various kinds of second messengers, depending on the type and context of the original signaling events (McConkey *et al.*, 1994; Osborne *et al.*, 1994, Mountz *et al.*, 1995). However, apoptosis molecules have not been fully defined for the types and conditions of all cells, and in addition to signal transduction molecules, various products of protooncogenes, oncosuppressor genes, and transcription factors such as bcl-2, bax, myc, p53, nur77, AP-1, c-myc, and so forth, have been known to operate at different points along the path to apoptosis (Osborne *et al.*, 1994; Mountz *et al.*, 1995).

The biological activation of immunocompetent cells is generally associated with the production of immune mediators and the activation of the cell cycle processes, and so some of them inevitably proceed to the apoptosis process, which is regulated by a number of closely related cyclin proteins, cyclin-dependent protein kinases, and cyclin-dependent kinase inhibitors (Peninger and Mak, 1994; Mountz *et al.*, 1995).

Although glucocorticoid-induced death of T lymphocytes is well known to be a typical model of apoptosis, the mechanism of apoptotic reaction has not yet been fully elucidated (Qwens *et al.*, 1991; Dicken and Miesfeld, 1992; Osborne *et al.*, 1994).

A23187 and PKC activators induced IL-2 production of lymphocytes and IL-2 increased ODC mRNA and activity, and on the other hand, cAMP post-transcriptionally augmented ODC activity (Otani *et al.*, 1990). And Kahn *et al.* (1992) showed that the role of the Na^+-K^+ pump in the regulation of polyamine transport is linked with protein kinase C activity. By the way, it has been reported that polyamines can modulate various G-protein coupled signaling processes: inhibit adenylyl cyclase, phospholipase C, phospholipase A_2 and protein kinase C; activate cAMP phosphodiesterase and Ca^{2+}-channels (Moruzzi *et al.*, 1987; Caldarera *et al.*, 1990).

Polyamines have been known to contribute to the stability of chromatin, the resistivity of DNA to DNAse, and the fidelity of transcription (Snyder, 1989; Heby and Persson, 1990). Furthermore, Brüne *et al.*, (1991) reported that polyamines inhibited DNA fragmentation of permeabilized hepatocytes as well as MGBG-induced fragmentation of thymocyte DNA, and that both of the glucocorticoid-induced apoptosis and the Ca^{2+}-induced apoptosis of thymocytes were inhibited by spermine. Recently, Brooks (1995) reported that because cell cycle progression is allowed by co-ordination of polyamine expression with DNA transcription, histone synthesis and acetylation, and nuclear protein phosphorylation, inappropriate cell-cycle expression of polyamine synthesis can lead to apoptosis of non-viable cells at G1/S transition.

And our recent studies showed that the *in vivo* apoptosis reaction of mouse thymocytes to dexamethasone was enhanced by DFMO but inhibited by DHEA, aminoguanidine, and nimodipine, respectively.

However, inhibition of ODC by treatment with DFMO caused depletion of the putrescine and spermidine contents of L1210 cells, and the antiproliferative effect of DFMO was neutralized by polyamine uptake (Heby *et al.*, 1990). Therefore, in order to study the role of polyamines in the apoptosis biology, we evaluated the effect of polyamines on the dexamethasone-induced cell death of mouse thymocytes treated with inhibitors of polyamine synthesis, such as DFMO and MGBG, referring the effects of A23187, DHEA, and aminoguanidine.

The present study showed that dexamethasone did not affect the fractional size of necrotic thymocytes in primary culture and produced the about quintuple increase of apoptotic cells, but A23187 increased the fractions of both necrotic cells and apoptotic cells by about 4~5 times; DFMO and MGBG also significantly decreased the thymocyte viability; thymocyte DNA fragmentation was significantly enhanced by dexamethasone, A23187, and DHEA but not affected polyamines and polyamine sythesis in-

hibitors; and dexamethasone-and A23187-induced decreases of thymocyte viability were not affected by DFMO or MGBG but significantly attenuated by DHEA and spermine. But polyamines and aminoguanidine could not decrease the viability of the cells. DFMO and MGBG, decreased the thymocyte viability and their effects were significantly attenuated by spermine, and furthermore, the decreases of thymocyte viability induced by combined treatments of dexamethasone with DFMO and MGBG tended to be inhibited by polyamines; particularly, putrescine and spermine significantly attenuated the decrease of thymocyte viability induced by [dexamethasone+DFMO] and [dexamethasone+MGBG], respectively.

DHEA, a weak androgenic precursor of testosterone, can be implicated in the dexamethasone-dependent cell death of necrosis as well as apoptosis, as a partial agonist on glucocorticoid receptors (Riley *et al.*, 1990). In this study, the antiapoptotic effect of DHEA was also consistent with those of previous reports (Riley *et al.*, 1990; Blauer *et al.*, 1991). And the inhibitory effects of spermine and other polyamines on the inhibitory effects of DFMO and MGBG on thymocyte viability were also very similar to the data of Brüne *et al.* (1991), convincing that polyamines are essential for the thymocyte resistivity against apoptosis as well as cell growth, proliferation and differentiation (Pegg, 1986; Brooks, 1995). By the way, the inhibitors of polyamine synthesis such as DFMO and MGBG have been thought to induce the development of apoptosis in thymocytes by the effect of decreasing the cellular polyamine contents, associated with the direct effects on the cellular processes, and especially, DFMO may stimulate the polyamine uptake into the cells (Kahn *et al.*, 1989; Morton and Pegg, 1995). And Kahn *et al.*, (1994) presented that the existence of differences in the polyamine transporter depended on the variety of the cell types. To reconfirm the antiapoptotic effect of polyamines, we evaluated the effect of exogenous polyamines on the dexamethasone-induced apoptosis of thymocytes pretreated with DFMO or MGBG in this study. Consequently, the obtained results showed that the apoptotic effectiveness of polyamines on the thymocytes simultaneously treated with dexamethasone and polyamine synthesis inhibitors was somewhat different from that on the apoptosis induced with single treatment of DFMO or MGBG. So to speak, spermine inhibited the DFMO-induced reduction of thymocyte viability by about 48%, but inhibited the viability reduction by combined treatment of dexamethasone with DFMO by only about 20%, and on the other hand, spermine inhibited the MGBG-induced reduction of thymocyte viability by only about 26%, but inhibited the viability reduction by combined treatment of dexamethasone with DFMO by about 33%. If that is true, it follows that the intracellular polyamine contents of thymocytes could depend not only on the synthesis rate, but also on the rate of uptake from the extracellular medium and on the rate of efflux (Grillo *et al.*, 1990). However, the difference of the antiapoptotic effectiveness of spermine on the thymocytes induced by single treatment of DFMO or MGBG and on the thymocytes induced by combined treatment of dexamethasone with DFMO or MGBG, suggests that the mode of spermine uptake by thymocytes treated with DFMO seems not to be in line with that of thymocytes treated with MGBG; if not so, the modes of spermine uptake by thymocytes treated with DFMO and MGBG, respectively, can be differently regulated by dexamethasone. In our future works, it would be a subtle subject to be defined in the experiment for thymocyte uptake of polyamines via specific membrane transporters using [^{3}H]-labelled substrates (Grillo *et al.*, 1990; Kahn *et al.*, 1992; Boglf *et al.*, 1994).

In conclusion, the results obtained from this study suggest that polyamines play some protective role in the thymocyte apoptosis induced by dexamethasone or A23187, and that the antiapoptotic activity of polyamines can be ascribed in part to the polyamine uptake of thymocytes via specific membrane transporters.

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=국문초록=

Dexamethasone에 의한 생쥐 흉선의 Apoptosis에서 Polyamine의 역할

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세포내 polyamine은 DNA 구조 뿐 아니라 전사과정, 세포의 성장, 분화, 및 증식 등에 간여하는 바, 배양 흉선세포의 apoptosis 을 억제한다고 한다.

따라서 dexamethasone에 의한 생쥐 흉선세포의 apoptosis 반응에 대한 polyamine의 억제작용을, polyamine 생성과 대사억제제들로 처치한 흉선세포의 일차배양실험에서 관찰하여, 그 결과를 A23187과 DHEA의 작용과 비교하였다.

1) 흉선세포 생존율이 dexamethasone, DHEA, A23187, DFMO, MGBG들에 의하여 직접 현저히 억제되며, aminoguanidine, putrescine, spermidine, 및 spermine들에 의해서는 영향을 받지 않았다.

2) 흉선세포 DNA의 분절화가 dexamethasone과 A23187에 의하여 유의하게 증강되어 있으며 DHEA에 의하여도 다소 증가되었으나, DFMO, MGBG, aminoguanidine, putrescine, spermidine, 및 spermine들에 의하여는 크게 영향을 받지 않았다.

3) Dexamethasone에 의한 흉선세포의 apoptosis는 DHEA에 의하여 억제된 반면, DFMO, MGBG, 및 aminoguanidine에 의하여는 영향을 받지 않았다.

Spermine은 dexamethasone과 A23187에 의한 세포생존율 감소를 유의하게 억제하였으며, A23187에 의한 세포생존율 감소는 putrescine과 spermidine에 의하여도 억제되는 경향을 보였다.

4) DFMO 및 MGBG에 의한 흉선세포 생존율 감소는 spermine에 의해 현저히 억제되었으나, putrescine과 spermidine에 의하여는 영향을 받지 않았다.

5) Dexamethasone을 DFMO 또는 MGBG와 병합처치하여 나타나는 흉선세포 생존율 감소는 각각 spermine과 putrescine에 의하여 유의하게 억제되었으나, aminoguanidine 또는 DHEA와 dexamethasone의 병합처치에 의한 생존율 감소는 polyamine 전처치에 의해 감소되지 않았다.

이상의 결과는 polyamine이 흉선세포의 apoptosis 반응을 억제할 수 있고, 이같은 억제효과의 일부가 $[Ca^+]_i$ 증가에 관련되는 신호전달과정과 연관될 뿐 아니라, 세포막의 polyamine transporter를 통한 polyamine 섭취가 이들의 생합성 또는 유리기능과 함께 세포내 polyamine 함량을 조정하므로, 흉선세포의 apoptosis에 억제적으로 작용할 수 있음을 시사하는 것으로 사료된다.