

Association of Dexamethasone-induced Apoptosis and G₁-Arrest of Human Leukemic CEM Cells with Polyamine Deficit

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The effects of DFMO or/and putrescine on the dexamethasone-induced apoptosis of CEM cells were studied to investigate the role of polyamines in anti-leukemic glucocorticoid action. Dexamethasone-induced apoptosis was preceded by significant decreases of cellular polyamine contents and putrescine uptake activity. But DFMO produced decreases of putrescine and spermidine contents and marked increase of putrescine uptake activity, but did not induce apoptosis. However, dexamethasone and DFMO, respectively, induced G₁-arrest in cell cycle and hypophosphorylation of pRb, resulting in the increase of G₁ to S ratio and decrease of CEM cell count. DFMO enhanced the dexamethasone-induced apoptosis and G₁-arrest. On the other hand, putrescine little affected the apoptotic and G₁-arresting activities of dexamethasone, but almost suppress the effects of DFMO and also the DFMO-dependent enhancement of dexamethasone effects. These results suggested that the dexamethasone-induced apoptosis to be associated with pRb hypophosphorylation and G₁-arrest in CEM cells might be ascribed to the concomitant decreases of cellular polyamine contents and putrescine uptake activity.

Key Words: Leukemic cell, Dexamethasone, DFMO, Polyamine, Putrescine uptake, Apoptosis, pRb

INTRODUCTION

Glucocorticoids have therapeutic potentials of anti-inflammatory and immunosuppressive effects, and induce apoptosis of lymphocytes (Diasio & LoBuglio, 1996). And the lympholytic activity of glucocorticoids has been proved to be related to their apoptotic activity on lymphocytes (Wyllie, 1980). Dexamethasone has been well known to lead prompt remission in acute lymphoblastic leukemia (Chabner et al, 1996).

Polyamines are essential for cell growth, proliferation, and differentiation (Tabor & Tabor, 1984; Pegg, 1986; Heby & Persson, 1990), and so have some roles in apoptosis (Brooks, 1995). Polyamines, particularly spermine, have been reported to show the inhibitory effect on the apoptosis induced by dexamethasone

(LaVoie & Witorsch, 1995), Ca²⁺ ionophore (Brune et al, 1991), and topoisomerase inhibitors (Solary et al, 1996; 1994; Bertrand et al, 1993) in thymocytes and lymphocytes of leukemia and lymphoma.

On the contrary, several reports demonstrated that excessive polyamine accumulation might induce apoptotic cell death (Pegg et al, 1995; Poulin et al, 1995), and that a stable polyamine analogue also induced both growth inhibition and apoptosis of cancer cells (McCloskey et al, 1995). And in the mouse myeloma cells to overexpress ornithine decarboxylase (ODC), the addition of ornithine to the culture medium increased cellular putrescine content, resulting in apoptotic cell death (Tobias & Kahana, 1995).

In the early phase of the rat thymocyte apoptosis induced by glucocorticoid, heat shock, and γ -irradiation, an increased expression of ODC gene appears with the decrease of cellular polyamine contents,

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before laddering of genomic DNA (Desiderio et al, 1995; Grassilli et al, 1995).

The cellular levels of polyamines are regulated by the activities of polyamine transporters (Pegg, 1988; Lessard et al, 1995) as well as by the activities of synthetic enzymes, ODC and S-adenosylmethionine decarboxylase (SAM-DC), spermidine/spermine N^1 -acetyltransferase, and oxidases (Pegg, 1988; Seiler, 1987; Casero & Pegg, 1993).

This study was carried out to investigate the role of polyamines in the dexamethasone-induced apoptotic cell death of CCRF-CEM cells, a human T lymphoblastic leukemia cell line (Foley et al, 1965) which has been considered to be labile to dexamethasone cytotoxicity (Norman & Thompson, 1977; Yuh & Thompson, 1989), referring the effect of the polyamine depletion by DL- α -difluoromethylornithine (DFMO) (Mamont et al, 1978).

METHODS

Cell culture and drug treatment

CEM cells (ATCC CCL 119) were cultured in RPMI 1640 medium (Gibco) containing 10% fetal bovine serum (Gibco) under humidified atmosphere of 5% CO₂ at 37°C. Two hours after beginning of cell culture (seeding density of $2 \pm 0.2 \times 10^5$ cells/ml), DFMO (1×10^{-3} M; control, PBS 0.1% v/v of medium; Marion Merrell Dow) or dexamethasone (1×10^{-6} M; control, ethanol 0.1%) was added to the culture medium, and the polyamine contents and putrescine uptake activity of CEM cells were measured. And 2 hours, 14 hours, and 16 hours after beginning of cell culture, DFMO, putrescine (1×10^{-5} M; control, PBS 0.1%), and dexamethasone were respectively added to the culture medium, and then the studies of MTT assay, LDH release, DNA fragmentation, cell cycle analysis, and phosphorylation of Rb protein were performed with 24 hour intervals or at 72 hour after dexamethasone treatment.

Measurement of cellular polyamine contents

The CEM cells of ($2 \pm 0.2 \times 10^6$) were washed twice with PBS. The washed pellet was extracted with 200 μ l of 0.6 M perchloric acid at 4°C for 30 min and then centrifuged at $12000 \times g$ for 5 min. The supernatant of 100 μ l was evaporated to dryness. For

derivatization of polyamines, the dry residue dissolved in 100 μ l of 1 M sodium carbonate was mixed with 300 μ l of FNBT reagent (1% 4-fluoro-3-nitrobenzotrifluoride solution in DMSO; Aldrich) and incubated at 60°C for 20 min. At the end of the reaction, 40 μ l of 1 M histidine in 1 M sodium carbonate was added to the solution, and the reaction was continued for further 5 min. After cooling the reaction mixture, the polyamine derivatives extracted twice with 2 ml of 2-methylbutane (Aldrich) were dried and dissolved in methanol. The methanol solution was applied on an ODS reverse phase column of HPLC, which was eluted by an isocratic mobile phase of 80% acetonitrile, monitoring at 242 nm (Spragg & Hutchings, 1983).

[¹⁴C]Putrescine uptake measurement

The CEM cells ($1 \pm 0.1 \times 10^6$ /ml) were resuspended in PBS and then incubated with 1 μ M~20 μ M of [¹⁴C]putrescine (Amersham) at 37°C for 60 min under 5% CO₂ atmosphere. At the end of incubation, cold PBS of 1 ml containing 1 mM putrescine was added to the cell suspension and centrifuged at $2000 \times g$ for 5 min. The pellet was washed twice with PBS and dissolved in 200 μ l of 1 N NaOH at 60°C for 60 min. The solution was neutralized with 200 μ l of 1 N HCl, and the radioactivity was measured by β -scintillation counter (Porter et al, 1985; Lessard et al, 1995). The uptake parameters were determined by Edie-Scatchard plot.

MTT assay

The culture medium (100 μ l) containing suspended CEM cells was incubated with 10 μ l of MTT labeling reagent (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), 5 mg/ml in PBS; Boehringer Mannheim), at 37°C for 4 hours. And the formazan crystal produced by cellular dehydrogenases was resolved in 100 μ l of solubilizing solution (10% SDS in 0.01 M HCl; Boehringer Mannheim) at 37°C for 12 hours, and then the absorbance was measured at 600 nm (Mosmann, 1983).

LDH release assay

The supernatant (50 μ l) of culture medium was reacted with 50 μ l of LDH substrate solution (Promega)

for 30 min, and the reaction was stopped by addition of 50 μ l of 1 M acetic acid. The activity of LDH released was measured as the absorbance at 492 nm (Korzeniewski & Callewaert, 1983; Decker & Lohmann-Matthes, 1988).

DNA fragmentation assay

The CEM cells ($2 \pm 0.2 \times 10^6$) washed with PBS were digested in 100 μ l of lysis buffer (20 mM EDTA, 100 mM Tris, pH 8.0, and 0.8% sodium lauryl sarcosine) and 50 μ l of RNase A (1 mg/ml) at 37°C for 90 min, and then incubated overnight with 50 μ l of proteinase K (20 mg/ml) at 50°C. The genomic DNA was extracted once with phenol-chloroform-isoamylalcohol (25:24:1) and twice with chloroform-isoamylalcohol (24:1). DNA was precipitated by adding ammonium acetate upto 2.5 M and absolute ethanol. Being incubated for more than 2 hours at 4°C, the DNA was pelleted by centrifugation 12000 \times g for 15 min and washed with 70% ethanol. The DNA pellet was dried and dissolved in TE buffer (pH 8.0), and the fragmented DNA of 10 μ g was separated on a 2% agarose gel, which was stained with ethidium bromide and visualized under UV light.

Flow cytometric analysis of cell cycle

The PBS-washed CEM cells ($1 \pm 0.1 \times 10^5$) were fixed in 2 ml of cold 70% ethanol at 4°C for 60 min, and then washed with PBS and resuspended in 0.5 ml of PBS. To this cell suspension, 0.5 ml of RNase (1 mg/ml in PBS) and 1 ml of propidium iodide (100 μ g/ml in PBS) were added. And the cellular distributions to G₁, S, and G₂/M phases were analyzed by a flow cytometer (Nicoletti et al, 1991).

Western blot analysis of phosphorylated Rb protein

The CEM cells ($6 \pm 0.2 \times 10^6$) washed with PBS were lysed in SDS-PAGE sample buffer containing dithiothreitol, and boiled for 5 min. The protein content of PBS-washed cells was measured by Bradford's method (1976). Each lysate was electrophoresed on a 7.5% SDS-PAGE gel and transferred into PVDF membrane. Rb immunoblot was probed using the anti-human Rb monoclonal Ab (Pharmingen), and detected with ECL-blotting system containing HRP-labelled secondary Ab of mouse Ig (Amersham).

RESULTS

Effects of DFMO and dexamethasone on the polyamine metabolism in CEM cells

The change of polyamine contents was measured after DFMO and dexamethasone treatment to evaluate the time-dependent change of polyamine metabolism by DFMO or dexamethasone in CEM cells (Fig. 1). Putrescine content was almost depleted to less than 2% of control after 8 hours of DFMO treatment, and by dexamethasone, it was significantly decreased to about 50% of control at 8 hour and nearly depleted to that by DFMO after 24 hours. Spermidine content was almost depleted after 24 hours of DFMO

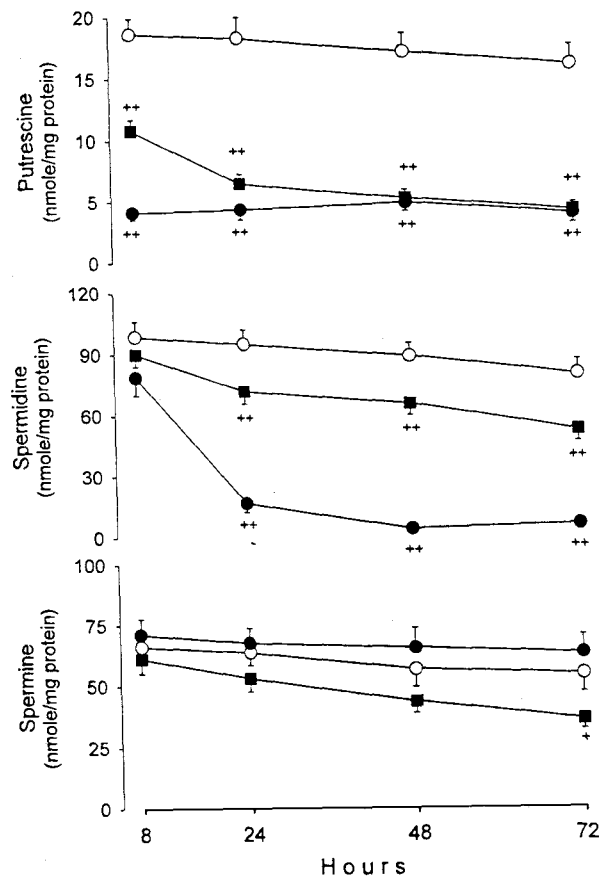


Fig. 1. Effects of DFMO (10^{-3} M) and dexamethasone (DX: 10^{-6} M) on the polyamine contents of CEM cells. ○: Control, ●: DFMO, ■: DX. Each point represents mean \pm S.E. of 4 independent measurements. + and ++ indicate $p < 0.1$ and $p < 0.05$, in comparison to the control group.

Table 1. Effects of DFMO and dexamethasone on the [¹⁴C]putrescine uptake of CEM cells

Treatment	Km (uM)	Vmax (nM/hr/mg protein)
Control	22.42 ± 2.30	2.14 ± 0.19
DFMO 12 hr	8.31 ± 1.21**	7.69 ± 0.81**
DFMO 24 hr	6.85 ± 0.89**	15.24 ± 1.17**
DFMO 48 hr	6.96 ± 0.75**	27.93 ± 2.13**
DFMO 72 hr	7.05 ± 0.79**	1.53 ± 1.96**
DX 24 hr	17.16 ± 1.94	1.12 ± 0.21**
DX 48 hr	18.31 ± 1.95	1.20 ± 0.29**
DX 72 hr	17.20 ± 1.87	1.14 ± 0.27**
DFMO 36 hr & DX 24 hr	9.48 ± 0.86	16.98 ± 1.71**
DFMO 60 hr & DX 48 hr	2.15 ± 1.02**	9.71 ± 0.89**
DFMO 84 hr & DX 72 hr	12.61 ± 0.93**	9.29 ± 1.32**

** indicates $p < 0.05$

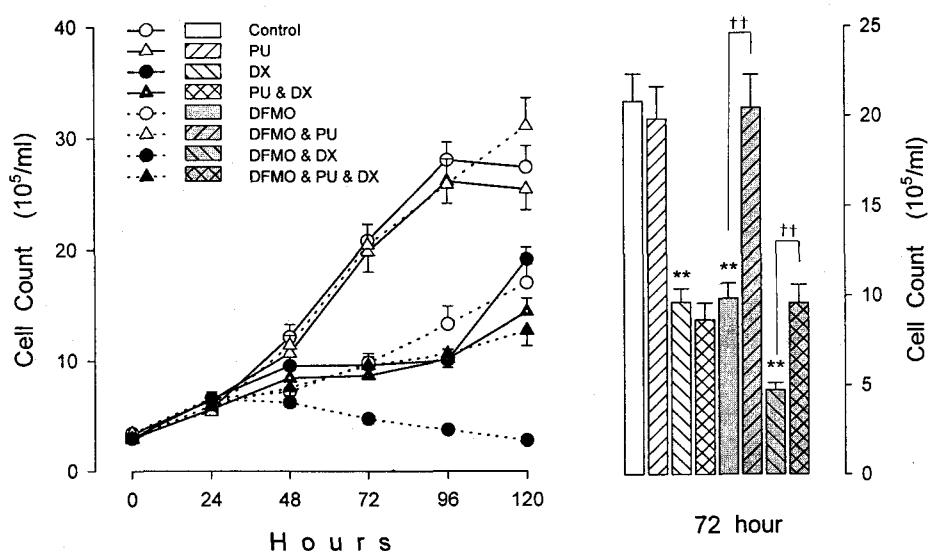


Fig. 2. Effects of DFMO and putrescine (PU, 10^{-5} M) on the change of CEM cell count by dexamethasone.

** indicates $p < 0.05$, in comparison to the control count at 72 hour.

treatment, and by dexamethasone, it was decreased to less than 80% of control after 24 hours. Finally, spermine content was not affected by DFMO, and by dexamethasone, the decrease of spermine content was continued till 72 hour after treatment.

CEM cells showed Km and Vmax of putrescine uptake as follows; 22.4 ± 2.3 μ M and 2.14 ± 0.19 nM/hour/mg protein, respectively (Table 1). DFMO significantly decreased the Km at 12, 24, 48, and 72

hour, and significantly increased the Vmax at 12 hour and further increased that after 24 hours. But dexamethasone slightly decreased the Km at 24, 48, and 72 hour, and significantly decreased the Vmax. DFMO, treated at 12 hour prior to dexamethasone, enhanced the slight decrease of Km by dexamethasone, and reversed the decrease of Vmax by dexamethasone.

Effects of DFMO and putrescine on the inhibition of cellular proliferation by dexamethasone

Cell counting and MTT assay were performed to evaluate the effects of DFMO and putrescine on the change of cellular proliferation by dexamethasone (Fig. 2 & 3).

The cell count and the MTT value of CEM cells increased for 4 days, and this increase was significantly inhibited by dexamethasone. DFMO also significantly inhibited the increase of cell count and MTT value, and enhanced the inhibitory effects of dexamethasone. Otherwise, putrescine had little effect on the increase of cell count and MTT value of CEM cells, and little affected the inhibitory effect of dexamethasone on the cell count and MTT value. But putrescine almost reversed the inhibitory effect on the cell count and MTT value by DFMO, and also reversed the enhancement of dexamethasone effects by DFMO. The MTT value of culture well may represent the count of viable cells in the well, and the

absorbance per viable cell, which declined slowly, was affected by neither dexamethasone nor DFMO (Fig. 3-lower).

Effects of DFMO and putrescine on the apoptotic cell death by dexamethasone

LDH release and DNA fragmentation were measured to examine the effects of DFMO and putrescine on the apoptotic cell death induced by dexamethasone (Fig. 4 & 5).

The LDH release from CEM cells and the fragmentation of DNA appeared not to be significant for 72 hours, but dexamethasone significantly increased LDH release after 48 hours and induced evident fragmentation of DNA at 72 hour. DFMO had little effect on LDH release and induced little fragmentation of DNA. But DFMO significantly enhanced the increase of LDH release by dexamethasone (Fig. 4-lower), and also enhanced the DNA fragmentation by dexamethasone. Putrescine had little effect on the LDH release and DNA fragmentation of CEM cells, and little affected the increase of LDH release and

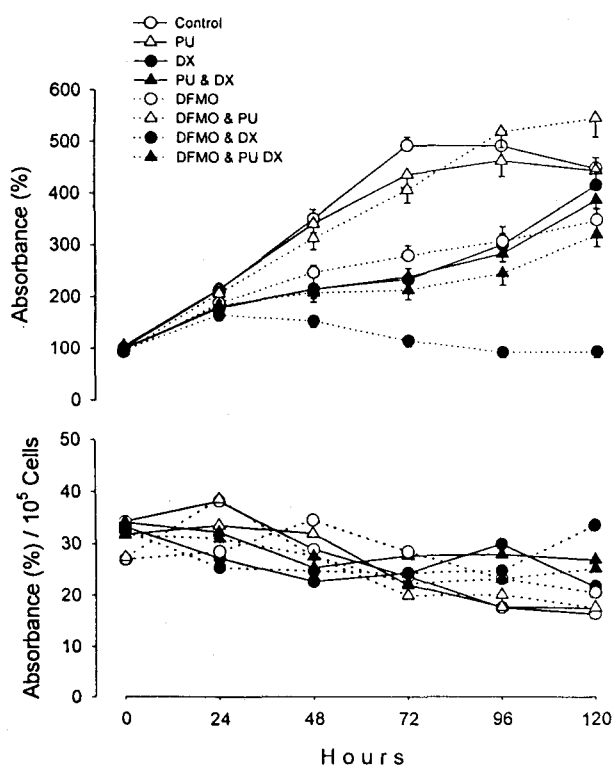


Fig. 3. Effects of DFMO and putrescine on the change of MTT value of CEM cells by dexamethasone.

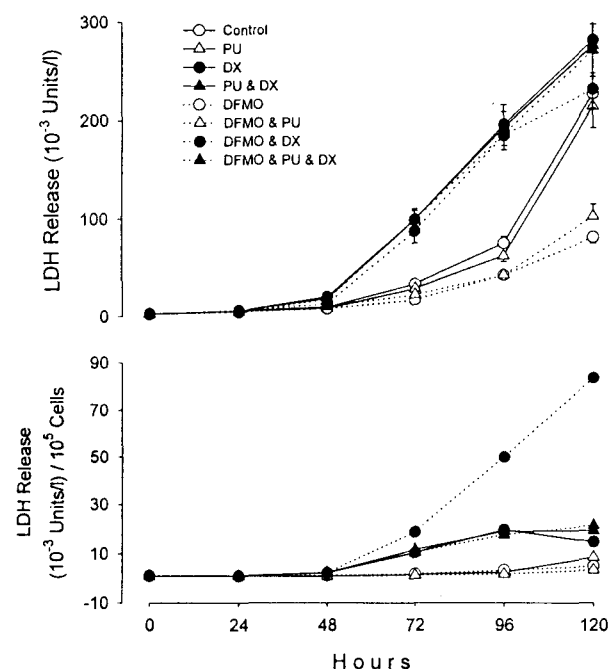


Fig. 4. Effects of DFMO and putrescine on the increase of LDH release from CEM cells by dexamethasone.

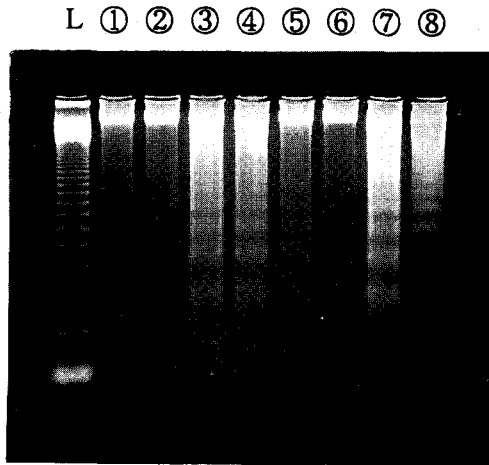


Fig. 5. Effects of DFMO and putrescine on the change of DNA fragmentation at 72 hour after dexamethasone treatment.

lane L: Authentic marker of 123 bp DNA ladder,
 lane 1: Control, lane 2: PU,
 lane 3: DX, lane 4: PU & DX,
 lane 5: DFMO, lane 6: DFMO & PU,
 lane 7: DFMO & DX, lane 8: DFMO & PU & DX

DNA fragmentation by dexamethasone. But putrescine almost reversed the enhancement of dexamethasone-induced LDH release (Fig. 4-lower) and DNA fragmentation by DFMO.

Effects of DFMO and putrescine on change of cell cycle by dexamethasone

Cell cycle profile was analyzed to assess the effects of DFMO and putrescine on the cell cycle arrest by dexamethasone and to elucidate the relation between the cell cycle arrest and subsequent development of apoptosis.

Cell cycle distributions of CEM cells were as follows; $36.6 \pm 1.5\%$ in G₁, $48.1 \pm 1.9\%$ in S, and $15.4 \pm 1.2\%$ in G₂/M phase at 72 hour, and dexamethasone significantly increased the cellular distribution in G₁ phase and reciprocally decreased the distribution in S phase, with persistent percentage in G₂/M phase. DFMO also increased the ratio of cellular distribution in G₁ phase to the distribution in S phase, with no change in G₂/M phase, and DFMO enhanced the G₁-arrest by dexamethasone. Putrescine little affected the cell cycle progression as well as the G₁-arrest by

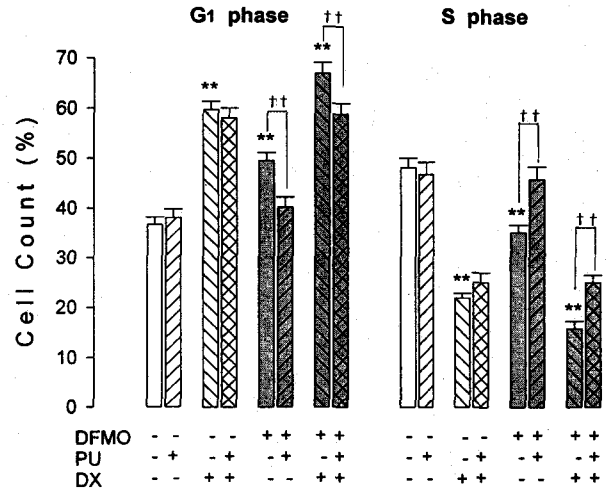


Fig. 6. Effects of DFMO and putrescine on the change of cell cycle distribution of CEM cells by dexamethasone.

Table 2. Effects of dexamethasone, DFMO, and putrescine on the ratio of G₁ to S phase distribution of CEM cells

Treatment	(-) Putrescine	(+) Putrescine
Control	0.77 ± 0.06	0.83 ± 0.08
DX	$2.75 \pm 0.20^{**}$	2.37 ± 0.25
DFMO	$1.42 \pm 0.11^{**}$	$0.89 \pm 0.09^{++}$
DFMO & DX	$4.41 \pm 0.56^{**}$	$2.39 \pm 0.23^{++}$

** indicates $p < 0.05$ in comparison to the control, and ++ indicates $p < 0.05$ in comparison to the (-) putrescine.

dexamethasone, but it almost reversed the G₁-arrest by DFMO and also reversed the enhancement of dexamethasone-induced G₁-arrest by DFMO (Fig. 6 & Table 2).

Effects of DFMO and putrescine on the change of pRb phosphorylation by dexamethasone

The phosphorylation of pRb has been known to closely relate to the cell cycle progression through G₁ phase, and we analyzed the phosphorylation status of pRb to explain aspects of the effects of DFMO and putrescine on G₁-arrest induced by dexamethasone.

The pRb of CEM cells was highly phosphorylated

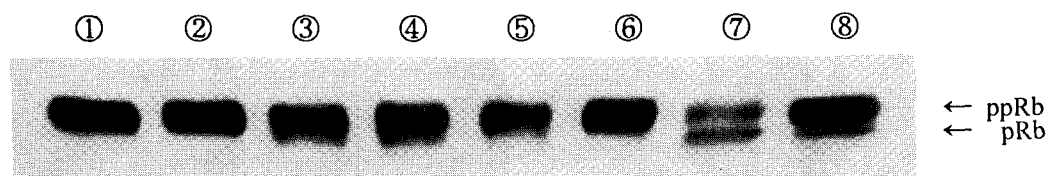


Fig. 7. Effects of DFMO and putrescine on the change of Rb protein phosphorylation at 72 hour after dexamethasone treatment.

lane 1: Control, lane 2: PU,
 lane 3: DX, lane 4: PU & DX,
 lane 5: DFMO, lane 6: DFMO & PU,
 lane 7: DFMO & DX, lane 8: DFMO & PU & DX

with normal cell cycle progression, and dexamethasone hypophosphorylated pRb at 72 hour of treatment (Fig. 7). DFMO also inhibited the phosphorylation, and enhanced the hypophosphorylation of pRb by dexamethasone to display an evident band of hypophosphorylated. Putrescine had little effect on the normal hyperphosphorylation and dexamethasone-induced hypophosphorylation, but putrescine reversed the hypophosphorylation of pRb by DFMO and also reversed the enhancement of dexamethasone-induced hypophosphorylation by DFMO.

DISCUSSION

The ODC and polyamines have been known to involve in dexamethasone-induced apoptosis of thymocytes (Desiderio et al, 1995; Grassilli et al, 1995), and the apoptosis was significantly inhibited by addition of polyamines to the culture medium (Brune et al, 1991).

In this study, DFMO almost depleted cellular putrescine and spermidine but little affected spermine content of CEM cells. Dexamethasone produced acute depletion of cellular putrescine, followed by gradual decrease of spermidine and spermine contents. DFMO enhanced the putrescine uptake activity within 12 hours of treatment, but dexamethasone depressed the V_{max} of putrescine uptake although the putrescine content was almost depleted. Though the polyamine transporters have been known to be upregulated by the decrease of cellular polyamine contents (Grillo et al, 1989), little resource of polyamines in culture medium made the transporters to be useless and then the putrescine and spermidine contents remained to

be depleted in DFMO-treated CEM cells. However, the spermine content was little changed by DFMO in this study, and this result seemed to be related to previous studies which showed the different responsivenesses of the cellular spermine contents to DFMO in different cell lines (Pera et al, 1986; Dorhout et al, 1995; Walters & Wojcik, 1994).

The dexamethasone-induced acute depletion of cellular putrescine and the following decrease of higher polyamine contents in this study might be associated with the reduction of ODC activity in CEM cells. Also, ODC expression was inhibited in the TGF β_1 -induced apoptosis of L1210 cells (Grzelkowska et al, 1995). But several reports opposed above suggestion; the early expression and increase in activity of ODC associated with the decrease of polyamine contents were preceded to the appearance of DNA laddering in dexamethasone-induced apoptosis of thymocytes (Desiderio et al, 1995; Grassilli et al, 1995), and the c-Myc-induced apoptosis was associated with the expression and activation of ODC (Packham & Cleveland, 1994; 1995; Packham et al, 1996). Nevertheless, there are several evidences that the decrease of cellular polyamine contents is one of the characteristic features of dexamethasone-induced apoptosis (Desiderio et al, 1995; Grassilli et al, 1995). There may be putative contributors to decrease polyamine contents in the process of apoptosis, besides the changes of activities of the synthetic enzymes (Desiderio et al, 1995); reduced cellular availability of ornithine and arginine as substrates, oxidative metabolism by diamine and polyamine oxidases (Buttke & Sandstrom, 1994), increased acetylation facilitating the excretion of polyamines (Casero & Pegg, 1993), activation of transglutaminase (Piacentini et al, 1991; Martin et al,

1994) which can use polyamines as substrates (Fesus et al, 1991).

The increase of putrescine uptake activity by DFMO was in accordance with the other studies using leukemia and other cell lines (Kramer et al, 1993; Byers & Pegg, 1989; Walters & Wojcik, 1994), and the decrease of putrescine uptake activity can be considered to be one feature in dexamethasone-induced apoptotic cell death. However it is remained to be clarified whether the decreases of polyamine contents and putrescine uptake activity are primary or secondary to the apoptosis induced by dexamethasone.

CEM cells continued to proliferate for 4 days without refresh of culture medium and showed slight decline of cellular activity in MTT assay after 24 hours. Both of DFMO and dexamethasone inhibited the proliferation of CEM cells, and dexamethasone increased LDH release from the cells and fragmentation of DNA, which were enhanced by DFMO. In addition, exogenous putrescine reversed the enhancement of dexamethasone-induced actions by DFMO as well as the antiproliferative action of DFMO. These results seemed to be in accordance with the previous studies, in which exogenous putrescine reversed the DFMO-induced suppression of DNA synthesis in lymphocytes and leukemic cells (Seyfried & Morris, 1979; Gallo et al, 1986; Endo et al, 1988). And these findings showed that DFMO produced antiproliferative effect on CEM cells without cell damages, and the antiproliferative action was exclusively dependent on the depletion of cellular polyamines and predisposed to the enhancement of apoptotic cell death induced by dexamethasone.

G₁/S transition is the critical point in cell cycle and is one of the steps to be regulated in apoptosis, and the hyperphosphorylation of pRb is appeared in G₁/S transition of cell cycle (Chiarugi et al, 1994; Kranenburg et al, 1995). ODC activity is strictly regulated during cell cycle, showing the highest peak in G₁ phase, and so polyamines have been known to be essential in cell cycle progression (Heby & Persson, 1990; Stimac & Morris, 1987; Kaminska et al, 1990). DFMO arrested the cell cycle at G₁ phase, and the G₁-arrest was definitely associated with DFMO-induced decrease of putrescine and spermidine contents in L1210 cells (Dorhout et al, 1995). Similarly in IL-3 dependent myeloid cells, the G₁-arrest induced by IL-3 deprivation also appeared to involve in the down-regulation of ODC and c-myc expression (Askew et al,

1991). In this study, the antiproliferative action of DFMO and the apoptotic action of dexamethasone described above appeared to be associated with the hypophosphorylation of pRb representing arrest of cell cycle in G₁ phase. The mechanism of hypophosphorylation of pRb induced by depletion of cellular polyamines is under study.

In conclusion, dexamethasone-induced apoptosis seems to be ascribed to cellular polyamine depletion associated with the inhibition of G₁/S transition.

REFERENCES

- Askew DS, Ashmun RA, Simmons BC, Cleveland JL. Constitutive c-myc expression in an IL-3-dependent myeloid cell line suppresses cell cycle arrest and accelerates apoptosis. *Oncogene* 6: 1915–1922, 1991
- Bertrand R, Solary E, Jenkins J, Pommier Y. Apoptosis and its modulation in human promyelocytic HL-60 cells treated with DNA topoisomerase I and II inhibitors. *Exp Cell Res* 207: 388–397, 1993
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. *Anal Biochem* 72: 248–254, 1976
- Brooks WH. Polyamine involvement in the cell cycle, apoptosis, and autoimmunity. *Med Hypotheses* 44: 331–338, 1995
- Brune B, Hartzell P, Nicotera P, Orrenius S. Spermine prevents endonuclease activation and apoptosis in thymocytes. *Exp Cell Res* 195: 323–329, 1991
- Buttke TM, Sandstrom PA. Oxidative stress as mediator of apoptosis. *Immunol Today* 15: 7–10, 1994
- Byers TL, Pegg AE. Properties and physiological function of the polyamine transport system. *Am J Physiol* 257: C545–C553, 1989
- Casero RA Jr, Pegg AE. Spermidine/spermine N¹-acetyltransferase - the turning point in polyamine metabolism. *FASEB J* 7: 653–661, 1993
- Chabner BA, Allegra CJ, Curt GA, Calabresi P. Antineoplastic agents. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG ed, *Goodman & Gilman's the Pharmacological Basis of Therapeutics*. 9th ed. McGraw-Hill, p 1233–1287, 1996
- Chiarugi V, Magnelli L, Cinelli M, Basi G. Apoptosis and the cell cycle. *Cell Mol Biol Res* 40: 603–612, 1994
- Decker T, Lohmann-Matthes ML. A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. *J Immunol*

- Methods* 115: 61–69, 1988
- Desiderio MA, Grassilli E, Bellesia E, Salomoni P, Franceschi C. Involvement of ornithine decarboxylase and polyamines in glucocorticoid-induced apoptosis of rat thymocytes. *Cell Growth Differ* 6: 505–513, 1995
- Diasio RB, LoBuglio AF. Immunomodulators: immunosuppressive agents and immunostimulants. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG ed, *Goodman & Gilman's the Pharmacological Basis of Therapeutics*. 9th ed. McGraw-Hill, p 1291–1308, 1996
- Dorhout B, te Velde RJ, Ferwerda H, Kingma AW, de Hoog E, Muskiet FA. In vivo effects of 4-amidinoin-dan-1-one 2'-amidinohydrazone (CGP 48664A) and α -difluoromethylornithine (DFMO) on L1210 growth, cell-cycle phase distribution and polyamine contents. *Int J Cancer* 62: 738–742, 1995
- Endo Y, Matsushima K, Onozaki K, Oppenheim JJ. Role of ornithine decarboxylase in the regulation of cell growth by IL-1 and tumor necrosis factor. *J Immunol* 141: 2342–2348, 1988
- Fesus L, Davies PJA, Piacentini M. Apoptosis: molecular mechanisms in programmed cell death. *Eur J Cell Biol* 56: 170–177, 1991
- Foley GE, Lazarus H, Farber S, Uzman BG, Boone B, McCarthy R. Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. *Cancer* 18: 522–529, 1965
- Gallo CJ, Koza RA, Herbst EJ. Polyamines and HeLa-cell DNA replication. *Biochem J* 238: 37–42, 1986
- Grassilli E, Desiderio MA, Bellesia E, Salomoni P, Benatti F, Franceschi C. Is polyamine decrease a common feature of apoptosis? Evidence from γ rays- and heat shock-induced cell death. *Biochem Biophys Res Commun* 216: 708–714, 1995
- Grillo MA, Colombatto S, Fulgosi B, Fasulo L. Transport and metabolism of polyamines in primary cell culture. In: Goldemberg SH, Algranati ID ed, *The Biology and Chemistry of Polyamines*, ICSU Symposium Series. vol 12. Oxford Univ Press, p 171–180, 1989
- Grzelkowska K, Motyl T, Malicka E, Ostrowski J, Trzeciak L, Filipecki M. Effect of orotic acid on TGF β_1 -induced growth inhibition of L1210 leukemic cells. *Int J Hematol* 61: 23–33, 1995
- Heby O, Persson L. Molecular genetics of polyamine synthesis in eukaryotic cells. *Trends Biochem Sci* 15: 153–158, 1990
- Kaminska B, Kaczmarek L, Grzelakowska-Sztabert B. The regulation of the G₀-S transition in mouse T lymphocytes by polyamines. *Exp Cell Res* 191: 239–245, 1990
- Korzeniewski C, Callewaert DM. An enzyme-release assay for natural cytotoxicity. *J Immunol Methods* 64: 313–320, 1983
- Kramer DL, Miller JT, Bergeron RJ, Khomutov R, Khomutov A, Porter CW. Regulation of polyamine transport by polyamines and polyamine analogs. *J Cell Physiol* 155: 399–407, 1993
- Kranenburg O, van der Eb AJ, Zanema A. Cyclin-dependent kinases and pRb: regulators of the proliferation-differentiation switch. *FEBS Letters* 367: 103–106, 1995
- LaVoie HA, Witorsch RJ. Investigation of intracellular signals mediating the anti-apoptotic action of prolactin in Nb2 lymphoma cells. *Proc Soc Exp Biol Med* 209: 257–269, 1995
- Lessard M, Zhao C, Singh SM, Poulin R. Hormonal and feedback regulation of putrescine and spermidine transport in human breast cancer cells. *J Biol Chem* 270: 1685–1694, 1995
- Mamont PS, Duchesne MC, Grove J, Bey P. Anti-proliferative properties of DL- α -difluoromethylornithine in cultured cells. A consequence of the irreversible inhibition of ornithine decarboxylase. *Biochem Biophys Res Commun* 81: 58–66, 1978
- Martin SJ, Green DR, Cotter TG. Dicing with death: dissecting the components of the apoptosis machinery. *Trends Biochem Sci* 19: 26–30, 1994
- McCloskey DE, Casero RA Jr, Woster PM, Davidson NE. Induction of programmed cell death in human breast cancer cells by an unsymmetrically alkylated polyamine analogue. *Cancer Res* 55: 3233–3236, 1995
- Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 65: 55–63, 1983
- Nicoletti I, Migliorati G, Pagliacci MC, Grignani F, Riccardi C. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. *J Immunol Methods* 139: 271–279, 1991
- Norman MR, Thompson EB. Characterization of a glucocorticoid-sensitive human lymphoid cell line. *Cancer Res* 37: 3785–3791, 1977
- Packham G, Cleveland JL. Ornithine decarboxylase is a mediator of c-Myc-induced apoptosis. *Mol Cell Biol* 14: 5741–5747, 1994
- Packham G, Cleveland JL. The role of ornithine decarboxylase in c-Myc-induced apoptosis. *Curr Top Microbiol Immunol* 194: 283–290, 1995
- Packham G, Porter CW, Cleveland JL. c-Myc induces apoptosis and cell cycle progression by separable, yet overlapping, pathways. *Oncogene* 13: 461–469, 1996
- Pegg AE, Poulin R, Coward JK. Use of aminopropyltransferase inhibitors and of non-metabolizable analogs to study polyamine regulation and function. *Int J Biochem Cell Biol* 27: 425–442, 1995
- Pegg AE. Polyamine metabolism and its importance in

- neoplastic growth and a target for chemotherapy. *Cancer Res* 48: 759–774, 1988
- Pegg AE. Recent advances in biology of polyamines in eukaryotes. *Biochem J* 234: 249–262, 1986
- Pera PJ, Kramer DL, Sufirin JR, Porter CW. Comparison of the biological effects of four irreversible inhibitors of ornithine decarboxylase in two murine lymphocytic leukemia cell lines. *Cancer Res* 46: 1148–1154, 1986
- Piacentini M, Fesus L, Farrace MG, Ghibelli L, Piredda L, Melino G. The expression of "tissue" transglutaminase in two human cancer cell lines is related with the programmed cell death (apoptosis). *Eur J Cell Biol* 54: 246–254, 1991
- Porter CW, Cavanaugh PF Jr, Stolowich N, Ganis B, Kelly E, Bergeron RJ. Biological properties of N^4 - and N^1, N^8 -spermidine derivatives in cultured L1210 leukemia cells. *Cancer Res* 45: 2050–2057, 1985
- Poulin R, Pelletier G, Pegg AE. Induction of apoptosis by excessive polyamine accumulation in ornithine decarboxylase-overproducing L1210 cells. *Biochem J* 311: 723–727, 1995
- Seiler N. Inhibition of enzymes oxidizing polyamines. In: McCann PP, Pegg AE, Sjoerdsma A ed, *Inhibition of Polyamine Metabolism - Biological Significance and Basis for New Therapies*. Academic Press, p 49–77, 1987
- Seyfried CE, Morris DR. Relationship between inhibition of polyamine biosynthesis and DNA replication in activated lymphocytes. *Cancer Res* 39: 4861–4867, 1979
- Solary E, Bertrand R, Pommier Y. Apoptosis induced by DNA topoisomerase I and II inhibitors in human leukemic HL-60 cells. *Leuk Lymphoma* 15: 21–32, 1994
- Solary E, Dubrez L, Eymin B, Bertrand R, Pommier Y. Apoptosis of human leukemic cells induced by topoisomerase I and II inhibitors. *Bull Cancer Paris* 83: 205–212, 1996
- Spragg BP, Hutchings AD. High-performance liquid chromatographic determination of putrescine, spermidine and spermine after derivatization with 4-fluoro-3-nitrobenzotrifluoride. *J Chromatogr* 258: 289–291, 1983
- Stimac E, Morris DR. Messenger RNAs coding for enzymes of polyamine biosynthesis are induced during the G_0 - G_1 transition but not during traverse of the normal G_1 phase. *J Cell Physiol* 133: 590–594, 1987
- Tabor CW, Tabor H. Polyamines. *Annu Rev Biochem* 53: 749–790, 1984
- Tobias KE, Kahana C. Exposure to ornithine results in excessive accumulation of putrescine and apoptotic cell death in ornithine decarboxylase overproducing mouse myeloma cells. *Cell Growth Differ* 6: 1279–1285, 1995
- Walters JD, Wojcik MS. Polyamine transport in human promyelocytic leukemia cells and polymorphonuclear leukocytes. *Leukemia Res* 18: 703–708, 1994
- Wyllie AH. Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 284: 555–556, 1980
- Yuh YS, Thompson EB. Glucocorticoid effect on oncogene/growth gene expression in human T lymphoblastic leukemic cell line CCRF-CEM. Specific c-myc mRNA suppression by dexamethasone. *J Biol Chem* 264: 10904–10910, 1989
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