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Effects of difluoromethylornithine on proliferation, polyamine content and plating efficiency of cultured human carcinoma cells*

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Summary. We investigated the effects of an irreversible inhibitor of ornithine decarboxylase, difluoromethylornithine (DFMO), on the proliferation, polyamine content, and plating efficiency of five human adenocarcinoma cell lines in vitro. DFMO inhibited the growth of all five lines when added at concentrations between 0.1 and 5.0 m*M*. The cell lines varied in their sensitivity to DFMO-induced cytostasis, HuTu-80 being most sensitive and HT-29 being most resistant. These differences appeared to be related to the ability of DFMO to prevent continued production of putrescine in the treated cells. Exogenous putrescine (5–50 μ*M*) reversed the growth inhibition for all five cell lines when added 48 h after DFMO treatment. The lowest concentration of exogenous putrescine (5 μ*M*) only restored intracellular polyamine content of DFMO-treated cells to control levels for the first 24 h after its addition. After that time, spermidine content declined once again to that observed for cells treated with DFMO alone. The higher concentrations of exogenous putrescine restored the content of all three polyamines to control levels for as much as 3 days after its addition, but did not cause a greater increase in cell growth rates than did 5 μ*M* putrescine. These data suggest that human adenocarcinoma cell proliferation is dependent on continued polyamine biosynthesis, but that the basal content of intracellular polyamines is greatly in excess of the minimum level required to support cell growth. In the 1.0–5.0 m*M* concentration range, DFMO treatment for 48 h caused a slight, but statistically significant, reduction in plating efficiency for three of our cell lines, and had no significant effect on the two others.

Introduction

The use of inhibitors of polyamine biosynthesis to investigate both polyamine function and regulation of this biosynthetic pathway has attracted considerable recent research efforts [5, 10, 13, 23]. Of the many questions amenable to investigation with this approach, two are of particular interest to this laboratory. First, numerous studies have attempted to characterize the function(s) of polyamines in regulation of cell proliferation and cell cycle traverse (for reviews see: [4, 15]). Secondly, many experiments testing the potential utility of inhibitors of polyamine biosynthesis in the chemotherapy of neoplastic and other proliferative diseases have recently been reported (for reviews see [1, 6, 13, 16]). We and other groups are investigating the potential utility of modifying tumor cell responses to antineoplastic drugs by depleting intracellular polyamine content. Our approaches to both these problems rely on the use of difluoromethylornithine (DFMO), a specific enzyme-activated irreversible inhibitor of ornithine decarboxylase (ODC, E.C. 4.1.1.17) [12], to inhibit polyamine production in cultured human tumor cells.

In this initial report, we present our observations regarding the time course and dose dependence of the effects of DFMO on proliferation and intracellular polyamine content of five human adenocarcinoma cell lines grown in monolayer culture. We also demonstrate that these effects are a specific consequence of the inhibition of ODC by DFMO, since exogenous putrescine (Pu), the product of ODC, reverses the effects of DFMO. Finally, we document the effect of DFMO pretreatment alone on the plating efficiency of human adenocarcinoma cells.

Materials and methods

Chemicals. Pu dihydrochloride and 1,7-diaminoheptane (DAH) were obtained from Sigma Chemical Co. (St. Louis, Mo). Sd trihydrochloride and spermine (Sp) tetrahydrochloride were purchased from Calbiochem-Behring (La Jolla, Calif). DFMO was a generous gift of the Merrell Research Center, Merrell-Dow Pharmaceuticals, Cincinnati, Ohio. Stock solutions of DFMO (3–150 m*M*) and of Pu (0.1–10 m*M*) were prepared in Dulbecco's modified Eagle's medium (DMEM), adjusted to pH 7.2–7.4, sterilized by membrane filtration (0.2 μm pores), and stored at −20 °C. All other chemicals used were of analytical grade and were obtained from standard commercial sources.

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Abbreviations: ANOVA, analysis of variance; DAH, diaminoheptane; DFMO, α-difluoromethylornithine; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; ODC, ornithine decarboxylase; Pu, putrescine; SD, standard deviation; Sd, spermidine; Sp, spermine

Cell culture and drug treatment. Starter stocks of the cell lines used here were either obtained from the laboratory of Dr. W. L. Washtein of this institution (HuTu-80 and HT-29) or were purchased from the American Type Culture Collection, Rockville, Md (ME-180, MCF-7, and A-427). The tissues of origin of these five human carcinoma cell lines are: HuTu-80, stomach [17]; HT-29, colon [2]; ME-180, cervix [22]; MCF-7, breast [21]; and A-427, lung [3]. Characterization of these cell lines has been described in the papers cited above. Cultures were routinely monitored and found free of mycoplasma infection according to the Mycotect™ assay (BRL Inc., Gaithersburg, Md).

All cell lines were cultured in DMEM plus 10% fetal bovine serum (FBS, Dutchland Laboratories, Denver, Pa) at 37 °C in a humidified atmosphere of 9% CO_2/91% air. Antibiotics and other medium additives were not used. Experiments were initiated by seeding cells (5×10^5 to 1×10^6) harvested from 4-day-old cultures (mid to late exponential growth) into 75 cm^2 tissue culture flasks (Corning) in 14.5 ml DMEM + 10% FBS. After an initial 24-h incubation (to allow for cell attachment and the start of exponential cell growth) cells were treated with 0.5 ml 30-fold concentrated solution of DFMO in DMEM. Control flasks received 0.5 ml DMEM at the same time. In experiments testing the ability of exogenous Pu to reverse the effects of DFMO, 150 µl 100-fold concentrated Pu in DMEM was added 24–48 h after DFMO treatment. At the times indicated below (see *Results*), cells were harvested by trypsinization and dispersed into single-cell suspensions in fresh DMEM + 10% FBS, and the cell densities were then measured with a Model ZBI Coulter cell counter (Coulter Electronics, Inc., Hialeah, Fla).

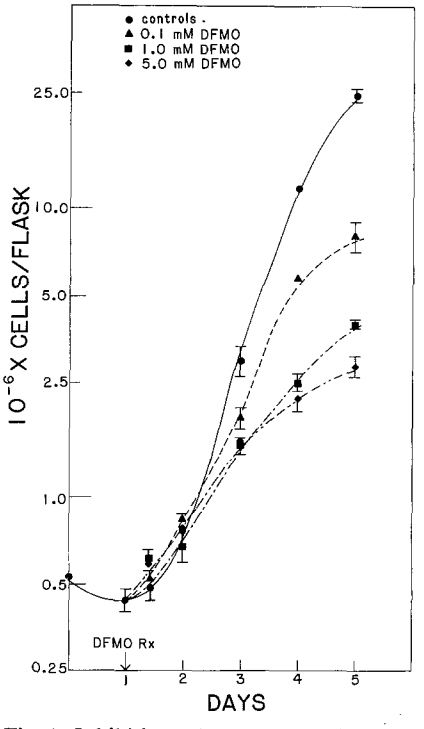

Fig. 1. Inhibition of HuTu-80 cell proliferation by DFMO. Cultures were seeded on day 0 and treated with 0.1 to 5.0 mM DFMO on day 1. Three replicate cultures at each concentration of DFMO, and three untreated controls, were harvested, dispersed and counted (as described under Materials and methods) each day. Points are the means (±SD) of three replicate determinations

Polyamine analysis. Aliquots of the cell suspensions were pelleted by centrifugation (500–600 *g*, 10 min at 0–4 °C) and washed by resuspension in ice-cold phosphate-buffered saline (free of Ca^{2+} – and Mg^{2+}) and a second centrifugation. Washed cell pellets were extracted by sonication (10–15 s at settings 1.5–2, Branson Sonifier Model 185 Cell Disruptor) in 75 µl per 10^6 cells (with a minimum volume of 150 µl) of 0.2 *N* $HC10_4$ containing 20 µ*M* DAH as an internal standard for estimation of recovery. Cell homogenates were kept at 0 °C for at least 30 min, after which cell debris was removed by centrifugation (1400 *g*, 20 min at 0–2 °C). Polyamine concentrations in aliquots (30–75 µl) of the supernatant solutions were determined by means of a minor modification of the reverse-phase paired-ion high-pressure liquid chromatography method of Seiler and Knodgen [20].

Plating efficiency assay. Clonogenicity of cells cultured in the presence or absence of various concentrations of DFMO was determined at 48 or 72 h after the drug's addition to 1-day-old cultures. The cells were trypsinized, dispersed, and counted as above, and 50–200 single cells were seeded into 60-mm culture dishes in a total of 6 ml fresh DMEM + 10% FBS. Five replicate dishes at each of three seeding densities were prepared from each culture tested. After incubation of the dishes for 11–14 days as above, colonies were simultaneously fixed and stained with 0.5% methylene blue (w/v) in 50% aqueous methanol (v/v). Clonogenicity was calculated as the ratio of colonies (of 50 or more cells) observed to cells seeded. Preliminary experiments showed no improvement of control clonogenicity in the presence of over 10% FBS or of feeder layers of heavily irradiated cells, or after longer incubation periods.

Results

We observed a dose-dependent inhibition of proliferation for each of the five cell lines in the presence of DFMO. Figure 1 shows representative growth curves obtained for one cell line, HuTu-80, in the absence and in the presence of 0.1–5.0 m*M* DFMO. Statistically significant differences between control and treated cultures in the number of cells per flask were only observed after 24–48 h of incubation with the ODC inhibitor. Similar results were obtained for all five cell lines. Table 1 lists the yield of cells per flask on the 5th day of incubation with 0–5 m*M* DFMO for the five lines investigated. The cell lines differed in their sensitivity to DFMO-induced cytostasis, HuTu-80 and ME-180 being more sensitive and HT-29, MCF-7, and A-427, less so.

The time course of changes in intracellular polyamine content for these two cell lines is depicted in Fig. 2. For both HuTu-80 and HT-29, all three concentrations of DFMO depleted Pu content below our detection limit (0.05 nmol/10^6 cells) by 12–24 h after treatment (panels IA and IIA). Spermidine was maximally depleted for both lines by 24 h after treatment with 1.0 or 5.0 m*M* DFMO. For HuTu-80, but not for HT-29, the lowest concentration (0.1 m*M*) also brought about maximal depletion of Sd within the 1st day of treatment. All three concentrations resulted in increased Sp content for the first 24 h of treatment with both cell lines. For HuTu-80 cells, Sp levels declined below controls by 48 h of treatment with all three doses, while only the two higher doses reduced HT-29 Sp content below that of controls. Results obtained for the

Table 1. Inhibition of human carcinoma cell proliferation by DFMO

Cell line	10^{-6} × Number of cells per flask[a] Concentration of DFMO[b]			
	Controls	0.1 m*M*	1.0 m*M*	5.0 m*M*
HuTu-80	24.74 ± 1.23	8.09 ± 0.97	3.99 ± 0.12	2.86 ± 0.24
HT-29	14.63 ± 0.50	16.92 ± 0.15	8.46 ± 0.62	7.12 ± 0.33
ME-180	9.90 ± 0.54	5.85 ± 0.34	3.63 ± 0.25	3.09 ± 0.08
MCF-7	20.31 ± 0.42	19.57 ± 1.23	9.23 ± 0.76	7.49 ± 0.56
A-427	18.16 ± 1.03	17.32 ± 0.11	5.54 ± 0.43	4.37 ± 0.09

[a] Measured 5 days after seeding 5×10^5 to 1×10^6 cells per flask. All values are the means (± SD) of three replicates

[b] Added 1 day after seeding

other three cell lines fell between those found for HuTu-80 and HT-29 (data not shown).

To verify that the growth inhibition caused by DFMO was a direct consequence of its effects on polyamine biosynthesis, we investigated the ability of exogenous Pu to reverse the growth inhibition resulting from treatment with the ODC inhibitor. We added Pu 48 h after treatment with DFMO, to maximize the degree of polyamine depletion before attempting to reverse the growth inhibition. Figure 3 shows growth curves for HuTu-80 cells treated with DMEM or 5 m*M* DFMO 1 day after seeding and then with DMEM or 5 μ*M* Pu 2 days later. While Pu has no effect on the growth of control cells, this low concentration of the diamine was able to restore the ability of cells treated with 5 m*M* DFMO to proliferate at close to control growth rates. There appeared to be little or no lag period between addition of exogenous Pu and resumption of cell proliferation. Essentially identical observations were made for each of the five cell lines we used. Table 2 summarizes the cell yields obtained on the 6th day of incubation from untreated cultures and from cultures treated with 5 m*M* DFMO, 5 μ*M* Pu, or the sequential combination of both treatments. Exogenous Pu did not stimulate cell growth for cultures not treated with DFMO in any of our cell lines. The growth inhibition produced by 5 m*M* DFMO was comparable to that seen in our initial dose–response experiments with DFMO (Table 1). In all cases 5 μ*M* exogenous Pu was able to restore the DFMO-treated cells' ability to proliferate. There were, however, quantitative differences between the cell lines in the degree to which cell growth was restored by 5 μ*M* Pu in DFMO-treated cultures.

Measurement of intracellular polyamine content in the above reversal experiments showed that by 48 h after addition of 5 μ*M* Pu to DFMO-treated cells both Pu and Sd levels were no longer greater than those in cells treated with DFMO alone (Fig. 4). Identical results were obtained in two separate experiments for each of our five cell lines regarding this transient replenishment of Sd content of DFMO-treated cells by 5 μ*M* Pu. Sp levels were invariably raised above those of controls on the 1st day after addition of 5μ*M* to 5 m*M* DFMO-treated cultures, but declined to about the same as in controls for the remaining 2 days. In contrast to the above results, when 50 μ*M* Pu was used to reverse the effects of 5 m*M* DFMO, intracellular contents of all three polyamines were restored to close to or slightly higher than control levels for all 3 days after Pu addition (data not shown). However, we observed no significant difference between 5 μ*M* and 50 μ*M* exogenous Pu in the ability to reverse the growth inhibition caused by 5 m*M* DFMO (data not shown).

We measured the plating efficiency of controls and of cells cultured in the presence of 1.0 and 5.0 m*M* DFMO for 48 h (Table 3). The tabulated data were collated from three to five separate experiments for each cell line, with one or two replicate cultures at each concentration of DFMO per experiment and 6–15 replicate dishes counted from each culture tested. We observed considerable variance in plating efficiency for each cell line at each DFMO concentration (including 0), both between different experiments and between replicate cultures within an experiment. To test for a significant effect of DFMO on plating efficiency over and above this background variance, plating efficiencies were compared by a mixed model analysis of variance (ANOVA) (BMDP 3 V; [7]), replicate cultures and experiments being treated as random factors. This analysis was done in the arcsine-square root scale to stabilize the variance, with plating efficiencies weighted by the number of cells seeded per petri dish. The mean values [± standard deviations (SD)] listed in Table 3, however, are unweighted means. For three of the cell lines (HuTu-80, HT-29, and A-427) small but statistically significant reductions in plating efficiency were observed for the DFMO-treated cultures. For these three cell lines, exogenous Pu (50–100 μ*M*) restored the plating efficiency of DFMO-treated cultures to control levels (data not shown).

Discussion

DFMO inhibits the proliferation of all five human carcinoma cell lines we have investigated (Fig. 1, Table 1). The degree of cytostasis observed is related to DFMO concentration and to the drug's ability to block de novo production of polyamines. Further, growth inhibition is only observed once cells are significantly depleted of Sd content (Fig. 2). These observations, together with the ability of very low concentrations of exogenous Pu to restore the proliferation of DFMO-treated cells (Fig. 3, Table 2), convincingly argue that DFMO-induced growth inhibition in these human carcinoma cell lines is a specific consequence of the effects of DFMO on intracellular polyamine biosynthesis and content. These results are thus qualitatively similar to and confirm those previously reported using other rodent and human tumor cell lines [5, 8–10, 13, 19].

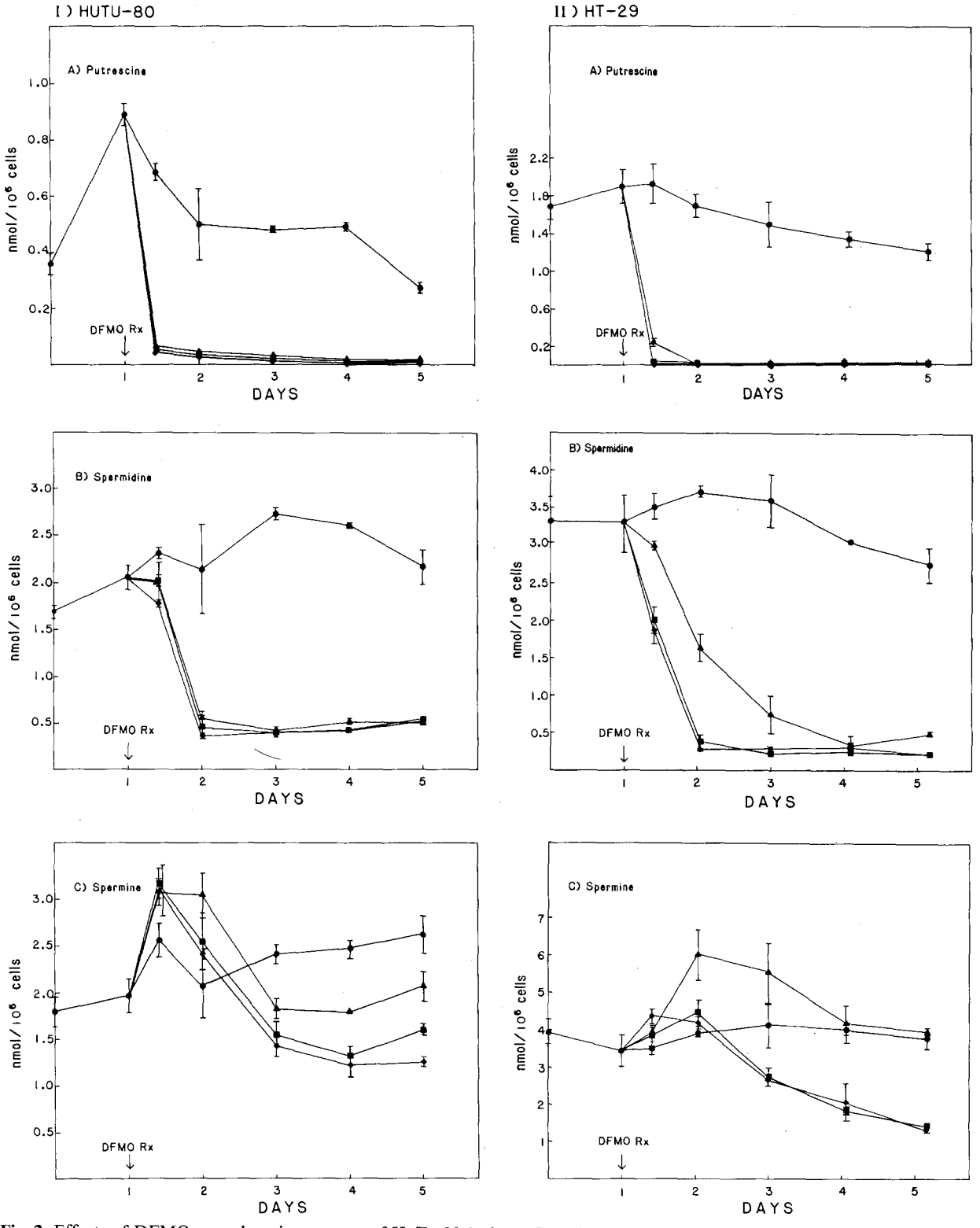

Fig. 2. Effects of DFMO on polyamine content of HuTu-80 (column I) and HT-29 (column II) cells. Cultures were seeded on day 0 and treated with 0 (●), 0.1 (▲), 1.0 (■), or 5.0 (◆) mM DFMO on day 1. Each day, three replicate cultures were harvested, dispersed, counted and pelleted as described (see Materials and methods). Cell pellets were extracted and polyamine concentrations of the extracts were determined by reverse-phase HPLC, also as described. Putrescine content, panels IA and IIA; spermidine content, panels IB and IIB; spermine content, panels IC and IIC. Points are the means (±SD) of three replicate determinations

The data of Fig. 3 and Table 2 demonstrate conclusively that as little as 5 μ*M* exogenous Pu is able to reverse the growth inhibition produced by 5 m*M* DFMO. Furthermore, Fig. 3 shows that the growth rate of cells treated with 5 μ*M* Pu after 48 h in the presence of 5 m*M* DFMO is nearly the same as that of untreated controls. Table 2 also shows that most of the cell lines can achieve nearly the same final cell density in Pu-reversed DFMO-treated cul-

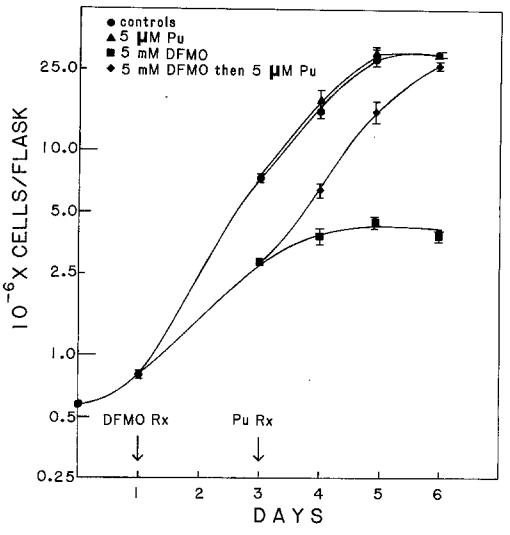

Fig. 3. Reversal of the DFMO-induced growth inhibition of Hu-Tu-80 cells by exogenous putrescine. Cultures were seeded on day 0 and treated on day 1 with 0 or 5 mM DFMO. Half of each group had 5 μM Pu added on day 3. Three replicate cultures were harvested from each treatment group at the times shown. Points are the means (±SD) of three replicate derminations

Table 2. Reversal of the growth inhibition produced by 5 m*M* DFMO in the presence of 5 μ*M* exogenous Pu

Cell line	10^{-6} × Number of cells per flask[a] Treatment			
	Controls	5 μ*M* Pu	5 m*M* DFMO[b]	5 m*M* DFMO[b] then 5 μ*M* Pu
HuTu-80	29.9 ± 0.7	29.7 ± 1.1	3.84 ± 0.25	26.6 ± 1.2
HT-29	19.6 ± 0.2	20.6 ± 0.9	4.47 ± 0.24	10.1 ± 0.4
ME-180	16.4 ± 0.4	16.3 ± 1.0	4.02 ± 0.13	11.7 ± 1.1
MCF-7	23.0 ± 0.3	22.5 ± 1.0	8.52 ± 0.43	17.5 ± 0.3
A-427	31.4 ± 0.7	30.0 ± 1.5	6.26 ± 0.10	27.4 ± 0.9

[a] Measured 6 days after seeding 5×10^5 to 1×10^6 cells per flask; data are the means (±SD) of three replicates

[b] DFMO was added 1 day after seeding; controls recieved an equal volume of vehicle at the same time. Pu was added 3 days after seeding (i.e., 2 days after DFMO treatment); controls and DFMO only cultures received an equal volume of vehicle at the same time

Table 3. Plating efficiencies of human carcinoma cells cultured in the presence or absence of DFMO

Cell line	Plating efficiency (%)[a]		
	Controls	1.0 m*M* DFMO[b]	5.0 m*M* DFMO[b]
HuTu-80	49.8 ± 12.4 (n = 103)	41.5 ± 10.7[c] (n = 120)	30.9 ± 10.9[c] (n = 100)
HT-29	64.9 ± 13.2 (n = 79)	70.7 ± 16.9 (n = 90)	53.1 ± 15.2[c] (n = 86)
ME-180	46.4 ± 13.1 (n = 66)	39.6 ± 18.8 (n = 71)	38.6 ± 17.5 (n = 72)
MCF-7	42.1 ± 14.6 (n = 88)	61.6 ± 18.9 (n = 86)	50.6 ± 21.1 (n = 79)
A-427	36.9 ± 8.6 (n = 77)	28.9 ± 8.1 (n = 81)	29.5 ± 14.6 (n = 65)

[a] Measured for cells harvested 3 days after seeding 5×10^5 to 1×10^6 cells per 75-cm^2 flask. Tabulated data are the means (±SD) of the indicated numbers of replicate 60-mm petri dishes

[b] DFMO treatment was at 24 h after seeding

[c] Significantly different from control plating efficiency, $P < 0.05$

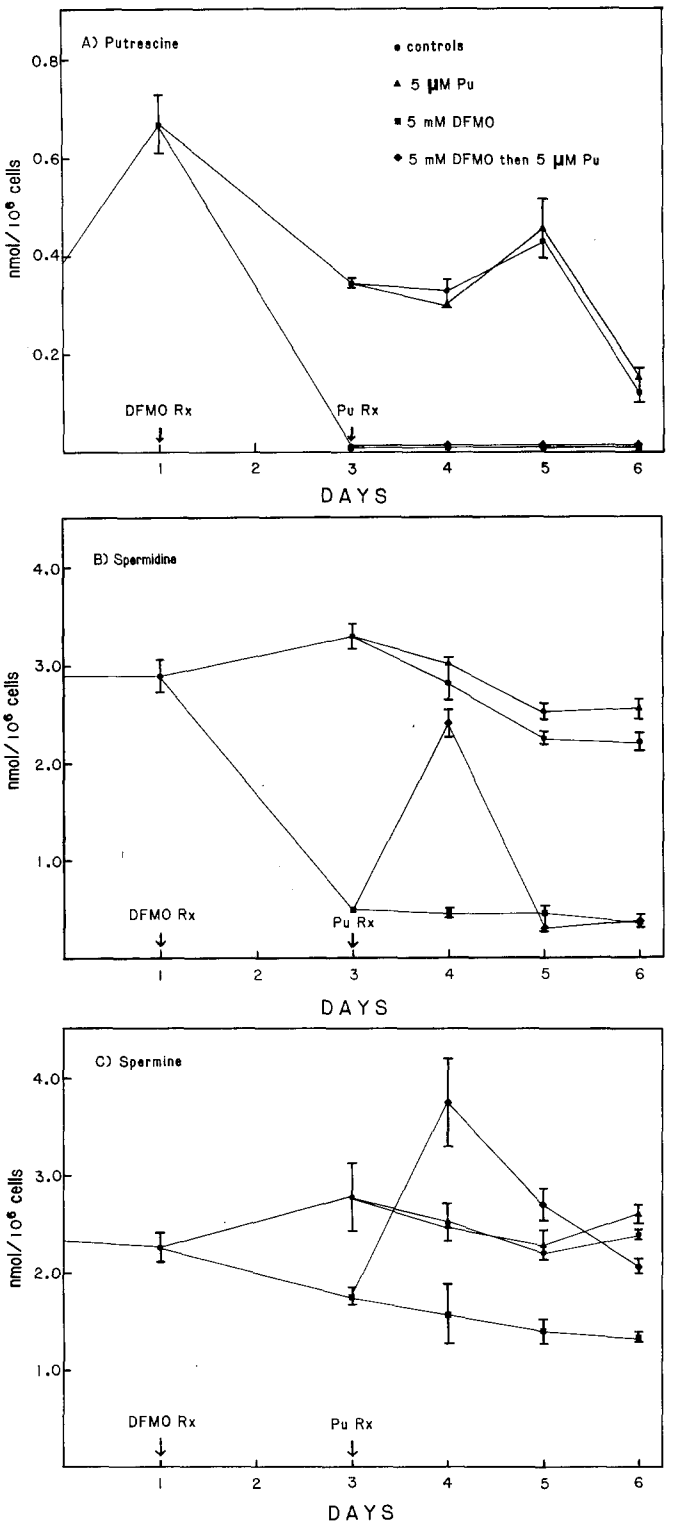

Fig. 4A–C. Effects of exogenous putrescine on polyamine content of DFMO-treated and control A-427 cells. Cultures were seeded on day 0 and treated with 0 or 5 mM DFMO on day 1. Half of each group received 5 μM Pu on day 3. Three replicate cultures from each treatment group were harvested at the times shown, and washed cell pellets were extracted and assayed for polyamine content by reverse-phase HPLC. Putrescine content **A**; spermidine content **B**; spermine content **C**. Points are the means (±SD) of three replicate determinations

tures as in completely untreated controls. These observations are similar to those we reported earlier for 9L cells [19] and to those reported for other cell lines [5, 8–10, 13]. Although these observations suggest that DFMO-induced cytostasis is thus a specific consequence of polyamine depletion, a second possibility remains. It has been shown that an additional consequence of inhibition of ODC by DFMO is a rather drastic increase in the intracellular content of decarboxylated adenosylmethionine [11, 14]. Since decarboxylated adenosylmethionine may inhibit a number of intracellular transmethylation reactions, it is at least possible that the growth inhibition in DFMO-treated cells is due to the accumulation of this sulfonium nucleoside rather than to the depletion of polyamines. Reversal of the growth inhibition by exogenous polyamines did not resolve this issue, since it also reversed the increase in intracellular decarboxylated adenosylmethionine. In both studies, however, the concentrations of exogenous Pu or Sd used to reverse the growth inhibition also restored intracellular Sd content to near control levels. With our cell lines, 5 μ*M* Pu supports continued cell proliferation even though Sd content (on days 5 and 6) is not different from that of cells treated with 5 m*M* DFMO alone (Fig. 4, panel B). It would thus be of great interest to study changes in decarboxylated adenosylmethionine content of these cell lines after addition of 5 μ*M* Pu to DFMO-treated cultures. Such experiments are planned for the near future. The observation of near-normal growth rates on days 4 through 6 of the recovery experiments in spite of the very low Sd levels (see Fig. 3 and 4) also implies that the basal level of intracellular Sd content (as well as virtually all the intracellular Pu) is far in excess of the minimum needed to support continued proliferation. This contention is also supported by the fact that reversal experiments with 50 μ*M* Pu, in which concentrations of all three polyamines were restored to control levels (data not shown), showed no enhancement of growth rate over that observed in reversal of DFMO-induced growth inhibition by 5 μ*M* Pu.

The data of Fig. 2 show that by 2 days after the addition of 1.0–5.0 m*M* DFMO to our human carcinoma cell cultures the cells were maximally depleted of Pu and Sd. In addition, the Sp content of the DFMO-treated cells was also reduced to somewhat more than 50% of control levels. Since longer incubations with DFMO do not appear to produce a more substantial reduction of polyamine content, we feel that our investigations of chemosensitization of human tumor cells to antineoplastic agents by polyamine depletion can best be performed with human carcinoma cells pretreated with 1.0–5.0 mM DFMO for 48 h. The data of our Pu reversal studies demonstrate that a 12–24 h period in the presence of 50–100 μ*M* Pu is needed to restore concentrations of all three polyamines in DFMO-pretreated cells to near control levels. Accordingly, reversal experiments for any significant synergism we find will require addition of 50–100 μ*M* Pu 12–24 h before treatment with the second drug. The above dose ranges and treatment times for both DFMO and Pu also seem optimal for our future studies of cell cycle traverse in polyamine-deficient human carcinoma cells.

For several of our human carcinoma cell lines, a 48 h pretreatment with DFMO caused a significant reduction in plating efficiency (Table 3). The drug did not, however, cause the one to several logs of cell kill that can be produced by most of the antineoplastic agents currently in

clinical use, even at the high concentrations we studied (1.0–5.0 m*M*). Our data thus support previous assessments of DFMO as a relatively nontoxic cytostatic (as opposed to cytocidal) agent [5, 8–10, 13, 18, 19]. Our present observations do demonstrate, however, that surviving fractions of DFMO-pretreated cultures after treatment with a second (cytocidal) agent must be calculated with reference to controls treated with DFMO alone, rather than to totally untreated controls.

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References

1. Coward JK (1982) Polyamine metabolism-recent developments and implications for the design of new chemotherapeutic agents. Annu Rep Med Chem 17: 253
2. Fogh J, Trempe G (1975) New human tumor cell lines. In: Fogh J (ed). Human cells in vitro. Plenum, New York, p 115
3. Giard DJ, Aaronson SA, Todaro GJ, Arnstein P, Kersey JH, Dosik H, Parks WP (1973) In vitro cultivation of human tumors: Establishment of cell lines derived from a series of solid tumors. J Natl Cancer Inst 51: 1417
4. Heby O, Andersson G (1980) Polyamines and the cell cycle. In: Gaugas JM (ed) Polyamines in biomedical research. Wiley, Chichester, p 17
5. Heby O, Janne J (1981) Polyamine antimetabolites: Biochemistry, specificity, and biological effects of inhibitors of polyamine synthesis. In: Morris DR, Marton LJ (eds) Polyamines in biology and medicine. Dekker, New York, p 243
6. Janne J, Poso H, Raina A (1978) Polyamines in rapid growth and cancer. Biochim Biophys Acta 473: 241
7. Jenrich R, Sampson P (1981) General mixed model analysis of variance. In: Dixon WJ (ed) BMDP statistical software. University of California Press, Berkeley, p 413
8. Mamont PS, Duchesne MC, Grove J, Bey P (1978a) 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
9. Mamont PS, Duchesne MC, Joder-Ohlenbusch AM, Grove J (1978b) Effects of ornithine decarboxylase inhibitors on cultured cells. In: Seiler N, Jung MJ, Koch-Weser J (eds) Enzyme activated irreversible inhibitors. Elsevier/North Holland Biomedical, Amsterdam, p 43
10. Mamont PS, Bey P, Koch-Weser J (1980) Biochemical consequences of drug-induced polyamine deficiency in mammalian cells. In: Gaugas JM (ed) Polyamines in biomedical research. Wiley, Chichester, p 147
11. Mamont PS, Danzin C, Wagner J, Siat M, Joder-Ohlenbusch AM, Claverie N (1982) Accumulation of decarboxylated S-adenosyl-L-methionine in mammalian cells as a consequence of the inhibition of putrescine biosynthesis. Eur J Biochem 123: 499
12. Metcalf BW, Bey P, Danzin C, Jung MJ, Casara P, Vevert JP (1978) Catalytic irreversible inhibition of mammalian ornithine decarboxylase by substrate and product analogues. J Am Chem Soc 100: 2551
13. Pegg AE, McCann PP (1982) Polyamine metabolism and function. Am J Physiol 243: C212
14. Pegg AE, Poso H, Shuttleworth K, Bennett RA (1982) Effect of inhibition of polyamine synthesis on the content of decarboxylated S-adenosylmethionine. Biochem J 202: 519
15. Rupniak HT, Paul D (1981) Polyamines and the control of the cell cycle in animal cells. In: Morris DR, Marton LJ (eds) Polyamines in biology and medicine. Dekker, New York, p 315
16. Scalabrino G, Ferioli ME (1982) Polyamines in mammalian tumors: II. Adv Cancer Res 36: 1
17. Schmidt M, Good RA (1975) Transplantation of human cancers to nude mice and effects on thymus growth. J Natl Cancer Inst 55: 81
18. Seidenfeld J, Deen DF, Marton LJ (1980) Depletion of intracellular polyamine content does not alter the survival of 9L rat brain tumor cells after X-irradiation. Int J Radiat Biol 38: 223
19. Seidenfeld J, Gray JW, Marton LJ (1981) Depletion of 9L rat brain tumor cell polyamine content by treatment with DL-α-difluoromethylornithine inhibits proliferation and the G_1 to S transition. Exp Cell Res 131: 209
20. Seiler N, Knodgen B (1980) High-performance liquid chromatographic procedure for the simultaneous determination of the natural polyamines and their monoacetyl derivatives. J Chromatogr 221: 227
21. Soule HD, Vasquez J, Lang A, Albert S, Brennan M (1973) A human cell line from a pleural effusion derived from a breast carcinoma. J Natl Cancer Inst 51: 1409
22. Sykes JA, Whitescarver J, Jernstrom P, Nolan JF, Byatt P (1970) Some properties of a new epithelial cell line of human origin. J Natl Cancer Inst 45: 107
23. Williams-Ashman HG, Canellakis ZN (1979) Polyamines in mammalian biology and medicine. Perspect Biol Med 22: 421

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