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Induction of Major Chromosome Aberrations in Chinese Hamster Ovary Cells by α-Difluoromethylornithine[1]

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ABSTRACT

DL-α-Difluoromethylornithine (DFMO) is a specific irreversible inhibitor of ornithine decarboxylase (EC 4.1.1.17) and has antitumor effects. In this paper, we show that DFMO inhibits the growth of and causes severe chromosomal damage in Chinese hamster ovary cell strain A7 which grows without serum but has deficient arginase activity and therefore requires ornithine or polyamines for continuous replication. In ornithine-containing medium, the A7 cells had very few chromosome aberrations, but incubation of these cells with 0.5 mM DFMO for 7 days induced chromosome aberrations in 12 to 46% of the mitoses. Depletion of polyamines by omitting ornithine from the medium also caused chromosome aberrations. The chromosomal damage found after DFMO treatment alone and ornithine deprivation alone were of similar nature. In addition to chromosome breaks, there were chromosome fragmentation and structurally changed chromosomes including rings, chromatid exchange configurations, and chromosome elongations. A phenomenon resembling premature chromosome condensation was also seen. Double-minute chromosomes were visible in some mitoses, and the chromosome elongations sometimes gave an impression of homogeneously staining regions.

INTRODUCTION

The decarboxylation of ornithine by ODC[3] (EC 4.1.1.17) leads to formation of putrescine which is the precursor of the higher polyamines spermidine and spermine. Marked increase in ODC activity and rapid accumulation of polyamines are characteristically associated with rapid growth. Inhibition of the ODC activity by a specific irreversible inhibitor, DFMO (16), retards the cellular proliferation (8, 14, 19) and has antitumor effects. It prolongs survival of mice bearing L1210 leukemia (19) and retards the growth rate of EMT6 tumors (22). It also reduces the number of papillomas induced on mouse skin by tumor promoters (27, 29). Moreover, DFMO potentiates the antitumor effects of various cytostats (1, 15). Administration of DFMO in combination with a differently acting polyamine inhibitor, methylglyoxal bis(guanylhydrazone), had some therapeutic effects in leukemic children (25). In most experiments, DFMO appeared to be nontoxic. However, in newborn rats, DFMO was found to delay both the intestinal mucosal maturation and recovery from injury (12). Prolonged DFMO treatment of rat reduced the number of leukocytes, RBC, and platelets (13). This drug also markedly suppressed marrow recovery in rats with chemotherapy-induced bone marrow hypoplasia (13). There is some indication that DFMO may cause chromosomal damage. DFMO increased the number of sister chromatid exchanges by 1,3-bis(2-chloroethyl)-1-nitrosourea (28), and studies on viscoelasticity indicated that DFMO may cause conformational changes in DNA (7). In addition, DFMO-treated mitotic HeLa cells had diminished ability to induce premature chromosome condensation when fused with interphase cells (26). In this paper, we show that DFMO causes severe chromosomal damage in a Chinese hamster ovary cell line A7, which grows without serum but because of deficient arginase activity requires ornithine or polyamines for continuous replication (5, 6, 20).

MATERIALS AND METHODS

Cell Culture. A7 cells were cultured on collagen-coated Petri dishes in a 1:1 mixture of minimal essential medium and nutrient mixture F12 supplemented with 0.1% bovine serum albumin (20). In the experiments, nutrient mixture without putrescine was used. The bovine serum albumin was dialyzed against 1 M NaCl:0.2 M $MgCl_2$, and finally against 0.9% NaCl in order to remove traces of polyamines found as impurities. Medium was changed every 3 to 4 days. Cells were regularly checked for mycoplasmas. All tests were negative.

Study of Chromosome Aberrations. The cells were detached with trypsin, washed twice with minimal essential medium and incubated for 2 hr in this medium together with deacetylmethylcolchicine (Colcemid; 0.05 μg/ml; Ciba). The medium was then replaced with 0.075 M potassium chloride and incubated for 15 min at 37°. The cells were fixed with a mixture of ethanol:acetic acid, 3:1 overnight. The slides were prepared by the air-drying technique, and three 10-day-old preparations were stained with Giemsa. The person scoring the chromosome aberrations was unaware which of the preparations contained DFMO and/or ornithine.

Chemicals. DL-α-Difluoromethyl ornithine (MDL 71.782 A) was a gift from the Merrell Research Center, Cincinnati, OH. L-Ornithine monohydrochloride was purchased from Fluka AG, Buchs, Switzerland.

RESULTS

Effect of DFMO on Growth of Cells. Addition of DFMO to a culture of A7 cells growing in the presence of ornithine decreased the rate of growth of the cells considerably. In DFMO-containing cultures, started with 650 cells/sq cm, the cell density after 10 days was only about one-sixtieth of that reached by the control cultures incubated without DFMO (Chart 1). Omission of ornithine from the culture medium inhibited the cellular growth almost to the same extent as did that of DFMO.

Effect of DFMO on Chromosomes. Analysis of chromosome aberrations was made in cultures growing with and without DFMO in the presence and absence of ornithine. Table 1 shows that ornithine-containing cultures without DFMO had high mitotic index, and the frequency of chromosome aberrations was very low. In addition to gaps, there were only a few chromosome

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[3] The abbreviations used are: ODC, ornithine decarboxylase; DFMO, α-difluoromethylornithine.

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Table 1

Effect of DFMO and ornithine starvation on chromosome aberrations

Cells were seeded on Petri dishes (diameter, 3 cm) in ornithine- and DFMO-free medium, and to the indicated cultures ornithine and DFMO were added. After different time intervals, the cells were prepared for inspection of chromosomes. For each value except one, 50 mitoses were scored. For mitotic index, 1000 cells were counted.

		Additions		Chromosome aberrations (%)						
Duration of incubation (days)		Ornithine (0.1 mM)	DFMO (0.5 mM)	Gap	Break[a]	Ring[b]	CTE[c]	Major aberrations[d]	Total (gaps excluded)	Mitotic index (%)
Experiment 1	3	+	−	4	0	0	0	0	0	6.2
	3	+	+	12	12	0	0	0	12	3.4
	3	−	−	24	0	0	0	8	8	1.2
	3	−	+	4	0	0	0	12	12	0.8
	5	+	−	6	4	0	0	0	4	5.2
	5	+	+	12	4	0	8	24	36	1.6
	5	−	−	20	8	0	0	12	20	0.4
	5	−	+	4	0	0	0	16	16	0.4
	7	+	−	8	2	0	0	0	2	4.8
	7	+	+	8	16	4	0	16	36	0.8
	7	−	−	24	0	0	0	16	16	1.0
	7	−	+	12	8	0	4	4	16	0.4
Experiment 2	5	+	−	6	2	0	0	0	2	8.0
	5	+	+	8	14	0	0	0	14	3.8
	5	−	−	20	6	2	4	4	16	2.4
	5	−	+	16	4	0	0	16	20	1.8
	7	+	−	6	0	0	0	0	0	4.4
	7	+	+	26	8	0	2	2	12	2.0
	7	−	−	10	6	0	4	22	32	0.2
	7[e]	−	+	16	8	2	4	20	34	<0.1
Experiment 3	7	+	−	8	0	0	0	0	0	5.4
	7	+	+	10	18	4	4	20	46	1.0
	7	−	−	12	6	2	4	26	38	0.6
	7	−	+	6	20	16	10	30	76	<0.1
	10	−	+	2	18	2	2	68	90	<0.1
	10	−	−	4	24	0	4	38	66	<0.1

[a] Mitoses with break(s); 30% of the mitoses had more than one break.
[b] Mitoses with a single ring or with a ring and break(s).
[c] CTE, chromatid exchange configuration; mitoses with a single chromatid exchange configuration, with a chromatid exchange configuration and break(s), or with a chromatid exchange configuration, break(s), and a ring.
[d] All mitoses contained chromosome fragmentation; in addition, 70% of the mitoses had ring(s) and/or chromatid exchange configuration(s), 20% of the mitoses had chromosome elongation(s), and 15% of the mitoses had chromosome pulverization.
[e] Twenty-five mitoses were scored.

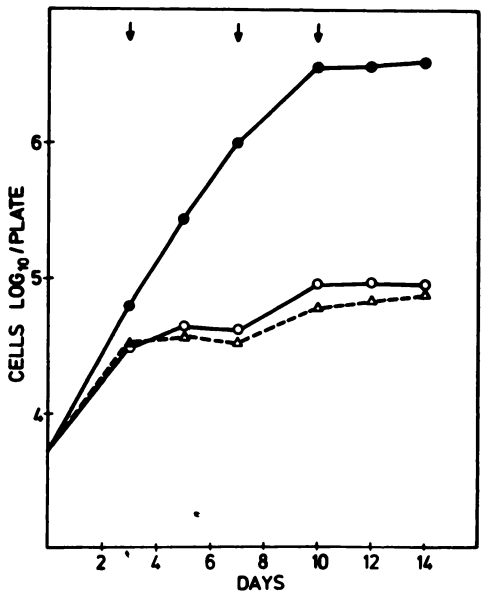

Chart 1. Five thousand cells were seeded on Petri dishes (diameter, 3 cm) in ornithine- and DFMO-free medium. To the indicated cultures, ornithine and DFMO were added, and the cells were counted at different time intervals in an electric cell counter (Coulter Counter). *Arrows*, medium changes. ●, ornithine, 0.1 mM. △, ornithine, 0.1 mM and DFMO, 0.5 mM. ○, no additions.

breaks. No chromosome fragmentation, rings, or chromatid exchange configurations were visible. However, when the ornithine-containing cultures were incubated with DFMO for 7 days, the mitotic index usually decreased several-fold, and 12 to 46% of the mitoses expressed chromosome aberrations, gaps excluded. Moreover, in every experiment a certain fraction of the mitoses showed major chromosome aberrations consisting of chromosome fragmentation with or without acentric fragments, dicentrics, rings, chromosome exchange configurations, and chromosome elongations (Table 1; Fig. 1). Some mitoses also had chromosome pulverization resembling premature chromosome condensation. The results indicate that DFMO induces severe chromosome aberrations in the A7 cells. In each experiment, the difference between the DFMO-containing and the control cultures after 7 days' incubation was significant ($p < 0.001$, according to the Brandt-Snedecor χ^2 method). Omission of ornithine from the culture medium had an effect similar to that of addition of DFMO; the chromosome aberrations were of the same nature, and they occurred roughly at the same frequency. Combining DFMO treatment with ornithine deprivation did not significantly increase chromosome aberrations. It is of interest that mitoses in DFMO-treated and ornithine-starved cultures contained frequently double-minute chromosomes, and there were also chromosome elongations sometimes giving an impression of homogeneously staining region (Fig. 1, *B* and *C*).

DISCUSSION

The results presented in this paper indicate that DFMO, an irreversible inhibitor of ODC, induces severe chromosomal damage in the ornithine-dependent A7 cells grown in ornithine-containing medium. We also confirm earlier observations that polyamine depletion by deprivation of ornithine and polyamines causes chromosomal damage in these cells (10, 19). Analysis of the chromosome aberrations revealed that, in addition to breaks, there were structurally changed chromosomes, including rings and chromatid exchange configurations. This indicates that some kind of repair mechanism is operating in polyamine-depleted cells, induced either by DFMO or by ornithine starvation. The occurrence of double-minute chromosomes and chromosome elongations, giving sometimes an impression of homogeneously staining region, is interesting although there is no information about their possible connection with gene amplification (23).

There is an excellent stereospecific fit between polyamines spermidine and spermine with the DNA double helix (11), and lack of polyamines causes retardation of DNA synthesis (3, 4, 18, 24). Considering that only one-half of the negative charges in the DNA are neutralized by histones, one is tempted to speculate that polyamines might neutralize, at least part, of the remaining negative charges. Depletion of polyamines could make the chromatin structure more fragile and thus give rise to chromosome aberrations. This would explain the irreversibility of the effect of prolonged polyamine starvation (5).

It is notable that chromosome aberrations found in *Mycoplasma*-infected cultures (2, 17) resemble the abberations induced by DFMO and ornithine starvation. It is conceivable that the mechanism in both cases is the same. Mycoplasmas are thought to damage the cells mainly by depleting arginine from the medium (9). Because arginine is the precursor of ornithine and consequently of polyamines, its disappearance would cause polyamine depletion in the cells, and this in turn might lead to chromosome aberrations.

The results presented in this paper show that DFMO can cause severe damage to chromosomes and thus prevent the correct transfer of genetic information from one cell generation to another. Therefore, DFMO may act as a mutagen and a carcinogen.

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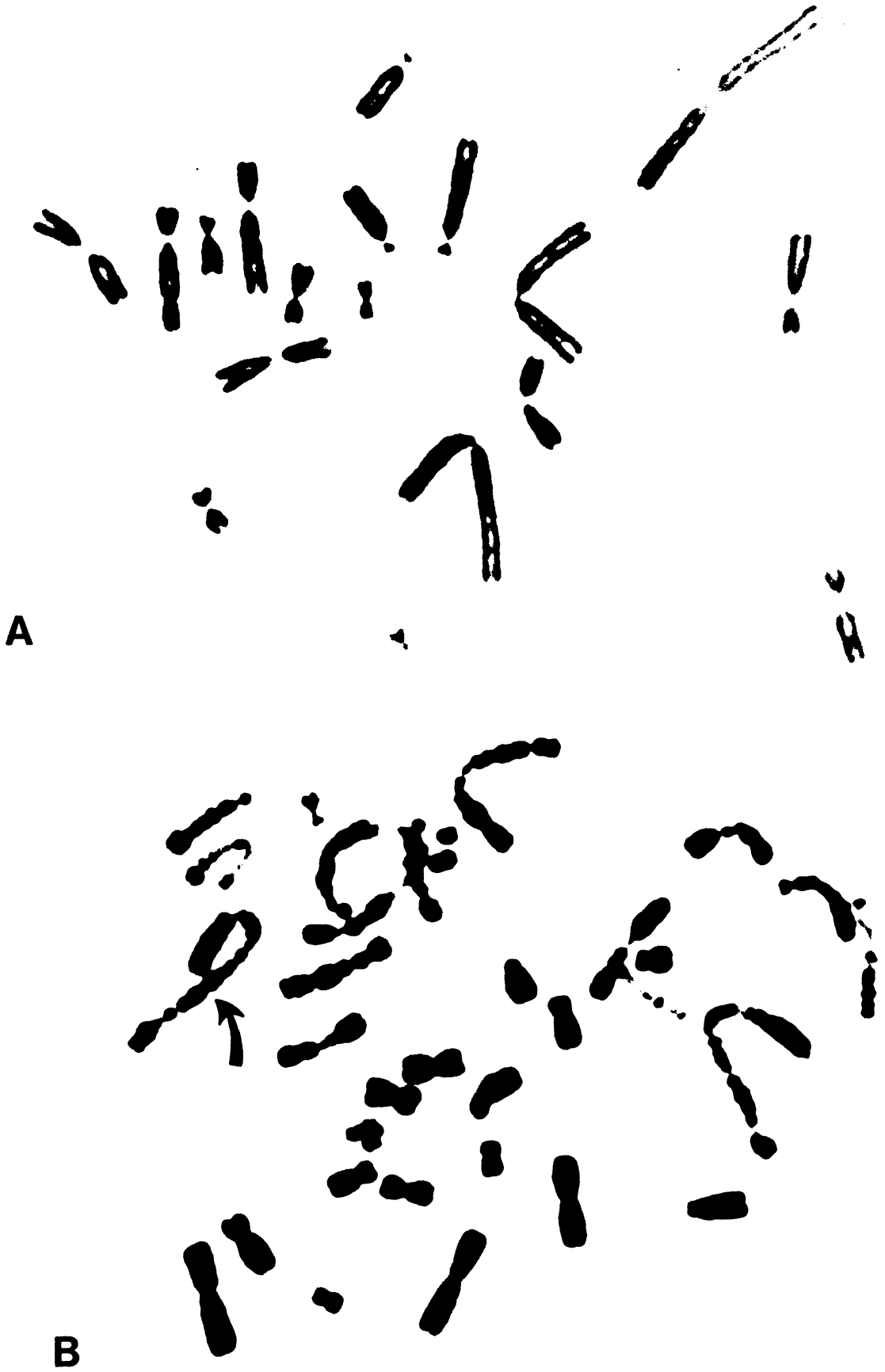

Fig. 1. For chromosome studies, the cells were stained with Giemsa. *A*, metaphase from a control culture grown in the presence of ornithine shows no chromosome aberrations. *B* to *D*, the most typical chromosome aberrations in cultures treated with DFMO (0.5 mM) in the presence of ornithine (0.1 mM) for 7 days. *Arrows*, some chromosome aberrations. *B*, chromosome elongation or homogeneously staining region. *C*, structurally altered chromosomes such as rings, dicentrics, and acentric fragments. *D*, chromosome breakage, chromatid exchange configurations, and double-minute chromosomes.