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ORNITHINE DECARBOXYLASE INHIBITORS INCREASE THE CELLULAR CONTENT OF THE ENZYME: IMPLICATIONS FOR TRANSLATIONAL REGULATION

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SUMMARY: Ehrlich ascites tumor cells grown in the presence of inhibitors of ornithine decarboxylase (EC 4.1.1.17) exhibited an elevated content of this enzyme. The increase could not solely be explained by a decrease in the degradation rate of the enzyme. Instead a stimulation of enzyme synthesis, probably mediated via the polyamine-depleting properties of the inhibitors, is suggested. The enhancement of cellular ornithine decarboxylase content was not accompanied by any significant changes in the amount of ornithine decarboxylase mRNA, indicating a regulation at the level of translation.

The polyamines putrescine, spermidine and spermine are essential for cell growth and differentiation (1-4). Inhibition of polyamine biosynthesis using reversible inhibitors of ornithine decarboxylase (ODC), e.g. DL-α-hydrazino-ornithine and DL-α-methylornithine (MO), results in increased activity of this enzyme, when measured in diluted or dialyzed extracts (5-9). The increase is, at least partly, due to stabilization of the enzyme. The binding of an inhibitor to the active site is believed to render the enzyme less susceptible to proteolytic degradation (10). However, it is also conceivable that the inhibitors, via their polyamine-depleting effect, stimulate the synthesis of ODC (11).

DL-α-Difluoromethylornithine (DFMO) is an extremely potent enzyme-activated irreversible inhibitor of ODC (12). Since this inhibitor binds irreversibly to the active site one would expect it to be at least as effective as the reversible inhibitors in stabilizing ODC against degradation. Thus far, such a study has been hampered due to the irreversible loss of ODC activity. This obstacle has now been overcome by the generation of

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monospecific antibodies against ODC (13-16) and the development of radioimmunoassays for the enzyme (15,16).

In the present report the effects of MO and DFMO on the induction of ODC in Ehrlich ascites tumour cells has been thoroughly investigated using a radioimmunoassay as well as a newly isolated cDNA probe for ODC (17). The results indicate that these inhibitors, probably via their polyamine-depleting properties, stimulate ODC expression at the translational level.

MATERIALS AND METHODS

Materials. MO and DFMO were generous gifts from the Merrell Dow Research Institute (Strasbourg, France). L-(1-^{14}C)Ornithine was obtained from New England Nuclear. Iodine-125 and ^{32}P-dCTP were purchased from Amersham. cDNA (pODC 934) encoding mouse kidney ODC was a kind gift of Dr. Franklin G. Berger (17).

Cells . Ehrlich ascites tumor cells were grown in a 1:1 mixture of Eagle's minimum essential medium and Ham's F12 medium (lacking putrescine) supplemented with 0.2 % bovine serum albumin and antibiotics. The cells were seeded at a density of 1.0 x 10^5 cells/ml in the absence or presence of 5 mM MO or 5 mM DFMO.

Analytical methods. Extracts for measurement of ODC activity and ODC protein were prepared by sonicating the cells in 10 mM Tris-HCl (pH 7.2) containing 0.5 mM Na_2EDTA, 5 mM dithiothreitol and 50 µM pyridoxal 5'-phosphate, followed by centrifugation at 20,000 x g for 20 min. ODC activity was determined by measuring the release of $^{14}CO_2$ from L-(1-^{14}C)ornithine in the presence of saturating levels of pyridoxal 5'-phosphate (0.1 mM) and L-ornithine (0.5 mM) (18). The amount of ODC protein was measured with a radioimmunoassay essentially as described by Seely and Pegg (15), using monospecific mouse kidney ODC antibodies (14) and purified mouse kidney ODC (19) that had been iodinated with the chloramine T method (20).

For polyamine analysis we used an amino acid analyzer equipped with fluorescence detection. Total RNA (20 µg), isolated by the guanidinium/cesium chloride method (21), was fractionated in formaldehyde containing 1% agarose gels (22), transferred onto Gene-Screen (New England Nuclear) and hybridized to pODC 934 labeled by nick translation (23). The relative amounts of ODC mRNA were measured by scanning the autoradiographs, and by cutting out the bands and counting the radioactivity in a liquid scintillation spectrometer.

RESULTS

A typical standard curve for the ODC radioimmunoassay is given in Fig. 1. The sensitivity of the technique enabled us to measure even very low amounts of ODC protein in the cells.

Dilution-induced growth of the cells was accompanied by a large increase in ODC activity, which attained a peak value 1 day after seeding (Fig. 2). This increase in enzyme activity was paralleled by an accumulation of

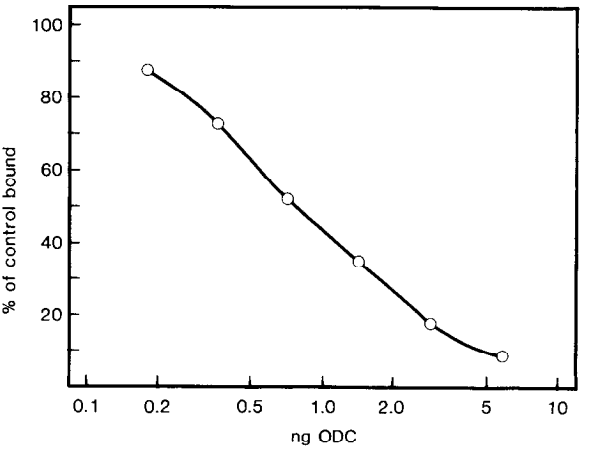

Fig. 1. Standard curve of ODC radioimmunoassay. Iodinated enzyme was incubated with the antiserum (diluted 1:100,000) and the indicated amounts of purified mouse kidney ODC. Antibody-bound radioactivity was determined after precipitation with bacterial protein A adsorbent.

enzyme protein (Fig. 2). Addition of MO to the medium did not change the temporal pattern of ODC induction. However, the increases in ODC activity as well as in ODC protein were 6-7 fold greater in the presence of the inhibitor (Fig. 2). As expected, a marked decrease in ODC activity was observed when the medium was supplemented with DFMO. Nevertheless, the measurement of ODC protein revealed a large increase and a peak value of 3-4 times that obtained without DFMO was seen after 2 days of treatment (Fig. 2). Subsequently the amount of ODC protein declined slowly.

The additional increase in ODC activity caused by MO was partly due to stabilization of the enzyme (Fig. 3). When cycloheximide was added to cells

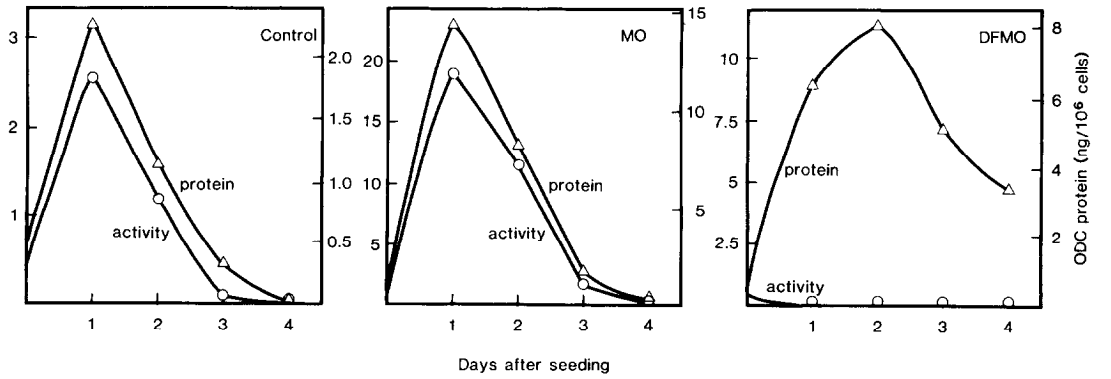

Fig. 2. Effects of MO and DFMO on the induction of ODC in Ehrlich ascites tumor cells. ODC activity (○), 1 unit = 1 nmol CO_2/h; ODC protein (△).

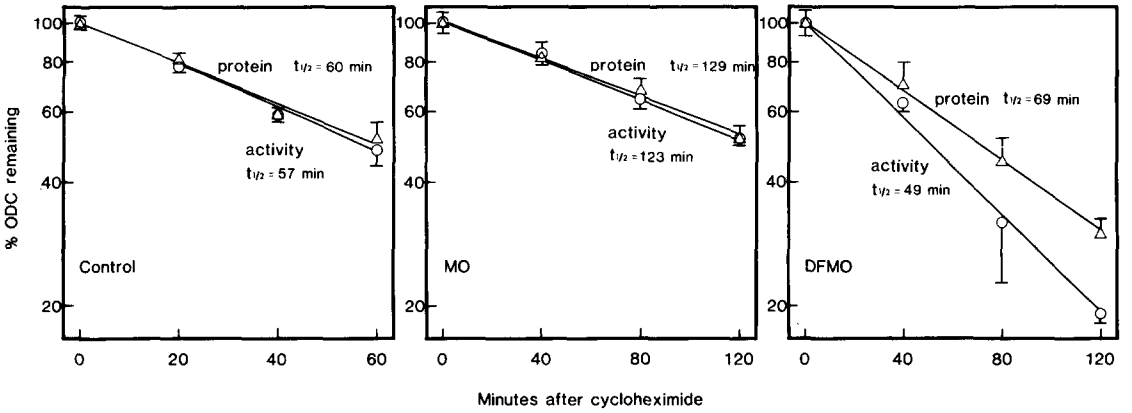

Fig. 3. Effects of MO and DFMO on the turnover of ODC in Ehrlich ascites tumor cells. Cycloheximide (50 µg/ml) was added after 1 day of treatment with MO or DFMO. Results are expressed as per cent of the value obtained at the time of cycloheximide administration. The initial ODC activities (units/10^6 cells) were 2.0, 7.1 and 0.03; and the initial ODC protein contents (ng/10^6 cells) were 1.2, 7.1 and 4.3 for control, MO-treated and DFMO-treated cells, respectively. ODC activity (○); ODC protein (△). Mean ± S.E.M., n = 3.

grown in the presence of MO the ODC activity disappeared with a half-life of 123 minutes as compared to a half-life of 57 minutes when cells were grown in the absence of MO. The ODC protein was shown to turn over at about the same rate as the ODC activity, in MO-treated and in control cells (Fig. 3). Interestingly, the ODC protein was not stabilized to any greater extent by the binding of DFMO to the active site. In this case ODC protein turned over with a half-life of 69 minutes, whereas the half-life of the remaining enzyme activity was estimated to be about 50 minutes (Fig. 3). The latter value is a rough estimate since less than 1 % of the ODC molecules were catalytically active.

Hence, it is apparent that the increase in ODC content caused by DFMO treatment cannot be explained by a decreased rate of degradation. Neither can the 2-fold increase in half-life of ODC be solely responsible for the 6-7 fold increase in ODC content observed after the addition of MO. Thus, part of the effect of MO and DFMO on the amounts of ODC must be exerted by a change in the synthesis of ODC.

In order to determine whether the presumable change in enzyme synthesis caused by the inhibitors was related to a change in the level of ODC

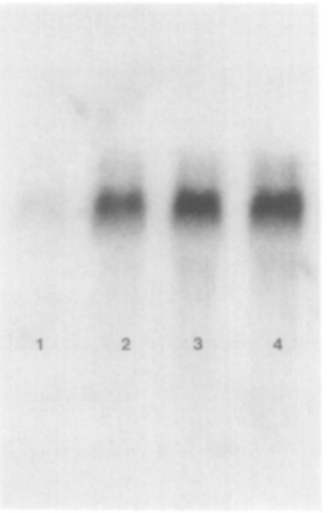

Fig. 4. Effects of MO and DFMO on ODC mRNA levels in Ehrlich ascites tumor cells. Before seeding (lane 1); 1 day after seeding (lane 2); 1 day after seeding and addition of MO (lane 3); 1 day after seeding and addition of DFMO (lane 4). The relative amounts of ODC mRNA were 1:3.2:3.5:3.6.

message, total RNA from the cells was examined by Northern blot analysis using a cDNA encoding mammalian ODC (17). As seen in Fig. 4 a striking increase in the content of ODC mRNA was observed when the cells were diluted in fresh medium. The addition of MO or DFMO to the medium did not markedly affect the levels of ODC mRNA, indicating an effect on the translational rather than the transcriptional level (Fig. 4).

Cell growth after seeding was accompanied by an increased polyamine content (Fig. 5). The addition of MO or DFMO to the medium inhibited this

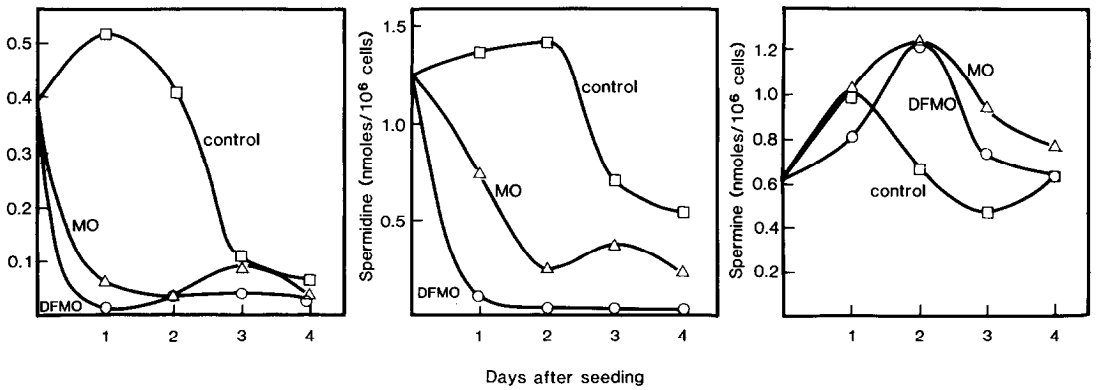

Fig. 5. Effects of MO and DFMO on the polyamine content of Ehrlich ascites tumor cells. Control (□); MO (△); DFMO (○).

accumulation and gave rise to a marked decline in the putrescine and spermidine content (Fig. 5). The spermine content, on the other hand, was not reduced by treatment with the inhibitors (Fig. 5). In fact, an increase in cellular spermine content was observed after 2 and 3 days of treatment.

DISCUSSION

The present results clearly demonstrate that the increase in ODC content caused by MO or DFMO cannot solely be explained by a decrease in the degradation rate of the enzyme. In the case of DFMO, ODC was hardly stabilized at all. This is somewhat surprising since treatment with the reversible inhibitors always results in a prolongation of the half-life of the enzyme (5-8). The mechanism of this stabilization is not yet fully understood. It is believed that the presence of the inhibitor in the active site renders the enzyme less susceptible to proteolytic degradation (10). The binding of DFMO, however, does not seem to give this stabilization of ODC. This is also indicated by the work of Seely and Pegg (24) who demonstrated that ODC labeled with radioactive DFMO turns over at the same rate as the native enzyme in the mouse kidney.

Hence, the effects of the inhibitors appear to also involve an increased synthesis of ODC. This is, however, not accompanied by any changes in the amount of ODC mRNA, indicating a regulation at the level of translation. In this connection it should be mentioned that no effect on total protein synthesis was observed after treatment with MO or DFMO (not shown). The stimulation of ODC synthesis by the inhibitors is most likely mediated via their polyamine-depleting properties. Evidence is accumulating that the polyamines exert a negative feed-back control of ODC (1-4).

Prolonged treatment with DFMO has recently been shown to give rise to resistant cell lines that overproduce ODC (25-28). However, in these cases the overproduction appears to be caused by amplification of the ODC gene which results in elevated levels of the ODC message (27-29).

ACKNOWLEDGEMENT

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