α-DIFLUOROMETHYLORNITHINE INHIBITS THE GROWTH OF FIBROSARCOMA IN RATS

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SUMMARY

α-Difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase (ODC) and polyamine synthesis retarded the growth of rat fibrosarcoma. Rats were given a 2% solution of DFMO starting 24 hr after implantation of fibrosarcoma cells. This resulted in an 80% inhibition of tumor weight gain on day 17 as well as a 70% decrease in putrescine and spermidine levels in tumors. Hepatic levels of these amines were also reduced. DFMO treatment partially prevented increase in liver γ-glutamyl transpeptidase (GGTP) which was seen as a response to the extra hepatic tumor. However the levels of ornithine, proline and urea were unaffected in both tumor and liver on treatment with DFMO.

INTRODUCTION

POLYAMINES putrescine, spermidine, spermine are important body constituents mediating a large number of processes. Increase in ornithine decarboxylase, the first and rate-limiting enzyme in polyamine biosynthesis and accumulation of polyamines are associated with rapid cell proliferation and tumor growth[1]. α-Difluoromethylornithine (DFMO) an irreversible inhibitor of ODC has been developed as an effective anticancer agent. It was shown to be very effective both in combination and by itself in experimental tumor models, leukemia and other solid cancers[2, 3]. Its potent effect on tumors is due to its inhibition of ODC which results in decrease in the putrescine and spermidine levels in tumors.

The presence of tumor alters the functions of distal non-tumor tissues, such as the altered polyamine metabolism in liver, spleen and kidney of leukemia bearing mice[4]. Numerous liver enzymes are also known to be affected by extra hepatic tumors. γ-glutamyl transpeptidase is one of the enzymes which has been found to undergo several-fold increase in response to fibrosarcoma[5].

As liver is the organ processing most of the drugs glutathione levels are perturbed during drug metabolism[6]. Furthermore DFMO could affect ornithine levels, due to its inhibition of ODC. This might reflect in altered levels of either ornithine, proline or urea, the metabolites into which ornithine could possibly be channeled.

In the present paper we report the effect of DFMO on tumor growth and also the metabolic changes in the liver and tumor which might reflect the efficacy of the drug.

MATERIALS AND METHODS

α-difluoromethylornithine was a generous gift from the Merrell Dow Research Centre, Cincinnati, Ohio, USA. Putrescine, spermine, γ-glutamyl-*p*-nitroanilide, dithiobisnitrobenzoid acid, ninhydrin were purchased from Sigma Chemical Company, U.S.A. Dowex AG 1 × 8 (200–400 mesh) Dowex AG 50 × 8 (200–400 mesh) were from Bio. Rad. All other chemicals were of analytical grade.

Fibrosarcoma tumor

Fibrosarcoma was induced in Wistar rats by subcutaneous implantation of Millipore filter disc impregnated with a 5% suspension of 20-methylcholanthrene in paraffin oil[7]. Tumors appeared in about 4 weeks after implantation and were highly localised and maintained by serial transplantation. For serial transplantation the excised tumor, free of necrotic and muscle tissues, was minced and suspended in normal saline. A suspension of about 1×10^5 cells in 0.2 ml saline was injected subcutaneously. The tumor became palpable in 6–8 days time, grew steadily up to the end of second week after which necrosis set in and the animal eventually died in about 4 weeks. Animals were housed in plastic cages and fed food and water *ad libitum*. Animals were divided into two batches after injection of fibrosarcoma tumor cells. One batch was given a solution of DFMO as the sole drinking fluid starting from day 1. An 1% solution of DFMO was used from day 1 to day 8. From day 9 a 2% solution of DFMO was given till the end of the experiment. The other batch was given tap water. The daily fluid intake was calculated.

Measurement of tumor weight

Tumor weight was approximately calculated by two-dimensional measurements using the fomula[8] length × width^2/2. Tumor weight obtained from vernier calipers measurements of length and width and actual weight measurement of the tumor were found to be nearly the same.

Animals were killed on day 17 by ether anaesthesia. The tumor and liver were rapidly excised and weighed. A piece of tissue free from necrosis was weighed and immediately homogenised in ice cold 5% perchloric acid. The perchloric acid extract was used for measuring ornithine, proline, urea and polyamines as described earlier[9].

Glutathione levels were estimated using dithiobisnitro benzoic acid in extracts obtained by homogenising tissues in 4% sulfosalicylic acid[10]. γ-glutamyl transpeptidase was assayed in liver homogenates using γ-glutamyl *p*-nitroanilide as substrate[11].

RESULTS

The volume of DFMO solution consumed was about 13 ml/rat/day. For a 100 g rat this was equal to a dose of 1.29 g/kg/day of DFMO in the first half and, 2.13 g/kg/day in the second half of the experiment.

As shown in figure 1, there was an 80% inhibition of tumor growth as measured on day 17. On this day the average tumor weight of control rat was approximately 5 times that of treated animals. Figure 2 shows the changes in polyamines levels in the liver and tumors of DFMO treated and untreated rats. Putrescine levels were very low in treated rats. Spermidine showed a 73% decrease in tumor in treated animals as measured on day 17. Spermine levels did not show any appreciable alteration. Spermidine was increased in the liver of tumor bearing rat also. DFMO treatment caused a 30% decrease of spermidine level. As in the case of tumor, DFMO treatment did not alter spermine levels in liver.

Table 1 shows changes in liver weight, GSH and GGTP levels as measured on day 17. Liver weight was not affected by the presence of tumor and was therefore unchanged by DFMO. Glutathione levels were slightly decreased in the liver of tumor bearing animals. Drug-treated animals showed almost normal GSH levels. There was a 2.2 fold increase in GGTP. DFMO treatment partially prevented the increase in GGTP and it was about 40% of the value seen in tumor bearing animals.

The effect on ornithine and other related metabolites in the tumor and liver of treated and untreated

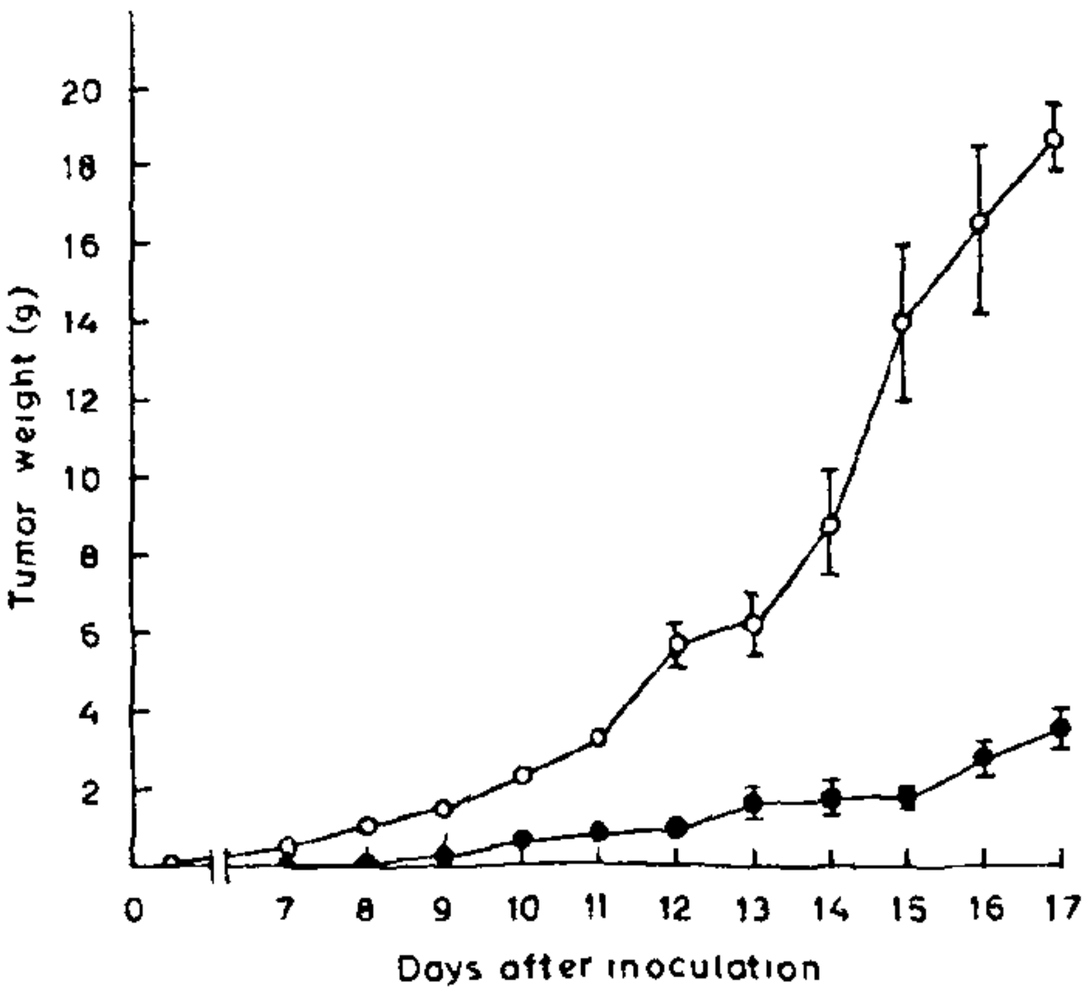

Figure 1. Inhibition of growth of tumor in rats by DFMO administered in drinking water. Other details of feeding schedule are as mentioned in text. Values represent Mean ± SEM of 4–6 rats. o–o untreated ●–● treated.

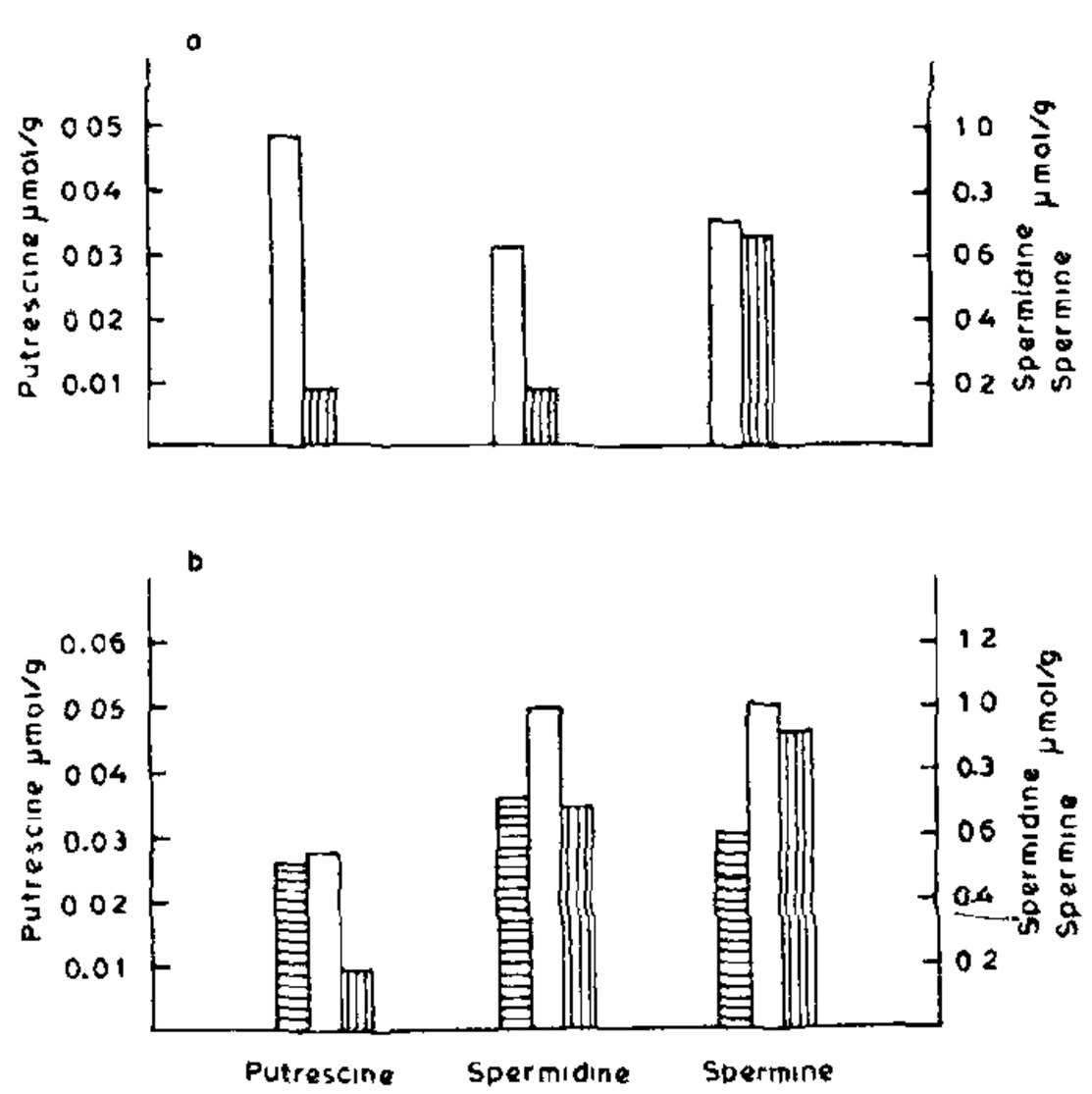

Figure 2. Effect of DFMO on polyamine concentrations in (a) tumor and (b) liver of rat as measured on day 17. Values represent mean of 4 rats. normal ▤ -untreated ▢ -treated ▥.

fibrosarcoma bearing rat is shown in table 2. Ornithine and urea levels remained unaffected both in the presence of tumor as well as during drug treatment. Proline levels were not altered by the drug in the tumor

Table 1 *Effect of DFMO on hepatic GSH and GGTP in fibrosarcoma tumor bearing rats*

	Liver weight(g)	Glutathione μmol/g	GGTP μmol/g/min
Day 0	4.86 ± 0.33	5.62 ± 0.23	38.3 ± 8.5
Day 17 (untreated)	4.38 ± 0.37	4.51 ± 0.14	85.0 ± 23.0
Day 17 (treated)	4.39 ± 0.40	4.97 ± 0.48	51.7 ± 6.1

Values are mean ± SD of 4 rats. GGTP assay was performed in liver homogenates as mentioned in text. GSH was assayed with DTNB[10].

whereas there was a 40% decrease in the liver of drug treated rats.

DISCUSSION

We have shown DFMO to be effective in retarding tumor growth when started immediately after injection of fibrosarcoma tumor cells. Earlier reports on other solid tumors have shown inhibition by DFMO at concentrations ranging from 3–5%. We have seen DFMO to be unable to prevent the growth of tumor when a 2% solution of DFMO was started on day 8, the time during which the tumor became palpable. It appears that its growth inhibitory properties are dependent more on the time at which the treatment is started rather than on the dosage used. Perhaps a continuous infusion of DFMO may bring about better response during later stages of tumor growth.

There is no complete depletion of polyamines because ODC is known to have a rapid turnover in tissues[12] and spermine is unaffected due to the stimulatory action of DFMO on S-Adenosylmethionine decarboxylase activity[2].

DFMO is non-toxic even at high concentrations, and this property is finding important use in combination chemotherapy and as an adjuvant during chemotherapy with cytotoxic drugs[13]. Recent results obtained with DFMO in combination with interferon in a mouse melanoma model are promising[14].

Increase in hepatic γ-glutamyl transpeptidase has been noticed in animals with a variety of tumors[5, 15]. This work shows GGTP to be drug responsive and may be a good marker to test efficacy of anticancer drugs.

Maintenance of normal levels of ornithine and urea and decrease in proline levels by the drug in liver may possibly due to the fact that DFMO is specific only for ODC and does not inhibit by other ornithine metabolising enzyme[16]. We plan work using radiolabeled aminoacids to identify the channeling of these metabolities.

ACKNOWLEDGEMENT

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1. Janne, J., Poso, H., Raina, A., *Biochem. Biophys. Acta*, 1978, **473**, 241.
2. Prakash, N. J., Schechter, P. J., Grove, J. and Koch-Weser, J., *Cancer Res.*, 1978, **38**, 3059.
3. Prakash, N. J., Schechter, P. J., Mamont, P. S., Grove, J., Koch-Weser, J. and Sjoerdsma, A., *Life Sci.*, 1980, **26**, 181.
4. Heby, O. and Russell, D. H., *Cancer Res.*, 1973, **33**, 159.
5. Koss, B. and Greengard, O., *Cancer Res.*, 1982, **42**, 2146.
6. Boyland, E. and Chasseand, L. F., *Adv. Enzymol.*, 1969, **32**, 173.
7. Nagarajan, B. and Sankaran, S., *Indian J. Cancer*, 1973, **10**, 83.
8. Geran, R. I., Greenberg, N. H., Macdonald, M. M., Schumacher, A. M. and Abbott, B. J., *Cancer Chemotherapy Rep.*, 1972, **3**, 1.

Table 2 *Concentrations of ornithine, proline and urea in tumor and liver of fibrosarcoma bearing rats. Effect of treatment with DFMO*

	Ornithine		Proline		Urea	
	Tumor	Liver	Tumor	Liver	Tumor	Liver
Day 0	—	0.222 ± 0.034	—	0.274 ± 0.05	—	2.87 ± 0.253
Day 17 (untreated)	0.05 ± 0.02	0.204 ± 0.053	2.4 ± 0.15	0.593 ± 0.17	3.61 ± 0.83	3.5 ± 1.09
Day 17 (treated)	0.041 ± 0.013	0.206 ± 0.04	2.16 ± 0.56	0.358 ± 0.076	3.13 ± 0.942	3.2 ± 0.6

Values are expressed in μmol/g and is the mean ± SD of 4 rats.

9. Gopalakrishna, R. and Nagarajan, B., *Anal. Biochem.*, 1980, **107**, 3181.
10. Ellman, G. L., *Biochem. Biophys.*, 1959, **82**, 70.
11. Chandar, N. and Nagarajan, B., *Biochem. Int.*, 1984, **8**, 41.
12. Russell, D. H. and Snyder, S. H., *Mol. Pharmacol.*, 1969, **5**, 253.
13. Bartholeyns, J. and Koch-Wesser, J., *Cancer Res.*, 1981, **41**, 5158.
14. Sunkara, P. S., Prakash, N. J., Mayer, G. D. and Sjoerdsma, A., *Science*, 1983, **219**, 851.
15. Herzfeld, A. and Greengard, O., *Cancer Res.*, 1977, **37**, 231.
16. Sjoerdsma, A., *Clin. Pharmacol. Ther.*, 1981, **30**, 3.

NEWS

BEARDS AND MOUSTACHES: HAZARDOUS TO YOUR HEALTH?

. . . A team of scientists from the USSR Academy of Medical Sciences' Research Inst. of General & Communal Hygiene analyzed the quality of air inhaled by men with moustaches and beards. The air inhaled by these men "was found to contain several dozen toxic substances including phenol, benzene, toluene, ammonia, sulphuretted hydrogen, acetone, isoprene and acetic acid. . . . Most probably, the substances were exhaled by the subjects themselves, absorbed by the hair surface and then released again, having been inhaled. . . . The average pollution index of inhaled air in the case of moustached subjects equalled 4.2 maximum permissible concentrations [MPC] as against less than one MPC in clean air. A beard does not increase pollution so much—only to 1.9 MPC. However, if both a moustache and a beard are worn the index goes up to 7.2—if the person is a non-smoker. In the case of smokers, the figures 24.7 (moustache alone), 18.2 (beard alone) and 49.3 (both)."

[Mikhail Dmitriyev in *Sputnik* (12): 145-6, Dec. 84. (From *Priroda*) Reproduced with permission from Press Digest, *Current Contents*®, No. 4, January 28, 1985, p. 11. (Published by the Institute for Scientific Information®, Philadelphia, PA, USA.)]

DEPRESSED? CALL SHRINK LINK

. . . "A new form of practice: counselling strangers who are anxious, depressed, or just plain worried. And the patients supply their own couches. A telephone service called Shrink Link has been operating in New York since October [1984]. Anyone who has a problem can call . . . and talk to a psychiatrist or psychologist at a cost of $15 for each 10 minutes, payable by credit card. . . . Howard I. Glazer [Payne Whitney Psychiatric Ctr., Cornell U.] says using the phone has advantages. He says people don't hesitate to use it before problems get severe enough to drive them to make an office appointment. . . . Callers present a wide range of problems. Many have financial worries. Single women often complain that all the attractive men they meet are married or gay. Callers who are under stress and mainly tense may get coached in relaxation exercises. Chronic pain that restricts activity is a frequent complaint, and these people are referred to pain clinics. 'We have to be very careful,' says Kathryn Hahner [Shrink Link project director]. 'If a problem appears to be even slightly medical, we refer the caller to a physician or a clinic.' But some who need referral for psychotherapy are reluctant to accept it, often for financial reasons, Hahner says. To such people she suggests resources offering services for fees adjusted according to income."

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