An essential role for polyamines in tumor metastases

Prasad S. Sunkara, Nellikunja J. Prakash and Andrea L. Rosenberger

Merrell Dow Research Center, 2110 East Galbraith Road, Cincinnati, OH 45215, USA

Received 29 October 1982

Polyamine *Tumor metastasis* *α-Difluoromethyl* *Ornithine*

1. INTRODUCTION

Investigations into the role of polyamines in cellular proliferation have been greatly facilitated by the availability in recent years of specific inhibitors of polyamine biosynthetic enzymes [1,2]. Difluoromethyl ornithine (DFMO) is a specific irreversible inhibitor of ornithine decarboxylase [3] and causes rapid depletion of intracellular putrescine and spermidine in a number of cells grown in culture [4–6]. Inhibition of polyamine biosynthesis by DFMO results in inhibition of tumor growth in a number of transplanted and chemically induced animal tumors [7,8]. Although these experimental observations are indicative of an essential role for polyamines during tumor cell proliferation, there is no evidence available for their role in tumor metastases. The ability of primary tumors to metastasize is a major obstacle to curing human cancers. In the present study we have examined the effect of DFMO on the growth and pulmonary metastasis of Lewis lung (3LL) tumor in mice. The Lewis lung tumor of C57 BL mice provides an excellent experimental model for studying tumor metastases. The primary tumor grows rapidly and metastasizes to lungs in ~3 weeks after tumor transplantation; these pulmonary foci can easily be counted. These results indicate that inhibition of polyamine biosynthesis by DFMO results in a 40–50% inhibition of growth of the primary tumor and ~80% inhibition of pulmonary metastasis. Further, the effects of DFMO could easily be reversed by simultaneous administration of putrescine to the animals along with DFMO, suggesting an essential role for polyamines in tumor growth and metastasis.

2. MATERIALS AND METHODS

2.1. *Animals*

C57 BL/6J mice (18–20 g) from Charles River were used for the transplantation of the tumor. The animals were housed in stainless steel cages with free access to food and water.

2.2. *Tumor*

Lewis lung carcinoma (3LL) was kindly supplied by Mason Research Institute. The tumor was propagated and maintained in vivo by serial transfers of dissociated tumor cells in C57 BL/6J mice. Rapidly dividing 2-week-old tumors were excised from the animals and trypsinised. The resulting cell suspension was passed through sterile gauze. The viability of the tumor cells in the supernatant was determined by Trypan blue dye exclusion method. Tumors were induced in mice by subcutaneous injection of 1×10^6 viable cells at the interscapular region. Tumors started to appear within a week. Mice bearing tumors were killed on the 18th day, the primary subcutaneous tumor was excised, and a portion of the tissue frozen at $-70°C$ for polyamine analysis. The lungs were examined for metastases by injecting diluted India ink into the trachea before fixation of the whole lung [9]. Metastases appeared as white nodules against black normal lung.

2.3. *Drugs*

D,L-α-Difluoromethyl ornithine (DFMO) was

Published by Elsevier Biomedical Press
00145793/82/0000—0000/$2.75

synthesized in our laboratories as in [3]. DFMO was administered in the drinking water as a 2%-aqueous solution. The mean daily intake of the drug was found to be about 3 g.kg^{-1}.day. Putrescine was administered intraperitoneally at 100 mg.kg^{-1}.day for 18 days.

2.4. *Polyamine analysis*

The tumor tissues were homogenized in 0.4 M perchloric acid, and the supernatants obtained after centrifugation were used for polyamine determination by dansylation and subsequent thin-layer chromataography as in [10].

3. RESULTS

The effect of DFMO on the growth and polyamine content of Lewis lung tumor is presented in fig. 1. Administration of DFMO to animals bearing tumors resulted in a 69% and a 75% decrease in the intracellular levels of putrescine and spermidine, respectively, without any change in the spermine concentrations. The decreased polyamine levels were also associated with an inhibition of tumor growth by 43%. Inhibition of polyamine biosynthesis as a result of DFMO administration not only inhibited growth of the primary tumor but also dramatically decreased the secondary pulmonary metastases by 79% with 25% of the animals showing no visible metastases (table 1). Further, this inhibition of tumor growth and metastases could be reversed by simultaneous administration of putrescine to the animals (fig. 1 and table 1). Administration of putrescine to animals receiving DFMO did not result in any significant elevation of tumor putrescine concentration although spermidine concentrations were substantially elevated compared to tumors from animals which received DFMO alone. The lack of any effect on tumor putrescine concentration following exogenous putrescine administration to DFMO-treated animals is most likely due to its rapid conversion to spermidine. Since polyamine depletion in tumor cells leads to an increase of *S*-adenosyl-L-methionine decarboxylase [7,12], the rate-limiting enzyme involved in the conversion of putrescine to spermidine, DFMO treatment is likely to facilitate the conversion of exogenous putrescine to spermidine in the solid tumor employed here.

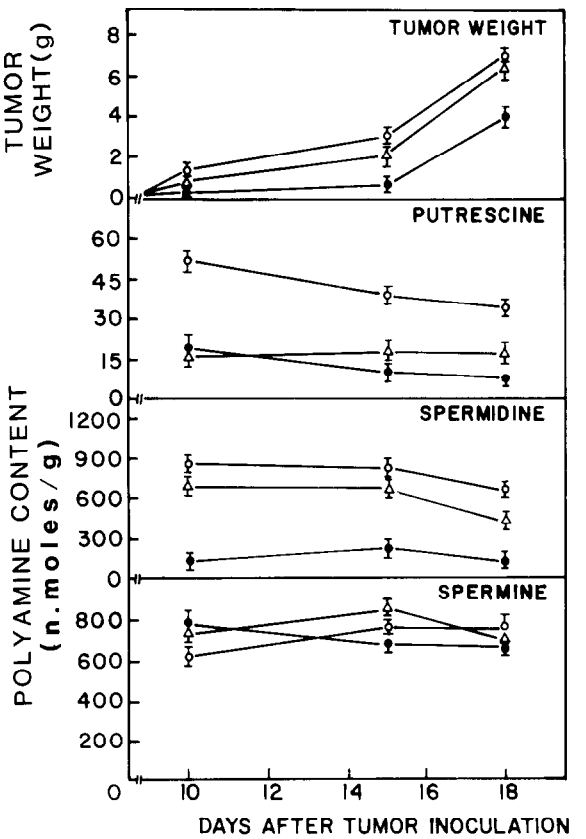

Fig. 1. Effect of DFMO on the growth and polyamine levels of Lewis lung tumor (3LL) in mice. Tumors were induced in C57 BL/6J mice by subcutaneous injection of 1×10^6 viable 3LL cells/mouse in the intrascapular region. DFMO was administered in the drinking water as a 2%-aqueous solution (~3 g.kg^{-1}.day^{-1}). Putrescine was administered intraperitoneally at 100 mg.kg^{-1}.day^{-1}. Points represent mean ± SEM: (○) untreated animals; (●) DFMO-treated; (△) DFMO + putrescine-treated animals.

4. DISCUSSION

The novel finding of this study is that inhibition of polyamine biosynthesis resulted in a decrease in the spread of the tumor cells to distant sites; i.e., metastases. The fact that the inhibition of metastases could be reversed by simultaneous administration of putrescine suggests that polyamines may play an important role not only in tumor growth [7,8] but also in the process of tumor metastases. Although the mechanism by which DFMO inhibits tumor metastases is not yet clear,

Table 1

Effect of DFMO on the inhibition of growth and pulmonary metastases of Lewis lung carcinoma in mice

Treatment	Tumor weight (g) (Mean ± SE; $n = 20$)	% Inhibition	No. animals showing no visible metastases	Metastatic foci (Mean ± SE; $n = 20$)	% Inhibition
Control	7.01 ± 0.39		0/20	21.38 ± 5.8	
DFMO	4.01 ± 0.46[b]	43	5/20	4.60 ± 1.43[b]	79
DFMO + Put.	6.54 ± 0.72[a]	7	0/20	24.80 ± 5.10[a]	0

[a]not significant; [b]significant at $P < 0.01$

1×10^6 3LL tumor cells/animal were injected subcutaneously at the intrascapular region. DFMO was administered as 2%-aqueous solution as the sole drinking fluid (~3 g/kg/day). Putrescine 100 mg/kg was given intraperitoneally daily, starting day 1–18. At the end of 18 days the animals were killed, tumors were excised and weighed. Pulmonary metastases were determined as in [9]

one can envisage that the depletion of cellular polyamines in the primary tumor could have affected:

(i) The invasion of the tumor cells into the lymphatics or blood vessels and their transport into distant organs;

(ii) The establishment of a microenvironment unfavorable to the growth of pulmonary metastases [11].

These results indicate that DFMO and other specific polyamine antimetabolites merit consideration as potential therapeutic agents in the clinical management of metastasis.

ACKNOWLEDGEMENTS

The authors thank Dr A. Sjoerdsma for his helpful discussions; Mr P. Lachmann for technical help and Mrs L. Parrott for secretarial assistance.

REFERENCES

[1] Cohen, S.S. (1971) Introduction to Polyamines, p. 96, Prentice-Hall, Englewood Cliffs, NJ.

[2] Janne, J., Poso, H. and Raina, A. (1978) Biochim. Biophys. Acta 473, 241–293.

[3] Metcalf, B.W., Bey, P., Danzin, C., Jung, M.J., Casara, P. and Vevert, J.P. (1978) J. Am. Chem. Soc. 100, 2551–2553.

[4] Mamont, P.S., Duchesne, M.C., Grove, J. and Bey, P. (1978) Biochem. Biophys. Res. Commun. 81, 58–66.

[5] Sunkara, P.S., Fowler, S.K., Nishioka, K. and Rao, P.N. (1980) Biochem. Biophys. Res. Commun. 95, 423–430.

[6] Luk, G.D., Civin, C.I., Weissman, R.M. and Baylin, S.B. (1982) Science 216, 75–77.

[7] Prakash, N.J., Schechter, P.J., Mamont, P.S., Grove, J., Koch-Weser, J. and Sjoerdsma, A. (1980) Life Sci. 269, 181–194.

[8] Fozard, J.R. and Prakash, N.J. (1982) Naunyn-Sehiedeberg's Arch. Pharmacol. 320, 72–77.

[9] Wexler, H. (1966) J. Natl. Cancer Inst. 36, 641–645.

[10] Seiler, N. (1970) Methods Biochem. Anal. 18, 259–334.

[11] Poste, B. and Fidler, I.J. (1980) Nature 283, 139–146.

[12] Mamont, P.S., Duchesne, M.C., Grove, J. and Tardif, C. (1978) Exp. Cell Res. 115, 387–393.