Eur J Cancer Clin Oncol, Vol. 23, No. 8, pp. 1103–1107, 1987.
Printed in Great Britain

0277-5379/87$3.00+0.00

Phase I–II Clinical Trial with Alpha-difluoromethylornithine—An Inhibitor of Polyamine Biosynthesis

YOAV HORN,* PAUL J. SCHECHTER† and LAURENCE J. MARTON‡

**Department of Oncology, Assaf Harofeh Medical Center, Sackler School of Medicine, Zerifin, Israel, †Merrell Dow Research Institute, Strasbourg, France and ‡Department of Laboratory Medicine and the Brain Tumor Research Center of the Department of Neurological Surgery, School of Medicine, University of California, San Francisco, U.S.A.*

Abstract—*Alpha-difluoromethylornithine (DFMO) is an enzyme-activated, irreversible inhibitor of ornithine decarboxylase, the first enzyme in the synthesis of the polyamines putrescine, spermidine and spermine. DFMO has been shown to have a cytostatic and cytotoxic effect against various human tumor cell lines. The present study was designed to evaluate the toxicity and efficacy of this compound when administered orally at a dose of* 1.7 g/m sq. *t.i.d. added to conventional chemotherapy to* 38 *patients with carcinoma of the breast, stomach, prostate, female genital organs or metastatic carcinoma of unknown origin. A control group of* 32 *patients with similar malignancies received conventional chemotherapy only.*

Gastrointestinal, hematologic and biochemical abnormalities caused by DFMO were negligible. Reasonable ototoxicity was the major toxic effect caused by DFMO and resulted in discontinuation of therapy in 6 *of* 38 *patients* (15.8%). *No differences in disease progression were seen between those patients receiving DFMO plus conventional chemotherapy and those receiving only conventional chemotherapy.*

INTRODUCTION

ALPHA-DIFLUOROMETHYLORNITHINE (DFMO) is an enzyme-activated, irreversible inhibitor of ornithine decarboxylase [1], the enzyme catalyzing the first step in the synthesis of the polyamines putrescine, spermidine and spermine. These three aliphatic bases are essential for cell growth and differentiation [2, 3]. In most cell lines studied, DFMO depletes cells of putrescine and spermidine with little or no effect on spermine levels [4]. DFMO usually causes a decrease in growth without displaying significant cytotoxicity, although cytotoxicity against human small cell carcinoma [5], 9L rat brain tumor cell grown as spheroids [6] and murine B 16 melanoma [7] has been demonstrated. A number of human cancer cell lines have been shown to be affected *in vitro* by treatment with DFMO [8–10]. In addition to effects of DFMO alone, combination therapy with other known cancer chemotherapeutic agents appears to be a promising area of investigation. Potentiation of the cytotoxicity of cell-cycle-specific [11–13] and non-specific agents [14, 15] as well as interferon [7] has also been reported. An increased production of polyamines, as reflected in physiological fluid levels, has been correlated with early relapse and disease activity in patients with a variety of neoplastic disorders [16–20]. With increasing interest in the use of DFMO in the therapy of cancer, we conducted a phase I–II trial with this agent to define both qualitative and quantitative characteristics of the drug's toxicity and to assess any possible activity in a variety of malignancies.

The objectives of the present study were to determine the tolerance and efficacy of multiple oral doses of DFMO when given in addition to "conventional" chemotherapy.

PATIENTS AND METHODS

Patients with histologically confirmed diagnoses of carcinoma of the breast, stomach, prostate, female genital organs or metastatic carcinoma of unknown origin, in whom chemotherapy or hormone therapy was indicated, administered either as adjuvant for local advanced disease or as therapy for regional or distant metastatic disease. The primary tumor sites listed were selected based on previous data that showed significant differences in the urinary polyamine level between active and non-active disease

Accepted 19 January 1987.

in these malignancies [18, 19]. Prior to entry into the protocol, patients had a complete history, physical examination, biochemical levels (serum urea, bilirubin, total protein, albumin, alkaline phosphatase, SGOT and serum and urine creatinine) and complete blood counts. Based on these, and on relevant X-rays and radionuclide scans, disease status was determined. An informed consent form was signed by every patient. According to the order of referral, patients were alternatively assigned into one of two groups: group 1 conventional chemotherapy alone; group 2 conventional chemotherapy plus DFMO. Chemotherapy consisted of specific drug combinations for each tumor site identical for the DFMO and control groups and established according to departmental policies (see Table 2). DFMO (DL-alpha-difluoromethylornithine hydrochloride monohydrate) was administered orally at a dose of 1.7 g/sq.m t.i.d.; this dose was based on previous clinical experience [16, 21]. In the DFMO group, this drug was added to the cytotoxic regimen, while in the control group chemotherapy was given without DFMO. During the time of treatment every patient was evaluated at 3–4 week intervals. At each evaluation patients were examined, questioned for symptoms related to toxicity and blood was obtained for biochemistry and hematology. Hematologic toxicity was scored on a 0–4 scale based on the severity of toxicity as follows.

Grade of toxicity	WBC count (cells/mmsq.)	Platelets (cells/mmsq.)
0	> 4000	> 125,000
1	3000–3999	100,000–124,999
2	2000–2999	75,000–99,999
3	1500–1999	50,000–74,999
4	< 1500	< 50,000

Patients went off protocol either if evaluation revealed progression of disease or if 6 months had elapsed. In patients who completed the 6-month DFMO treatment period without incident and who were still in remission or who were stable, DFMO was continued with close monitoring until progression.

RESULTS

A total of 70 patients were evaluated. Thirty-eight patients received combined chemotherapy plus DFMO and 32 patients received chemotherapy without DFMO and served as controls.

Table 1 summarizes the patients' age and their primary tumor sites. In the DFMO group 29 of the patients were males and 9 were females. In the control group 10 were males and 22 were females. The clinical stage, based on commonly used international staging methods, revealed that 5.3, 28.9 and 65.8% of the patients were stages 2, 3 and 4, respectively, in the DFMO group, and that 18.8, 37.5 and 43.7% of the patients were stages 2, 3 and 4, respectively, in the control group.

Table 1. Patient data

	DFMO				Control			
Primary site	No.	Sex M	Sex F	Mean age	No.	Sex M	Sex F	Mean age
Breast	17	1	16	56.3	14	0	14	55.6
Stomach	4	2	2	57.5	6	4	2	56.7
Prostate	9	9	0	66.9	5	5	0	68.8
Gynecologic	5	0	5	59.4	5	0	5	61.2
Metastatic	3	2	1	58.3	2	1	1	67.5
Total	38	29	9	59.7	32	10	22	62.0

Table 2 shows the current "conventional" chemotherapy which was used in the different tumors with or without DFMO. The following drug combinations were used:

1. Breast
   a. Adjuvant therapy—tamoxifen citrate, 10 mg b.i.d.
   b. Advanced disease—cyclophosphamide 5 mg/kg, methotrexate 0.5 mg/kg 5-fluorouracil 10 mg/kg, i.v., repeated at 3 week intervals (CMF).
2. Stomach and metastatic carcinoma of unknown origin—cyclophosphamide 5 mg/kg days 1 and 5, vincristine sulfate (Oncovin) 0.025 mg/kg days 2 and 5, methotrexate 0.5 mg/kg days 1 and 4, 5-fluorouracil 10 mg/kg days 1 to 5 i.v., repeated at monthly intervals (COMF).
3. Prostate—doxorubicine hydrochloride (Adriamycin) 1.5 mg/kg, cyclophosphamide 5 mg/kg, i.v., repeated at 3 week intervals (AC).
4. Female genital—doxorubicine hydrochloride (Adriamycin) 1.5 mg/kg, cyclophosphamide 5 mg/kg, *cis*-platinum 1.0 mg/kg, i.v., repeated every 3 weeks.

Table 3 summarizes hematologic toxicity.

Comparison of the white blood cell and platelet counts in patients receiving DFMO with those of the control group revealed no significant difference between the groups. Subjective symptoms were recorded at each follow up examination. In previous studies anorexia, nausea, vomiting and diarrhea were associated with DFMO treatment [16, 21, 22]. These symptoms were absent or minimal in the majority of individuals in both groups (Table 4). Patients in the DFMO group showed a slightly higher degree of these subjective symptoms when present than did the control patients. Symptoms were always reversible with discontinuation of

Table 2. Current therapy

Primary site	DFMO CMF	Horm	COMF	AC	ACP	Control CMF	Horm	COMF	AC	ACP
Breast	14	3	—	—	—	9	5	—	—	—
Stomach	—	—	4	—	—	—	—	6	—	—
Prostate	—	—	—	9	—	—	—	—	5	—
Gynecologic	—	—	—	—	5	—	—	—	—	5
Metastatic	—	—	3	—	—	—	—	2	—	—
Total	14	3	7	9	5	9	5	8	5	5

CMF: cyclophosphamide, methotrexate, 5-fluoruracil.
Horm: tamoxifen citrate.
COMF: cyclophosphamide, vincristine sulfate, methotrexate, 5-fluorouracil.
AC: doxorubicin hydrochloride, cyclophosphamide.
ACP: doxorubicin hydrochloride, cyclophosphamide, *cis*-platinum.
See text for doses and schedules.

Table 3. Highest hematologic toxicity level

	Toxicity	DFMO (n = 38) N	%	Control (n = 32) N	%
WBC	0	27	71.1	26	81.3
	1	9	23.7	5	15.6
	2	2	5.2	1	3.1
	3	0	0.0	0	0.0
	4	0	0.0	0	0.0
Platelet	0	27	71.1	25	78.1
	1	5	13.2	4	12.5
	2	4	10.5	1	3.1
	3	1	2.6	2	6.3
	4	1	2.6	0	0.0

Periodic audiograms were performed throughout the course of therapy in the DFMO treated patients (Table 5). Normal audiograms were found in 11 (28.9%) of the 38 patients. Minimal, moderate or severe decreases were found in 7.9, 21.0 or 18.5% of the patients, respectively. All ototoxic symptoms were reversible within a period of several weeks after discontinuation of DFMO.

The biochemical tests carried out on every patient at each follow up visit were within normal limits in all patients prior to study. During treatment no significant differences were noted between control and DFMO-treated patients. A few patients had transient elevation of liver function tests, but no single test ever exceeded 150% of normal.

Table 4. Gastrointestinal toxicity

Toxicity	Anorexia DFMO	Control	Nausea DFMO	Control	Vomiting DFMO	Control	Diarrhea DFMO	Control
None	16/38 (42.1%)	21/32 (65.6%)	8/38 (21.1%)	15/32 (46.9%)	19/38 (50.0%)	23/32 (71.9%)	20/38 (52.6%)	28/32 (87.5%)
Minimal	20/38 (52.6%)	10/32 (31.3%)	28/38 (73.6%)	17/32 (53.1%)	18/38 (47.4%)	9/32 (28.1%)	18/38 (47.4%)	4/32 (12.5%)
Moderate	2/38 (5.3%)	1/32 (3.1%)	2/38 (5.3%)	0/32 (−)	1/38 (2.6%)	0/32 (−)	0/38 (−)	0/32 (−)
Severe	0/38 (−)	0/32 (−)	0/38 (−)	0/32 (−)	0/38 (−)	0/32 (−)	0/38 (−)	0/32 (−)

DFMO either with or without symptomatic treatment.

The major DFMO related toxicity was high frequency hearing loss. Table 5 shows that 55.3% of patients in the DFMO group had no change in their subjective hearing capacity while minimal, moderate or severe decrease was found in 18.4, 23.7 and 2.6%, respectively. No subjective hearing loss was found in the control group.

The protocol stipulated a 6-month period of treatment and evaluation. However, earlier discontinuation of treatment occured occasionally due to the reasons summarized in Table 6. Progression of disease before the end of the 6-month period occurred in 10 DFMO-treated patients (26.3%) and in 10 control patients (31.3%). Toxicity, resulting in discontinuation of treatment, was found in 6 DFMO-treated patients (15.8%) and in none of the

Table 5. Audiotoxicity and audiograms

	Subjective hearing decrease			Audiogram	
	DFMO (n = 38)	Control (n = 32)	Hearing loss	DFMO (n = 38)	Control (n = 32)
None	21 (55.3%)	32 (100.0%)	Normal (0–25 dB)	11 (28.9%)	0 (−)
Minimal	7 (18.4%)	0 (−)	Minimal (25–35 dB)	3 (7.9%)	0 (−)
Moderate	9 (23.7%)	0 (−)	Moderate (35–55 dB)	8 (21.0%)	0 (−)
Severe	1 (2.6%)	0 (−)	Severe (55–80 dB)	7 (18.5%)	0 (−)
			Not done	9 (23.7%)	32 (100.0%)

Table 6. Reasons for discontinuation of therapy and status of patients at end of 6 months

	DFMO (n = 38)	Control (n = 32)
Progression	10 (26.3%)	10 (31.3%)
Toxicity	6 (15.8%)	0 (−)
Refused therapy	1 (2.6%)	1 (3.1%)
Off therapy at 6 months	0 (−)	21 (65.6%)
Death due to cancer	2 (5.3%)	0 (−)
Death not due to cancer	1 (2.6%)	0 (−)
Still on therapy	18 (47.4%)	0 (−)

Table 7. Number of patients without progression at 6 months by tumor type

Primary site	DFMO	Control
Breast	8	9
Stomach	3	4
Prostate	7	3
Female genital	5	4
Metastatic	2	1
Total	25 (65.8%)	21 (65.6%)

controls. Only 18 patients (47.4%) in the DFMO group completed the 6-month period while still in remission and, therefore, without a need to discontinue therapy.

Table 7 summarizes the activity of disease 6 months after initiating therapy for both DFMO and control groups. There were no differences in the number of patients who progressed and in the number of patients with stable disease between the 2 groups.

DISCUSSION

The present study was aimed at evaluating the qualitative and quantitative characteristics of the toxicity caused by DFMO when added to other chemotherapy as well as the possible efficacy of such treatment. The prescribed daily dose of 1.75 g/m sq. was generally well tolerated with minimal gastrointestinal toxicity and only occasional hematologic or biochemical abnormalities none of which resulted in discontinuation of treatment. The major toxicity noted in our patients was ototoxicity which was reversible upon discontinuation of DFMO. Of 38 patients in the DFMO group, 17 patients (44.7%) presented with minimal to severe hearing loss. These subjective findings correlated with a high frequency hearing loss, evaluated audiographically in 18 patients (47.4%). In 6 patients the ototoxicity necessitated discontinuation of DFMO therapy. The mechanism of this ototoxicity is unknown.

In the tumor types studied and at the doses of DFMO used, we could find no evidence of a beneficial effect of DFMO added to conventional chemotherapy. Thus, the percentage of patients with stable disease after 6 months was identical whether or not DFMO was added. It should be noted, however, that by chance the patients allocated to the DFMO treatment group had, on the average, a more advanced stage of disease. It must be made clear that the promising results from animal tumor models showing potentiation of the cytotoxic effects of chemotherapeutic agents by DFMO, were optimized for that purpose. The study reported here was optimized to look at the toxicity of DFMO when added to "conventional chemotherapy" and was not directed towards a specific laboratory defined DFMO/chemotherapeutic agent interaction. As such our failure to increase efficacy of therapy is not surprising and the possibility

remains that alternate combinations of agents or more potent inhibitors of polyamine biosynthesis may prove efficacious.

Acknowledgements—Supported in part by NIH Program Project Grant CA-13525, and NCI National Cooperative Drug Discovery Grant CA-37606 and by Merrell Research Institute, Strasbourg, France.

REFERENCES

1. Metcalf BW, Bey P, Danzin C, Jung MJ, Casara P, Vevert JP. Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E.C.4.1.1.17) by substrate and product analogues. *J Am Chem Soc* 1978, **100**, 2551–2553.
2. Pegg AE, McCann PP. Polyamine metabolism and function: a brief review. *Am J Physiol* 1982, **243**, C212–C221.
3. Heby O. Role of polyamines in the control of cell proliferation and differentiation. *Differentiation* 1981, **19**, 1–20.
4. Marton LJ. Polyamines and cancer therapy. In: *Polyamines in Clinical Disorders*. D.F. Tierney, Moderator. *West J Med* 1985, **142**, 63–73.
5. Luk GD, Abeloff MD, Griffin CA, Baylin SB. Successful treatment with D,L-alpha-difluoromethylornithine in established human cell variant lung carcinoma implants in athymic mice. *Cancer Res* 1983, **43**, 4239–4243.
6. Sano Y, Deen DF, Oredsson SM, Marton LJ. Effects of growth and potentiation of 1,3-bis-(2-chloroethyl)-1-nitrosourea cytotoxicity by alpha-difluoromethylornithine in 9L rat brain tumor spheroids. *Cancer Res* 1984, **44**, 577–581.
7. Sunkara PS, Prakash NJ, Mayer GD, Sjoerdsma A. Tumor suppression with a combination of alpha-difluoromethylornithine and interferon. *Science* 1983, **219**, 851–853.
8. Luk GD, Civin CI, Weissman RM, Baylin SB. Ornithine decarboxylase: essential in proliferation but not in differentiation of human promyelocytic leukemia cells. *Science* 1982, **216**, 75–77.
9. Luk GD, Goodwin G, Marton LJ, Baylin SB. Polyamines are necessary for the survival of human small-cell carcinoma in culture. *Proc Natl Acad Sci USA* 1981, **78**, 2355–2358.
10. Mamont PS, Duchesne M-C, Grove J, Bey P. Antiproliferative properties of DL-alfa-difluoromethylornithine in cultured cells. A consequence of the irreversible inhibition of ornithine decarboxylase. *Biochem Biophys Res Commun* 1978, **81**, 58–66.
11. Prakash N, Sunkara PS. Combination chemotherapy involving alpha-difluormethylornithine and 1-beta-D-arabinofuranosylcytosine in murine L1210 leukemia. *Cancer Res* 1983, **43**, 3192–3196.
12. Sunkara PS, Fowler SK, Nishioka K, Rao PN. Inhibition of polyamine biosynthesis by alpha-difluoromethylornithine potentiates the cytotoxic effects of arabinosyl cytosine in HeLa cells. *Biochem Biophys Res Commun* 1980, **95**, 423–430.
13. Sunkara PS, Fowler SK, Nishioka K. Selective killing of transformed cells in combination with inhibitors of polyamine biosynthesis and S-phase specific drugs. *Cell Biol Int Rep* 1981, **5**, 991–997.
14. Hung DT, Deen DF, Seidenfeld J, Marton LJ. Sensitization of 9L rat brain gliosarcoma cells to 1,3-bis-(2-chloroethyl)-1-nitrosourea by alpha-difluoromethylornithine, an ornithine decarboxylase inhibitor. *Cancer Res* 1981, **41**, 2783–2785.
15. Marton LJ, Levin VA, Hervatin SJ, Koch-Weser J, McCann PP, Sjoerdsma A. Potentiation of the therapeutic effects of 1,3-bis-(2-chloroethyl)-1-nitrosourea by alpha difluoromethylornithine, an ornithine decarboxylase inhibitor. *Cancer Res* 1981, **41**, 4426–4431.
16. Warrell RP, Coonley CJ, Burchenal JH. Sequential inhibition of polyamine synthesis. A phase I trial of DFMO (alfa-difluoromethylornithine) and methyl-GAG [methylglyoxal-bis(guanylhydrazone)]. *Cancer Chemother Pharmacol* 1983, **11**, 134–136.
17. Durie BGM, Salmon SE, Russel DH. Polyamines as markers of response and disease activity in cancer chemotherapy. *Cancer Res* 1977, **37**, 214–221.
18. Horn Y, Beal SL, Walach N, Lubich WP, Spigel L, Marton LJ. Further evidence for the use of polyamines as biochemical markers for malignant tumors. *Cancer Res* 1982, **42**, 3248–3251.
19. Horn Y, Beal SL, Walach N, Lubich WP, Spigel L, Marton LJ. Relationship of urinary polyamines to tumor activity and tumor volume in patients. *Cancer Res* 1984, **44**, 4675–4678.
20. Fulton DS, Levin VA, Lubich WP, Wilson CB, Marton LJ. Clinical correlation of cerebrospinal fluid polyamine levels. In: *Neurobiology of Cerebrospinal Fluid*, Wood JH ed. New York, Plenum Press 1983, 441–452.
21. Abeloff MD, Slavik M, Luk GD *et al.* Phase I trial and pharmacokinetic studies of alpha-difluoromethylornithine—an inhibitor of polyamine biosynthesis. *J Clin Oncol* 1984, **2**, 124–130.
22. Maddox AM, Keating NJ, McCredie KE, Estey E, Freireich EJ. Phase I evaluation of intravenous difluoromethylornithine—a polyamine inhibitor. *Invest New Drugs* 1985, **3**, 287–292.