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Evaluation of continuous-infusion alpha-difluoromethylornithine therapy for colorectal carcinoma*

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Summary. A total of 32 evaluable patients with measurable advanced colorectal carcinoma were treated with continuous-infusion alpha-difluoromethylornithine (DFMO) at a median daily dose of 8 g/m^2 (range, 6–14 g/m^2). DFMO was infused over 24 h daily for 28 days, followed by a rest period of 7 days. Of the 32 patients, 14 had received no prior chemotherapy. A total of 65 courses was given, with the median being 2 (range, 1–9 courses). None of the patients achieved a partial or complete response; however, 3 patients achieved a minor response and 14 had stable disease. The frequent toxic effects of DFMO included thrombocytopenia (which was dose-limiting), malaise, nausea, vomiting, reversible hearing loss, and diarrhea. Our data suggest that continuous-infusion DFMO therapy is feasible and results in only mild gastrointestinal toxicity. Although DFMO proved to be ineffective as a single agent in this trial, it could probably best be used in combination with cytotoxic agents known to enhance its antitumor activity in a preclinical setting.

Introduction

The polyamines putrescine, spermidine, and spermine have been associated with cell growth and differentiation [15]. Putrescine levels are known to increase during cell growth and to diminish when cell growth is inhibited [15, 24, 25, 27]. Thus, inhibitors of polyamine synthesis provide a unique class of agents with potential properties of cytoreduction. Synthesis of putrescine is regulated by the availability of ornithine and the activity of ornithine decarboxylase (ODC) [1]. Among several irreversible inhibitors of ODC, alpha-difluoromethylornithine (DFMO) has been extensively studied. DFMO is an enzyme-activated, irreversible inhibitor of ODC [13–15, 21] that acts by covalently binding to the enzyme [13, 14]. DFMO has been observed to reduce intracellular levels of putrescine consistently in a variety of in vitro systems [11, 12, 16–18, 20]. In addition, in transplantable tumor models it causes a reduction in tumor growth as well as polyamine levels [6]. DFMO has also been shown to be cytotoxic to small-cell carcinoma cell lines [9, 15].

In clinical phase I studies, DFMO given orally (either alone or in combination with other agents) has resulted in significant gastrointestinal toxicity [1, 22, 26]. However, another phase I study using continuous infusion of DFMO has demonstrated amelioration of severe gastrointestinal toxic effects [10]. Thrombocytopenia and ototoxicity have also been noted as significant toxic effects.

Because ODC has a rapid rate of turnover, with a half-life of only a few minutes [15], intermittent administration of DFMO might not result in sustained suppression of ODC activity and, thus, of polyamine synthesis. To maximize ODC suppression and sustain inhibition of polyamines, we used continuous i.v. infusion of DFMO to overcome the potential disadvantages of intermittent administration. This report evaluates continuous-infusion DFMO in a group of patients with advanced colorectal carcinoma.

Patients and methods

In all, 34 patients with histologically proven advanced colorectal carcinoma were enrolled in this study. All patients were required to have measurable carcinoma and to be >18 years of age. Other patient criteria included normal pretreatment serum creatinine and serum bilirubin level, normal peripheral blood counts, normal pretreatment audiograms, and a performance status of 2 or less on the Zubrod scale. A total of 14 eligible patients who had not received prior chemotherapy were entered in the study to assess the effectiveness and tolerance of DFMO. Prior radiotherapy was allowed if it had not compromised signal measurable lesions. Informed consent was obtained from all patients.

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Table 1. Patient characteristics

Total patients (*n*)	34
Evaluable for toxicity	33
Evaluable for response	32
Median age in years (range)	60 (35–76)
Performance status:	
0	2
1	26
2	4
3	1
Sex ratio (M: F)	18:15
Prior therapy:	
No prior chemotherapy	14
Radiotherapy	16
Chemotherapy	18

Table 2. Nonhematologic toxicity of DFMO

Toxicity	Toxicity grade per course[a]:		
	1	2	3
Malaise	8	9	0
Nausea and vomiting	7	5	0
Hearing loss	2	5	1
Diarrhea	6	1	1

[a] Toxicity criteria according to Ajani et al. [3]

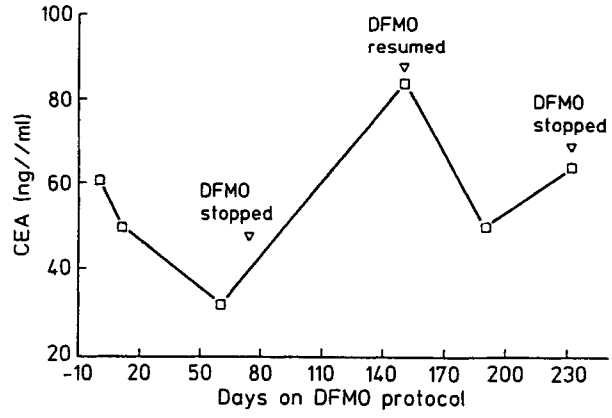

Fig. 1. Sequential changes in serum CEA levels in a patient who underwent intermittent DFMO therapy

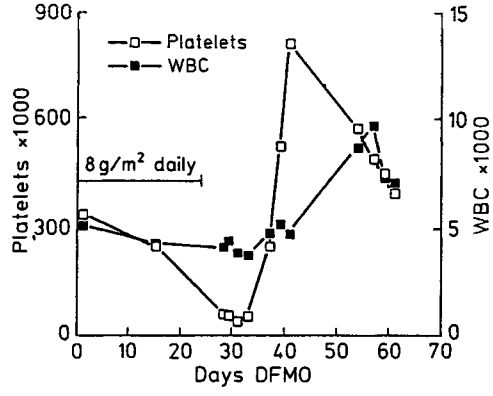

Fig. 2. Graph showing the representative changes in a patient's leukocyte and platelet counts and their relationship to DFMO infusion

All patients underwent pretreatment evaluation that included computerized tomographic scan of the abdomen and pelvis, chest radiograph, electrocardiogram, audiogram, urinalysis, determination of serum levels of carcino embryonic antigen (CEA), serum chemistry and electrolyte evaluations, and complete blood and platelet counts. Baseline plasma DFMO levels and erythrocyte and plasma polyamine levels were also determined in 25 patients.

During therapy, complete blood, differential, and platelet counts were carried out once a week. CEA levels (if the baseline was abnormal) were measured, serum chemistry analyses were done, and audiograms, chest radiographs, and other appropriate radiographs were repeated at the end of each course (and more frequently when necessary) to document response and toxic effects.

Plasma DFMO levels were determined on days 14 and 28 of infusion in 25 patients and, in addition, every day for the first 5 days in 5 patients. Plasma and erythrocyte polyamine levels were determined on days 0, 7, 21, 28, and 35 in 25 patients.

DFMO was provided by Merrell-Dow Research Institute, Cincinnati, Ohio. Central venous access was required in all patients. The starting daily dose of DFMO was 6 g/m^2 mixed in approximately 150 ml normal saline solution and infused over a 24-h period using a Pancretec pump in an outpatient setting. DFMO was given daily for 28 days, followed by a rest period of 7 days; thus, one cycle of chemotherapy required a total of 35 days.

Therapy was discontinued if a patient developed unacceptable toxic effects or if tumor progression occurred. The daily dose of DFMO for subsequent courses was increased or decreased by 2 g/m^2 according to the predetermined dose modification criteria. In cases in which the absolute granulocyte count fell to <500 cells/μl, the platelet count fell to <50,000/μl, or grade 3 nonhematologic toxicity occurred [3], the daily dose of DFMO was reduced by 2 g/m^2 for the subsequent course. However, during 28-day DFMO administration, if a patient's platelet count fell to <50,000/μl, if clinical hearing loss developed, or if other grade 3 nonhematologic toxic effects occurred, the DFMO infusion was terminated for that course and the dose level for the subsequent course was modified as described above. The daily DFMO dose was increased by 2 g/m^2 in the subsequent course if the lowest absolute granulocyte count was >1,000 cells/μl, if the platelet count was >100,000/μl, or if the nonhematologic toxic effects were less severe than grade 1. For the subsequent course, DFMO was not resumed unless all toxic reactions had resolved.

The criteria for defining response were standard. A complete response was defined as the complete disappearance of all measurable disease. A partial response represented a reduction of ≥ 50% in the sum of the product of the two longest dimensions of the measurable tumor and the absence of new lesions. A minor response was defined as any tumor reduction less than that required for 3 partial response in the absence of new lesions, and stable disease, as no change in measurable disease. Progressive disease represented any progression in measurable disease. The duration of response was calculated from the time of response to the time of disease progression. Toxic reactions were graded according to criteria developed at our institution [3].

Results

Of 34 patients enrolled in this study, 33 were evaluable for toxicity and 32 were evaluable for response to DFMO therapy. One patient was registered but did not receive therapy, and one patient was diagnosed as having carcinoma of the pancreas. Patient characteristics are shown in Table 1.

In all, 65 courses of DFMO were given, with the median number per patient being 2 (range, 1–9 courses). The

median daily dose of DFMO was 8 g/m^2 (range, 6–14 g/m^2). None of the patients achieved a complete or partial response; however, three patients (all were previously treated with chemotherapy) showed minor responses (lymph node metastases in two patients and liver metastases in one) lasting <3 months. A total of 14 patients had tumor stabilization for a median duration of 2 months (range, 2–10 months).

Of 21 patients with elevated levels of serum CEA prior to initiation of therapy, 4 who demonstrated a substantial drop (50% in 2 cases and 40% in another 2) had either a minor response or stable disease. The biologic effect of DFMO was exemplified in one patient whose CEA level dropped by 40% following two courses of DFMO. When his treatment was interrupted for an urgent carotid endarterectomy, his CEA level rose above the pretreatment level, but it dropped again following the third course of DFMO (see Fig. 1). In 12 patients who had either progressive or transiently stable carcinoma, elevated pretreatment serum CEA levels continued to rise despite DFMO therapy. In all, 16 patients had progressive disease following therapy.

Changes in red blood cell and plasma polyamine levels and DFMO pharmacokinetics recorded in 25 patients have been described elsewhere [2]. The steady-state plasma DFMO levels correlated with the nadir platelet count, suggesting the potential utility of DFMO levels for predicting the severity of thrombocytopenia. The steady-state level of DFMO was reached at 24 h and was maintained throughout the infusion (Nishioka et al., manuscript in preparation).

DFMO given by daily continuous infusion for 28 days was well tolerated by most patients. Common nonhematologic toxic effects, shown in Table 2, included malaise, nausea, vomiting, diarrhea, and hearing loss. Reversible ototoxicity requiring DFMO dose reduction as well as delays in resumption of the next treatment course occurred in eight patients; complete recovery occurred within 4 weeks after discontinuation of DFMO. Uncommon toxic effects included headache, anorexia, stomatitis, and tinnitus. None of the patients had grade 4 nonhematologic toxic effects, and only one had grade 3 diarrhea.

The predominant hematologic toxicity was thrombocytopenia, although some degree of granulocytopenia and anemia also occurred. Blood transfusions were rarely required. The median nadir absolute granulocyte count noted at all DFMO dose levels was 2,600 cells/μl (range, 0–13,400 cells/μl); the median nadir platelet count was 62,000/μl (range, 12,000–221,000/μl). Of 65 courses, 20 resulted in nadir platelet counts of <50,000/μl; however, none of the patients suffered morbidity from thrombocytopenia. Thrombocytopenia usually increased in severity after 20 days of DFMO, but platelet counts recovered with an overshoot at 4 or 5 days following discontinuation of DFMO (see Fig. 2). In seven patients, DFMO was discontinued when platelet counts fell to <50,000/μl. In three patients whose platelet counts fell to <50,000/μl during the 28-day infusion, DFMO administration was not interrupted because the platelet count results could not be reviewed immediately; none of these three patients suffered additional morbidity. Only five courses resulted in absolute granulocyte counts of <1,000 cells/μl. Variations in individual tolerance to DFMO were observed; for example, one patient tolerated a daily dose of 14 g/m^2 without developing myelosuppression or ototoxicity.

Discussion

DFMO, predominantly a cytostatic agent, has been studied extensively in oral treatment regimens. Our data suggest that continuous-infusion DFMO reduces the severity of the gastrointestinal toxicity that accompanies oral doses and produces thrombocytopenia as the major dose-limiting toxicity. Loss of hearing is a transient, nonsevere problem that occurs in some patients. However, at doses that resulted in significant normal tissue toxicity (suggesting that the dose levels studied were biologically active), DFMO did not produce any major responses in patients with colorectal carcinoma. Minor activity against tumors was observed in three patients, suggesting DFMO's possible useful role when combined with agents with which it has demonstrated synergistic antitumor activity in preclinical models. DFMO has been shown to enhance the cytotoxicity of various anticancer agents (for example, methylglyoxal-bis-guanylhydrazone [8]) in vitro and in vivo, making it an attractive drug for further development in combination chemotherapy [4, 5, 19, 23]. However, the possibility of synergistic or additive toxicity exists.

In rats bearing colon carcinoma or fibrosarcoma, we have recently demonstrated [7] that DFMO-induced thrombocytopenia was eliminated or reduced in severity when ornithine was concomitantly infused with the continuous-infusion DFMO. Thus, in a rat model, concomitant ornithine infusion at a molar ratio between 0.2 and 0.5 resulted in protection of the host from thrombocytopenia, and, more importantly, tumor growth inhibition by DFMO was unaffected. This suggests that a similar strategy is worth investigating in patients.

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References

1. Abeloff MD, Slavik M, Luk GD, Griffin CA, Hermann J, Blanc O, Sjoerdsma A, Baylin SB (1984) Phase I trial and pharmacokinetic studies of alpha-difluoromethylornithine. An inhibitor of polyamine synthesis. J Clin Oncol 2: 124–130
2. Ajani JA, Ota DM, Grossie VB, Levin B, Nishioka K (1989) Alterations in polyamine metabolism during continuous intravenous infusion of alpha-difluoromethylornithine: correlation of thrombocytopenia with alpha-difluoromethylornithine plasma levels. Cancer Res 49: 5761–5765
3. Ajani JA, Welch SR, Raber MN, Fields WS, Krakoff IH (1989) Comprehensive criteria for therapy-induced toxicity. Cancer Invest 8: 141–153
4. Bjerkvig R, Oredsson SM, Marton LJ, Linden M, Deen DF (1983) Cell cycle age response of 9L cells to 1,3-bis(2-chloroethyl)-1-nitrosourea and modification by alpha-difluoromethylornithine. Cancer Res 43: 1497–1500

5. Ducore JM, McNamara L (1986) Effect of alpha-difluoromethylornithine on L-phenylalanine mustard-induced cytotoxicity and DNA interstrand cross-linking in a human cell line in vitro. Cancer Res 46: 1068–1072
6. Grossie VB, Ota DM, Ajani JA, Nishioka K (1987) Effect of intravenous alpha-difluoromethylornithine on the polyamine levels of normal tissue and transplantable fibrosarcoma. Cancer Res 47: 1836–1840
7. Grossie VB, Ota DM, Ajani JA, Chang TH, Patenia D, Nishioka K (1989) Reduction of difluoromethyl-ornithine-induced thrombocytopenia with ornithine while maintaining anti-tumor activity. Cancer Res 49: 4159–4162
8. Herr HW, Kleinert EL, Relyea NM, Whitmore WF (1984) Potentiation of methylglyoxal-bis-guanylhydrazone by alpha-difluoromethylornithine in rat prostate cancer. Cancer 53: 1294–1298
9. Luk GD, Goodwin G, Marton LJ, Baylin SB (1981) Polyamines are needed for the survival of human small-cell lung carcinoma in culture. Proc Natl Acad Sci USA 78: 2355–2358
10. Maddox AM, Keating MJ, McCredie KE, Estey E, Freireich EJ (1985) Phase I study of intravenous difluoromethylornithine – a polyamine inhibitor. Invest New Drugs 3: 287–292
11. Mamont PS, Duchesne MC, Grove J, Bey P (1978) Antiproliferative properties of DL-alpha-difluoromethyl ornithine in cultured cells. A consequence of the irreversible inhibition of ornithine decarboxylase. Biochem Biophys Res Commun 81: 58–66
12. Mamont PS, Siat M, Joder-Ohlenbusch AM, Bernhardt A, Casara P (1984) Effect of (2*R*, 5*R*)-6-heptyne-2,5-diamine, a potent inhibitor of L-ornithine decarboxylase, on rat hepatoma cells cultured in vitro. Eur J Biochem 142: 457–463
13. McCann PP, Pegg AE, Sjoerdsma A (1987) Inhibition of polyamine metabolism. Biologic significance and basis for new therapies. Academic Press, Orlando, Florida, pp 1–371
14. Pegg AE (1986) Recent advances in the biochemistry of polyamines in eukaryotes. Biochem J 234: 249–262
15. Pegg AE (1988) Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. Cancer Res 48: 759–774
16. Pegg AE, McCann PP (1982) Polyamine metabolism and function. Am J Physiol 243: C212–C221
17. Pegg AE, Wechter RS, Clark RS, Wiest L, Erwin BG (1986). Acetylation of decarboxylated *S*-adenosylmethionine by mammalian cells. Biochemistry 25: 379–384
18. Pera PJ, Kramer DL, Sufrin JR, Porter CW (1986) Comparison of the biological effects of four irreversible inhibitors of ornithine decarboxylase in two murine lymphocytic leukemia cell lines. Cancer Res 46: 1148–1154
19. Seidenfeld J, Komar KA (1985) Chemosensitization of cultured human carcinoma cells to 1,3-bis(2-chloroethyl)-1-nitrosourea by difluoromethylornithine-induced polyamine depletion. Cancer Res 45: 2132–2138
20. Seidenfeld J, Gray JW, Marton LJ (1981) Depletion of 9L rat brain cell polyamine content by treatment with D,L-alpha-difluoromethylornithine inhibits proliferation and the G1 to S transition. Exp Cell Res 131: 209–216
21. Sjoerdsma A, Schechter PJ (1984) Chemotherapeutic implications of polyamine biosynthesis inhibition. Clin Pharmacol Ther 35: 287–300
22. Splinter TAW, Romjin JC (1986) Phase I study of alpha-difluoromethylornithine and methyl-GAG. Eur J Clin Oncol 22: 61–67
23. Sunkara PS, Fowler SK, Nishioka K, Rao PN (1980) Inhibition of polyamine biosynthesis by alpha-difluoromethylornithine potentiates the cytotoxic effects of arabinosyl cytosine in HeLa cells. Biochem Biophys Res Commun 95: 423–430
24. Tabor CW, Tabor H (1984) Polyamines. Annu Rev Biochem 53: 749–790
25. Tabor CW, Tabor H (1985) Polyamines in microorganisms. Microbiol Rev 49: 81–99
26. Warrell RP, Coonley CJ, Burchenal JH (1983) Sequential inhibition of polyamine synthesis: a phase I trial of DFMO (alpha-difluoromethylornithine) and methyl-GAG [methylglyoxal-bis(guanylhydrazone)]. Cancer Chemother Pharmacol 11: 134–136
27. Williams-Ashman HG, Canellakis ZN (1979) Polyamines in mammalian biology and medicine. Perspect Biol Med 22: 421–453