Toxicity Evaluation of Difluoromethylornithine: Doses for Chemoprevention Trials[1]

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Abstract

This intergroup trial was developed to determine the toxicity of relatively low doses of difluoromethylornithine (DFMO) administered to humans for 1 year. The goal was to find an appropriate DFMO dose for use in human chemoprevention trials. Patients with resected superficial bladder cancers were studied. Following stratification, they were randomized to daily DFMO doses of 0.125, 0.25, 0.5, or 1.0 g/day for a planned period of 1 year.

Patients were followed closely for evidence of drug toxicity. Seventy-six patients were evenly randomized (19 per group) to receive each dose of DFMO. Forty-nine patients received DFMO for more than 200 days while 35 received the drug for ≥350 days. No substantial drug-related toxicity was observed at any dose.

DFMO doses of ≤1 g/day for periods up to 1 year appear to be without significant toxicity in most patients. This dose range may be appropriate for use in future human cancer chemoprevention trials.

Introduction

DFMO[3] is an irreversible inhibitor of ornithine decarboxylase, an enzyme which appears to play an important role in the process of tumor promotion (1–3). DFMO has been demonstrated to inhibit carcinogenesis in multiple animal tumor model systems. Specifically, DFMO inhibits 12-O-tetradecanoylphorbol-13-acetate-induced mouse skin tumors (4), afla-

toxin-induced rat hepatomas (5), axoxymethane-induced rat intestinal tumors (6, 7), nitrosamine- or dimethylhydrazine-induced rat mammary tumors (8–10), nitrosamine-induced rat bladder tumors (11, 12), and also N-methyl-N-nitrosourea-induced rat bladder tumors (13).

Oral DFMO has been studied in humans. In a Phase I trial, the recommended maximum tolerated dose for cancer treatment was 9 g/m²/day (14). Dose-limiting toxicity has usually been thrombocytopenia and ototoxicity. Ototoxicity appears to correlate with the total cumulative DFMO dose when relatively high daily DFMO doses are used (15). Thrombocytopenia has been a problem almost exclusively in patients who have received prior systemic chemotherapy.

It is well appreciated that minimal, or a complete absence of, toxicity is required for drugs to be used as chemoprevention agents (16). Thus, if DFMO is to have a role in human cancer chemoprevention, a relatively nontoxic dose needs to be defined. The present study was designed to assess the toxicity of low doses of DFMO. At the time the study was initiated, available data had demonstrated that DFMO doses of 1–2 g/m²/day resulted in reversible ototoxicity after approximately 7–9 weeks of treatment (17). Not knowing how much less than this would be tolerable, we developed a randomized toxicity study designed to evaluate single daily DFMO doses of 0.125, 0.25, 0.5, and 1.0 g/day.

Patients with resected superficial bladder cancer were chosen as the subjects for this study because (*a*) they are at considerable risk for developing disease recurrence; (*b*) there are data which demonstrate that DFMO can inhibit bladder cancer carcinogenesis (11–13) and the growth of established cultured human bladder cancer cells (18); and (*c*) the large majority of DFMO is excreted unchanged in the urine (19), allowing it to incubate with urothelial cells for prolonged periods of time.

Materials and Methods

This study was conducted by the North Central Cancer Treatment Group and the ECOG. Patients with an ECOG performance status of 0 or 1 who had undergone transurethral resection of newly diagnosed or recurrent superficial (stage T_a) or superficially invasive (T_1) transitional cell carcinoma of the bladder were eligible for this study if all visible tumor had been removed, and if the attending urologist had determined that the standard clinical management of this patient would be an expectant observation without further planned surgery, intravesical therapy, or systemic chemotherapy. Because this study was an assessment of toxicity and tolerance, the time interval between the transurethral resection of the index tumor and study entry were intentionally not defined. However, patients with their index tumor resected more than 8 weeks before registration were required to be cystoscopically and cytologically free of transitional cell carcinoma before they were eligible for entry. Eligibility requirements included WBC counts >4,000

Table 1 Distribution of baseline factors

Baseline factors	Treatment arm				P	Total (n = 76)
	0.125 g DFMO (n = 19)	0.25 g DFMO (n = 19)	0.5 g DFMO (n = 19)	1.0 g DFMO (n = 19)		
Sex (%)					0.94	
Male	68	68	74	79		72
Female	32	32	26	21		28
Tumor grade (%)					0.99	
I	42	42	37	37		40
II	53	47	53	53		51
III	5	11	10	10		9
Tumor stage (%)					0.88	
T_a	84	79	79	79		80
T_1	11	21	21	21		19
T_{is}	5	0	0	0		1
Focus (%)					0.96	
Unifocal	53	63	53	58		57
Multifocal	47	37	47	42		43
Institution (%)					0.99	
Mayo	21	16	21	26		21
North Central Cancer Treatment Group	21	26	26	21		24
ECOG	58	58	53	53		55
History (%)					0.99	
Initial	47	47	53	47		49
Recurrent	47	47	42	47		46
Missing	5	5	5	5		5
Age (yr)					0.77	
Median	60	60	62	66		62
Mean	64	61	60	64		62

mm^3, platelet counts >100,000 mm^3, hemoglobin >11.0 mg/dl, and serum creatinine and aspartate aminotransferase levels <1.5 times the upper normal limit. Patients were ineligible if they had prior systemic chemotherapy or radiation therapy for malignant disease; second primary tumors outside of the bladder within the previous 3 years other than basal or squamous cell carcinomas of the skin; clinically significant hearing impairment defined as an inability to hear 25 decibels at any tested frequency <2000 Hertz; or other significant medical or psychiatric problems which would have made them a poor research study candidate. Patients who had previously received intravesical therapy for transitional cell carcinoma but met other entry criteria and were no longer actively receiving therapy were eligible.

At the time of study initiation, all patients had to undergo a history and physical examination, complete blood count, chemistry evaluation, urinalysis, screening audiogram, cystoscopy, and urine or bladder wash cytology. All patients provided written informed consent according to federal guidelines. At study registration, patients were stratified by sex, tumor grade, tumor stage, tumor multiplicity (unifocal *versus* multifocal), and disease history (initial diagnosis *versus* recurrent bladder cancer). They were then randomized to receive one of the following four daily doses of DFMO: 0.125, 0.25, 0.50, or 1.0 g/day given as a single daily dose for a planned period of 1 year.

While on study therapy, patients underwent a history and physical examination every 3–6 months and were followed monthly between clinic visits by alternating nurse phone calls and patient questionnaires to inquire about toxicity. Repeat cystoscopic procedures and audiograms were administered at 3–6-month intervals, and blood work was done at 1–3-month intervals. Additional audiograms were to be administered if the patient complained of any hearing impairment.

Protocol treatment was to be stopped for significant toxicity or recurrent bladder cancer. The toxicity of primary concern was hearing impairment. Significant hearing impairment was defined to be a hearing loss, compared with pretreatment hearing levels, of either: (a) 10 decibels or more at two different frequencies in each ear or (b) 15 decibels or more at one frequency in each ear. For such toxicity, the DFMO was to be discontinued permanently.

The trial was designed to be a single-stage, four-armed, randomized Phase II study with toxicity as the primary end point. Since the four treatments differed only in the prescribed DFMO daily dose, analysis plans were to estimate the unacceptable toxicity rate in each arm individually and to look at all four arms collectively for evidence of a dose-response effect on toxicity.

Binomial point and confidence interval estimators were used to estimate the unacceptable and total toxicity rates in each treatment arm. Nonparametric Fisher's exact tests and Wilcoxon rank sum tests were used to compare the toxicity incidence and severity distributions among the treatment arms. Recurrence-time distributions were estimated with Kaplan-Meier survival curves and compared among treatment groups and other subsets with log rank tests.

Results

A total of 76 patients were randomized into this study from March 17, 1992 through January 14, 1994. All were eligible. The four treatment groups were equal in size (19/arm) and well balanced with respect to the baseline factors (Table 1). Six patients (8%) refused to complete a full year of DFMO treatment (not dose related) while 32 (42%) took less than 1 year of DFMO because of disease recurrence. Forty-nine patients (64%) remained on DFMO for >200 days, and 35 patients
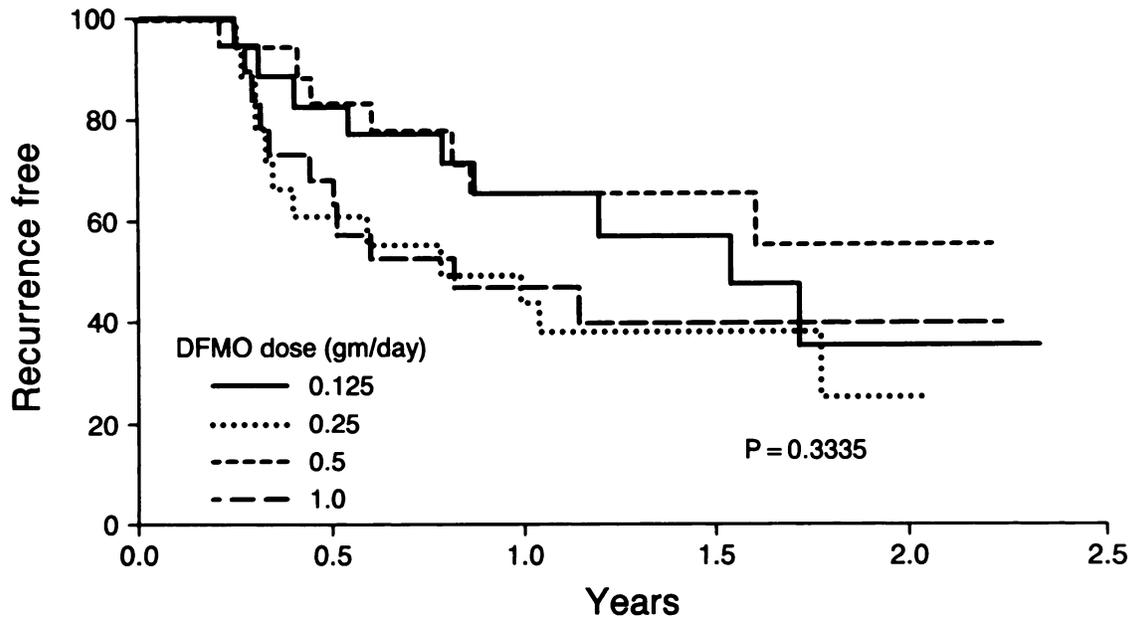
Fig. 1. Times to disease recurrence for protocol patients grouped by DFMO dose level.

(46%) remained on study for >350 days. The median number of days on study was similar in all four study arms.

No evidence of DFMO-induced ototoxicity was found in this study with the exception that one patient on the highest DFMO dose did develop hearing loss after a head cold. At the time of the hearing loss, this patient had bilateral middle ear effusions. After the DFMO was stopped, the effusions cleared and the hearing loss resolved. DFMO was not restarted at the patient's request.

No other substantial toxicity was noted in this study despite searching for such. Mild nausea was noted in 7% of the patients, and mild vomiting occurred in two patients. Mild to moderate levels of tinnitus, diarrhea, dermatitis, lethargy, and musculoskeletal pain were recorded for one or two patients each, and mild grades of several other toxicities were reported for single patients. Since none of these side effects appeared to be dose related, it is not clear that any of them were actually caused by DFMO. Furthermore, toxicity as measured by the total number of toxicities recorded for each DFMO dose (1, 4, 10, and 3, respectively, for lowest to highest DFMO doses) provided no evidence of a dose-response effect.

Sixty-five study participants (86%) completed at least one of the quarterly patient questionnaires. In response to the query, "Are you experiencing any side effects from the DFMO?" a "yes" answer on at least one questionnaire was given by 22% of respondents (13%, 44%, 22%, and 7%, respectively, for lowest to highest DFMO doses), giving a 95% exact binomial confidence interval ranging from 12.3 to 33.5% for the perceived toxicity incidence rate.

The recurrence-time curves for the four study arms are shown in Fig. 1. Although there is a suggestion that there are two distinct curves, the fact that the two best curves correspond to the lowest and second highest daily doses does not support either a dose-response or an optimal-dose interpretation.

Discussion

The present study supports the concept that DFMO doses of 0.125–1.0 g/day are well tolerated for periods of up to 1 year.

This is consistent with data from Love *et al.* (20) which showed that DFMO doses of 0.5 g/m²/day were well tolerated. (A 0.5 g/m²/day would equal a dose of 0.85 g/day for a 1.7-m² person.) It should be remembered, however, that this study required relatively normal hearing capability, and thus the safety of this dose of DFMO has not been studied in patients with clinically significant hearing impairment.

We did see symptoms of minor "toxicities" (*e.g.*, nausea, fatigue) in this trial and a 22% positive response on a question asking "Are you experiencing any side effects from DFMO?" It is not clear whether these toxicities were actually caused by DFMO or whether they were from a "reverse placebo effect." A double-blind, placebo-controlled randomized clinical trial would be required to determine whether these low doses of DFMO caused any more toxicity than a placebo would. With respect to this point, uncontrolled trials suggested that vitamin E caused a number of toxicities, whereas subsequent placebo-controlled trials demonstrated that these toxicities were just from "background noise" (21).

Other studies have demonstrated that DFMO doses of 1.5 g/day or greater cause toxicity which would be prohibitive in a chemoprevention study (17), suggesting that a daily dose of ≤1.0 g/day (or 0.5 g/m²/day) should be chosen for future chemoprevention trials in humans. A single daily dose appears reasonable, since the biological effect of DFMO on tissue ornithine decarboxylase activity is prolonged (22), and this schedule might be associated with greater patient compliance than more frequent administration schedules.

Currently, there is little reported experience with the use of DFMO for periods longer than 1 year. Hopefully, longer term use of DFMO will not cause toxicity, but this should be studied in future trials. It should be noted that, in trials where higher daily doses of DFMO were used, ototoxicity was observed in about 10% of the patients at total DFMO doses of about 150 g/m²/day while approximately 75% of the patients developed clinically demonstrable hearing loss at total DFMO doses >250 g/m² (15). The total DFMO dose in the patients using our

highest daily dose for 1 year was approximately 215 g/m^2 for an average size person.

Although the current study does not rule out the possibility that these DFMO doses could cause ototoxicity in some patients, the incidence of such toxicity should be quite low. Nonetheless, given that chemoprevention drugs are likely to be administered to relatively healthy subjects, a low incidence of a significant toxicity such as ototoxicity could represent a substantial impediment to the successful use of a chemoprevention agent. Thus, future patients receiving low doses of DFMO will need to be closely followed for this toxicity.

This current trial was not designed to determine whether DFMO has chemopreventative or cytotoxic activity against bladder cancer. The relatively small numbers of patients in each dose group and the interpatient diversity make it impossible to assess drug efficacy in patients with superficial bladder cancer.

These data suggesting that DFMO doses ≤1.0 g/day are relatively nontoxic in most people, prompt the question, do DFMO doses of ≤1 g/day have biological activity in humans? In response, doses in this range can inhibit 12-*O*-tetradecanoylphorbol-13-acetate-induced ornithine decarboxylase activity in human skin (20, 23). In addition, DFMO doses between 0.1 and 1.0 g/m^2/day cause biological activity in human colonic tissue (24). Thus, further investigation of such doses of DFMO as a chemopreventative agent may be justified.

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