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https://escholarship.org/uc/item/7wc0b55d

Journal

Journal of the National Cancer Institute, 90(16)

ISSN

0027-8874

Authors

Meyskens, Frank L
Gerner, Eugene W
Emerson, Scott
et al.

Publication Date

1998-08-19

DOI

10.1093/jnci/90.16.1212

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Effect of α-Difluoromethylornithine on Rectal Mucosal Levels of Polyamines in a Randomized, Double-Blinded Trial for Colon Cancer Prevention

Frank L. Meyskens, Jr., Eugene W. Gerner, Scott Emerson, Daniel Pelot, Theodore Durbin, Karen Doyle, Westley Lagerberg

Background: Polyamines (e.g., putrescine, spermidine, and spermine) are required for optimal cell growth. Inhibition of polyamine synthesis suppresses carcinogen-induced epithelial cancers, including colon cancer, in animal models. In a short-term phase IIa trial, we determined that low doses of α-difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase (an enzyme involved in polyamine synthesis), reduced the polyamine content of normal-appearing rectal mucosa of subjects with a prior history of resected colon polyps. In a follow-up study, we have attempted to determine the lowest dose of DFMO that can suppress the polyamine content of rectal mucosa over a course of 1 year with no or minimal side effects. *Methods:* Participants were randomly assigned to daily oral treatment with a placebo or one of three doses (0.075, 0.20, or 0.40 g/m^2) of DFMO. Baseline and serial determinations of polyamine levels in rectal mucosa and extensive symptom monitoring (including audiometric measurements, since DFMO causes some reversible hearing loss at higher doses) were performed over a 15-month period. *Results:* DFMO treatment reduced putrescine levels in a dose-dependent manner. Following 6 months of treatment, doses of 0.20 and 0.40 g/m^2 per day reduced putrescine levels to approximately 34% and 10%, respectively, of those observed in the placebo group. Smaller decreases were seen in spermidine levels and spermidine:spermine ratios. Polyamine levels increased toward baseline values after discontinuation of DFMO. Although there were no statistically significant differences among the dose groups with respect to clinically important shifts in audiometric thresholds and nonaudiologic side effects, statistically significant higher dropout and discontinuation rates were observed in the highest dose group. *Conclusions:* Polyamine levels in rectal mucosa can be continuously suppressed by daily oral doses of DFMO that produce few or no side effects. A dose of 0.20 g/m^2 can be used safely in combination phase IIb or single-agent phase III chemoprevention trials. [J Natl Cancer Inst 1998;90:1212–8]

Polyamines (e.g., putrescine, spermidine, and spermine) are required for optimal growth of bacteria, yeast, and animal cells *(1–3)*. Activation of ornithine decarboxylase (ODC) is required for carcinogenesis and subsequent tumor development in model tumor systems *(4–7)*. This enzyme regulates the initial step in the synthesis of polyamines (e.g., putrescine and spermidine), including those in which polyps and cancers form *(7)*. ODC activity and, in some cases, polyamine contents are elevated in several human precancerous conditions, including co-

lon polyps *(8,9)*, Barrett's esophagus *(10,11)*, and cervical intraepithelial neoplasia *(12)*.

α-Difluoromethylornithine (DFMO) is an enzyme-activated, irreversible inhibitor of ODC and causes a depletion in the intracellular concentrations of putrescine and its derivative, spermidine *(13)*. Levels of spermine, which is derived from spermidine, are not as markedly affected by the enzyme inhibition. DFMO was initially synthesized for therapeutic anticancer usage, but it was found not to be an active cytotoxic agent in chemotherapy trials against any human cancer *(14)*, except perhaps demonstrating moderate activity in the treatment of malignant brain tumors *(15)*. In general, the compound was nontoxic, with the significant exception of hearing loss, which was reversible after the drug treatment was discontinued *(16)*. The onset of the hearing loss could be associated with total cumulative dose *(17)*.

In experimental animal models, DFMO is a potent inhibitor of carcinogenesis that is especially active in preventing carcinogen-induced epithelial cancers of many organs, including those of the colon *(4–7)*. DFMO acts late in the tumor-promotion phase in animals, but the precise mechanism by which it inhibits the development of polyps and cancers is unknown. Effects on cell transformation, invasion, and angiogenesis have been shown [reviewed in *(18)*]; for example, overexpression of ODC enhances cellular transformation and invasion *(19)*. A study *(20)* from our laboratory shows that DFMO can inhibit the expression of matrilysin (a protein important in metastasis) in one human colon cancer-derived cell line.

The current trial was designed to determine if DFMO could be used to test the hypothesis that inhibition of polyamine synthesis could decrease colorectal or other organ carcinogenesis in humans. A number of pilot and phase I trials have demonstrated that low doses of DFMO can be given to human populations for up to 1 year, with minimum toxicity *(21–26)*. Several years ago, we reported the results of a short-term phase IIa dose de-escalation trial of DFMO in patients with prior colon polyps and

demonstrated that polyamine content in rectal mucosa was depleted after only 1 month of DFMO at doses as low as 0.10 g/m^2 per day *(26)*. The current trial was designed to determine whether polyamine content in rectal mucosa could be suppressed (without a rebound) for 1 year with doses lower than 0.50 g/m^2 per day and whether dose-limiting side effects, particularly audiometric, would occur at these low doses. A randomized, placebo-controlled phase IIb trial of 1-year duration was therefore undertaken; 118 participants received either placebo or DFMO (0.075, 0.20, or 0.40 g/m^2 per day) and were evaluated serially for polyamine content of rectal mucosa, audiometric changes, and other side effects.

Patients, Materials, and Methods

Patient Characteristics

This phase IIb clinical trial was planned to obtain follow-up measurements after 1 year of treatment of at least 25 participants in each of four dosage groups. Thus, 123 patients treated at the University of California, Irvine (UCI Medical Center and Long Beach Veterans Administration [VA] Medical Center) were randomly assigned in a double-blinded fashion to receive either daily oral placebo or one of three doses of DFMO (0.075, 0.20, or 0.40 g/m^2 per day). Five of those subjects (one in the placebo group, two in the 0.075-g/m^2 per day group, and two in the 0.20-g/m^2 per day group) withdrew from the study immediately after randomization but prior to taking any doses of their assigned study drug. No follow-up information is available on these subjects; therefore, the analysis reported in this manuscript is restricted to the 118 subjects who started on treatment.

Eligibility requirements included men and women, aged 40–80 years, who had had an adenomatous colon polyp(s) (>3 mm) removed within 5 years of entering the study. Patients were ineligible if they had familial polyposis, a colon resection of greater than 40 cm, or a resection of the ileocecal valve. The patients also had to be in generally good health, have a Karnofsky performance score of greater than 70, and have no severe chronic or life-threatening diseases, including no history of invasive cancer within 5 years. To be eligible for this trial, patients could not have a history of abnormal wound healing. Although problems of wound healing have not been reported in previous short-term trials of DFMO, action of DFMO on proliferating cells indicates that patients prone to cutaneous or internal abscess (for whatever reason) should be excluded from study. A complete blood cell count had to show a hematocrit level of greater than 35%, a white blood cell (WBC) count greater than 4000 cells/mm^3, and a platelet count greater than 100 000 cells/mm^3. Discriminating chemical laboratory values were serum creatinine levels less than 1.5 mg/100 mL, bilirubin levels less than 2.0 mg/100 mL, and asparate amniotransferase levels less than 2.0 times normal. Urinalysis had to show less than 1+ protein, 0–3 urinary casts, and 0–5 WBCs and no red blood cells. Prestudy requirements also included acceptable results of pure-tone audiometry (<20 decibel [dB] baseline thresholds for frequencies 250, 500, 1000, and 2000 Hz). Patients could not be on a special diet that precluded compliance with study requirements. Patients' diets were not monitored during the course of the DFMO treatment. Patients were not permitted to take salicylates (>81/mg per day) and calcium supplements (>500 mg/day) or to regularly take peptic ulcer medication, corticosteroids, nonsteroidal anti-inflammatory drugs, or anticoagulants. All patients signed a consent form approved by the University of California, Irvine, or the Long Beach VA Medical Center Institutional Review Boards.

Potentially eligible participants were identified in the endoscopy clinics of the two institutions, on referral from community gastroenterologists, and from self-referrals generated by public advertising. No incentives were provided to study participants. More males than females entered in the study because of the sex distribution of the participants recruited from the VA Medical Center.

We obtained eight colorectal biopsy specimens at each time point: prior to the start of DFMO treatment, after 6 and 12 months of treatment, and 3 months after treatment cessation. Polyamine contents were evaluated using three of these biopsies selected randomly. Detailed quality control studies, addressing biopsy size, processing, and other interpatient and intrapatient variability affecting measurements of these polyamine parameters have been published elsewhere *(27)*.

At the end of treatment, serum samples were also collected. To avoid possible effects of diurnal variations in laboratory end points, all biopsies were performed between 7:30 AM and 11:30 AM. Losses during transportation and laboratory equipment malfunction caused some loss of polyamine measurements. Thus, pre-DFMO polyamine content measurements in biopsy specimens were available for only 114 of the 118 participants. Participant dropout following randomization and initiation of treatments resulted in further sample loss. After 6 and 12 months on DFMO and then 3 months off study, polyamine measurements were available for 106, 95, and 92 participants, respectively.

Adherence Information

Participant adherence to the 52-week DFMO treatment schedule was recorded by self-report. Of the participants completing the 1-year study, 80% reported taking their DFMO doses for more than 80% (292 days) of the year, and 71% reported taking their DFMO doses for more than 90% (328 days) of the year. The distribution of adherence by dose group is shown in Fig. 1. There was a statistically significant trend toward lower adherence in the higher dose groups when defined by 80% adherence (two-sided $P = .004$) or by 90% adherence (two-sided $P = .028$). This result is driven largely by the higher study dropout in the 0.40-g/m^2 per day dose group (*see* ''Results''). Among those participants still on study at any given time, there was no statistically significant difference among the dose groups with respect to adherence, whether defined by 80% adherence ($P = .767$) or by 90% adherence ($P = .074$).

Symptom Monitoring

Participants were monitored every 3 months in person (months 3, 6, 9, 12, and 15) and by phone for the intervening 6-week periods. In addition to an open-ended interview process, the participant was also asked specifically about the occurrence of nausea, (epigastric) pain, diarrhea, poor appetite (anorexia), blood in stool, oral soreness, tiredness (fatigue), headaches and CNS symptoms, dizziness, hearing loss, ringing in the ears (tinnitus), and tingling/numbness (peripheral nervous system). All responses were recorded. Since we anticipated that the total number of symptoms in any category was likely to be small, we developed a grading system based on the rationale for early drug modification or discontinuation: grade 0—unrelated to symptoms (e.g., moving, job travel, inconvenience of the study visits, etc); grade 1—participant cited symptoms that were not judged to be related to DFMO therapy; grade 2—clinical symptoms
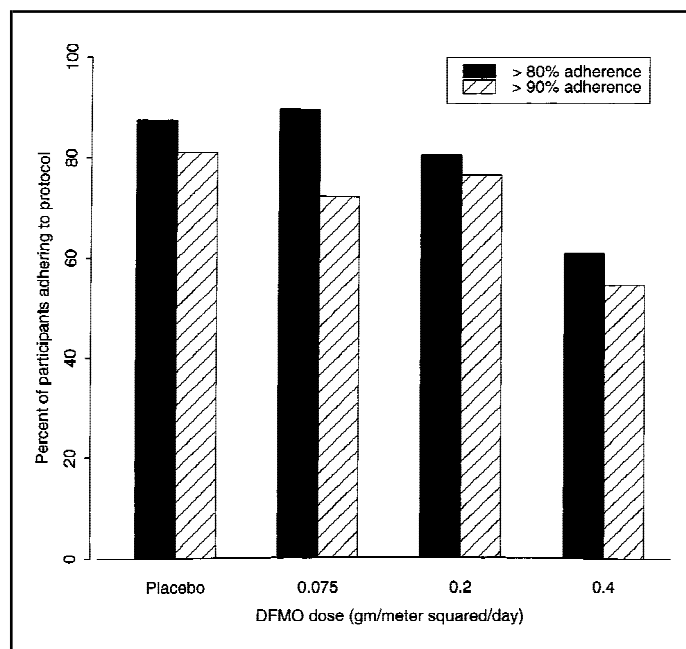

Fig. 1. Adherence of participants by different α-difluoromethylornithine dosage groups. ■ = >80% adherence; ▨ = 90% adherence.

suggested careful evaluation and the participant decided to stop the study; and grade 3—clinical symptoms and physician judgment suggested that the participants should stop the study drug.

Polyamine Analysis

Polyamine contents were determined as described earlier (28). Polyamine contents were evaluated using three of the eight rectal mucosal biopsy specimens (randomly selected). We adhered to quality control parameters as described in an earlier study (27), addressing biopsy size, processing, and other interpatient and intrapatient variability affecting measurements of mucosal polyamine concentrations. Our methods measure putrescine, cadaverine, histamine, spermidine, spermine, and monoacetyl derivatives of putrescine, spermidine, and spermine. On the basis of these quality control studies, we used three of eight biopsy specimens per patient to determine polyamine contents at each time point monitored. Quality assurance procedures included regular measurements of standard polyamine preparations and use of internal standards in assessing polyamine amounts. Briefly, three individual biopsy specimens, each weighing 5–30 mg, were minced in 300 μL of 9.2 N perchloric acid ($HClO_4$), homogenized with a tight-fitting Teflon pestle homogenizer, and vortexed vigorously. These lysates were stored at 4 °C overnight, rehomogenized, and revortexed the following day. This treatment precipitates the majority of cell proteins, while free polyamines are soluble in 0.2 N $HClO_4$. The acid-insoluble fraction was collected by centrifugation in a Sorvall model MC 12V minifuge with a model F12/M.18 rotor at 12 000 rpm for 10 minutes (Sorvall Manufacturing, Wilmington, DE). Polyamines in the acid-soluble fraction were determined by high-performance liquid chromatography using the method of Seiler and Knodgen (29) and were normalized to protein content in the acid-insoluble fraction. Protein levels were assessed using the bicinchoninic acid method, according to the manufacturer's instructions (Pierce Chemical Co., Rockford, IL). In a study of the sources of error in measuring colorectal tissue polyamine contents in patients not treated with DFMO, we found that the ratios of spermidine to spermine varied less than protein-normalized polyamine contents (27). We found similar results in our phase IIa trial (26). We have analyzed all of our results both as protein-normalized polyamine contents and as the ratios of spermidine to spermine. DFMO depletes putrescine and spermidine but not spermine contents in animal cells and tissues, thus suppressing the ratios of spermidine to spermine.

Audiometry

Bilateral pure-tone air conduction audiograms were performed by an audiologist at baseline and 1, 3, 6, 9, and 12 months after the onset of DFMO treatment in all study participants and 3 months after drug discontinuation. Thresholds were measured at 250, 500, 1000, 2000, 3000, 4000, 6000, and 8000 Hz. The study required that patients who experienced a more than 20 dB decrease from baseline threshold would be taken off the drug and their audiometric recovery documented by serial testing. Following completion of the trial, baseline thresholds were compared to 12-month thresholds by taking the mean and standard deviation of changes (in dB) for each DFMO dosage group.

Statistical Methods

Estimates of the variation of polyamine levels in the patients in the phase IIa study (26) suggested that a sample size of 25 per group would have a power of 0.80 to detect a 15% decrease and a power of 0.95 to detect a 20% decrease in the spermidine : spermine ratio. Subjects were randomly assigned in blocks of eight within each clinical site to minimize confounding DFMO dose with any time trends that might exist. Polyamine measurements were logarithmically transformed to remove skewness. Descriptive statistics are therefore presented as the geometric mean and 95% confidence intervals. The data were analyzed primarily by analysis of variance, with tests for trend by DFMO dose and adjustment for baseline and other covariates performed using linear regression. Audiometric results were similarly analyzed by analysis of variance and linear regression. All other side effects were measured according to their absence or presence, and due to low event rates, permutation tests were used to assess statistically significant differences among the treatment groups. All *P* values are two-sided.

All analyses were by intent-to-treat insofar as possible. While participants needing or desiring to discontinue DFMO due to adverse symptoms were encouraged to continue follow-up for polyamine measurements and audiometry, most often the subjects declined all further participation in the study. Hence, the analyses of polyamine measurements and audiometry can only be generalized to the subjects on study at any given time. One subject with nondetectable levels of spermidine and spermine at the 12-month biopsy was deleted from the analysis of the spermidine : spermine ratio.

Table 1. Polyamine levels of normal-appearing rectal mucosa of subjects given α-difluoromethylornithine (DFMO) or placebo by dose group and time

Time	Dose, 0 g/m² per day		Dose, 0.075 g/m² per day		Dose, 0.2 g/m² per day		Dose, 0.4 g/m² per day		Analysis of variance *P*†
	No. of subjects	Mean (95% CI)*	No. of subjects	Mean (95% CI)*	No. of subjects	Mean (95% CI)*	No. of subjects	Mean (95% CI)*	
Putrescine									
Baseline	32	0.52 (0.39–0.69)	29	0.46 (0.31–0.69)	25	0.36 (0.18–0.73)	28	0.27 (0.11–0.63)	.3484
6 mo	30	0.71 (0.53–0.97)	28	0.25 (0.12–0.54)	23	0.24 (0.12–0.47)	25	0.07 (0.02–0.20)	.0001
12 mo	28	0.61 (0.29–1.30)	26	0.71 (0.47–1.07)	21	0.42 (0.20–0.90)	20	0.07 (0.02–0.36)	.0019
Off DFMO	27	0.98 (0.69–1.40)	26	0.78 (0.43–1.43)	21	0.63 (0.30–1.31)	18	0.96 (0.69–1.33)	.6058
Spermidine									
Baseline	32	2.97 (2.54–3.48)	29	3.17 (2.69–3.74)	25	3.13 (2.69–3.65)	28	3.11 (2.51–3.85)	.9518
6 mo	30	3.07 (2.61–3.61)	28	2.51 (2.22–2.84)	23	2.23 (1.78–2.80)	25	2.39 (1.97–2.90)	.0489
12 mo	28	3.01 (2.57–3.53)	26	2.75 (2.38–3.18)	21	2.33 (1.74–3.11)	20	1.33 (0.60–2.99)	.0152
Off DFMO	27	2.54 (2.21–2.91)	26	2.18 (1.15–4.15)	21	2.85 (2.49–3.27)	18	2.57 (2.18–3.04)	.7802
Spermine									
Baseline	32	7.17 (5.94–8.66)	29	7.61 (6.57–8.82)	25	7.94 (6.60–9.55)	28	7.19 (6.04–8.55)	.8146
6 mo	30	6.88 (5.99–7.90)	28	7.75 (6.92–8.67)	23	6.71 (5.64–7.97)	25	7.11 (5.72–8.83)	.5794
12 mo	28	5.67 (4.58–7.02)	26	7.12 (5.97–8.48)	21	6.52 (5.32–7.99)	20	3.79 (1.51–9.56)	.1811
Off DFMO	27	5.95 (5.10–6.95)	26	4.49 (2.19–9.21)	21	5.11 (3.86–6.76)	18	5.96 (4.87–7.29)	.7372
Spermidine : spermine ratio									
Baseline	32	0.41 (0.35–0.49)	29	0.42 (0.35–0.49)	25	0.39 (0.32–0.49)	28	0.43 (0.36–0.51)	.9183
6 mo	30	0.45 (0.37–0.54)	28	0.32 (0.28–0.37)	23	0.33 (0.26–0.42)	25	0.34 (0.25–0.45)	.0800
12 mo	28	0.53 (0.43–0.65)	26	0.39 (0.33–0.45)	21	0.36 (0.25–0.51)	19‡	0.33 (0.28–0.40)	.0149
Off DFMO	27	0.43 (0.37–0.49)	25‡	0.47 (0.39–0.57)	21	0.56 (0.42–0.73)	18	0.43 (0.36–0.51)	.1856

*Mean = geometric mean; CI = confidence interval.

†Test for linear trend.

‡Ratio measurements could not be done in all specimens because of undetectable levels of respective polyamines. Change in 'n' in all dosage groups over time is observed because of participant dropout.

Results

Polyamine Content in Colorectal Biopsy at Baseline

One hundred eighteen participants, 18 females and 100 males, ranging in age from 44 to 80 years (mean ± standard deviation, 63.3 ± 8.5) were treated on this study. Older subjects tended to have lower levels at baseline for all polyamine measurements, but in all cases that trend was not statistically significant (P>.15). There was no statistically significant association between sex and baseline polyamine levels. Table 1 shows the geometric means of the various polyamine measurements within dose groups according to time on study. Also presented is the P value from the analysis of variance testing for statistically significant differences among the dose groups. No adjustment was made for the multiple comparisons inherent in considering multiple times during the study. It should be noted that the dose 0 group (placebo) did generally show nonsystematic changes in polyamine values over the course of the study. Three months after treatment cessation, putrescine measurements were statis-

tically significantly higher (P = .0003) and spermine measurements were statistically significantly lower (P = .046) than the respective measurements at baseline. Such changes can result from within person variation over time or laboratory drift; however, because of the randomized study design, the comparisons across dose groups at each biopsy time are valid.

Fig. 2 presents graphs of the geometric means and standard errors for each of the polyamine values by DFMO dose for each measurement time during the study. Also presented is the P value of a test for linear trend by dose at each time point.

DFMO inhibited putrescine levels in a dose-dependent manner, an effect that was evident by 6 months and showed a consistent proportion decrease. At a daily dose of DFMO of 0.40 g/m^2, putrescine levels were decreased to approximately 10% of those of the placebo group. Similar declines were seen for spermidine levels; the spermidine : spermine ratios decreased with dose and time as well. All polyamine levels in general returned toward the baseline after discontinuation of DFMO. Neither age nor sex was a statistically significant predictor of change in polyamine levels. Because patients who dropped out of the study
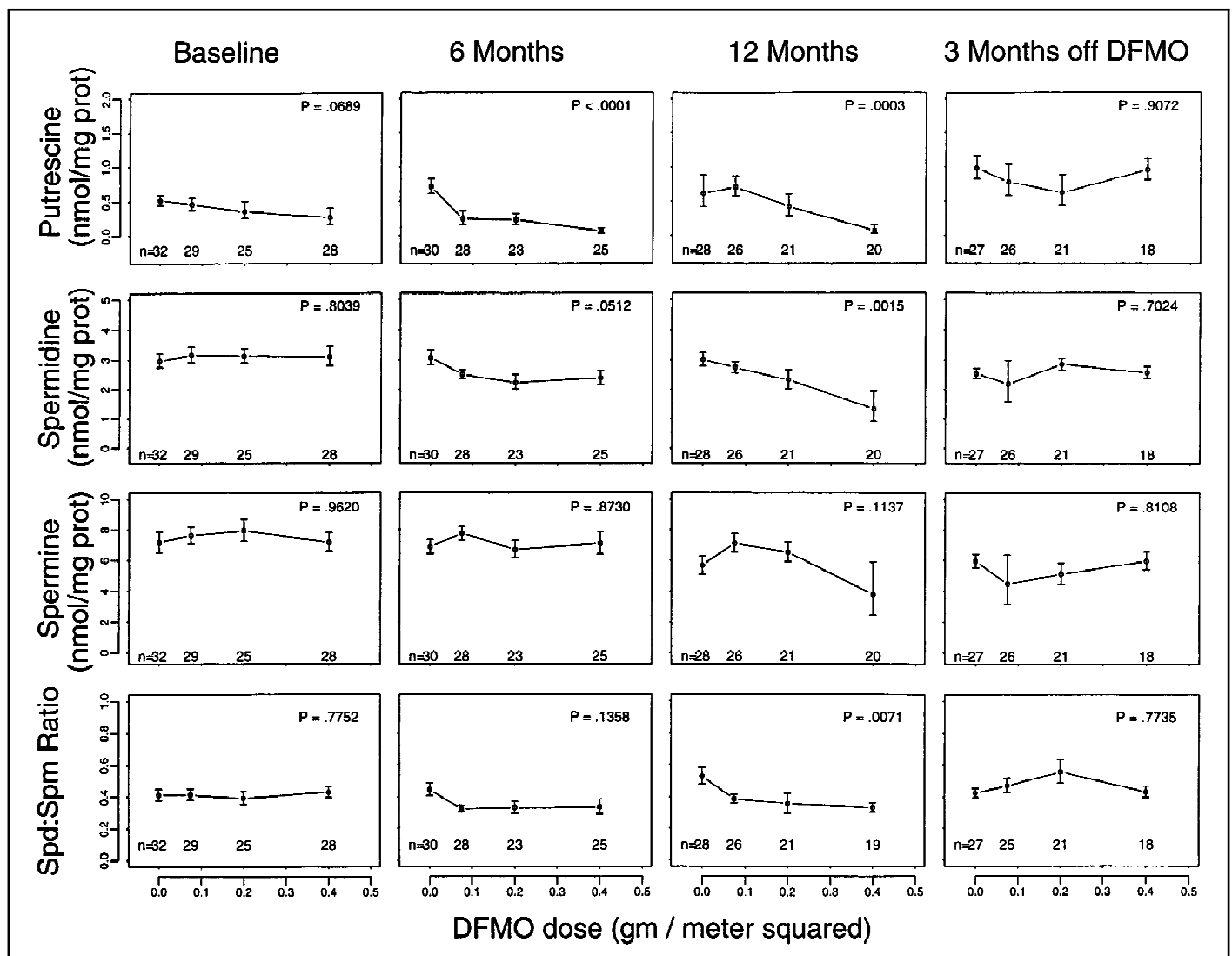

Fig. 2. Polyamine content in rectal mucosal biopsy specimens by different α-difluoromethylornithine (DFMO) dosage groups (0.075, 0.20, and 0.40 g/m^2 per day) over time. Values shown for putrescine, spermidine, and spermine are in mmol/mg protein; Spd : Spm = spermidine : spermine ratio; Prot = protein. P values are given for the test for linear trend.

generally did so before providing the 6-month biopsy specimen, we have insufficient data to be able to assess any trends in the polyamine levels among such patients.

Fig. 3 presents graphs of the geometric means for each dosage group over time. In this descriptive figure, the measurements at each time point have been normalized to the dose-0 group's measurement, and all analyses were adjusted for baseline values.

Symptom Monitoring, Side Effects, and Dropout Rates

All participants were carefully monitored for symptoms as described above. There were no statistically significant differences among the treatment groups with respect to any single symptom. Since the number of symptoms being monitored was large and their occurrences were infrequent, we grouped the symptoms together in our evaluation. The number of participants with side effects by dosage group is summarized in Table 2. The numbers were comparable in the placebo and highest dosage group, and no dose–response effect was evident. A total of 22 participants stopped taking the drug earlier than the planned 12-month duration, and an additional five subjects temporarily (<2 weeks) stopped taking the drug at some point during the study while their symptoms were evaluated. Ten of these individuals discontinued the study for grade 0 reasons (i.e., not related to symptoms). The number of participants with grade 1–2 toxicity or higher and their relationship to dosage group and treatment modification of early discontinuation are shown in Table 3. This table presents the frequency of side effects restricted to grade 1 or 2 or higher. Although there is a statistically significant higher dropout rate due to more severe side effects in the highest dose group (0.40 g/m^2 per day) ($P = .006$), there did not seem to be a linear increase in incidence of side effects with dose among the other three dose levels. In particular, the placebo group had relatively higher rates of grade 0 side effects compared with the other groups (Table 2). The reasons for dropouts with grades 2- and 3-level side effects included four patients with dizziness and imbalance and one patient with audiometric hearing loss in the 0.40-g/m^2 per day dose group, one patient with speech difficulties in the 0.075-g/m^2 per day dose group, and one patient with forgetfulness and one patient with a severe rash in the placebo group.

Table 2. Subjective evaluation of side effects by grade and dosage group of subjects given α-difluoromethylornithine (DFMO) or placebo*

	DFMO, g/m^2 per day			
Grade	0 (n = 32)	0.075 (n = 29)	0.20 (n = 26)	0.40 (n = 31)
≥0	28 (88%)	22 (76%)	19 (73%)	23 (74%)
≥1	11 (34%)	7 (24%)	6 (23%)	13 (42%)
≥2	5 (16%)	4 (14%)	0 (0%)	7 (23%)
3	3 (9%)	1 (3%)	0 (0%)	4 (13%)

*All symptoms and grading scale are listed in the "Patients, Materials, and Methods" section. Numbers represent participants with one or more symptoms. The differences in side effects in different dosage groups were not statistically significant as determined by Monte Carlo permutation test.

Table 3. Early discontinuation of α-difluoromethylornithine (DFMO) treatment and dose modification by grade and dosage group

	DFMO, g/m^2 per day			
Grade	0 (n = 32)	0.075 (n = 29)	0.20 (n = 26)	0.40 (n = 31)
≥1*	3 (9%)†	1 (3%)	0 (0%)	8 (26%)‡
≥2	2 (6%)	1 (3%)	0 (0%)	5 (16%)

*Grade of side effects was determined according to the scale as defined in the "Patients, Materials, and Methods" section.

†Additionally, two participants (one in dose 0 and one in dose 0.075) stopped study drug temporarily (<2 wks) for grade 1 side effects, and three participants (one in dose 0 and two in dose 0.075) stopped study drug temporarily for grade 2 side effects. All five of these participants resumed their assigned dose level and completed the study.

‡The concentration of dropouts in the highest dosage group is not random Monte Carlo permutation test (two-sided $P = .006$); test for trend (two-sided $P = .045$).
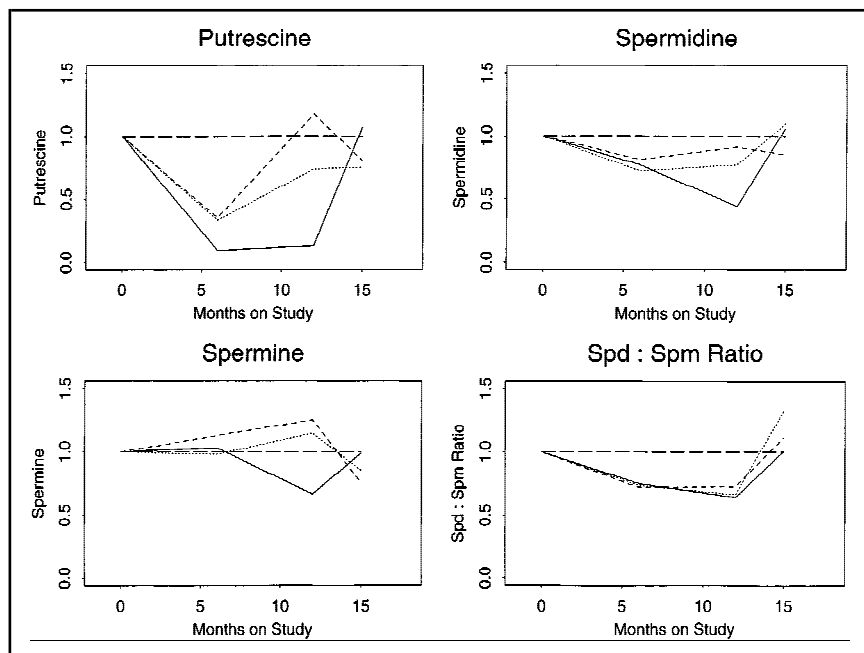
Audiometric Measurements

Table 4 shows the number of patients in each DFMO dose group who demonstrated clinically significant changes in audiometric thresholds at any frequency. The placebo group had three participants with a greater than 15 dB decrease in thresholds at one frequency compared with four participants in the highest dosage group. At three frequencies, 250 Hz in the right and left ears, 3000 Hz in the right ear, and 500 Hz in the left ear, statistical trends toward threshold elevation were found; however, no statistically significant trends were found for the other frequencies tested. The lack of trends across frequencies suggests that no major audiotoxicity occurred in the dosage groups as a whole.

Fig. 3. Polyamine content in rectal mucosal biopsy specimens over time (adjusted for baseline). – – – = 0 g/m^2 per day; ---- = 0.075 g/m^2 per day; ..... = 0.20 g/m^2 per day; and —— = 0.40 g/m^2 per day.

Table 4. Overall audiometric changes* in subjects in different α-difluoromethylornithine (DFMO) dosage groups†

Pre-treatment/post-treatment comparison, increase in threshold levels	DFMO, g/m² per day			
	0	0.075	0.20	0.40
	No. tested‡			
	(n = 32)	(n = 28)	(n = 26)	(n = 27)
≥15 dB	3 (9%)	3 (11%)	2 (8%)	4 (15%)
≥20 dB	1 (3%)	3 (11%)	0 (0%)	2 (7%)
≥25 dB	0 (0%)	1 (4%)	0 (0%)	1 (4%)

*Change at any frequency.

†The difference in audiometric thresholds were not statistically significant as determined by Monte Carlo permutation test.

‡Every patient did not get the test each time, and therefore comparisons could only be done for those who had both the pre- and the post-treatment evaluations.

Discussion

The results from this placebo-controlled, randomized trial demonstrate that the oral administration of low doses of DFMO can suppress polyamine content in biopsy specimens of colorectal mucosa for at least 1 year without a rebound effect or after it has been stopped. Additionally, by 3 months after being off the study, the polyamine content in biopsy specimens had returned to normal. It is likely that even longer-term suppression will be sustainable and can be accomplished at the low doses used. Equally important is that continuous or intermittent administration of DFMO will be necessary to lower the risk of colon cancer in this population. DFMO also depletes polyamine contents in other gastrointestinal tissues, but the degree of depletion is tissue specific and reflects the relative proliferative status and expression of ODC (30).

For putrescine content, a dose–response effect of the DFMO was evident at both 6 and 12 months; spermidine content was also lowered (Fig. 3). Both putrescine and spermidine contents were consistently lowered more by a dose of 0.40 g/m² per day compared with a dose of 0.20 g/m² per day. However, of considerable importance in assessing the relative efficacy of 0.20 or 0.40 g/m² per day in producing a biologic effect was that the decrease in spermidine : spermine ratio was similar for the two doses after both 6 and 12 months of therapy. This suggests that both doses are comparable in lowering polyamine count in rectal mucosal tissue and a similar biologic outcome would be reasonable to expect. The similarity of these two doses in lowering polyamine content is also important inasmuch as the data suggest that the constellation of symptoms and the decreased adherence produced at the higher dose could lead to an increased incidence of dosage modification and/or trial discontinuation over a long term. This observation would argue for a dosage closer to 0.20 g/m² per day of DFMO for long-term chemoprevention trials, since adherence decreases the longer participants are on trial.

Our finding that DFMO suppresses colorectal putrescine and spermidine contents indicates that decarboxylation of endogenous ornithine is a significant source of polyamines in this tissue in humans. Studies (30) in rodent models show that colorectal tissues can also take up luminal polyamines produced by enteric bacteria. Further reductions in colorectal tissue polyamine contents may be achievable by limiting this source of polyamines and could increase the efficacy of polyamine depletion as a strategy for colon cancer chemoprevention (31).

The lack of consistent audiometric changes at all frequencies tested is of considerable importance for the continued development of this compound as a chemoprevention agent. Possible explanations for lack of hearing loss with DFMO treatment need to be offered, since in previous clinical trials of DFMO, hearing loss has been described. At large doses (2–12 g/m² per day) given over periods of up to 50 weeks, DFMO produced hearing loss at 500, 1000, 2000, 4000, and 8000 Hz (17). In particular, hearing loss was present in up to 75% of patients who received a cumulative dose of more than 250 g/m². In another study, audiotoxicity, defined as a greater than 20-dB loss at two frequencies, was noted at total daily doses greater than 1.0 g/m², with cumulative total doses of greater than 90 g/m² (23). While both of these studies employed high DFMO doses, evidence, including the present study, indicates that doses of DFMO less than 0.5 g/m² per day do not produce hearing loss. Pasic et al. (24) administered DFMO in daily doses of 0.5, 1, 2, 3, or 5 g/m² for 6–12 months to 27 individuals. None of 12 people receiving 0.5 g/m² per day developed hearing loss, while all individuals receiving 2, 3, or 5 g/m² per day experienced hearing loss. However, the hearing loss was mild (12.0-dB change in threshold across all frequencies) at the three larger doses and improved in all patients tested after DFMO administration was stopped. This study, which finds no DFMO audiotoxicity at any dose up to 0.40 g/m² per day, is consistent with earlier findings. Whether the hearing changes seen in trials involving higher doses of DFMO is a result of dose rate or cumulative dose of DFMO cannot be answered by the current trial. It is encouraging that no consistent hearing changes were documented, even in the high-dose group, since a total dose (cumulative dose of DFMO, 144 g) was achieved at which hearing losses might have been anticipated. However, since one patient was taken off study secondary to worsening pure-tone thresholds, this side effect should be clinically monitored in future trials, but intervals between audiometric tests could be lengthened.

Although there are notable exceptions, most phase III chemoprevention trials to date have been based on epidemiologic or animal data without systematic pilot, phase I, or phase II trials (32–35). In some cases (e.g., β-carotene for prevention of lung cancer in smokers), an adverse effect has been seen (36,37). The systematic clinical development of DFMO as a chemoprevention agent by our group and others (8,10,17,21,23–27) provides a useful platform for the study of this compound in a number of organ sites, including the cervix, in which considerable apparent activity against cervical intraepithelial neoplasia has been reported (38). A number of compounds with a mechanism of action different from that of DFMO have shown promise as colon cancer chemoprevention agents (39). Preclinical models have consistently demonstrated that low doses of combinations of agents are more effective in preventing cancer than either agent alone, and with decreased toxicity (40). The combination of DFMO plus other agents (e.g., calcium and nonsteroidal anti-inflammatory drugs) or accompanied by dietary manipulations (decreased fat, increased fiber) would seem a logical next step. Alternatively, a phase III trial for the prevention of colon polyps/cancer using DFMO alone would be reasonable.

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