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ARTICLES

Dose De-escalation Chemoprevention Trial of α-Difluoromethylornithine in Patients With Colon Polyps

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Background: **α-Difluoromethylornithine (DFMO) is a potent inhibitor of carcinogenesis in experimental animal models. In these animal models, DFMO has been especially active in preventing carcinogen-induced epithelial cancers, including those of the skin, colon, breast, and urinary bladder. Although DFMO is known to exert its diverse biological effects by suppressing intracellular pools of the polyamines putrescine and spermidine, the precise mechanism by which polyamine depletion, induced by DFMO, suppresses carcinogenesis is unknown.** ***Purpose:*** **The specific aim of our study was to determine the lowest dose of DFMO that would deplete target tissue (colorectal mucosa) levels of these polyamines in humans who had undergone prior removal of colon polyps while producing minimal toxic effects.** ***Methods:*** **A dose de-escalation chemoprevention trial of DFMO was conducted in 111 patients (36 female and 75 male) who were in generally good health, aged 39-79, and who had undergone colonoscopy for surgical removal of an adenomatous colon polyp greater than 3 mm within 5 years prior to entering the study. Groups of patients (12-20 patients per group) were orally treated with single, daily doses of DFMO ranging from 3.0 to 0.1 g/m^2 for 4 weeks (28 days). Prior to initiation of DFMO treatment and at the end of treatment, six colorectal biopsy specimens were collected from each patient, along with serum samples. All biopsies were performed between 9 AM and noon to avoid possible effects of diurnal variations in laboratory end points. Samples for analysis of plasma DFMO levels were also collected during this time period on the day after the last day of drug administration.** ***Results:*** **DFMO caused a decrease in both putrescine content and the ratio of spermidine to spermine for all dose groups down to 0.25 g/m^2. Both putrescine content and the ratio of spermidine to spermine and changes in these parameters as a function of DFMO treatment decreased as a function of donor age. None of the 30 patients receiving either 0.25 or 0.5 g/m^2 experienced any clinical ototoxicity in this trial.** ***Conclusions:*** **DFMO is both safe and effective in reducing colorectal mucosal polyamine contents when it is administered orally to patients at doses as low as 0.25 g/m^2 for 28 days. No ototoxicity was observed at doses up to twice this amount.** ***Implications:*** **If DFMO is also found to be effective in suppressing polyamine contents in other target tissues, it may be useful in preventing a wide range of human epithelial cancers, including those of the prostate and breast. [J Natl Cancer Inst 86:1122-1130, 1994]**

α-Difluoromethylornithine (DFMO) is an enzyme-activated, irreversible inhibitor of ornithine decarboxylase, the first enzyme in polyamine synthesis (*1*). Polyamines are ubiquitous polycations that are essential for optimal growth of bacteria (*2*), yeast (*3*), and animal cells (*4*). Inhibition of ornithine decarboxylase by DFMO causes a depletion in the intracellular concentrations of putrescine, the decarboxylation product of ornithine, and its derivative spermidine (*5*). Levels of spermine, which is derived from spermidine, are not as markedly affected by the inhibitor.

DFMO treatment generally slows the growth of, but is not toxic to, animal cells (*6*). In animal studies, DFMO has been found to cause few toxic effects (*7*), although it can induce audiogenic seizures in a susceptible strain of mice (*8*). DFMO has been investigated as a therapeutic anticancer agent in humans. Clinical studies in humans support the conclusion that DFMO is generally nontoxic, with a significant exception being

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See "Notes" section following "References."

that high doses of the drug can induce ototoxicity, a high-frequency hearing loss, which has been reversible when drug treatment was discontinued (*9*). DFMO has not been found to be an active cytotoxic agent in any human cancer chemotherapy trial, although this drug is reported to have some activity as a cytostatic agent in the treatment of malignant brain tumors (*10*). DFMO is a potent inhibitor of carcinogenesis in experimental animal models (*11*). It is especially active in preventing carcinogen-induced epithelial cancers, including those of the skin (*12*), colon (*13*), breast (*14*), and bladder (*15*). The precise mechanism or mechanisms by which polyamine depletion, induced by DFMO, suppresses carcinogenesis are unknown. DFMO appears to act late in tumor promotion in experimental animals (*16*), suggesting that inhibition of cell proliferation is not its primary mechanism of action. For example, recent studies from our laboratory show that DFMO can inhibit the expression of matrilysin in one human colon cancer-derived cell line (Wallon UM, Shassetz LR, Cress AE, et al.: manuscript submitted for publication). DFMO is of current interest as a possible cancer chemopreventive agent in humans as a consequence of these and other studies (*17-20*). Ornithine decarboxylase activity and, in some cases, polyamine contents are elevated in several human precancerous conditions, including colon polyps (*17,18*) and Barrett's esophagus (*19,20*).

This study was designed to be part of a larger strategy to determine if DFMO could be used to test the hypothesis that inhibition of polyamine synthesis could decrease colorectal carcinogenesis in humans. DFMO is known to exert its diverse biological effects by suppressing intracellular pools of putrescine and spermidine, while generally not affecting the size of the spermine pool (*5*). Thus, a decrease in the spermidine pool size, as a consequence of DFMO treatment, can be measured either as a decrease in the tissue spermidine content normalized to tissue protein content or wet weight (two independent measurements and two independent sources of error) or as a decrease in the ratio of spermidine to spermine (one independent measurement and only one source of error). In a preliminary report of a subset of patients entered in this trial (*21*), we have reported DFMO-dependent decreases in putrescine and spermidine, but not in spermine. In a detailed quality-control study of measurements of polyamine contents in human colorectal mucosa (*22*), we concluded that the ratio of spermidine to spermine was less susceptible to measurement errors from several sources. Thus, we have now evaluated the effects of de-escalating DFMO doses on colorectal mucosa polyamine contents by analyzing changes in the ratio of spermidine to spermine in biopsy specimens obtained before and after DFMO treatments lasting 4 weeks. From this analysis, we wanted to determine the lowest dose of DFMO that would deplete target tissue (i.e., colorectal mucosa) levels of these polyamines in humans who had undergone prior removal of colon polyps.

Materials and Methods

Patient Characteristics

One hundred eleven patients treated at the University of California, Irvine, were entered in this trial. Groups of patients received daily doses of DFMO administered orally, ranging from 3 to 0.1 g/m^2, for 4 weeks. Eligibility requirements included men or women, aged 39-80, 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 or nonpolyposis or had a colon resection of greater than 40 cm or a resection of the ileocecal valve. The patient 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. 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 aspartate aminotransferase or alanine aminotransferase levels less than 2.0 times normal. Urinalysis had to show less than 1+ protein, 0-3 urinary casts, and 0-5 WBCs and red blood cells. Prestudy requirements also included acceptable results of screening audiometry (<10 dB base-line loss for age at any frequency). Patients could not be on a special diet that precluded compliance with study requirements. Patient diets were not monitored during the course of DFMO treatment. Pretreatment diets were monitored in a subset of patients; no differences in pretreatment subject diets were found by dose groups. Patients were not permitted to take on a regular basis salicylates, antacids, calcium supplements, peptic ulcer medication, corticosteroids, nonsteroidal anti-inflammatory drugs, or anti-coagulants. All patients signed a consent form approved by the University of California, Irvine Institutional Review Board.

Prior to the start of DFMO treatment, we obtained six colorectal biopsy specimens, as described previously (*21*). At the end of treatment, six colorectal biopsy specimens were again collected, along with serum samples. By protocol and in order to avoid possible effects of diurnal variations in laboratory end points, all biopsies were performed between 9 AM and noon. Samples for analysis of plasma DFMO levels were also collected during this time period on the day after the last day of drug administration. The protocol initially called for polyamine determinations on three biopsy specimens and labeling indices (percent cells in mucosal crypt cells that were in S phase, as assessed by incorporation of [^{3}H]thymidine or bromodeoxyuridine [BrdUrd]) that were measured on three biopsy specimens at both base line and end of treatment. The protocol was later modified to omit the labeling index measurements; laboratory equipment malfunction caused some polyamine measurements to be lost. Thus, pre-DFMO and post-DFMO polyamine measurements are available for 94 patients, and labeling index measurements are available for 55 patients. Our original plan was to treat 15 patients at each dose level, beginning with the highest dose (3 g/m^2 per day), and then to proceed sequentially to the next lowest level. The actual number of patients assigned to each dose level ranged from 12 to 20, and more patients were assigned to the groups treated first to ensure that we had adequate patient numbers to discern possible differences in laboratory end points.

Adherence Information

Patient adherence to the 4-week (28-day) DFMO treatment schedule was documented. Ninety-eight percent of the patients took their DFMO doses for more than 21 days, 94% took their DFMO doses for more than 25 days (25 of 28 days = 89% compliance), and 83% took their DFMO doses for all 28 days of the scheduled treatment.

Laboratory Procedures

Polyamine analysis. Polyamine contents were determined in one of the two paired colorectal biopsy specimens, as described in an earlier publication (*21*). Briefly, biopsy specimens were minced, disrupted by sonication, and clarified by low-speed centrifugation (2000*g* for 10 minutes). Clarified lysates were then extracted in 0.2 N $HClO_4$. Polyamines in the acid-soluble fraction were determined by high-performance liquid chromatography (HPLC), using the method of Seiler and Knodgen (*23*), 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). In a study of the sources of error in measuring colorectal tissue polyamine contents in patients not treated with DFMO (*22*), we found that the ratios of spermidine to spermine varied less than protein-normalized polyamine contents. We found similar results in this trial. While we have analyzed all of our results both as protein-normalized polyamine contents and as the ratios of spermidine to spermine, we have chosen to express our results here as changes in the ratios of spermidine to spermine.

Table 2. Plasma DFMO levels* after 28 days of DFMO treatment

DFMO dose, g/m^2 per day	No. of patients	Mean	Standard deviation	Minimum	Maximum
3.00	17	40.01	43.74	2.9	151.4
1.50	15	41.29	56.40	ND†	164.7
1.00	15	18.01	15.34	ND†	52.2
0.75	16	7.85	4.79	1.1	17.0
0.50	9	13.81	19.64	1.0	63.0
0.25	13	4.97	4.82	1.0	18.8

*Plasma DFMO levels in nmol/mL.
†ND = none detected; limit of detection, 0.05 nmol/mL.

nonrandomized, unblinded study. These data may simply reflect a statistical regression to the mean. Indeed, there was a similar tendency for the lowest base-line values to correspond to the higher values after DFMO therapy. Labeling indices reported here represent pooled values determined by either the [^{3}H]thymidine or immunohistochemical BrdUrd methods. There was no significant difference observed in either base-line labeling index measurements (P = .18) or as a change from base line (P = .33), comparing these two measurements. There was no effect of treatment on labeling-index measurements, even after adjusting for method of measuring labeling index. Labeling index measurements were based on an average of 10.66 crypts counted (standard deviation, 8.57) per patient sample.

Toxic Effects Associated With DFMO Treatment

Table 3 shows the toxic effects associated with DFMO doses that de-escalate from 3 to 0.1 g/m^2. All toxic effects were self-limiting and did not require discontinuation of treatment, with the exception of one patient in the 0.25-g/m^2 dose group who had severe abdominal pain. This patient was seen in the emergency room; the only finding was gallstones on ultrasound. The pain resolved over the next day, and the patient successfully completed the clinical trial without further incident. In the "other" category in Table 3, one patient in the 1.5-g/m^2 group experienced a myocardial infarction and had to be withdrawn from the study. This event was reported to the National Cancer Institute as an adverse reaction. Also in the "other" category, other symptoms included abdominal cramping associated with diarrhea lasting 1 day; anal irritation lasting 3 days in a patient having visible blood in the stool; hypertension that arose after 1 week of DFMO, but was also coincident with discontinuation of cardiovascular medications; and one patient in the 1.5-g/m^2 group who had self-diagnosed atrial fibrillation and discontinued taking DFMO after 3 days of treatment. One patient in the 0.75-g/m^2 dose group reported tinnitus while receiving DFMO. This patient had experienced intermittently for 4-5 years tinnitus that was believed to be secondary to aspirin use. No other patients reported changes in their hearing. In the 0.1-g/m^2 dose group, one patient who was receiving lithium had leukocytosis (WBC count, 15.9 × 10^3 cells/mm^3), and one patient had an increased serum aspartate aminotransferase level of 124 mg/100 mL, which decreased to 33 mg/100 mL 1 week later. With the exception of the myocardial infarction (for which treatment was stopped), the hypertension (treatment of which was resumed), and the self-diagnosed atrial fibrillation (which was the stated reason for the patient's withdrawing from the trial), all symptoms were mild and transient (1 or 2 days) and required no special treatment.

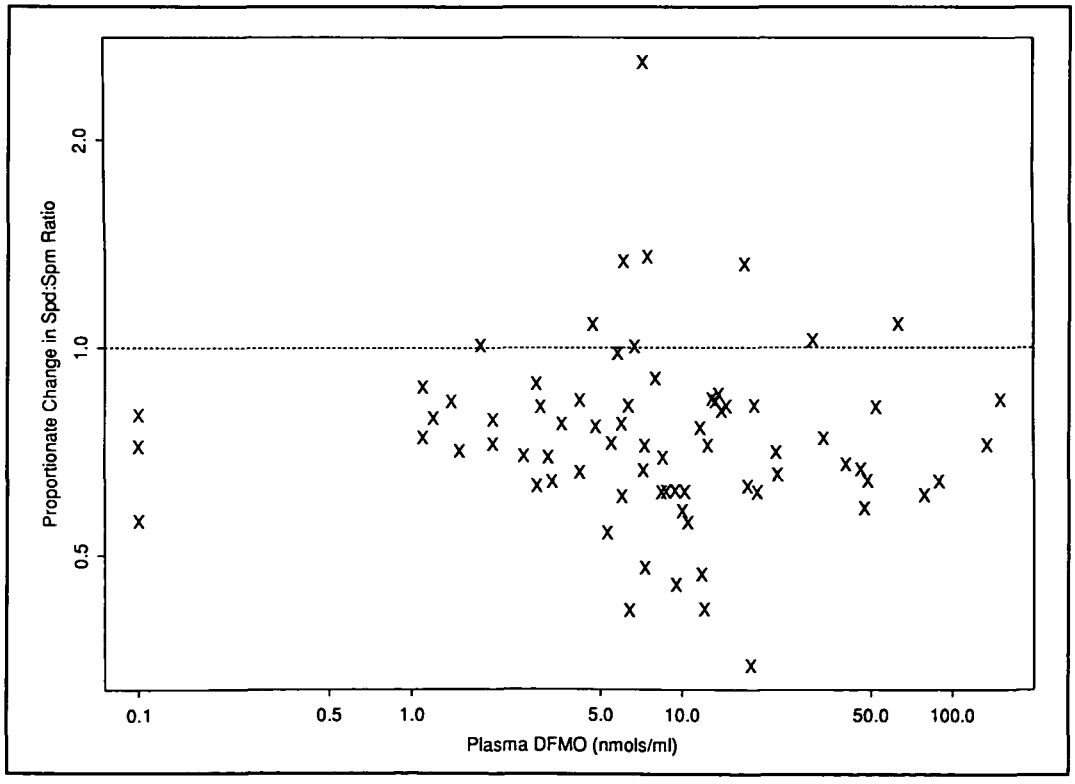

Fig. 2. Proportionate change in ratios of spermidine to spermine as a function of plasma DFMO concentration. Proportionate change of 1.0 means this parameter was the same before and after DFMO doses that resulted in the plasma DFMO concentrations as shown.

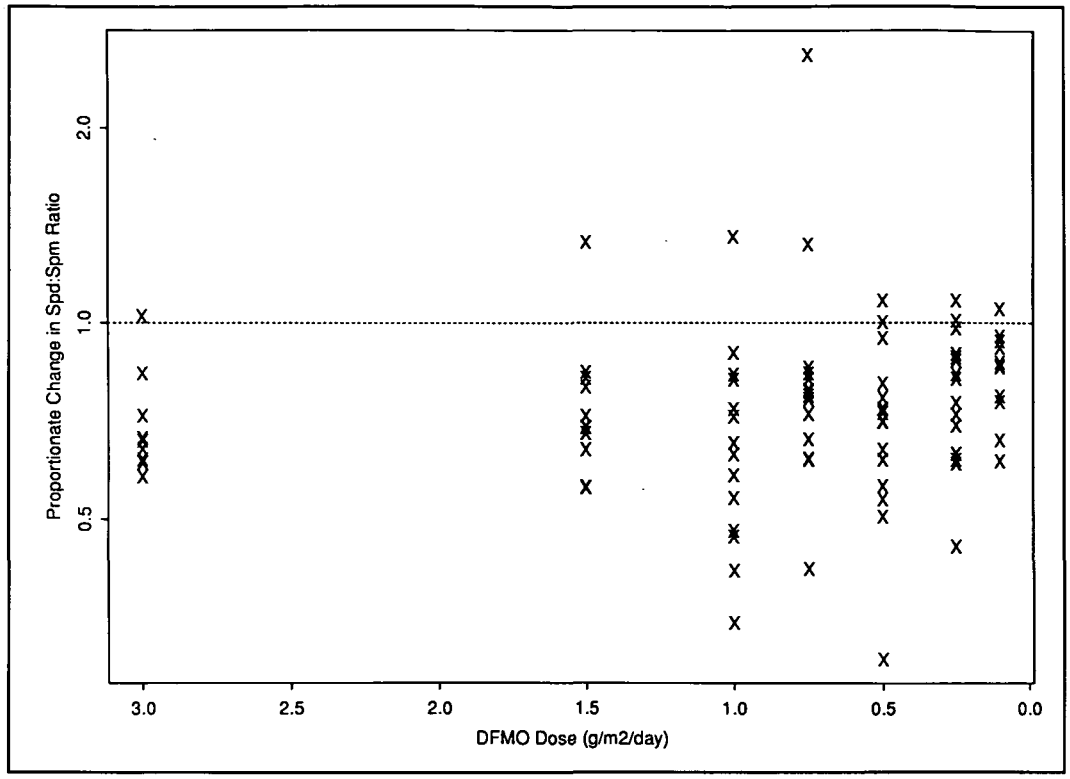

Fig. 3. Proportionate change in ratios of spermidine to spermine as a function of DFMO dose. This figure shows the distribution of changes in ratio of spermidine to spermine at each DFMO dose administered.

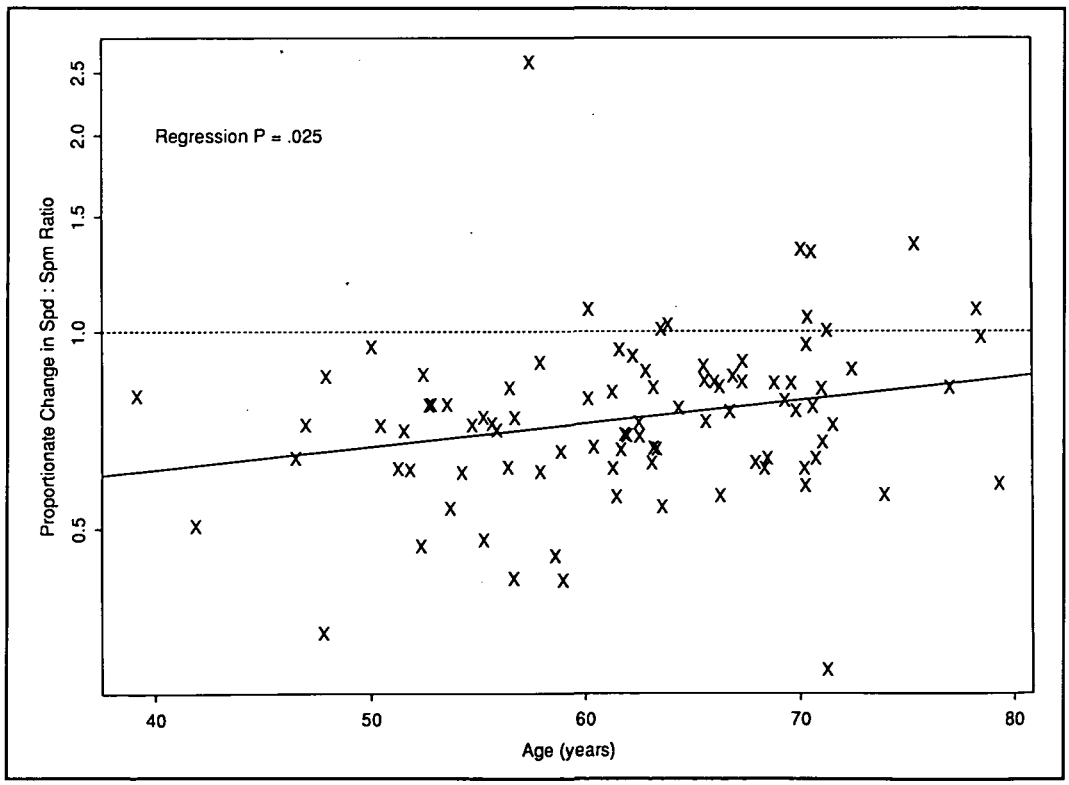

Fig. 4. Proportionate change in ratios of spermidine to spermine by age of the patient. Linear regression analysis shows that the proportionate decrease in this parameter decreases with increasing patient age.

Discussion

In this article, we describe the results of a dose de-escalation study to determine the minimum DFMO dose required to suppress colorectal mucosal tissue polyamine contents. In most phase I/II trials in clinical oncology, doses of an interventional agent are escalated to determine the maximum tolerated dose before antitumor efficacy of the agent is fully assessed. Cancer chemoprevention strategies differ from treatment strategies, in that patients at risk for the development of specific cancers, like those treated for colon polyp removal in this study, may be treated with an interventional agent for a long

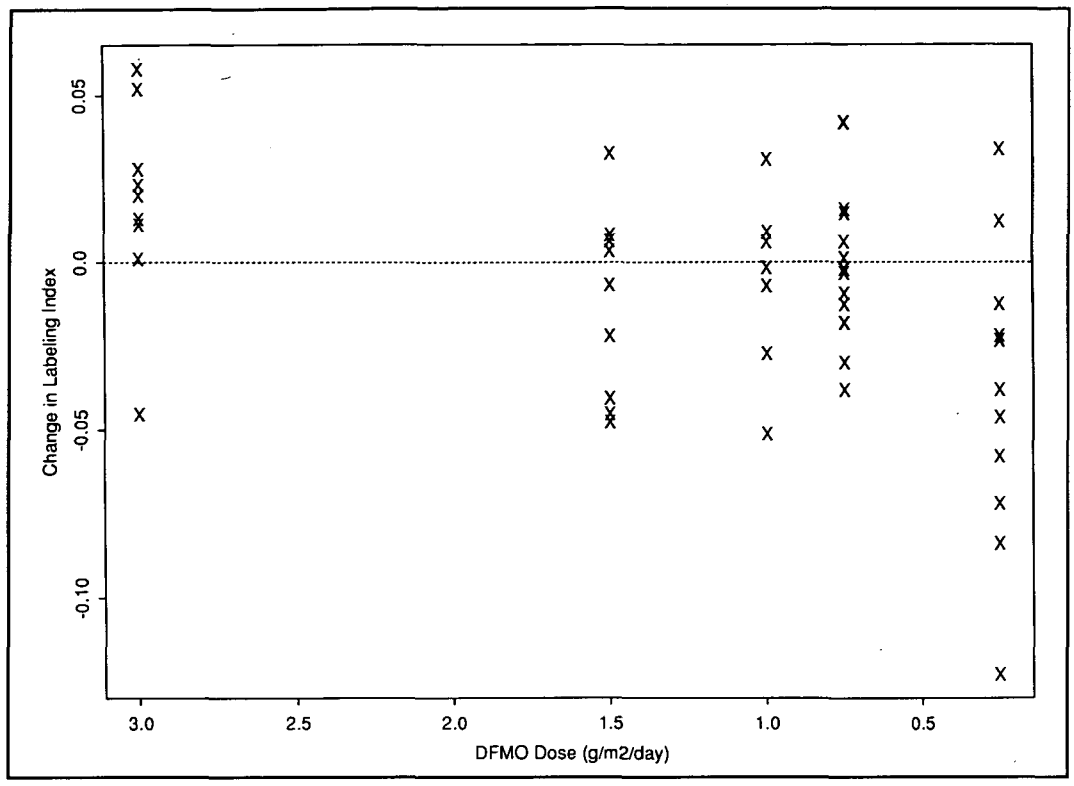

Fig. 5. Effect of DFMO on colorectal mucosal crypt cell labeling index. Absolute changes in label indices (number of cells incorporating either [^{3}H]thymidine or BrdUrd into their nuclei divided by the total cells evaluated) from pretreatment values as a function of DFMO dose administered are shown. 0 = no change.

period of time (years for chemoprevention, days–months for cancer treatment). Thus, identifying the lowest dose necessary to produce the desired effect, without producing significant toxic effects, is one important consideration of chemoprevention trials.

We used a fixed treatment time of 4 weeks in this trial and started with a dose of 3 g/m^2 DFMO per day. We knew from our previous study (*21*) that this dose was sufficient to reduce putrescine contents in colorectal mucosa of humans. We then explored the effect of DFMO treatment at several lower doses down to 0.1 g/m^2 per day. We found that doses down to 0.25 g/m^2 DFMO per day reduced putrescine contents and the ratios of spermidine to spermine in a highly statistically significant manner. At a dose of 0.1 g/m^2 per day of DFMO, the ratios of spermidine to spermine, but not putrescine contents, were also suppressed in a statistically significant manner.

Plasma DFMO levels measured in samples obtained within 24 hours of the final drug dose were directly related to the DFMO dose administered. Our measures of plasma DFMO most certainly underestimate maximum plasma DFMO concentrations during the 28-day treatment period, since another study has shown that the half-life of DFMO in plasma is 3-4 hours (*24*). This plasma DFMO value obtained 24 hours after the final DFMO dose was not functionally related to proportionate changes in the ratios of spermidine to spermine. Thus, this plasma DFMO parameter is not a useful measure of DFMO effect in the target colorectal tissue.

Table 3. Number of patients with toxic effects by dose group*

	DFMO dose, g/m^2 per day						
Toxic effect	3.0 (n = 20)	1.5 (n = 18)	1.0 (n = 15)	0.75 (n = 16)	0.5 (n = 15)	0.25 (n = 15)	0.1 (n = 12)
Any†	10	5	5	4	1	5	2
Nausea	0	2	1	2	1	0	0
Epigastric pain	0	3	2	3	1	2	1
Diarrhea	8	2	0	0	0	1	0
Blood in stool	2	0	1	0	0	0	0
Oral sores	1	0	1	0	0	0	0
Fatigue	1	0	1	0	0	1	0
Headache	0	0	0	0	0	1	1
Other	5	1	4	2	0	2	0

*Values = Number of patients.

†"Any" excludes toxic effects listed in the "other" category: abdominal cramping, irritation, patient-diagnosed atrial fibrillation, intestinal gas, vague malaise, wheezing, blurred vision, tinnitus, and low back pain.

We do not yet know the lowest dose of DFMO that will suppress polyamine contents in colorectal mucosa of humans treated orally with this drug. We do know from cell culture studies that it is possible to suppress ornithine decarboxylase activity over a broad range of nontoxic DFMO doses (5 m*M* to 50 μ*M*) (*27*). Thus, the lack of a dose-dependent effect on polyamine contents over the range from 3 to 0.25 g/m^2 per day is not necessarily surprising. On the basis of the results shown here for the 0.1-g/m^2 DFMO dose, we think we may be near a minimum oral dose required to deplete polyamine contents in this tissue. This question will be more definitively addressed in our subsequent phase IIb trial. In that study, we are evaluating three DFMO doses down to 0.05 g/m^2 per day administered for 1 year. Over longer time periods, doses lower than 0.25 g/m^2 per day may well deplete polyamine contents in colorectal mucosal tissue. The new study also includes a placebo arm.

Another motivation for conducting a dose de-escalation trial of DFMO was that this drug has been reported to induce ototoxicity, a high-frequency hearing loss, which has caused termination of DFMO treatment in previous human clinical trials (*9*). One aim in this trial was to determine if a DFMO dose could be identified that suppressed colorectal tissue polyamine contents without producing this toxicity. We found that only one patient in our trial, using DFMO doses of less than 3 g/m^2 per day for 4 weeks, showed any evidence of clinical ototoxicity. The tinnitus in that patient, who received a dose of 0.75 g/m^2 DFMO per day, was thought to be secondary to aspirin use. No post-treatment audiometry study was conducted; clinical evaluation concluded that this patient had Meniere's syndrome. Thus, we conclude that DFMO doses as low as 0.25, and possibly 0.1, g/m^2 per day for 4 weeks are effective in suppressing colorectal mucosal polyamine contents without causing acute ototoxicity or other toxic effects that might lead to discontinuation of DFMO treatment.

DFMO had no statistically significant effect on labeling indices of colorectal mucosal tissue in this study. This result shows that the labeling index is not a useful marker for assessing DFMO effects in trials such as the one reported here. DFMO is known to be an antiproliferative agent in experimental cell and animal systems (*5*). However, this drug does not generally inhibit cell proliferation; rather, it reduces the *rate* of cell growth. It has not been established that DFMO inhibits carcinogenesis in experimental models by inhibiting cell proliferation. Slaga et al. (*16*) observed that DFMO acts late in the promotion phase to inhibit skin carcinogenesis. Thus, cell proliferation leading to benign polyp formation occurred in this model during DFMO treatment. DFMO did inhibit cancer formation in models of skin (*16*) and colon (*13*) carcinogenesis. Recently, we have found that DFMO-mediated polyamine depletion suppressed the expression of matrilysin in a human colon tumor-derived cell line (Wallon UM, Shassetz LR, Cress AE, et al: manuscript submitted for publication). This protein is a secreted metalloproteinase implicated in tumor cell invasion (*28*), including invasion of colon cancer cells (*29*). Thus, DFMO may be acting to inhibit the expression of specific genes involved in the progression of benign to malignant tumors, in addition to slowing the rate of growth of proliferating cells.

The ratios of spermidine to spermine in colorectal mucosa decreased with the age of the patient, as did the quantitative change (decrease) in this parameter. These observations have both basic and practical implications. Others (*30*) have shown that ornithine decarboxylase activity decreases in senescent human fibroblasts. Decreased ornithine decarboxylase activity, mediated by either DFMO or biological regulatory mechanisms, results in a decrease in putrescine and spermidine, without a significant change in spermine. Thus, our findings of a decrease in the ratios of spermidine to spermine can be interpreted to occur as a consequence of an expected decrease in ornithine decarboxylase activity occurring with increasing age.

The practical implication of these findings is that toxicity in normal colorectal mucosa from DFMO therapy should decrease with increasing age, since the target enzyme for this drug, ornithine decarboxylase, is repressed as a normal consequence of senescence. Thus, we are encouraged that DFMO may be an agent that can be administered for extended periods of time (i.e., years) in cancer chemoprevention settings. DFMO is currently being investigated as a cancer chemopreventive agent in humans (*31*,*32*). Love et al. (*32*) have reported that DFMO, at a dose of 0.5 g/m^2 per day, can be safely used to treat patients for up to 6 months in a chemoprevention setting. The low toxicity of DFMO, combined with its significant activity as a chemopreventive agent in a variety of models of epithelial carcinogenesis in experimental animals, suggests that DFMO may have broad application in the prevention of common human cancers, including those of the colon, breast, and prostate. Future clinical trials will determine if this is the case.

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Notes

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