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Development of Difluoromethylornithine as a Chemoprevention Agent for the Management of Colon Cancer

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Abstract Experimental studies have demonstrated that carcinogenesis is a multistep process in which inappropriate proliferation of cells is a critical determinant. Polyamines support sustained growth and are highly regulated in all cells. The rate limiting enzyme for this pathway is ornithine decarboxylase (ODC), an enzyme that exhibits rapid turnover, and converts the amino acid ornithine to putrescine, which in turn is converted to the longer chain amines spermidine and spermine. In animal models of colon carcinogenesis, inhibition of ODC by difluoromethylornithine (DFMO), an enzyme-activated irreversible inhibitor, reduces the number and size of colon adenomas and carcinomas. DFMO was first ineffective when used clinically to treat acute leukemia or melanoma and caused clinically significant but reversible ototoxicity. Subsequently, we performed a series of analyses demonstrating that hearing loss was rare below a total cumulative dose of 150 gm/m^2 and increased with total cumulative dose of DFMO. The hearing loss was reversible with rapid reversion to baseline hearing. We and others have conducted Phase IIa trials to determine the lowest dose at which DFMO can decrease colon mucosa polyamine content, and found that an oral dose as low as 0.25 gm/m^2 per day (perhaps lower) decreases colon tissue putrescine content and lowers the spermidine/spermine ratio. We are currently conducting a long-term randomized Phase IIb trial which serially measures the long-term effect of several low doses (and placebo) of DFMO on sustaining polyamine depletion in colon mucosa, as well as carefully monitoring hearing by audiometry and other sophisticated tests. The eventual goal of these studies is to conduct a randomized Phase III trial of DFMO in preventing polyps development (as a surrogate for colon cancer) in patients with prior polyps, and second cancers in patients with resected low-stage colon cancers.

Key words: Chemoprevention, colon cancer, difluoromethylornithine, polyamine, polyps

Polyamines, cations ubiquitously distributed throughout prokaryotic and eukaryotic organisms, affect a number of biochemical processes in cells [1]. Depletion of polyamines results in a slowing of cellular growth and inhibition of carcinogenesis in essentially all models studied [2]; profound depletion of polyamines causes cell death. The polyamine regulatory pathways are complex and highly regulated [3]. A simple representation is provided in Figure 1. Polyamine synthesis begins with formation of putrescine from the amino acid ornithine by decarboxylation via the enzyme ornithine decarboxylation (ODC). Difluormethylornithine (DFMO), an enzyme-activated irreversible inhibitor of ODC, was synthesized in the mid-1970s and found to inhibit both normal and abnormal cellular growth [4]. However, its usefulness as a treatment modality was not demonstrated [5], probably because ODC is rapidly turned over and

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prohibitive doses were required to inhibit cellular tumor growth in an intact organism. Additionally, hearing loss occurred rapidly in many patients at the required high doses of DFMO; although hearing loss was reversed upon drug discontinuation, DFMO was shelved as a treatment approach to cancer.

During the 1980s experimental observations indicated that DFMO inhibited cancer formation especially in epithelial models, including those of the colon [6]. Particularly important was that inhibition of adenoma and colon cancer formation occurred without any measurable change in the normal adjacent mucosa or lesion labeling indexes, suggesting that changes in the rate of proliferation were sufficient to block or delay carcinogenesis. Additionally, a growing body of evidence suggests that proliferation only needs to return to normal, not be completely blocked to inhibit carcinogenesis. This has important implications for DFMO, which may slow growth to normal at very low, non-toxic doses and/or inhibit stimulation of proliferation by various carcinogens. We will summarize the developmental work to bring DFMO into clinical trials [7–9].

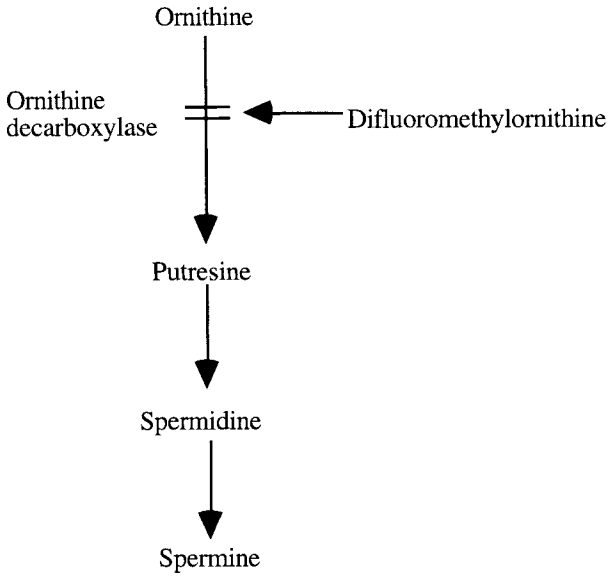

Fig. 1. Polyamine synthesis pathway.

CLINICAL LIMITATIONS

High doses of DFMO cause a number of side effects, including gastrointestinal upset, diarrhea, bloating, fatigue, arthralgias, insomnia, and a macular-papular rash [7,8,10]. It is unlikely that doses above 1.0-1.5 gm/m^2 po per day could be successfully used in long-term prevention trials.

Results from early treatment trials suggested that ototoxicity might also be a troublesome side effect in the clinical setting. Two types of toxicity were seen, including a vertigo-like syndrome (superficially resembling a Meniere-like syndrome) and hearing loss. Both side effects are reversible after drug discontinuation. Our current appreciation of the vertigo-like syndrome remains poorly understood; its characterization will be an important goal of ongoing longer term trials. In contrast, we have made substantial progress in understanding the nature of the hearing loss. We assessed the ototoxicity associated with DFMO in 58 patients with metastatic melanoma, analyzing the results of 179 sequential audiograms [10]. The key information from that study is shown in Figure 2. By regression analysis, cumulative DFMO dose showed a consistent and statistically significant positive relationship to hearing loss at multiple frequencies (500, 1,000, 2,000, 4,000, and 8,000 Hz). Patients with normal (threshold <30 db) baseline audiograms demonstrated more hearing loss than those with abnormal (threshold >= 30 db) baseline audiograms at the higher frequency levels. Of patients with normal prestudy hearing thresholds, 10% or less developed a demonstrable hearing deficit at cumulative DFMO doses below 150 g/m^2. Conversely, up to 75% of patients who received more than 250 g/m^2 developed a clinically demonstrable hearing loss. Other factors which worsened hearing loss included age, male gender, and the concomitant use of α2b-interferon. The effect on hearing was reversible after a few days to months, but recovery could not be completely assessed as many of the patients died of their illness or were quite ill. In our Phase IIa trial in patients with prior colon polyps, none of the 108 patients who received DFMO developed clinical hearing loss, but audiometry was not performed in this short-term (one month) study.

Love *et. al.* [7] has conducted a randomized Phase I chemoprevention dose-seeking study of DFMO using inhibition of ODC activity in human skin induced by 12-*O*-tetradecanoylphorbal-13-acetate as the indicator marker. Treatment-

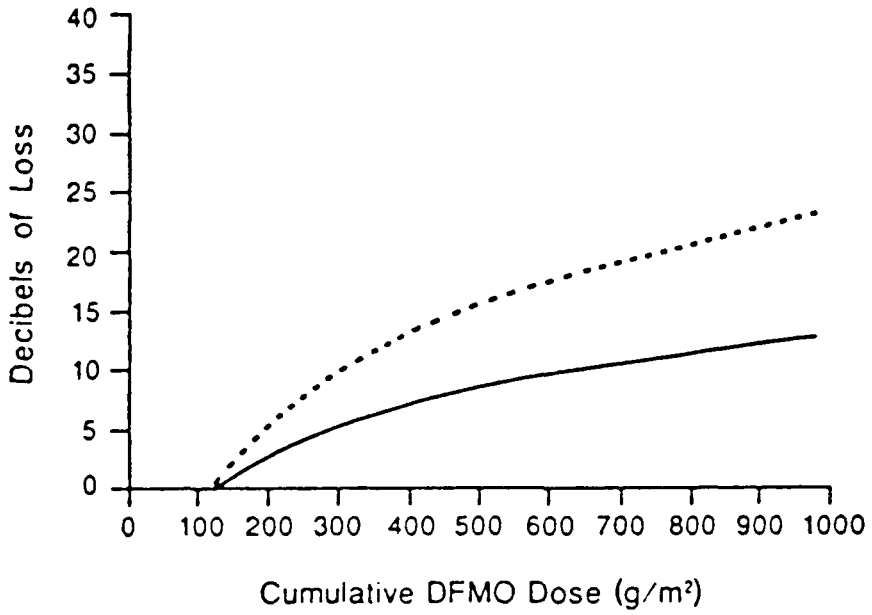

Fig. 2. Hearing loss associated with cumulative DFMO dose in males (dashed line) and females (solid line). Reproduced from Croghan *et al.*, 1991 with permission.

limiting audiotoxicity was observed at higher doses; however, seven patients treated with a dose of 0.5 gm/m^2/day DFMO had no audiotoxicity, even after six months of therapy, and suppression of ODC activity was maintained.

These results suggest that hearing loss may not develop at low doses of DFMO given over a long time period. Ongoing Phase IIb trials should help determine the frequency, intensity, and reversibility of the vertigo-like syndrome seen in some patients, as well as the lowest cumulative dose at which audiogram-detected hearing loss occurs, its reversibility after drug discontinuation, and whether the recovery is complete. Determining these measurements and features that affect these outcomes at the clinical level will obviously be important as well. In our current Phase IIb trial, patients receive serial completed pure tone audiometry and assessment of distortion product otacoustic emissions.

SURROGATE TISSUE

Performing serial biopsies of colon mucosa is obviously time consuming and uncomfortable, although not painful or dangerous. We therefore proposed to determine whether exfoliated buccal mucosal (EBM) cells could serve as a surrogate for polyamine effects in colon mucosal cells [12]. Buccal mucosal cells have been used to measure a number of biological and biochemical parameters, so using them as a relevant comparative tissue seemed reasonable. Our observations are summarized in Table I. We compared polyamine concentrations in rectal mucosal biopsies and in exfoliated buccal mucosal cells of five subjects before and after DFMO treatment. One month of 3 g/m^2/day of DFMO treatment caused a statistically significant decrease in putrescine and spermidine concentrations in rectal mucosa biopsy specimens but not in EBM samples. ODC activity in EBM was high (approximately 1 μM/min/mg protein), resistant to DFMO inhibition (K_i=4200 mM), dependent on GTP concentration (maximal at 0.1 mM), and had reduced enzyme activity concomitant with decreased oral bacterial concentration by antiseptic mouthwashing. Bacteria adherent to EBM were visible by electron microscopy; 40 bacterial colonies/ng protein were culturable from washed EBM samples. We conclude that use of EBM samples is inappropriate as a marker tissue of DFMO effect in the rectal mucosa. These results also raise the validity of EBM usage for any biochemical

TABLE I. Comparison of Properties of Polyamines in Bacteria, Eukaryotic Cells, and Exfoliated Buccal Mucosal Cells

	HPLC Profile	ODC Activity
Eukaryotic Cells	low putrescine no cadaverine spd/spm~	Low activity GTP independent Inhibited by DFMO
Exfoliated Buccal Cells	high putrescine high cadaverine spd/spm>>1	High activity GTP dependent not inhibited by DFMO
Bacteria	high putrescine high cadaverine spd/spm>>1	High activity GTP dependent not inhibited by DFMO

Adapted from [12].

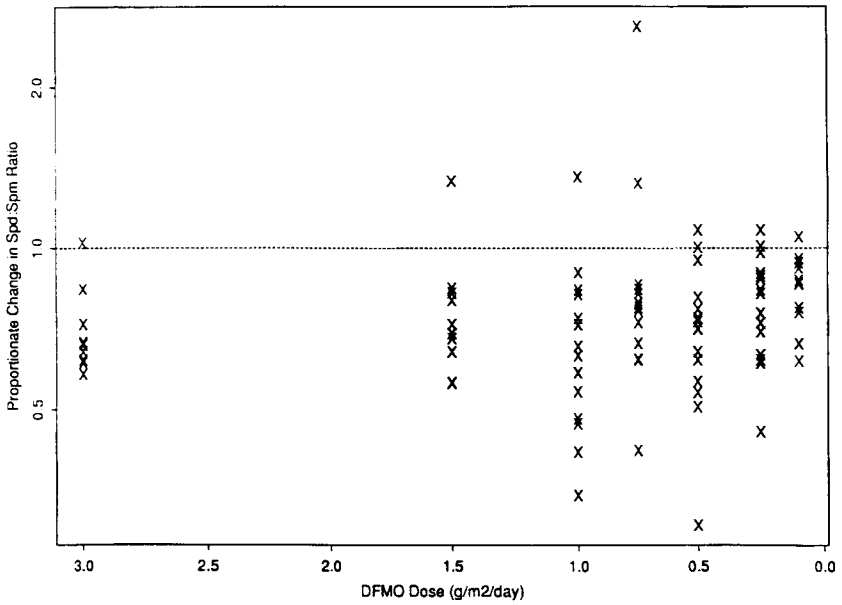

Fig. 3. Proportionate changes in ratios of spermidine to spermine as a function of DFMO dose. This figure shows the distribution of changes in ratio of spermidine to spermine at each DFMO dose administered. Reproduced for Meyskens *et al.*, 1994 with permission.

process that may be prominent in bacteria compared to mammalian cells.

DOSE DE-ESCALATION CHEMOPREVENTION TRIAL

The design and results of our Phase IIa chemoprevention trial are summarized below, and the key data relating the proportionate change in ratios of spermidine to spermine as a function of DFMO dose is reproduced here (Fig. 3). The specific aim of our study was to determine the lowest dose of DFMO that would deplete target tissue (colorectal mucosa) levels of polyamines while producing minimal toxic effects in humans who had undergone prior removal of colon pol-

TABLE II. Key Features of Phase IIb DFMO Chemoprevention Trial

Patients with prior resected polyp.
Randomized to placebo or one of three low doses of DFMO for one year.
Serial biopsies of rectal mucosa for polyamine content.
Serial audiometry.
Intensive side effects monitoring.

yps. A dose de-escalation chemoprevention trial of DFMO was conducted in 111 patients (36 female and 75 male) in generally good health, aged 39–79, who had undergone colonoscopy for surgical removal of an adenomatous colon polyp greater than 3 mm within five years prior to entering the study. Groups of patients (12–20 patients per group) were treated with single, daily oral doses of DFMO ranging from 3.0–0.1 g/m^2 for four weeks (28 days). Before 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 a.m. and noon to avoid possible effects of diurnal variations in laboratory endpoints. Samples for analysis of plasma DFMO levels were also collected during this time period on the day after the last day of drug administration. 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. These results and those of Love *et. al.* [7] suggest that a dose of 0.25–0.50 gm/m^2/day should deplete polyamines without producing clinical ototoxicity.

PHASE IIb CHEMOPREVENTION TRIAL

This ongoing one-year trial will determine whether polyamine depletion in colon mucosa can be sustained by a low dose of DFMO without hearing loss or other limiting side effects. Key elements of the study are summarized in Table II. Patients will be similar to those entered on the Phase IIa trial, randomized to receive one of four doses of DFMO (placebo, 0.075 gm/m^2/day, 0.20 gm/m^2/day, 0.40 gm/m^2/day) for one year. Pre-therapy, one month, six month, and post-therapy rectal mucosal biopsies will be obtained to measure polyamine content. Also, potential cochleotoxicity will be assessed at the same time-points, using pure-tone audiometry measurements and distortion product otacoustic emissions. If the trial demonstrates that polyamine content can be lowered without developing clinically significant hearing loss or a vertigo-like syndrome, a randomized Phase III trial will be conducted. Patients similar to those in the Phase IIb trial will have new polyp formation incidence as the endpoint; patients with surgically resected Dukes A or B_1, colon cancer will have second cancers and/or new polyps as the primary endpoint.

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