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BRIEF COMMUNICATIONS

α-Difluoromethylornithine and Polyamine Levels in the Human Prostate: Results of a Phase IIa Trial

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Prostate cancer is the most commonly diagnosed malignancy in men and the second leading cause of male cancer deaths in the United States *(1)*. As such, the prevention of prostate cancer is of national medical concern. One approach to prevention of prostate cancer is to suppress the polyamine levels in the prostate, an avenue suggested by studies indicating that ornithine decarboxylase (ODC), the first enzyme in the polyamine pathway, is overexpressed in human prostate cancer tissue *(2)* and is the target enzyme for α-difluoromethylornithine (DFMO). This inhibitor suppresses tissue contents of polyamines, which are required for optimal cell proliferation and differentiation.

Elevated levels of polyamine are associated with several malignant or premalignant lesions *(3–5)*. Prostate cancer seems a logical organ system for DFMO chemoprevention, since ODC activity and polyamine content are higher in prostatic tissue than in other mammalian tissues *(6)*. Also, investigators *(7–10)* have demonstrated marked polyamine suppression by DFMO in rodent prostates and prostate cell lines. Mohan et al. *(2)* measured ODC activity in benign and malignant tissues from the same patient and found the cancerous portion to have levels almost three times those of benign tissue. In addition, they evaluated the ODC activity of prostatic fluid obtained by massage and found that, in men with prostate cancer, levels were 50% higher than in men with benign hypertrophy. However, prior to proceeding with a DFMO prostate cancer chemoprevention trial, documentation of the effects of DFMO on human prostate *in vivo* was needed. Because that information was unpublished, this phase IIa trial was implemented. Subsequently, Messing et al. *(11)* published the results of a small placebo-controlled trial of DFMO on human prostate polyamine levels. Their study demonstrated reduced levels of putrescine only. Our study differs from the study by Messing et al. in that in our study the reduction of putrescine was greater, and there was also a statistically significant reduction in the levels of the polyamines spermidine and spermine.

The protocol for our study was approved by the investigational review board of the University of California, Irvine, and by the Long Beach Veterans Administration Medical Center, and subjects gave written informed consent. Men who were having a transrectal prostate needle biopsy underwent four additional core needle biopsies; the specimens obtained at these biopsies were frozen immediately. If the patient elected to undergo an invasive prostate procedure, he was asked to continue participation and to take oral DFMO at a dose of 0.5 g/m^2 once daily for 28 days before the second procedure. The dosage chosen was based on prior studies *(12,13),* in which patients with colon polyps were treated with a range of DFMO doses and polyamine contents in rectal mucosal biopsy specimens were assessed. This dose produced polyamine suppression without side effects *(12,13)*. Just before the surgical procedure, four transrectal core biopsy specimens were taken, frozen, and used for the polyamine analysis.

Polyamine analysis was performed with the use of standard reverse-phase, ion-paired high-performance liquid chromatography methods, described previously *(12–14)*. Polyamine levels are reported in nanomoles per milligram protein. The limit of detection of our method is 0.01 nmol/mg protein. Nondetectable levels correspond to less than 0.01 nmol/mg. For statistical analysis, 0.01 nmol/mg was imputed when polyamine levels were below the limit of detection. All *P* values were two-sided and were considered to be statistically significant at $P<.05$. We compared pre-DFMO and post-DFMO polyamine values using the Wilcoxon matched-pairs signed rank test. Prostate-specific antigen values and histologic descriptions of the prostate are provided in Table 1.

Fig. 1 compares the absolute values of putrescine, spermidine, spermine, and the spermidine/spermine ratio before and after DFMO treatment. Pretherapy putrescine was detectable before DFMO administration in six men and was nondetectable in three men. The average putrescine level was 0.42 nmol/mg (median, 0.22 nmol/mg; interquartile range, 0.01–0.78 nmol/mg). All men had undetectable levels of putrescine after DFMO treatment. For the six men with pre-DFMO putrescine levels of 0.01 nmol/mg or higher, the average percent decrease from baseline was at least 97.6% (median, 95.0%; $P = .031$). Spermidine was measurable in all specimens before DFMO administration. The average pretreatment level of spermidine was 1.21 nmol/mg (median, 0.81 nmol/mg; interquartile range, 0.51–1.47 nmol/mg). The average level of spermidine after therapy was 0.32 nmol/mg (median, 0.21 nmol/mg; interquartile range, 0.08–0.43 nmol/mg); in two specimens, the levels were undetectable. The average percent decrease from baseline was 73.6% (median, 69.0%; $P = .004$). The average spermine level before DFMO administration was 29.14 nmol/mg (median, 28.85 nmol/mg; interquartile range, 13.33–47.39 nmol/mg); after DFMO administration, it decreased in all specimens to an average level of 14.33 nmol/mg (median, 12.90 nmol/mg; interquartile range, 7.40–22.01 nmol/mg). The average percent decrease from baseline was 50.8% (median, 55.0%; $P = .004$). The spermidine/spermine ratio was calculated for each specimen. Eight of nine patients had a decrease in this ratio after DFMO was given. The average decrease from baseline was 50% (median, 52%; $P = .019$).

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See "Notes" following "References."

Table 1. Patient demographics*

	Patient No.								
	1	2	3	4	5	6	7	8	9
Routine PSA†	5.8	9.8	2.3	1.7	5.1	2.6	6.3	47.2‡	4.1
Pre-study PSA†	4.4	Not done	2.3	1.5	16.6§	11.1§	8.7	23.2	6.8§
Post-DFMO PSA†	3.7	7.5	2.5	1.4	5	2.3	6.8	35.5	8.2§
Initial pathology diagnosis‖,¶	Atypia	Gleason grade 5	Atypia, inflammation	Atypia	Atypia, inflammation	Gleason grade 6	Atypia, inflammation	Gleason grade 7	Gleason grade 6
Procedure#	Biopsy	RRP	Biopsy	Biopsy	TURP	RRP	Biopsy	RRP	RRP
Final pathology diagnosis‖,¶	Atypia	T3a, Gleason grade 6	Gleason grade 6	BPH	BPH, inflammation	T2b, Gleason grade 6	Inflammation	T4, Gleason grade 8	T2a, Gleason grade 6
Days of DFMO	28	28	35	30	30	28	25	29	21

*PSA = prostate-specific antigen; DFMO = α-difluoromethylornithine; RRP = radical retropubic prostatectomy; TURP = transurethral resection of prostate; BPH = benign prostatic hyperplasia.

†Three PSA values are given: 1) routine PSA—drawn prior to study participation as part of routine standard of care; 2) pre-study PSA—drawn the day of beginning the trial and starting DFMO; and 3) post-DFMO PSA—drawn the day of the second procedure after a month of DFMO.

‡PSA was drawn when patient presented to the emergency room with acute urinary retention. Such retention can elevate the PSA level.

§PSA was drawn within 6 weeks of biopsy; therefore, it may be falsely elevated.

‖Prostate cancer staging as per the TNM (tumor–node–metastasis) updated 1997 staging system *(18)* (American Joint Committee on Cancer 1997); histology as per Gleason Grading System *(19).* Atypia—findings are suspicious for cancer but the cytologic and/or architectural features are insufficient for a definitive diagnosis.

¶Initial pathology diagnosis = routine pathology report from the sextant prostate cores. Final pathology diagnosis = routine pathology report from the second prostate procedure, following DFMO.

#Procedures performed after participation in the trial included biopsy (repeat sextant biopsy for abnormal previous biopsy), RRP, and TURP.

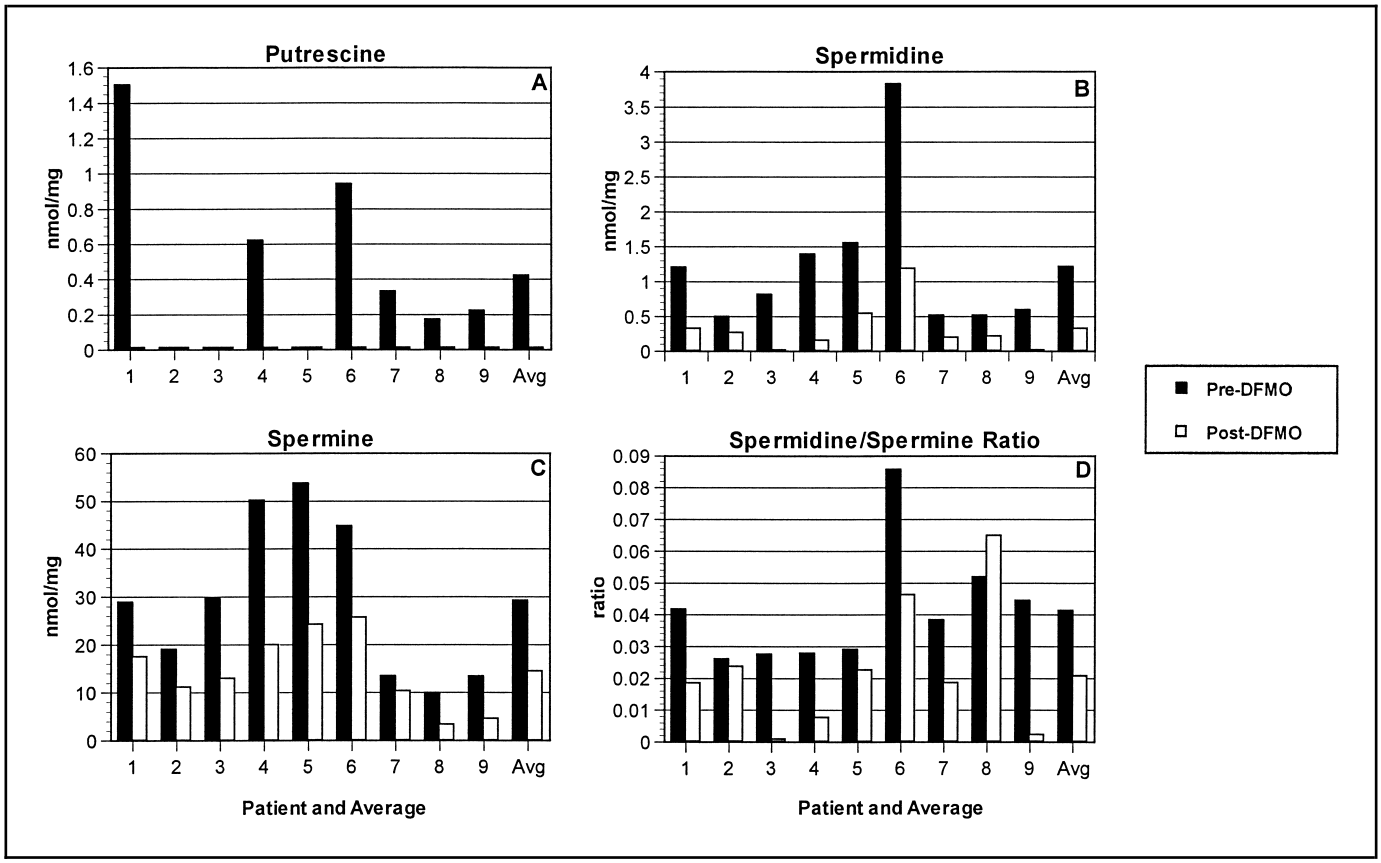

Fig. 1. Absolute values for polyamine content in prostatic tissue obtained by core biopsy before and after 28 days of α-difluoromethylornithine (DFMO) treatment. **A**) Putrescine. **B**) Spermidine. **C**) Spermine. **D**) Spermidine/spermine ratio.

In this short-term trial, we were able to demonstrate a statistically significant reduction in the levels of prostate polyamines after administration of oral DFMO at a dose of 0.5 g/m^2 daily for 28 days. In 1999, Messing et al. *(11)* published results of 2 weeks of oral administration of DFMO on human prostate polyamine levels. Men in that study were randomly assigned to receive either DFMO at a dose of 0.5 g/m^2 for 2 weeks (n = 15) or placebo (n = 10) prior to prostatec-

tomy. The mean putrescine levels were statistically lower in the DFMO-treated group (1.43 nmol/mg DNA versus 1.95 nmol/mg DNA; $P = .03$). There were no differences in ODC activity or in levels of spermidine or spermine measured in their study.

A brief review of the two trial designs points to study differences that can account for the discordant results between the two studies. Our study had the advantage that we elected to use each male as his own control for polyamine suppression by using samples from the same male before and after DFMO. Our data show a wide variation in polyamine levels among the subjects prior to manipulation. This variability makes it difficult to assess differences in a small control versus treatment group and may be the reason why only putrescine, with the smallest variability, was statistically significantly changed in the trial conducted by Messing et al. *(11).* A similar difficulty with the variability in polyamine levels was addressed by Mitchell et al. *(15),* who reported on polyamine levels in cervical cancer compared with levels in normal cervical tissue. These authors concluded that, because of the variability in the polyamine levels, large numbers of subjects would be needed to see a statistically significant result.

There are also processing issues related to the manner in which the tissues were managed between these two studies. We took cores prior to surgery, whereas Messing et al. *(11)* took cores after the prostate was removed. The impact of ischemia for 1–2 hours on the prostate is unknown as the prostate is systematically devascularized and removed. This confounder, therefore, was not an issue in our study. Another difference between the studies was the length of treatment. Our subjects received 4 weeks of DFMO, as opposed to 2 weeks in the study by Messing et al.

Administration of oral DFMO for 4 weeks reduces the levels of putrescine, spermidine, and spermine in a statistically significant manner in human prostate tissue. The relationship between overexpression of ODC, elevated levels of polyamines, and cancer risk has been explored [reviewed in *(16)*], and current chemoprevention trials with DFMO are ongoing in breast, cervix, colon, and skin *(17).* With the information from this trial, we plan to proceed with a prostate cancer chemoprevention trial with DFMO.

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NOTES

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