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Chemoprevention in Prostate Cancer: The Role of Difluoromethylornithine (DFMO)

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Abstract The polyamines are normal cell constituents considered to have an important role in the regulation of proliferation and differentiation. DFMO is an irreversible, enzyme-activated, suicide inhibitor of ornithine decarboxylase (ODC), the enzyme responsible for the first and rate-limiting step in mammalian polyamine synthesis. Preliminary data show that DFMO inhibits tumor cell growth *in vitro* and *in vivo*, and that it demonstrates chemopreventive activity in a variety of animal tumors. The prostate contains some of the highest concentrations of polyamines and of polyamine-synthetic enzymes (including ODC) in the mammalian organism. ODC activity in the prostate was shown to be more susceptible to DFMO inhibition than in other organs. We have found the ODC activity of the Dunning R3327 rat prostatic carcinomas to be as sensitive to inhibition by DFMO as the normal rat prostate. Furthermore, DFMO was inhibitory to the growth of the tumor both *in vitro* and *in vivo*. Given the slow growth rate and long latency period of human prostate cancer and the preliminary DFMO data, we suggest that clinical trials to evaluate the chemopreventive potential of DFMO in prostatic carcinoma deserve serious consideration.

Key words: chemoprevention, difluoromethylornithine (DFMO), polyamines, prostate carcinoma

Prostate cancer has become the most common malignancy in American males as well as the second leading cause of cancer mortality in this population. It was estimated that in 1991, 122,000 new cases of prostate cancer would be diagnosed and 33,000 patients would succumb to this disease [1]. Although effective therapy is available for localized prostate cancer (radical prostatectomy and radiation therapy), most men are diagnosed when the tumor is locally advanced or metastatic [2]. Since the early 1940s, hormonal therapy has been the mainstay of treatment for advanced prostate cancer [3]. Experience has taught us, however, that 20% to 30% of patients never respond to hormonal therapy, and over 95% of those who do respond fail within three years [4]. Traditional chemotherapy has produced a disappointing response rate of 20% to 30%, and the duration of these responses, generally, has been short (less than one year) [5].

Autopsy studies reveal the presence of histological prostate cancer in a staggering 30% of all males older than 50 years [6]. It has been estimated that ten million males in the U.S. harbor this form of disease [7]. The incidence of histological "autopsy" prostate cancer increases with each decade of life past 50 [8] and is similar in different populations around the world, a characteristic in sharp contradistinction to the marked variability in the incidence of clinical prostate cancer [9,10]. For example, in Japanese males the prevalence of histological prostate cancer is almost equal to that in U.S. males, but the age-adjusted incidence of clinical prostate cancer in the U.S. is 15- to 20-fold higher than in Japan [11]. In Japanese males who immigrate to the U.S., the incidence of clinical prostate cancer rises and becomes intermediate between the incidences in Japan and in the U.S. [11]. These epidemiological data suggest that the progression to clinical prostate cancer is a result of a complex interplay of genetic and environmental factors.

A considerable amount of experimental and clinical work suggests that carcinogenesis is a multistep process resulting from the consecutive accumulation of multiple genetic alterations [12,13]. Such changes recently have been documented for colon cancer [14]. Although the specific genetic alterations involved in prostate cancer have yet to be defined, Carter *et al.* [11]

in an elegant mathematical model have shown that a similar multistep process is compatible with the clinical observations in prostate cancer. Another distinguishing feature of clinical prostate cancer is its slow rate of progression. Several lines of evidence corroborate this statement. The first is the sporadic appearance of case reports in which 20 years or more were required for progression of the disease from stage A_1 to stage D_2. The second is the observation of the low mitotic index in well-differentiated tumors. This was substantiated by a low level of tritiated thymidine labeling (0.9%) as a measure of DNA replication [15]. The third and more recent source of evidence is the observation of the rise of serum prostate specific antigen (PSA) levels in untreated cases of prostate cancer [16]. It has been estimated that the doubling time of low-stage prostate cancer is in excess of 2 years [16]. Recognizing the fact that carcinogenesis is a multistep process, chemoprevention is a strategy aimed at reducing cancer deaths by intervention with compounds that can interfere with the various stages of cancer development. Although the ideal future chemopreventive agent may be antisense oligonucleotides targeted to activated oncogenes, many mechanistic questions related to cancer development must be explored before this approach becomes feasible. Presently, a number of pharmaceutical agents have shown promise in preclinical trials in animal tumor models as well as in limited clinical trials [17].

Prostate cancer, because of its long latency period, slow growth rate, slow doubling time and large number of histologic cancers poised for clinical progression, seems to lend itself well to a strategy of chemoprevention. This seems particularly true in the absence of effective chemotherapy for advanced disease, and because many patients currently are diagnosed with advanced disease.

What are the polyamines and what is their biological function? The aliphatic polyamines, putrescine, spermidine and spermine, are polycationic molecules present in all living organisms. They are synthesized by cells, and the pathways for their synthesis, degradation and interconversion are tightly regulated. Increased synthesis of polyamines is found universally to accompany cell proliferation and differentiation both in the developing embryo and in a variety of tumors. Although the exact role of these molecules has yet to be elucidated, it is clear that depletion of cellular polyamines results in the failure of cells to complete the S-phase of the cell cycle [18]. The polyamine biosynthetic pathways have been characterized. Since this topic is well covered in recent reviews [19,20], it will not be elaborated upon here. Suffice it to say that the enzyme ornithine decarboxylase (ODC) is responsible for the first and rate-limiting step in mammalian polyamine synthesis, namely, the conversion of ornithine to putrescine [21]. It is well established that an increase in ODC activity constitutes an early and essential event when cells are stimulated to proliferate [19].

Inhibition of ODC has been the target of intense investigation and has resulted in the synthesis of several such drugs. Perhaps the best known and best studied is DFMO [22]. Mechanistically, DFMO is a "suicide substrate"; it binds to ODC as a substrate and, once transformed by the enzyme, irreversibly inactivates it [22].

The prostate contains some of the highest concentrations of polyamines and of polyamine synthetic enzymes (including ODC) in the mammalian organism [23]. Although there are interspecies differences in prostatic polyamine content, the human and rat prostates are similar in their high polyamine levels [24]. In this context, it is interesting to note that the prostatic ODC activity in rats was shown to be more susceptible to *in vivo* inhibition by DFMO than the ODC activity in other organs [25]. In the same study, the amount of DFMO in the prostate did not differ from that in other tissues. In a separate study, administration of DFMO to adult rats caused a more than 50% reduction in prostate weight, while the weights of other organs were only slightly decreased [26]. Similar effects were observed in immature rats, and DFMO administration caused a marked reduction in the age-dependent increase in prostate weight as well as in its RNA and DNA content [26].

We studied the ODC activity of the various Dunning rat prostatic tumor variants and observed a distinct correlation between the growth rate of these tumors and their respective ODC activities [27]. Specifically, the ODC activity of the slow-growing Dunning R3327 H

Table I. The Effect of *in Vivo* Administered α-Difluoromethylornithine (DFMO) on ODC Activity of the R3327 Prostate-Derived Tumors

Tissue	ODC Activity[a]	% Inhibition With DFMO[b]	Tumor Doubling Time (days)
R3327 AT[c]	4,920 ± 510	91 ± 6	2
R3327 MAT-Lu	4,610 ± 470	92 ± 6	2
R3327 HIF	480 ± 60	82 ± 4	5
R3327	240 ± 20	86 ± 5	20
Dorsal Prostate	110 ± 15	88 ± 6	–
Ventral Prostate	1,400 ± 130	92 ± 5	–
Thymus	47 ± 10	10 ± 4	–

[a] Represents mean picomoles $^{14}CO_2$ released from ^{14}C-*l*-ornithine per hour per mg protein at room temperature in untreated controls. There are ten tumors per group. Mean ± SE.

[b] Three hours prior to sacrifice the animals were injected IP with DFMO (100 mg/kg) and the mean percent inhibition activity was determined by comparison with the ODC activity of PBS (1.0 ml/kg)-treated animals. There were ten individual tumors or tissues per group.

[c] The representative tumors are the following: R3327, originally reported by Dunning as slow-growing, androgen-responsive prostatic adenocarcinoma; R3327 HIF, androgen-independent, fast-growing adenocarcinoma derivative of the R3327; R3327 AT, anaplastic, fast-growing nonmetastatic autonomous R3327 derivative; R3327 MAT-Lu, metastatic derivative of the R3327 AT tumor that metastasizes to the lungs.

Table II. Effect of α-Difluoromethylornithine (DFMO) on the *in Vivo* Growth of the R3327, MAT-Lu Tumor

R3327 MAT-Lu Tumor[a]	PBS	DFMO
Tumor Wet Weight	1.39 ± 0.13 gm[b]	0.79 ± 0.18[c]
Total DNA Content	3.72 ± 0.42 mg	1.72 ± 0.44[c]
Total RNA Content	4.56 ± 0.35 mg	2.38 ± 0.46[c]

[a] One million MAT-Lu monodispersed cells were inoculated into both flanks of five control (PBS) or five treatment (DFMO) group animals. PBS (1 ml/kg) or DFMO (250 mg/kg) was injected IP at eight-hour intervals for the 18-day duration of the experiment. Three hours following the last injection, tumors were surgically removed, weighed and assayed for DNA and RNA content.

[b] Mean of ten tumors ± SE.

[c] Statistically different from control ($p < 0.05$) by Student's t-test.

was higher than that of the dorsal prostate which is considered its tissue of origin. As the tumor progressed towards a more anaplastic, rapidly growing phenotype, the ODC activity rose correspondingly. The most aggressive tumors (the R3327 AT and the R3327 MAT-Lu) had an ODC activity which was almost 20 times that of the well-differentiated, slow-growing R3327 H (see Table I).

In addition, DFMO was observed to cause a profound inhibition of the R3327 MAT-Lu growth *in vitro* at a concentration of 1 mM as judged by a clonogenic assay [27]. The effect of DFMO on the *in vivo* growth of the same tumor

was more modest, presumably because of a compensatory enhanced tumor uptake of serum polyamines (see Table II). Herr *et al.* [28], working with another variant of the Dunning tumor R3327 G, reported that DFMO acted synergistically with methylglyoxal-bis-guanylhydrazone (MGBG) to suppress tumor growth *in vivo*.

Because elevated ODC activity is associated with and antedates human colon tumors [29] and because it is commonly observed in carcinogen-induced animal tumors [30], there has been a growing interest in the use of DFMO for chemoprevention [17]. DFMO has demonstrated chemopreventive activity in rat mammary glands [31], in mouse skin tumor promotion [32], in the rat intestine [33] and in the mouse colon [34]. Like retinoids, it has been reported to induce differentiation in certain tumor cell lines, which may, in part, explain its usefulness as a chemoprevention agent [35,36]. It is well absorbed when administered orally and has relatively low toxicity. DFMO has been tried in a variety of human tumors including small cell lung cancer [37], colon cancer [37], metastatic melanoma [38] and acute leukemia [39]. It has also been used in combination therapy in brain tumors [40]. In phase I trials in humans, the maximum tolerated dose was 9 g/m^2/day. The dose-limiting toxicity was thrombocytopenia. Other side effects included diarrhea, hearing loss and anemia [37]. However, in an interesting animal study by Loprinzi and Verma [41], it was suggested that doses of DFMO equivalent to 100–150 mg/m^2/day are appropriate for chemoprevention trials. Furthermore, in the same study, a single daily dose was found to be adequate for ODC suppression in specific target organs. Other investigators have reported that inhibition of tumor formation can be brought about by lower concentrations of the inhibitor than are required to treat established neoplasms [42]. In an intriguing study, Luk *et al.* [43] have demonstrated that a cyclic regimen of DFMO administration successfully suppressed the growth of human small cell lung carcinoma implants in nude mice while reducing treatment toxicity.

Many questions concerning polyamine metabolism and the role of intervention with DFMO in prostate cancer remain unanswered. Is ODC activity in human prostate cancer elevated just as it is in the Dunning tumors? Does ODC elevation precede progression in early prostate cancer? Can this activity be effectively suppressed by oral DFMO and at what dose? If achieved, will this suppression translate into a lasting effect on tumor progression? In any case, the preliminary data suggest that DFMO deserves to be studied further for its chemopreventive potential in prostate cancer at least as intensely as in lung, colon or breast cancers.

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