Journal of Cellular Biochemistry, Supplement 16I:153–155 (1992)

A Prospective Clinical Trial of Difluoromethylornithine (DFMO) in Patients With Resected Superficial Bladder Cancer

Charles L. Loprinzi, MD[1] and Edward M. Messing, MD[2]

[1] Mayo Clinic, Rochester, Minnesota 55905
[2] Department of Surgery and Human Oncology, University of Wisconsin School of Medicine, Madison, Wisconsin 53792

Abstract Difluoromethylornithine (DFMO) is a promising chemopreventive agent which is excreted unchanged in the urine, is active *in vivo* against superficial bladder cancer in animal tumor model systems, and has cytotoxic activity *in vitro* against superficial bladder cancer cells. Thus, DFMO may be particularly efficacious in preventing the development of bladder tumors and/or for the therapy of established superficial bladder cancer. To examine this hypothesis, an intergroup clinical trial is currently accruing patients with cystoscopically resected superficial bladder cancer (who would otherwise simply be observed). While the primary goal of this protocol is to define a daily dose of DFMO having little or no toxicity for use in future randomized chemoprevention trials, the rate of recurrent bladder tumors will also be followed in the hope that DFMO will inhibit the development of recurrent bladder cancers.

Key words: bladder carcinoma, chemoprevention, DFMO

Over 50,000 new cases of bladder cancer will occur this year in the United States [1], almost all of which will be transitional cell carcinomas (TCCs) [2]. Approximately two-thirds of these will be superficial or superficially invasive (T_a, T_1, TIS) neoplasms. The vast majority of these will be papillary tumors of low histologic grade unlikely to progress to invasive or metastatic disease [3,4]. While individuals with aggressive superficial tumors (*e.g.*, those with flat [3], or high grade tumors [4,5] and/or carcinoma *in situ* [5,6], or recurrent low-grade papillary TCCs [5,6]) may benefit from intravesical therapy, such treatment is not usually offered to patients after their initial bladder tumor episode when only moderately or well differentiated papillary superficial tumors are encountered [4,5,7]. However, first time patients with low-grade, papillary tumors have a 50–70% chance of eventual tumor recurrence, usually within 12 months of the initial resection [6,7]. These individuals, therefore, comprise an ideal group in which to study preventive (*i.e.*, prophylactic) therapy.

Difluoromethylornithine (DFMO) is an irreversible inhibitor of the enzyme ornithine decarboxylase (ODC), which appears to play an important role in the process of tumor promotion [8,9]. DFMO inhibits carcinogenesis in animal tumor systems, such as 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced mouse skin tumors [10], aflatoxin-induced rat hepatomas [11], azoxymethane-induced rat intestinal tumors [12,13], and nitrosamine- or dimethylhydrazine-induced rat mammary tumors [14,15]. In addition, DFMO inhibits nitrosamine-induced rat bladder tumors [16,17] as well as *N*-methyl-*N*-nitrosourea (MNU)-induced rat bladder tumors [18]. NCI investigators have identified DFMO as a possible chemopreventive agent for human bladder cancer [19].

While normal and malignant human uroepithelial cells *in vitro* have similar baseline ODC activities, this activity is preferentially induced in TCC by epidermal growth factor and/or autologously produced growth stimulating and transforming substances [20]. A favorable therapeutic index may exist since normal urothelial cells require a 5- to 20-fold higher concentration of DFMO than TCC cells to achieve a similar degree of growth inhibition. In addition, the

TABLE I. Inhibition of Human Skin ODC Activity With Oral DFMO*

DFMO Dose ($gm/m^2/day$)	ODC During DFMO Treatment (Fraction of Pre-R Baseline)	95% Confidence Intervals	p
3.0	0.16	(0.07, 0.35)	<0.0001
1.0	0.40	(0.22, 0.72)	0.004
0.5	0.57	(0.29, 1.1)	0.10
0.25	0.70	(0.31, 1.6)	0.4

* TPA-induced human skin ODC activity was measured before (baseline) and after patients started oral DFMO. Post-treatment skin ODC values were averaged (geometric mean) and compared to baseline values [25].

majority of ingested DFMO is excreted unchanged in the urine [21] allowing it to bathe uroepithelial cells for prolonged periods of time. Thus, both biological and clinical properties of superficial TCC may render it especially susceptible to the effects of ODC blockade as well as provide a starting point for initial preventative efforts.

Oral DFMO has been studied in humans. In a phase I trial, the recommended maximum tolerated dose was 9 $gm/m^2/day$ (2.25 gm/m^2 every 6 hours). Dose-limiting toxicity has usually been thrombocytopenia (almost exclusively in patients who had received previous chemotherapy) and ototoxicity [22,23]. Ototoxicity appears to correlate with the total cumulative DFMO dose when relatively high daily DFMO doses are used [23]. Although definitive data are unavailable to demonstrate whether low, daily, oral doses of DFMO (*i.e.*, 0.125–1.0 gm/day) would eventually result in ototoxicity, early information suggests that lower doses (0.5 $gm/m^2/day$) can be administered for 10 months without significant side effects [24].

DFMO doses ranging from 0.25–3.0 $gm/m^2/day$ have been administered to 13 patients for 4–18 weeks without significant toxicity [25] and 0.5 gm $DFMO/m^2/day$ has been tolerated for 6–12 months [24]. In addition, a patient with metastatic melanoma received a DFMO dose of 6 $gm/m^2/day$ (in 3 divided doses) for 5 months without any permanent sequela [26]. It does not appear that the chronic administration of relatively low DFMO doses will result in any serious, irreversible toxicity.

Fortunately, DFMO doses much less than the maximum tolerated human dose have biological activity in humans [24,25]. Doses of 1–3 $gm/m^2/day$ significantly inhibit tumor promoter-induced ODC activity in human skin [25]. Table I illustrates the change in baseline skin ODC activity following various doses of DFMO.

Patients with completely resected, low-grade, superficial, or superficially invasive tumors represent a good population for testing the toxicity of prolonged low-dose DFMO since (1) standard management of these patients is often limited to careful observation after complete endoscopic resection, (2) approximately 50% of patients will develop recurrent disease, (3) patients are normally followed closely with frequent cystoscopic and cytologic examinations, (4) *in vitro* data demonstrate that bladder cancer cells are inhibited much more by DFMO than are normal bladder epithelial cells, and (5) there is no known long-term toxicity currently expected with DFMO.

In order to further evaluate DFMO in humans, a national intergroup trial (INT 89-0001) has been activated which involves patients with completely resected superficial bladder cancer for whom other treatment modalities (*e.g.*, cystectomy or intravesical therapy) are not recommended. Eligible patients are randomly assigned to receive relatively low daily DFMO doses (125 mg, 250 mg, 500 mg or 1000 mg) for one year. While the primary goal of this study is to determine the tolerability of long-term, low-dose, oral DFMO in humans, the rate of bladder cancer recurrence will also be followed.

If chronic, low-dose DFMO proves to be without significant toxicity, then placebo-controlled, randomized clinical trials with individuals at high risk for the development of a malignancy can be designed to definitively test whether or not DFMO has the same chemopreventive capabilities in humans as it does in many animal tumor model systems [10,18].

REFERENCES

1. Boring CC, Squires TS, Tong T: Cancer Statistics 1992. CA 42:19–38, 1992.
2. Koss L: Tumors of the urinary bladder. AFIP Fascile, Ser 2, No 11, 1975.
3. Cutler SJ, Heney NM, Friedell GH: Longitudinal study of patients with bladder cancer: Factors associated with disease recurrence and progression. In Bonney WW, Prout GR (eds): "Bladder Cancer," AUA Monographs, Vol 1. Baltimore, MD: Williams and Wilkins, 1982, pp 35–45.
4. Messing EM: Early stage bladder cancer. Wis Med J 86:14–17, 1987.
5. Messing EM, DeKernion JB: Chemotherapy of bladder cancer. In Zingg EJ, Wallace DMA (eds): "Bladder Cancer." Berlin: Springer-Verlag, 1985, pp 235–262.
6. Herr WH, Laudone VP, Whitmore WF Jr: Overview of intravesical therapy for superficial bladder tumors. J Urol 138:1363–1368, 1987.
7. Cox CE, Cass AS, Boyce WH: Bladder cancer: A 26 year review. J Urol 101:550–558, 1969.
8. Boutwell RK: Some biological aspects of skin carcinogenesis. Prog Exp Tumor Res 4:207–250, 1964.
9. Bryan GT: Chemical carcinogenesis in human subjects. In Kahn SD, Love RR, Sherman C Jr, Chakravorty R (eds): "Concepts in Cancer Medicine." New York, NY: Grune & Stratton, 1983, pp 45–66.
10. Takigawa M, Verma AK, Simsiman RC, Boutwell RK: Inhibition of mouse skin tumor promotion and of promoter-stimulated epidermal polyamine biosynthesis by alpha-difluoromethylornithine. Cancer Res 43:3732–3738, 1983.
11. Sondergaard D, Taylor F, Newberne PM: Effects of the irreversible ornithine decarboxylase inhibitor, alpha-difluoromethylornithine, aflatoxin B_1, and choline deficiency on hepatocarcinogenesis. Toxicol Pathol 13:36–49, 1985.
12. Nigro ND, Bull AW, Boyd ME: Importance of the duration of inhibition of intestinal carcinogenesis by difluoromethylornithine in rats. Cancer 35:153–158, 1987.
13. Nigro ND, Bull AW, Boyd ME: Inhibition of intestinal carcinogenesis in rats: Effect of difluoromethylornithine with piroxicam or fish oil. J Natl Cancer Inst 77:1309–1313, 1986.
14. Thompson HJ, Ronan AM: Effect of D,L-2-difluoromethylornithine and endocrine manipulation on the induction of mammary carcinogenesis by 1-methyl-1-nitrosourea. Carcinogenesis 7:2003–2006, 1986.
15. Thompson HJ, Herbst EJ, Meeker LD, Minocha R, Ronan AM, Fite R: Effect of D,L-α-difluoromethylornithine on murine mammary carcinogenesis. Carcinogenesis 5:1649–1651, 1984.
16. Matsushima M, Yagishita T, Ando K, Taguchi H, Bryan GT: Inhibition by sulfur-containing compounds of rodent vesical carcinogen-induced urinary ornithine decarboxylase (ODC) activity and bladder carcinogenesis. XIII International Cancer Congress 606, 1982.
17. Nowels K, Homma Y, Seidenfeld J, Oyasu R: Prevention of inhibitory effects of alpha-difluoromethylornithine on rat urinary carcinogenesis by exogenous putrescine. Cancer Biochem Biophys 8:257–263, 1986.
18. Homma Y, Ozono S, Numata I, Seidenfeld J, Oyasu R: Inhibition of carcinogenesis by alpha-difluoromethylornithine in heterotopically transplanted rat urinary bladders. Cancer Res 45:648–652, 1985.
19. Malone WF, Kelloff GJ, Pierson H, Greenwald P: Chemoprevention of bladder cancer. Cancer 60: 650–657, 1987.
20. Messing EM, Hanson P, Reznikoff CA: Normal and malignant human urothelium: *In vitro* response to blockade of polyamine synthesis and interconversion. Cancer Res 48:357–361, 1988.
21. Grove J, Fozard JR, Mamont PS: Assay of difluoromethylornithine in body fluids and tissues by automatic amino acid analysis. J Chromatogr 223:409–416, 1981.
22. Abeloff MD, Slavik M, Luk CD, Griffin CA, Hermann J, Blanc O, Sjoerdsma A, Baylin SB: Phase I trial and pharmacokinetic studies of alpha-difluoromethylornithine—an inhibitor of polyamine biosynthesis. J Clin Oncol 2:124–130, 1984.
23. Croghan M, Booth A, Aicken M, Meyskens F: Alpha-difluoromethylornithine as a chemoprevention agent: Dose related ototoxicity. Annual Meeting American Society of Preventive Oncology, 1989.
24. Love R, Carbone P, Verma A, Tutsh K, Carey P, Pomplan M, Wilding G, Gilmore D: Phase I study of difluoromethylornithine (DFMO): A chemopreventive agent. Proc Am Assoc Cancer Res 33:207, 1992.
25. Loprinzi CL, Love RR, Therneau TM, Verma AK: Inhibition of human skin ornithine decarboxylase activity by oral alpha-difluoromethylornithine. Cancer Therapy and Control 1:75–80, 1989.
26. Meyskens FL, Kingsley EM, Glattke T, Loescher L, Booth A, Dept. of Internal Medicine, Speech and Hearing Science, Cancer Center Division, University of Arizona, Tucson, AZ: A phase II study of alpha-difluoromethylornithine (DFMO) for the treatment of metastatic melanoma. Invest New Drugs 4:257–262, 1986.