Difluoromethylornithine Enhancement of Putrescine Uptake into the Prostate: Concise Communication

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We studied the effect of alpha-difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase, on putrescine uptake by the rat prostate. Using C-14 putrescine, we found a several-fold increase in uptake by both the dorsal and ventral prostates of DFMO-pretreated intact animals, compared with untreated controls. When previously castrated animals were treated with a combination of testosterone and DFMO, the prostatic uptake of exogenously administered C-14 putrescine increased more than tenfold. Under these conditions, DFMO enhanced the uptake by the prostate to a greater extent than by any other tissue studied.

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The aliphatic polyamines putrescine, spermidine, and spermine are normal, eukaryotic cell constituents (*1*). They are considered to have an important role in cell proliferation and differentiation (*1,2*). Polyamine levels, especially putrescine and spermidine, increase dramatically in rapidly growing cells in both normal and neoplastic tissues (*3–5*). The natural polyamines are found in high concentrations in the rat and human prostate (*3,6*). They are considered important for prostate-cell proliferation and for prostatic secretory function (*7,8*).

Nucleated eukaryotic cells have the ability to synthesize polyamines. Putrescine, the precursor of spermidine and spermine, is formed by direct decarboxylation of L-ornithine. The enzyme responsible for this reaction is ornithine decarboxylase (ODC). This constitutes the rate-limiting step in polyamine synthesis (*9*).

Mammalian cells in culture take up polyamines added to the culture medium by an active transport mechanism (*10,11*). Radiolabeled polyamines injected into intact rats were found to accumulate in the prostate to a greater extent than in many other tissues (*12,13*).

In 1977, Metcalf et al. (*14*) first synthesized difluoromethylornithine (DFMO)—an enzyme-activated irreversible inhibitor of ODC. By inactivating the ODC enzyme, DFMO blocked the rate-limiting step in polyamine biosynthesis. It was very effective in reducing the intracellular levels of polyamines, and this has been extensively documented (*7,15*).

More recently, Alhonen-Hongisto et al. reported that Ehrlich ascites carcinoma cells exposed in vitro to DFMO subsequently displayed a markedly enhanced uptake of exogenously added polyamines (*16*).

We were interested to see whether DFMO would have a similar effect on the prostatic uptake of polyamines in vivo. We examined the uptake of radiolabeled putrescine by the rat prostate following DFMO pretreatment alone, or in combination with androgen stimulation.

MATERIALS AND METHODS

Animals. Intact, mature, male Sprague-Dawley rats were used for the initial experiment. Mature, male Wistar-Furth rats were used for the parallel experiment, in which animals were castrated via the scrotal route, under ether anesthesia, seven days before androgen and DFMO administration. All the animals had access to a standard diet ad libitum and were kept on a schedule of 12 hr light and 12 hr darkness.

Chemicals. Alpha-difluoromethylornithine (DFMO), was generously provided.* The following reagents were procured commercially: dihydrotestosterone propionate†, sesame oil, scintillation cocktail‡, a protein reagent kit¶, L-[1-^{14}C]ornithine (59 mCi/mmole), [^{14}C]putrescine

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(116 mCi/mmole) NCS tissue solubilizer, and all other nonradioactive chemicals.

Tissue polyamine uptake determinations. Following various drug regimens, the rats were injected intravenously (dorsal vein of the penis) with 1.29 μCi C-14 putrescine per 100 g body weight. Three hours after this injection, blood was obtained by cardiac puncture, under light ether anesthesia. The rats were then killed by further ether inhalation. Tissues were removed, freed of fat and connective tissue, and weighed wet on a Mettler balance to the nearest mg. A 200–300-mg aliquot of tissue was taken, placed in a scintillation vial, and digested overnight with 1.5 ml of NCS tissue solubilizer at 50°C. The following day, 10 ml of scintillation cocktail were added to the digested samples, which were then counted in a liquid scintillation counter. Counts were corrected for quenching by the channels ratio technique (*17*).

Ornithine decarboxylase assay. To determine ODC, a 25% W/V tissue homogenate was prepared with the aid of a tissue homogenizer in a buffered solution (pH 7.0) of 25 m*M* phosphate, 5 m*M* dithiothreitol, 0.3 m*M* EDTA. The homogenate was centrifuged at 50,000 *g* for 2 hr. The supernatant was then decanted and assayed for ODC by the CO_2 trapping procedure using Beaven's modification (*18*) of the Kobayashi procedure (*19*). Protein was determined by the method of Bradford (*20*).

Experimental design. *A. Effect of DFMO on C-14 putrescine uptake by intact rats.* Thirty-two intact, mature, male Sprague-Dawley rats were included in this experiment. The animals were divided into the following four groups (eight animals per group), each receiving:

Group 1: sesame oil—0.2 ml subcutaneously + tap water.

Group 2: dihydrotestosterone propionate (DHTP) in sesame oil—2.0 mg/0.2 ml s.c. + tap water.

Group 3: sesame oil—0.2 ml s.c. + 2% DFMO drinking water.

Group 4: DHTP in sesame oil 2.0 mg/0.2 ml s.c. + 2% DFMO drinking water.

The injections were administered daily and were scheduled for the same time each day. The daily DFMO intake per animal was in the range 1.5–2.0 g/kg body weight (DFMO at this concentration did not reduce water consumption by the animals). Seventy-two hours after the initiation of treatment, all the animals were injected with 1.29 μCi C-14 putrescine per 100 g body weight. Three hours later, blood was obtained by cardiac puncture, and the rats were killed and assayed for putrescine uptake as described above.

B. Effect of DFMO on C-14 putrescine uptake by previously castrated rats. Thirty-four mature, male Wistar-Furth rats were castrated via the scrotal route under ether anesthesia. Seven days after castration, they were assigned to the same four treatment regimens as above. Groups 1 and 3 had six animals each. Groups 2 and 4 had 11 animals each. Seventy-two hours after the initiation of treatment, the animals were injected with C-14 putrescine, bled, then killed and their tissues assayed as described above.

Statistics. Statistical significance was determined by the two-tailed Student's t-test.

RESULTS

Oral administration of DFMO resulted in about a 90% inhibition of prostatic ODC activity (Table 1). The same degree of ODC inhibition was observed in the prostates of androgen-stimulated castrate animals and in those of intact animals, when compared with their respective controls.

In castrated animals, treatment with androgens increased the mean wet weight of the ventral and dorsolateral prostate by 91 and 94% respectively (Table 2). Concomitant DFMO administration significantly ($p < 0.05$) suppressed the androgen-induced weight gain in the ventral and dorsolateral prostate (Group 4 compared with Group 2). In intact animals, the magnitude of prostatic weight changes was considerably less. The changes in the dorsolateral prostate did not achieve statistical significance. In the ventral prostate, however, DFMO significantly suppressed the mild weight gain

TABLE 1. ORNITHINE DECARBOXYLASE ACTIVITY IN THE VENTRAL PROSTATE

	Intact Sprague-Dawley rats		Castrated Wistar-Furth rats	
Group	$^{14}CO_2$ released per mg protein per hr* (pmole)	% inhibition by DFMO	$^{14}CO_2$ released per mg protein per hr.* (pmole)	% inhibition by DFMO
1 CONTROL	520 ± 77	—	—	
2 DHTP	661 ± 59	—	1685 ± 59	—
3 DFMO	59 ± 6	88.7	—	
4 DHTP + DFMO	61 ± 10	90.8	174 ± 17	89.4

* Figures represent mean ± standard error of the mean.

TABLE 2. PROSTATE AND BODY WEIGHTS IN GRAMS*

	Intact Sprague-Dawley rats			Castrated Wistar-Furth rats		
Group	Body wt.	Ventral prostate	Dorsolateral prostate	Body wt.	Ventral prostate	Dorsolateral prostate
1 CONTROL	411 ± 12	0.695 ± 0.091	0.233 ± 0.011	410 ± 12	0.162 ± 0.006	0.130 ± 0.012
2 DHTP	441 ± 18	0.812 ± 0.033	0.278 ± 0.015	444 ± 18	0.310 ± 0.009	0.252 ± 0.014
3 DFMO	440 ± 7	0.624 ± 0.030	0.242 ± 0.013	374 ± 30	0.170 ± 0.008	0.128 ± 0.010
4 DHTP + DFMO	414 ± 16	0.659 ± 0.056	0.256 ± 0.017	414 ± 10	0.260 ± 0.008	0.216 ± 0.011

* Figures represent—mean ± standard error of the mean
Significantly ($p < 0.05$) different from respective control.

induced by androgen stimulation (Group 4 compared with Group 2; $p < 0.05$).

In intact animals, DFMO + DHTP pretreatment increased uptake of C-14 putrescine 5.4-fold in the ventral prostate and 4.4-fold in the dorsolateral (Fig. 1). The same drug combination in previously castrated animals resulted in a 10.7-fold mean increase in C-14 putrescine uptake in the ventral prostate and 5.8-fold in the dorsolateral (Fig. 2). DFMO pretreatment also enhanced the putrescine uptake into other organs examined (kidney, lung, spleen, liver), but not to the same extent as into the prostate (Figs. 1 & 2). The plasma levels of C-14 putrescine were in the range 0.001–0.0013% of the injected dose, and there were no statistically significant differences among the various groups.

When the uptake of C-14 putrescine was expressed as a ratio of organ to abdominal wall muscle (Fig. 3), this ratio was 30:1 for the ventral prostate and 25:1 for the dorsolateral prostate in animals castrated and pretreated with DFMO + DHTP. In noncastrated animals, the same treatment regimen resulted in 13:1 and 12:1 ratios for the ventral and dorsolateral prostate respectively.

DISCUSSION

The animals in our experiments were pretreated with DFMO for 3 days, since such a treatment period had been reported to produce profound polyamine depletion in tumor cells in vitro (*16,21*) and in prostatic tissue in vivo (*22,23*). The animals were then killed and the radioactivity in various organs was examined 3 hr after the intravenous injection of C-14 putrescine. We selected the 3-hr interval based on the study of Clark and Fair (*12*), who demonstrated that radioactive putrescine uptake by the prostate of intact rats peaked at 1 hr after injection and was still near its peak at 3 hr.

The animals received oral DFMO in a total daily dose of 1.5–2.0 g/kg body weight. We did not observe any toxicity. This is in accordance with other studies noting lack of DFMO toxicity in rodents (*24*) or humans (*25*).

Under the experimental conditions, DFMO effectively inhibited ODC activity in the rat prostate. This finding is in agreement with observations by Seiler et al. (*22*) and Danzin et al. (*23*). Danzin et al. have also shown that as

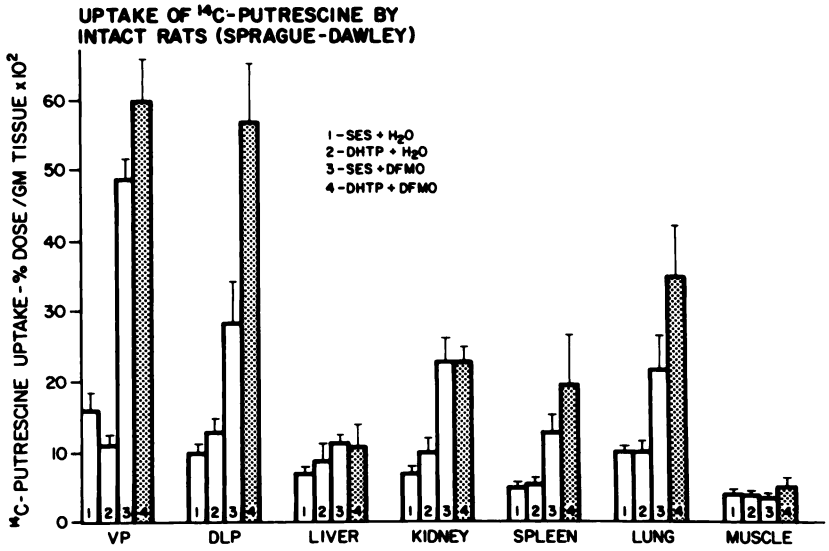

FIG. 1. Tissue uptake of C-14 putrescine in mature, intact, male Sprague-Dawley rats. Figures represent mean ± standard error of mean. Significant differences ($p < 0.05$ by Student's t test) were—VP (ventral prostate): 4 vs. 1, 4 vs. 2, 3 vs. 2; DLP (dorsolateral prostate): 4 vs. 1, 4 vs. 2, 4 vs. 3, 3 vs. 2; kidney, spleen and lung: 3 vs. 1, 3 vs. 2, 4 vs. 1, 4 vs. 2.

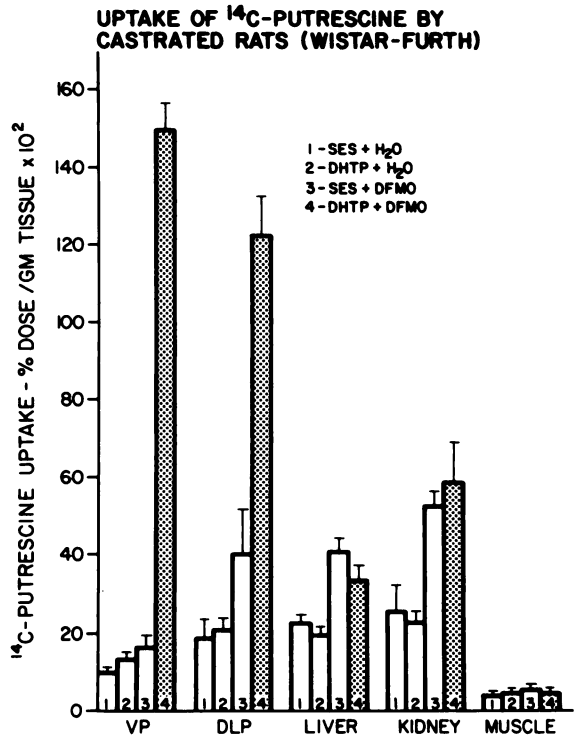

FIG. 2. Tissue uptake of C-14 putrescine in mature, castrated Wistar-Furth rats. Figures represent mean ± standard error of the mean. Significant differences ($p < 0.05$ by Student's t test) were VP (ventral prostate): 4 vs. 1, 4 vs. 2, 4 vs. 3; DLP (dorsolateral prostate): 4 vs. 1, 4 vs. 2, 4 vs. 3, 3 vs. 1; Liver and kidney: 4 vs. 1, 4 vs. 2, 3 vs. 1, 3 vs. 2.

a consequence of ODC inhibition, DFMO rapidly depletes the prostate of putrescine and spermidine (*15*). Polyamine-deprived cells in vitro attempt to restore their intracellular polyamine pools by markedly enhancing the uptake of exogenous polyamines (*16*). Our results suggest that a similar phenomenon occurs in vivo. Pretreatment of intact animals with DFMO resulted in a threefold increased uptake of C-14 putrescine into both the ventral and the dorsolateral prostate (Fig. 1; Group 3 vs. 1). In castrated animals, however, DFMO pretreatment alone had no detectable effect on prostatic C-14 putrescine uptake (Fig. 2; Group 3 vs. 1), and this is probably because the atrophic rat prostate has a low content of, and little requirement for, polyamines (*7*).

The most dramatically enhanced uptake of C-14 putrescine occurred in the prostate of animals treated with a combination of DFMO and androgen (Figs. 1 & 2, Group 4). This uptake was particularly enhanced in the prostates of previously castrated animals (Fig. 2), where the androgen-stimulated buildup of polyamines was blocked by DFMO (*7*).

The natural high uptake of radiolabeled polyamines by the prostate was used in the past to visualize the dog prostate on a positron emission tomographic scanner, using C-11 methylputrescine as tracer (*26*). After intravenous injection, a 10:1 prostate-to-skeletal-muscle ratio was achieved. Since we obtained a 30:1 prostate-to-skeletal-muscle putrescine uptake ratio following DFMO pretreatment and hormonal manipulation, we may guess that a similar protocol could be used to image the human prostate. For this purpose, use of an F-18 label ($T_{1/2}$ = 110 min) might be preferable to C-11, which has a 20-min half-life.

Preliminary experiments suggest that a tumor derived from rat prostate displays similarly enhanced putrescine uptake following DFMO pretreatment (*27*). If the same is true for human prostatic carcinoma, imaging of soft-tissue metastases might be possible using a combination of DFMO and radiolabeled putrescine analogs.

FOOTNOTES

* Merrel-National Laboratories, Cincinnati, OH through the courtesy of Drs. Peter McCann and William L. Albrecht.

† Steraloids, Wilton, NH.

‡ "Scintilene", Fisher Scientific Co., Fairlawn, NJ.

¶ Bio-Rad Laboratories, Richmond, CA.

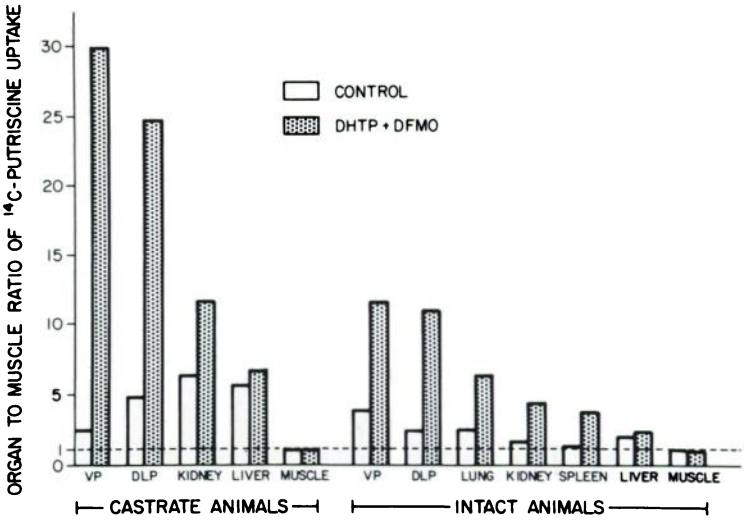

FIG. 3. Ratio (tissue to muscle) of C-14 putrescine uptake.

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REFERENCES

1. JANNE J, POSO H, RAINA A: Polyamines in rapid growth and cancer. *Bioch Biophys Acta* 473:241-293, 1978
2. HEBY O: Role of polyamines in control of cell proliferation and differentiation. *Differentiation* 19:1-20, 1981
3. TABOR H, TABOR CW: Spermidine, spermine and related amines. *Pharmacol Rev* 16:245-300, 1964
4. RUSSELL DH, LEVY CC: Polyamine accumulation and biosynthesis in a mouse L1210 leukemia. *Cancer Res* 31: 248-251, 1971
5. RENNERT O, MIALE T, SHUKLA J, et al: Polyamine concentrations in bone marrow aspirates of children with leukemia and other malignancies. *Blood* 47:695-701, 1976
6. WILLIAMS-ASHMAN HG, LOCKWOOD DH: Role of polyamines in reproductive physiology and sex hormone action. *Ann NY Acad Sci* 171:882-894, 1970
7. DANZIN C, JUNG MJ, CLAVEERIE N, et al: Effects of alpha-difluoromethylornithine, an enzyme-activated irreversible inhibitor of ornithine decarboxylase on testosterone-induced regeneration of prostate and seminal vesicles in castrated rats. *Biochem J* 180:507-513, 1979
8. MCKEEHAN WL, GLASS HA, ROSSER MP, et al: Prostatic binding protein, polyamine, and DNA synthesis in rat ventral prostatic cells. *The Prostate* 3:231-246, 1982
9. WILLIAMS-ASHMAN HG, COPPOC GL, WEBER G: Imbalance in ornithine metabolism in hepatomas of different growth rates as expressed in formation of putrescine, spermidine and spermine. *Cancer Res* 32:1924-1932, 1972
10. POHJANPELTO P: Putrescine transport is greatly increased in human fibroblasts initiated to proliferate. *J Cell Biol* 68: 512-520, 1976
11. KANO K, OKA T: Polyamine transport and metabolism in mouse mammary gland. *J Biol Chem* 251:2795-2800, 1976
12. CLARK RB, FAIR WR: The selective *in vivo* incorporation and metabolism of radioactive putrescine in the adult male rat. *J Nucl Med* 16:337-342, 1975
13. WELCH MJ, COLEMAN RE, STRAATMANN MG, et al: Carbon-11-labeled methylated polyamine analogs: uptake in prostrate and tumor in animal models. *J Nucl Med* 18:74-78, 1977
14. METCALF BW, BEY P, DANZIN C, et al: Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E.C. 4.1.1.17) by substrate and product analogues. *J Am Chem Soc* 100:2551-2553, 1978
15. DANZIN C, JUNG MJ, GROVE J, et al: Effect of alpha-difluoromethylornithine, an enzyme-activated irreversible inhibitor of ornithine decarboxylase on polyamine levels in rat tissues. *Life Sciences* 24:519-524, 1979
16. ALHONEN-HONGISTO L, SEPPÄNEN P, JANNE J: Intracellular putrescine and spermidine deprivation induces increased uptake of the natural polyamines and methylglyoxal bis (guanylhydrazone). *Biochem J* 192:941-945, 1980
17. HERBERG RJ: Channels ratio method of quench correction in liquid scintillation counting. *Packard Technical Bull* 15: 1-7, 1965
18. BEAVEN MA, WILCOX G, TERPSTRA GK: A microprocedure for the measurement of $^{14}CO_2$ release from (^{14}C) carboxyl-labeled amino acids. *Anal Biochem* 84:638-641, 1978
19. KOBAYASHI Y: Determination of histidine decarboxylase activity by liquid scintillation counting of $^{14}CO_2$. *Anal Biochem* 5:284-290, 1963
20. BRADFORD MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248-254, 1976
21. SEPPÄNEN P, ALHONEN-HONGISTO L, JÄNNE J: Death of tumor cells in response to the use of a system of stimulated polyamine uptake for the transport of methylglyoxal bis (guanylhydrazone). *Eur J Biochem* 118:571-576, 1981
22. SEILER N, DANZIN C, PRAKASH NJ, et al: Effects of ornithine decarboxylase inhibitors *in vivo*. In *Enzyme activated irreversible inhibitors*. Seiler, H., et al., Eds. New York, Elsevier/North Holland, 1978, pp 55-71
23. DANZIN C, CLAVERIE N, WAGNER J, et al: Effect on prostatic growth of 2-difluoromethylornithine, an effective inhibitor of ornithine decarboxylase. *Biochem J* 202:175-181, 1982
24. PRAKASH NJ, SCHECHTER PJ, GROVE J, et al: Effect of alpha-difluoromethylornithine, an enzyme-activated irreversible inhibitor of ornithine decarboxylase on L1210 leukemia in mice. *Cancer Res* 38:3059-3062, 1978
25. SIIMES M, SEPPÄNEN P, ALHONEN-HONGISTO L, et al: Synergistic action of two polyamine antimetabolites leads to a rapid therapeutic response in childhood leukemia. *Int J Cancer* 28:567-570, 1981
26. FAIR WR, MILLER TR, SIEGEL BA, et al: Radionuclide imaging of dog prostate. *Urology* 12:575-577, 1978
27. KADMON D, HESTON WDW, FAIR WR: Alpha-difluoromethylornithine (DFMO) can greatly enhance putrescine uptake by a prostatic tumor. *Surg Forum*, (in press)

Erratum

In the article entitled "An Efficient Method for Loading In-111 into Liposomes Using Acetylacetone," JNM Vol. 23, No. 9 (September); 810–815, 1982, the optimal concentration of ACac should be in m*M* and not μM as indicated.

Line four, P811 should read " . . . isotonic saline, containing 29.4 m*M* ACac at pH 7.6."

Line ten, P813, second paragraph should read " . . . m*M* ACac for SUV (SM/CH.2*M*:1*M*)."

Figure 1, P812 should indicate ACac, m*M*.

Figure 4, P813, caption line 3 should read " . . . 30m*M* ACac . . . "