

# INHIBITION OF N-BUTYL-N-(4-HYDROXYBUTYL) NITROSAMINE-INDUCED URINARY BLADDER TUMOR IN RATS BY $\alpha$ -DIFLUOROMETHYLORNITHINE

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The therapeutic effects of  $\alpha$ -difluoromethylornithine (DFMO) on rats with bladder tumors induced by N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) were examined. Eight-week-old male Wistar rats were given 0.05% BBN in their drinking water for a period of 4 weeks. Therapy (0.1% DFMO in their drinking water) was started at week 4 and all rats were killed at week 60. DFMO was seen to significantly reduce the incidence, the mean number and the total size of tumors. No side-effects of DFMO were noted, except alopecia, which started at month 7 of the therapy.

**Key words :**  $\alpha$ -Difluoromethylornithine, Rat bladder tumor, N-Butyl-N-(4-hydroxybutyl) nitrosamine

## INTRODUCTION

Neoplastic cells contain high levels of polyamines, which are necessary for their continued proliferation<sup>1,2)</sup>. Depletion of polyamines by inhibition of polyamine biosynthesis has been shown to reduce the growth of experimental animal tumors<sup>3-7)</sup>. DFMO is an enzyme-activated irreversible inhibitor of ornithine decarboxylase (ODC), which catalyzes the first step in polyamine synthesis<sup>8)</sup>. Inhibitory effects of DFMO on urinary bladder carcinogenesis were examined using the heterotopically transplanted rat urinary bladder model<sup>9)</sup>. Normal rat urine is capable of inducing ODC when tested with a rat bladder carcinoma cell line, 804 G cells<sup>10)</sup>. Using the heterotopically transplanted rat urinary bladder (HTB) system, whole rat urine were demonstrated to enhance N-methyl-N-nitrosourea (MNU)-initiated urinary bladder carcinogenesis<sup>10)</sup>.

Therefore, we thought it would be of some interest to examine the effects of

DFMO on the induction of urinary bladder cancer in rats exposed to BBN.

## MATERIALS AND METHODS

**Chemicals**  $\alpha$ -Difluoromethylornithine (DFMO) was supplied by Merrell Dow Pharmaceuticals, Inc., Cincinnati, Ohio, U.S.A. and given as a 0.1% solution in water contained in light-proof bottles. BBN was purchased from Iwai Chemicals Co., Tokyo, Japan and was given as a 0.05% solution in water contained in light-proof bottles.

**Animals** Male Wistar rats (Sankyo Laboratories, Tokyo, Japan) were obtained 2 weeks before the start of the experiment at the age of 5 weeks. They were kept 5 to a cage and fed a CE-2 pellet diet (Nihon Kurea Co., Tokyo, Japan) and water, with or without BBN and DFMO, *ad libitum*.

**Design of the study** Rats were randomly allocated to one of 4 groups: There were 5 rats in groups (1) and (2), and 25 in groups (3) and (4). Group (1) consisted of

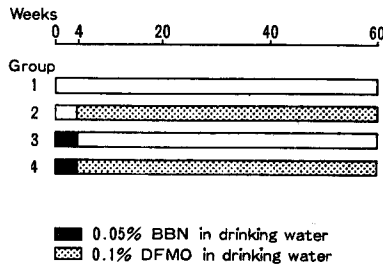


Fig. 1. Experimental protocol.

Table 1. Effect of  $\alpha$ -difluoromethylornithine on BBN-induced bladder tumors in male Wistar rats.

Group	No. of rats		No. of rats with gross tumor (%)	No. of tumors/rat (mean $\pm$ SD)	Total size of tumors (mm <sup>3</sup> )/rat (mean $\pm$ SD)
	Initial	Final			
1	5	5	0	0	0
2	5	5	0	0	0
3	25	20	20 (100)	2.1 $\pm$ 0.8	27.5 $\pm$ 33.1
4	25	25	16 (64)*	0.9 $\pm$ 0.9**	3.3 $\pm$ 6.4***

\* Significantly different from group 3 ( $P < 0.05$ )  
 \*\* Significantly different from group 3 ( $P < 0.01$ )  
 \*\*\* Significantly different from group 3 ( $P < 0.05$ )

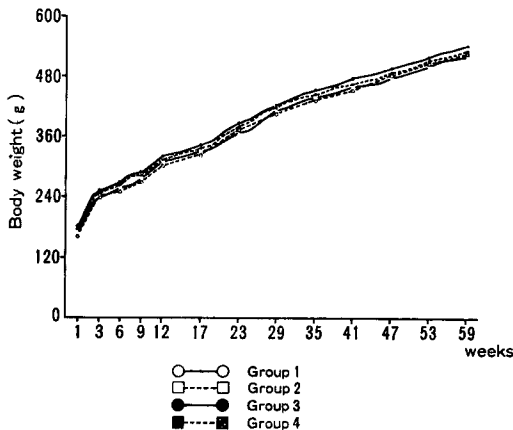


Fig. 2. Growth curves of all groups

the control rats (no treatment); in group (2) rats received 0.1% DFMO in their drinking water throughout the experiment; in group (3) rats received 0.05% BBN in their drinking water for 4 weeks; in group (4) rats received 0.05% BBN in their drinking water for 4 weeks and then were switched to receiving 0.1% DFMO in their drinking water for the rest of the experiment (Fig. 1).

Rats were killed at week 60, and their urinary bladders were distended

with 10% formaldehyde solution and a ligature was placed around the neck of each bladder to maintain proper distention. After fixation and counting of the number of gross tumors and measuring the size of each tumor, the bladder was sliced and prepared for light microscopy. Tissues were embedded in paraffin and stained with hematoxyline and eosin.

## RESULTS

Table 1 shows the incidence, the number and the total size of urinary bladder tumors among the various groups. DFMO had a marked effect; the incidence of gross urinary bladder tumors was reduced from 100% (group 3) to 64% (group 4) ( $p < 0.05$ ), the mean number of tumors per rat was reduced from 2.1 (group 3) to 0.9 (group 4) ( $p < 0.01$ ) and the mean total size of tumors per rat was reduced from 27.5 (group 3) to 3.3 (group 4) ( $p < 0.05$ ). No lesions were observed in group 1 (control) nor in group 2 (DFMO alone), nor were any calculi or parasites noted in any of the bladders examined.

The body weights of the rats in the 4 groups were almost the same (Fig. 2) and the side effect of alopecia was noted in group 2 and 4.

## DISCUSSION

These data demonstrate that DFMO in drinking water inhibits the formation of tumors in the urinary bladder of rats having been given BBN. DFMO has been shown previously by Kingsnorth et al. to inhibit dimethylhydrazine-induced colon tumors in mice<sup>5</sup>; by Kitagawa et al. to inhibit 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced skin tumors in mice<sup>6</sup> and by Kingsnorth et al. to inhibit the growth of experimental Wilms' tumors and renal adenocarcinoma<sup>7</sup>. Homma et al. demonstrated that long-term administration of DFMO inhibits bladder carcinogenesis initiated by MNU and promoted by urine in the HTB model<sup>8</sup>. Miyata et al. previously demonstrated that normal rat urine has a tumor-enhancing

cing effect on urothelial cells which have been briefly exposed to a chemical carcinogen<sup>11</sup>). Normal rat urine was also shown to be a strong ODC inducer<sup>10</sup>). These studies raised the possibility of inhibitory effects of DFMO on tumor promotion by urine *in vivo*. The results of the present experiment clearly show that DFMO effectively inhibits tumor promotion in rats.

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マウスにおける N-Butyl-N-(4-hydroxybutyl)nitrosamine 膀胱  
発癌に対する  $\alpha$ -Difluoromethylornithine の抑制効果

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マウスにおける N-Butyl-N-(4-hydroxybutyl)nitrosamine (BBN) 膀胱発癌に対する  $\alpha$ -Difluoromethylornithine (DFMO) の抑制効果を調べた。8週齢の Wistar 系雄性ラット50匹に0.05% BBN含有飲料水を、4週間与えた後、2群に分け、1群には0.1% DFMO含有飲料水を、他群には飲料水をそれぞれ自由に与えながら、CE-2 固型飼料で、60週

まで飼育後、屠殺して膀胱腫瘍の発生状況を観察した。その結果 DFMO は、BBN 膀胱発癌において、膀胱腫瘍発生率、膀胱腫瘍の数と膀胱腫瘍の大きさをそれぞれ有意に抑制することが判明した。DFMO の副作用は、実験開始7カ月頃より生じ始めた脱毛のみであった。