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Kyoto University
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\(^1\),\(^2\). Depletion of polyamines by inhibition of polyamine biosynthesis has been shown to reduce the growth of experimental animal tumors\(^3\)\(^-\)\(^7\). DFMO is an enzyme-activated irreversible inhibitor of ornithine decarboxylase (ODC), which catalyzes the first step in polyamine synthesis\(^8\). Inhibitory effects of DFMO on urinary bladder carcinogenesis were examined using the heterotopically transplanted rat urinary bladder model\(^9\). Normal rat urine is capable of inducing ODC when tested with a rat bladder carcinoma cell line, 804 G cells\(^10\). 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\(^10\).

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, \textit{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
Table 1. Effect of α-difluoromethylornithine on BBN-induced bladder tumors in male Wistar rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats initial</th>
<th>No. of rats final</th>
<th>No. of tumors/rat (mean ± SD)</th>
<th>Total size of tumors (mm³)/rat (mean ± SD)</th>
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<tr>
<td>1</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>20(100)</td>
<td>2.1 ± 0.8</td>
<td>27.5 ± 33.1</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>16(64)*</td>
<td>0.9 ± 0.9**</td>
<td>3.3 ± 6.4**</td>
</tr>
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</table>

* Significantly different from group 3 (P<0.05)
** Significantly different from group 3 (P<0.01)
*** Significantly different from group 3 (P<0.001)

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\(^5\); by Kitagawa et al. to inhibit 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-induced skin tumors in mice\(^6\) and by Kingsnorth et al. to inhibit the growth of experimental Wilms' tumors and renal adenocarcinoma\(^7\). Homma et al. demonstrated that long-term administration of DFMO inhibits bladder carcinogenesis initiated by MNU and promoted by urine in the HTB model\(^8\). Miyata et al. previously demonstrated that normal rat urine has a tumor-enhanc-
cing effect on urothelial cells which have been briefly exposed to a chemical carcinogen\textsuperscript{10}. Normal rat urine was also shown to be a strong ODC inducer\textsuperscript{10}. These studies raised the possibility of inhibitory effects of DFMO on tumor promotion by urine \textit{in vivo}. The results of the present experiment clearly show that DFMO effectively inhibits tumor promotion in rats.

\textbf{ACKNOWLEDGMENTS}

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マウスにおける N-Butyl-N-(4-hydroxybutyl)nitrosamine 腎結核に対する α-Difluormethylornithine の抑制効果

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マウスにおける N-Butyl-N-(4-hydroxybutyl)nitrosamine（BBN）腎結核に対する α-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、屠宰して腎結核の発生状況を観察した。その結果 DFMO は、BBN 腎結核において、腎結核発生率、腎結核の数と腎結核の大きさをそれぞれ有意抑制することが判明した。DFMO の副作用は、実験開始7ヵ月頃より生じ始めた脱毛のみであった。