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Effects of Single Chemotherapeutic Agents on Development of Urinary Bladder Tumor Induced by N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) in Rats

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Summary. Chemotherapeutic agents were evaluated for effect on the development of urinary bladder tumors induced by N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) in male Wistar strain rats. Seven hundred and two rats were given 0.05% BBN in drinking water for 8 weeks. After BBN treatment, the animals were divided into 26 groups to follow regimens of single chemotherapy. All drugs were administered intraperitoneally except in one group that was treated orally. In our experimental series, 5-fluorouracil (5-FU), N-(2-tetrahydrofuryl)-5-fluorouracil (FT-207), carbazilquinone (CQ), vincristine (VCR) and cis-diamminedichloroplatinum (CDDP) were effective in inhibiting the incidence of bladder tumor, however, adriamycin (ADM), mitomycin C (MMC), neocarsinostatin (NCS), cyclophosphamide (CPM) and bleomycin (BLM) were not effective.

Key words: Chemotherapy, Rat urinary bladder tumor, N-butyl-N-(4-hydroxybutyl) nitrosamine.

Introduction

If chemotherapy is to play an essential role in therapy for bladder carcinoma, the most effective drugs must be used and new agents evaluated as they become available. However, urologists or oncologists have little clinical data to select from many chemotherapeutic agents.

On the other hand, the clinician does have many methods of obtaining preliminaly data to assist the clinician in the selection of promising chemotherapeutic agents. There are many methods for laboratory chemosensitivity testing of bladder carcinoma [23]. For instance, tissue culture in vitro methods such as tumor stem cell assay [9], and animal in vivo methods such as nude mouse subcutaneous heterotransplantation model [22] were reported previously. One of the effective methods for screening many potential chemotherapeutic agents is to employ suitable animal tumor models.

Animal models of bladder tumor induced by specific bladder carcinogens such as N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) [14, 15], N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide (FANFT) [3, 5], and N-methyl-N-nitrosourea (MNU) [11]. Bladder tumors induced by BBN resemble their human counterparts both grossly and histologically and appear to be excellent for evaluating the effect of chemotherapeutic agents. BBN induces bladder neoplasms in a high percentage of mice [12], rats [14, 17, 18], and dogs [19] when administered orally. This compound is highly specific for the urothelium. We have preliminary data indicating a relation between the observation period following 8 weeks of treatment with 0.05% BBN and incidence of urinary bladder tumor in Wistar strain rats. Using this animal model, we evaluated the effect of individual chemotherapeutic agents on the incidence of bladder tumors induced by BBN.

Materials and Methods

Animals. Male Wistar strain rats (Fuji Animal Farm., Tokyo) weighing 150–200 g were used. All animals received a commercial stock diet (Oriental MF, Oriental Yeast Co., Osaka) and water ad libitum. Animals were housed five to a cage in an air conditioned room at 20 °C, and were weighed once a week.

Carcinogen. BBN (Izumi Chemical., Yokohama) was given as a 0.05% solution in the drinking water as described previously [17, 18].

Drugs. Ten chemotherapeutic agents were evaluated in this study: adriamycin (ADM), mitomycin C (MMC), cyclophosphamide (CPM), 5-Fluorouracil (5-FU), N-(2-tetrahydrofuryl)-5-fluorouracil (FT-207), neocarsinostatin (NCS), carbazilquinone (CQ), bleomycin (BLM), vincristine (VCR), cis-diamminedichloroplatinum (CDDP).

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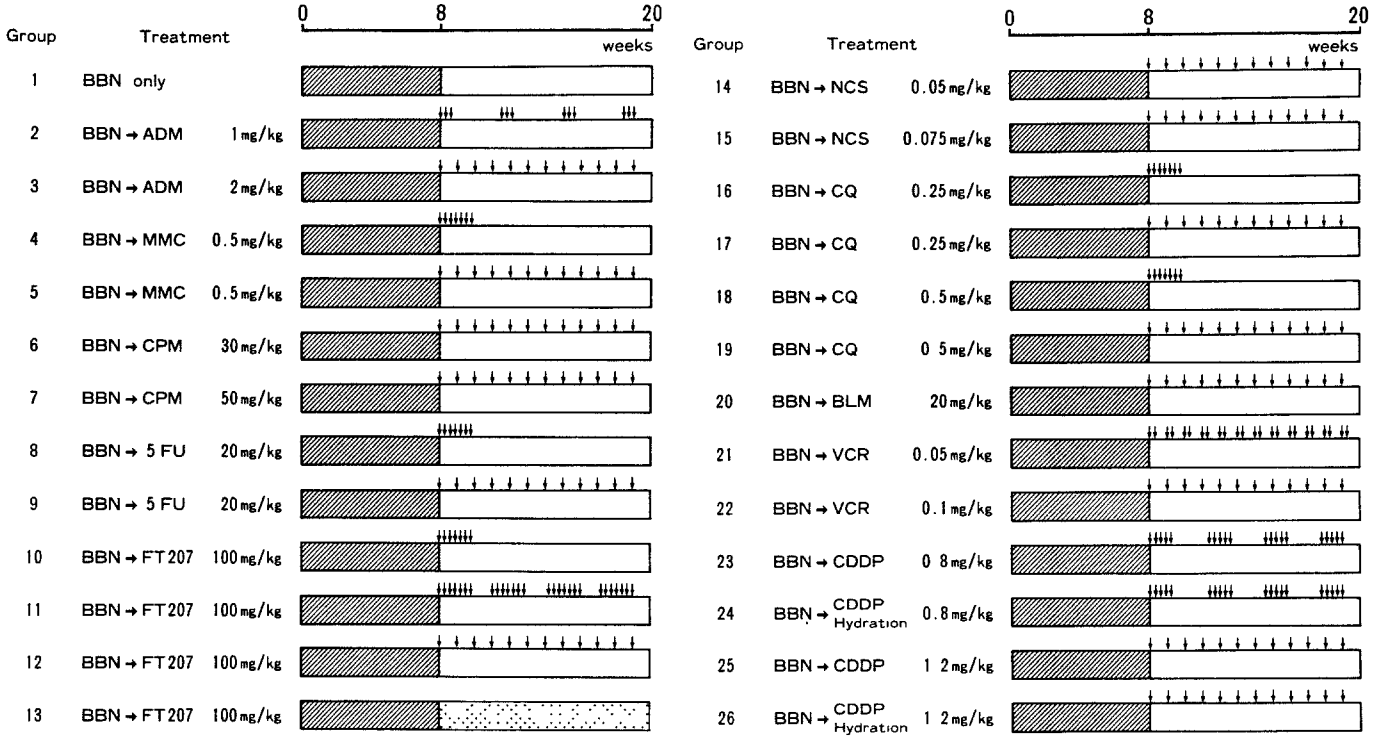

Fig. 1. Experimental design. ▭ basal diet; ▨ 0.05% BBN in drinking water; ▩ oral administration; ↓ intraperitoneal administration

Total administration dosages of each agent were about 10% of LD_{50} except 5FU, NCS and VCR groups. Dosages of these three agents were about 2% of LD_{50} because these agents were too toxic in preliminary experiments.

Experimental Groups. The experimental design is shown in Fig. 1. A total of 1,176 rats were used in this study. 702 rats received tap water containing 0.05% BBN for 8 weeks and the remaining 474 rats received tap water only. After BBN treatment, the 702 rats were divided into the following 26 groups. All chemotherapeutic agents were administered by intraperitoneal injection except group 13 (Fig. 1).

Group 1: After BBN treatment, 77 rats were given tap water only.
Group 2: Twenty rats administered 1 mg/kg body weight (B.W.) of ADM for a 3 day period every 3 weeks.
Group 3: Twenty rats administered 2 mg/kg B.W. of ADM once a week for 12 weeks.
Group 4: Twenty-four rats administered 0.5 mg/kg B.W. of MMC for initial seven days of the observation period.
Group 5: Twenty rats administered 0.5 mg/kg B.W. of MMC once a week for 12 weeks.
Group 6: Thirty rats administered 30 mg/kg B. W. of CPM once a week for 12 weeks.
Group 7: Fifteen rats administered 50 mg/kg B. W. of CPM once a week for 12 weeks.
Group 8: Sixteen rats administered 20 mg/kg B. W. of 5-FU for initial seven days.
Group 9: Twenty rats administered 20 mg/kg B. W. of 5-FU once a week for 12 weeks.
Group 10: Thirty rats administered 100 mg/kg B. W. of FT-207 for initial seven days.
Group 11: Eighteen rats administered 100 mg/kg B. W. of FT-207 for seven days at 3 weeks intervals.
Group 12: Eighteen rats administered 100 mg/kg B.W. of FT-207 once a week for 12 weeks.
Group 13: Sixteen rats given in their diet 100 mg/kg B.W./day of FT-207 for 12 weeks.
Group 14: Twenty-four rats administered 0.05 mg/kg B.W. of NCS once a week for 12 weeks.
Group 15: Forty rats administered 0.075 mg/kg B.W. of NCS once a week for 12 weeks.
Group 16: Twenty-seven rats administered 0.25 mg/kg B.W. of CQ for initial 7 days.
Group 17: Twenty-seven rats administered 0.25 mg/kg B.W. of CQ once a week for 12 weeks.
Group 18: Twenty-four rats administered 0.5 mg/kg B.W. of CQ daily for the initial 7 days.
Group 19: Twenty-four rats administered 0.5 mg/kg B.W. of CQ once a week for 12 weeks.
Group 20: Twenty rats administered 20 mg/kg of BLM once a week for 12 weeks.
Group 21: Twenty rats administered 0.05 mg/kg B.W. of VCR twice a week for 12 weeks.
Group 22: Thirty-six rats administered 0.1 mg/kg B.W. of VCR once a week for 12 weeks.
Group 23: Thirty-six rats administered 0.8 mg/kg B.W. of CDDP for 5 day periods at 3 weeks interval.
Group 24: Thirty-five rats administered 0.8 mg/kg B.W. of CDDP plus 20 ml of 0.9% NaCl solution containing 0.8 mg of furosemide for 5 day periods at 3 weeks interval.
Group 25: Thirty-one rats administered 1.2 mg/kg B.W. of CDDP once a week for 12 weeks.

Table 1. Effects of various chemotherapeutic agents on development of bladder tumors induced by BBN in rats

Group	Treatment	Effective no. of rats	Incidence of bladder lesions		*P*-value[c]
			Hyperplasia[a] (%)	Tumor[b] (%)	
1	BBN only	70	58 (82.9)	62 (88.6)	–
2	BBN ADM	15	12 (80.0)	12 (80.0)	n.s.
3	BBN ADM	11	10 (90.9)	11 (100.0)	n.s.
4	BBN MMC	12	8 (66.7)	9 (75.0)	n.s.
5	BBN MMC	16	15 (93.8)	13 (81.3)	n.s.
6	BBN CPM	28	24 (85.8)	22 (78.6)	n.s.
7	BBN CPM	8	7 (87.5)	6 (75.0)	n.s.
8	BBN 5FU	11	6 (54.5)	5 (45.5)	0.001
9	BBN 5FU	18	15 (83.3)	13 (72.2)	n.s.
10	BBN FT207	29	20 (68.9)	14 (48.3)	0.001
11	BBN FT207	14	11 (78.6)	6 (42.9)	0.001
12	BBN FT207	12	10 (83.3)	5 (41.7)	0.001
13	BBN FT207	16	14 (87.5)	9 (56.3)	0.01
14	BBN NCS	17	11 (64.7)	14 (82.4)	n.s.
15	BBN NCS	23	15 (65.2)	21 (91.3)	n.s.
16	BBN CQ	26	18 (69.2)	19 (73.1)	n.s.
17	BBN CQ	27	13 (48.1)	17 (63.0)	0.005
18	BBN CQ	20	16 (80.0)	8 (40.0)	0.001
19	BBN CQ	22	21 (95.5)	17 (77.3)	n.s.
20	BBN BLM	15	12 (80.0)	14 (93.3)	n.s.
21	BBN VCR	15	13 (86.7)	10 (66.7)	n.s.
22	BBN VCR	31	18 (58.1)	15 (48.4)	0.001
23	BBN CDDP	29	20 (69.0)	18 (62.1)	0.005
24	BBN CDDP + H	31	13 (41.9)	22 (71.0)	n.s.
25	BBN CDDP	29	12 (41.4)	16 (55.2)	0.001
26	BBN CDDP + H	25	23 (92.0)	20 (80.0)	n.s.

[a] Hyperplasia refers to papillary or nodular hyperplasia that is regarded as preneoplastic foci
[b] Papilloma plus carcinoma
[c] Significant difference in the incidence of tumor from rats in group 1
n.s. not significant

Group 26: Thirty-four rats administered 1.2 mg/kg B.W. of CDDP plus 20 ml of 0.9% NaCl solution containing 0.8 mg of furosemide once a week for 12 weeks.

474 rats without BBN were also divided into 25 groups and received chemotherapeutic agents by the same methods as in groups 2 to 26.

Animals that died within 20 weeks from the start of the experiment were excluded. At the end of the experiment, animals were sacrificed by ether inhalation. The carcass, liver, both kidneys, spleen, testes, and urinary bladder were weighted and samples were taken for a histological study. The urinary bladder was punctured at the urethro-vesical junction and a 0.5 ml of 10% buffered formaldehyde solution was injected into the bladder. After fixation, the bladder was carefully examined for tumors. All tissues were routinely stained with Hematoxylin and Eosin. Histopathological findings were classified into 3 types, hyperplasia, papilloma and carcinoma as reported previously [17, 18].

Statistical analysis of tumor incidence (papilloma plus carcinoma) was performed using Chi-square test. Statistical significance was accepted for $p < 0.05$.

Results

The average body weight of rats in all groups increased throughout the experiment, though weight gain in groups 2–5, 7–9, 13, and 20–22 were relatively small. No remarkable changes were seen in the liver, lung and gastrointestinal tract. All rats in groups 21–26 showed atrophy of the testes. Microscopic findings of the kindeys in groups 23 and 25 showed degeneration and necrosis of proximal tubules and interstitial edema. Histological findings in the urinary bladder in each of the groups are shown in Table 1.

Group 1 (BBN Only Group)

Hyperplasia of the urinary bladder epithelium was found in 58 rats (82.9%), and bladder tumor (papilloma plus carcinoma) in 62 (88.6%) of rats in this group.

Groups 2 and 3 (ADM Groups)

Among 15 rats of group 2, hyperplasia was seen in 12 rats (80.0%) and bladder tumor in 12 rats (80.0%). Ten (90.9%) of 11 rats in group 3 developed hyperplasia, and all 11 developed bladder tumor. The incidences of bladder tumor in either group was not significantly different from that of group 1.

Groups 4 and 5 (MMC Groups)

Hyperplasia developed in 8 (66.7%) of 12 rats in group 4 and in 15 (93.8%) of 16 rats in group 5. Bladder tumor was seen in 9 rats (75.0%) of group 4 and in 13 rats (81.3%) of group 5. The incidences of bladder tumor of these groups were not significantly different from that of group 1.

Groups 6 and 7 (CPM Groups)

Twenty-four (85.8%) of 28 rats in groups 6 and 7 (87.5%) of 8 rats in group 7 developed hyperplasia. Twenty-two rats (78.6%) in group 6 and 6 rats (75.0%) in group 7 developed bladder tumor. The incidences of bladder tumor of these groups were not significantly different from that of group 1.

Groups 8 and 9 (5-FU Groups)

Among 11 rats of group 8, hyperplasia was seen in 6 rats (54.5%) and bladder tumor in 5 rats (45.5%). Fifteen (83.3%) of 18 rats in group 9 developed hyperplasia, 13 rats (72.2%) developed bladder tumor. The incidence of bladder tumor in group 8 was significantly lower than that of group 1 ($p < 0.001$) (Table 1).

Groups 10, 11, 12 and 13 (FT-207 Groups)

Hyperplasia developed in 20 (68.9%) of 29 rats in group 10, in 11 (78.6%) of 14 rats in group 11, in 10 (83.3%) of 12 rats in group 12, and 14 (87.5%) of 16 rats in group 13. Bladder tumor was seen in 14 rats (48.3%) of group 10, 6 rats (42.9%) of group 11, 5 rats (41.7%) of group 12, and 9 rats (56.3%) of group 13. Bladder tumor incidences in group 10 ($p < 0.001$), group 11 ($p < 0.001$), group 12 ($p < 0.001$) and group 13 ($p < 0.01$) were significantly lower than that of group 1 (Table 1).

Groups 14 and 15 (NCS Groups)

Hyperplasia was seen in 11 (64.7%) of 17 rats in group 14 and in 15 (65.2%) of 23 rats in group 15. Bladder tumor was seen in 14 rats (82.4%) of group 14 and in 21 rats (91.3%) of group 15. These tumor incidences were not significantly different from that of group 1.

Groups 16, 17, 18 and 19 (CQ Groups)

Hyperplasia developed in 18 (69.2%) of 26 rats in group 16, 13 (48.1%) of the 27 rats in group 17, 16 (80.0%) of 20 rats in group 18, and 21 (95.5%) of 22 rats in group 19. Bladder tumor was seen in 19 rats (73.1%) of group 16, in 17 rats (63.6%) of group 17, in 8 rats (40.0%) of group 18, and in 17 rats (77.3%) of group 19. The tumor incidences of group 17 ($p < 0.005$) and group 18 ($p < 0.001$) were significantly lower than that of group 1 (Table 1).

Group 20 (BLM Group)

Hyperplasia developed in 12 (80.0%) of the 15 rats and bladder tumor in 14 (93.3%). The tumor incidence was not significantly different from that of group 1.

Groups 21 and 22 (VCR Groups)

Eighteen (58.1%) of 31 rats in group 21 and 13 (83.7%) of 15 rats in group 22 developed hyperplasia. Fifteen rats (48.4%) of group 21 and 10 rats (66.7%) of group 22 developed bladder tumor. The tumor incidence of group 21 was significantly lower than that of group 1 ($p < 0.001$) (Table 1). The tumor incidence of group 22 was not significantly different from that of group 1.

Groups 23, 24, 25 and 26 (CDDP Groups)

Hyperplasia was seen in 20 (69.0%) of the 29 rats in group 23, 13 (41.9%) of 31 rats in group 24, 12 (41.4%) of 29 rats in group 25, and 23 (92.0%) of 25 rats in group 26. Bladder tumor developed in 18 rats (62.1%) of group 23, in 22 rats (71.0%) of group 24, in 16 rats (55.2%) of group 25, and in 20 rats (80.0%) of group 26. The tumor incidences of group 23 ($p < 0.005$) and group 25 ($p < 0.001$), treated by CDDP without hydration, were significantly lower than that of group 1. The tumor incidences of groups 24 and 26, treated by CDDP with hydration, were not significantly different from that of group 1.

Discussion

Animal tumor models are being used more frequently to study tumor biology, metastatic growth, and to evaluate chemotherapeutic agents. Our bladder tumor model, based on induction by BBN, has allowed further biological evaluation and was suitable model for studying anti-tumor activity of drugs. Effective single agents were 5-FU, FT-207, CQ, VCR, and CDDP. ADM, MMC, CPM, NCS, and BLM, failed to inhibit tumor incidence effectively.

5-FU is a fluorinated pyrimidine and a pyrimidine antagonist. This drug is useful in the treatment of bladder carcinoma. In clinical cases, Pavon-Macaluso reported an objective response in 47% of cases treated only by 5-FU [20]. Other urologists have also reported anti-tumor effects utilizing this drug [4]. With an experimental model, however, Soloway reported that this drug was not effective when used as a single agent against transplantable transitional cell

tumor [22]. In our series, 5-FU and FT-207, which is a repository form of 5-FU, demonstrated definitive anti-tumor effect against bladder tumors induced by BBN. FT-207 has been synthesized in the Soviet Union [7, 8]. FT-207 is believed to act by conversion into 5-FU and to represent its chemical depot form [6]. Although FT-207 putatively requires liver-medicated activation, this drug also produces direct anti-tumor effects in vitro experiments. In this study, FT-207 showed remarkable anti-tumor effects when administered either orally or intraperitoneally. The effect of FT-207 may be stronger than that of 5-FU.

CQ is a derivatives of aziridinylbenzoquinone and has three biologically active groups, aziridinyl, carbamoyloxy, and quinonyl. Such a chemical structure can also be seen in MMC, which is well known anti-tumor agent [1]. Arakawa et al. [1, 2] reported that CQ produced inhibition of tumor growth in both transplantable and primary tumor in mice. In our series, administration of CQ markedly inhibited the incidence of urinary bladder tumors induced by BBN in groups 17 and 18. These our results suggest that CQ also have an anti-tumor effect against human bladder carcinoma.

VCR is a dimeric alkaloid similar in structure to vinblastine, which shows anti-tumor effects against leukemias, lymphomas, and a variety of solid tumors [13]. When tested against transplantable bladder carcinoma it was found to be more effective than alkylating agents such as CPM or MMC [13]. In our series, the tumor incidences in group 21 which was administered 1 mg/kg of VCR was lower than that in group 1 (Table 1). VCR had a greater anti-tumor effect on the BBN-induced bladder tumors than did CPM or MMC.

CDDP is considered the most active chemotherapeutic agent against transitional cell carcinoma of the bladder. Recently the effectiveness of CDDP in both human bladder carcinoma and carcinogen-induced animal bladder tumors is reported. Yagoda [24] and Herr et al. [10] found anti-tumor responses in 30–50% of high stage bladder carcinoma. Soloway [22] demonstrated that CDDP act as one of the most effective drugs inhibiting growth of both transplanted and primary tumor induced by FANFT. In our study, the tumor incidences in groups 23 and 25, which was administered weekly injection of 0.8 mg/kg and 1.2 mg/kg of CDDP without hydration, were significantly lower than the incidence of tumor in group 1, which was given BBN alone. CDDP demonstrated an inhibitory effect on bladder carcinogenesis induced by BBN, but microscopic examination of the kidneys in these groups showed a uniform presence of degeneration and necrosis of proximal tubules and interstitial edema due to platinum-induced nephrotoxicity. Nephrotoxicity of platinum compounds can be ameliorated or even prevented by hydration with saline solution [21]. Manitol and furosemide afford an additional protective effect of nephrotoxicity [21]. We tried to reduce platinum-induced nephrotoxicity by using injection of saline containing furosemide in groups 24 and 26. Microscopic examination of the kidneys in groups 24 and 26 were almost normal except for a little interstitial edema. However, the tumor incidences of groups 24 and 26, which were administered CDDP with hydration, were not lower than that of group 1. Thus, diuresis by saline containing furosemide may reduce anti-tumor effect of CDDP.

Some investigations [22, 24] indicated that ADM, CPM and MMC were effective against bladder cancer in both human and experimental cases. In our series, these agents showed no anti-tumor activity as single agent. It is well known that rat bladder tumor induced by BBN developed papillary and superficial tumor with a low grade of cellular atypia. Systemic administrations of ADM, CPM, and MMC may not be effective against low grade superficial bladder carcinoma. BLM and NCS were likewise not effective in our tumor model.

The chemotherapeutic agents evaluated in this study were administered as single treatments. Further evaluation with those agents in various combinations would be appropriate. Continued use of this murine bladder model is recommended for future screening of chemotherapeutic agents and for evaluation of treatment agents for bladder cancer. The baseline results from this study are reproducible and future studies in tumor model with chemotherapeutic combination can be evaulated.

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