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EFFECT OF INTRAVESICAL INSTILLATION OF ANTITUMOR DRUGS FOR THE DEVELOPMENT OF N-BUTYL-N-(4-HYDROXYBUTYL)NITROSAMINE-INDUCED BLADDER TUMORS IN RATS

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We examined the effects of intravesical instillation of adriamycin (ADM) and cis-diamminedichloroplatinum (CDDP) on the development of bladder tumors induced by 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine in 115 rats. Intravesical instillation was performed in two ways; continuous administration (5 times a week for 5 weeks) and intermittent administration (once a week). Group 1 (control group) did not receive intravesical instillation. Group 2 (ADM), group 3 (CDDP) and group 4 (physiological saline) were continuous type. Group 5 (ADM), group 6 (CDDP) and group 7 (physiological saline) received intermittent administration. Bladder weight was significantly higher in group 4 than in groups 1 or 7, and that in groups 2 and 3 than that in group 5 and 6. In groups 2, 3, 5 and 6 bladder weight was almost normal or higher than in the control group, and in group 3 histologically cancer was not seen in one rat. Physiological saline had promoting activity, and ADM and CDDP had both inhibitory and promoting activities. Also, intravesical instillation itself was suggested to promote tumor development under carcinogenic circumstances. We conclude that intermittent intravesical instillation should be performed to inhibit tumor recurrence and intravesical instillation therapy should not be performed clinically for a long period.

Key words: BBN-induced bladder tumor, Intravesical instillation

INTRODUCTION

In recent years, recurrence of tumors after a conservative operation such as transurethral resection (TUR) for superficial bladder tumors has been an important problem. Intravesical instillation of various antitumor drugs has been performed to inhibit tumor growth, and clinically effective results have been reported[1-2]. However, experimental studies suggested that antitumor drugs promote bladder carcinogenesis[3-4].

In the present study, we examined the inhibitory or promoting effects of intravesical instillation of adriamycin (ADM) and cis-diamminedichloroplatinum (CDDP) on the development of bladder tumors induced by 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) in rats, and we also studied the effects of the instillation interval and frequency.

MATERIALS AND METHODS

A total of 115 female F344 rats 6 weeks old (Charles River Japan, Inc., Kanagawa, Japan) were divided into 7 groups of 10 to 20 rats each. All rats were put into plastic cages each containing 5 rats and the floor mats were exchanged once a week. Rats in all groups were given drinking water containing 0.05% BBN (Izumi Chemical Co., Yokohama, Japan) for 20 weeks, and subsequently were supplied with normal drinking water for 20 weeks. Group 1 was the control group, which did

not receive intravesical instillation. Groups 2 and 5 received intravesical instillation of 1 mg/ml of ADM (Kyowa Hakko Co., Ltd., Tokyo), groups 3 and 6 received 1 mg/ml of CDDP (Bristol Myers Co., Ltd., Tokyo) and groups 4 and 7 received physiological saline. Groups 2, 3 and 4 received continuous treatment, 5 times a week for 5 weeks during administration of BBN. Groups 5, 6 and 7 received intermittent type treatment, once a week during the administration of BBN. Intravesical instillations were given a total of 20 times and the volume of ADM, CDDP solution and physiological saline were 0.3 ml (Fig. 1).

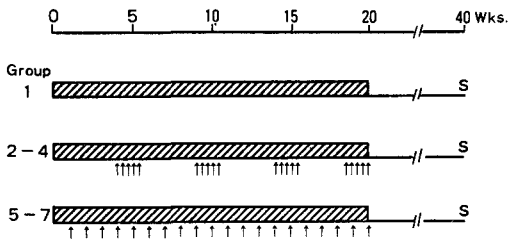

Fig. 1. Experimental design for examining the effects of intravesical instillation of antitumor drugs on BBN induced bladder tumor development. ▨: 0.05% BBN in drinking water, ↑: intravesical instillation, S: time of killing.

Intravesical instillation was performed under anesthesia with nembutal. Bladder urine was emptied by massage of the lower abdominal region, and the solution was transurethrally instilled by using a 21 or 23 gauge Teflon needle. After the solution was retained in the bladder for 1 hour, it was discharged by massage of the lower abdomen. Rats were killed under anesthesia with ether after 40 weeks. After urinary bladders were removed, their urine was emptied, their weights were measured, and they were fixed with 10% formalin solution. Lung, liver and kidney of rats in group 1 were removed and fixed with 10% formalin solution. Each bladder was cut with step section, stained with hematoxylin and eosin, and studied histologically. Other organs were grossly observed and in group 1 lung, liver and kidney were studied histologically.

RESULTS

Sixty one rats survived for 40 weeks, and were entered in this study, and the other rats which died before the 40th week were excluded.

Bladder weight (Table 1): The weight was 1.2±0.8 g/rat in group 1, 1.4±1.3 g/rat in group 2, 2.1±1.5 g/rat in group 3, 3.4±1.0 g/rat in group 4, 1.2±1.1 g/rat in group 5, 1.8±1.5 g/rat in group 6 and 0.9±0.3 g/rat in group 7 (means±SD). In group 4, which received intravesical instillation of physiological saline, bladder weight was significantly higher than that in groups 1 or 7 ($p<0.05$). No significant differences were detected among the other groups, but in groups 2, 3, 5 and 6, bladder weight was almost normal or increased. Bladder weight in groups 2 and 3 were higher than in groups 5 and 6.

Table 1. Findings in urinary bladder of F344 rats

Group	Treatment	Effective No. of rats	Bladder weight means±SD(g)	Rat body weight means±SD(g)
1	BBN	10	1.2±0.8	203.0±9.1
2	BBN+ADM	12	1.4±1.3	195.6±12.3
3	BBN+CDDP	12	2.1±1.5	198.7±7.1
4	BBN+Saline	3	3.4±1.0*	196.0±4.3
5	BBN+ADM	10	1.2±1.1	195.8±12.0
6	BBN+CDDP	11	1.8±1.5	198.1±7.5
7	BBN+Saline	3	0.9±0.3	199.3±5.2

* Statistically significant difference from Group 1 and 7 ($P<0.05$)

Histological findings (Table 2): Bladder lesions were classified according to the classification of Fukushima et al.[5] and General Rules for Clinical and Pathological Studies on Bladder Cancer[6]. Nodular hyperplasia without development of cancer was observed in only 1 rat in group 3 and all other rats showed transitional cell carcinomas. Transitional cell carcinoma with squamous cell carcinoma was noted in one case each of groups 5, 6 and 7 which all received intermittent instillation. Low stage (pTa, pT1) carcinoma was seen in groups 2, 4, 6 and 7, and low and high stage carcinomas were seen in groups 1,

3 and 5. Especially in group 5, pT3 was seen in one rat and pT4 in two rats, but no significant differences were seen between any two groups. Grade 2 was the most common type in all groups, but grade 1 was noted in 2 of the 12 rats which had received continuous intravesical instillation of ADM. Development of tumors other than those in the urinary bladder could not be detected, and tumors were not found in the lung, liver or kidney of the rats in group 1 histologically.

Table 2. Histological stage and grade of bladder cancer

Group	No	Stage					Grade		
		pTa	pT_1	pT_2	pT_3	pT_4	G1	G2	G3
1	10	2	7	1	0	0	0	8	2
2	12	2	10	0	0	0	2	10	0
3	11	0	9	1	0	1	0	10	1
4	3	0	3	0	0	0	0	3	0
5	10	0	6	1	1	2	0	8	2
6	11	1	10	0	0	0	0	10	1
7	4	0	3	0	1	0	0	2	2

DISCUSSION

Since Druckrey et al.[7] discovered that BBN has selective carcinogenicity for the urinary bladder, studies of experimental bladder tumors have been done extensively. When 0.05% BBN is administered mixed into drinking water for more than 12 weeks, tumor development occurs in almost 100% of the animals[8]. Histologically, 95.1% of the tumors are transitional cell carcinoma[5], and bladder tumors induced by BBN have become good experimental models for human bladder tumors.

A 40~70% recurrence of tumor is noted after therapy against superficial bladder tumors[2]. The possible causes are: 1) multifocal character, 2) viable tumor cells are implanted in bladder epithelium during the disturbance at the time of TUR, 3) TUR is incomplete, and 4) carcinogen exists in urine[9-11]. Clinically, prophylactic intravesical instillation of antitumor drugs has been performed in an attempt to inhibit these recurrences, and effective results have been reported[1-2]. We wanted to determine whether the intravesical instillation of ADM or CDDP inhibited or promoted the development of bladder tumors in rats under the carcinogenic circumstance of BBN administration. Bladder weight significantly increased in group 4 (physiological saline instillation) compared with the control group and group 7 (intermittent type), and no significant difference was noted in the other groups. In groups 2, 3, 5 and 6 the bladder weight was almost normal or increased. Histologically, high-stage was seen in groups 3 and 5 more than in control group 1 and low grade was observed more often in group 2 than in the other groups. In group 3 (CDDP instillation) nodular hyperplasia was observed in only one rat. These results suggest that physiological saline had promoting activity, and ADM and CDDP had both inhibitory and promoting activities in bladder carcinogenesis.

Akaza et al.[12] reported that bladder tumors indistinguishable morphologically from papillary transitional cell carcinoma were induced by noncarcinogenic substances, such as physiological saline and distilled water by continuous intravesical instillation using an osmotic mini-pump. Okajima et al.[13] reported that intravesical foreign matter had a promoting effect in carcinogenesis of bladder. Akaza et al.[14] reported that nodular or papillary hyperplasia was produced by transurethral fulguration from bladder mucosa of normal rats. We conclude that intravesical instillation itself produced the disturbances of the bladder epithelium and possibly promote the carcinogenesis of bladder under carcinogenic circumstances. Therefore, clinically intravesical instillation therapy should not be performed forcibly.

Ohtani et al.[3-4] performed intravesical instillation of ADM and MMC for a total of 12 times in rats after the administration of 0.05% BBN for 4 weeks, and found more rapid development of cancer in ADM and MMC groups than the group treated with physiological saline. They considered that ADM and MMC promoted the bladder carcinogenesis of rats. Our findings obtained using an experimental method different from theirs, suggested

that ADM and CDDP had both inhibitory and promoting activities in bladder carcinogenesis.

Inhibitory effects of tumor development were not shown between groups 2 and 5 and groups 3 and 6, but bladder weights in groups 2 and 5 were higher than in groups 3 and 6. A significant difference in bladder weight was noted between continuous and intermittent instillation of physiological saline group. Our experiments suggest that continuous intravesical instillation did more damage to the bladder epithelium and promoted carcinogenesis. Therefore, we conclude that intravesical instillation intermittently is more preferable to inhibit tumor recurrence clinically.

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和文抄録

ラットＢＢＮ膀胱腫瘍発生に対する抗腫瘍剤の効果について

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F334 ラット115匹に0.05% BBN を20週間経口投与し，同時に ADM, CDDP, 生食水の膀胱内注入療法を5週毎に5回連続群（type A, 計20回）と週1回群（type B, 計20回）に分け施行した．コントロール群は BBN 投与のみとした．40週後に屠殺し検討した．

Type A の生食群はコントロール群および type B の生食群に比較し有意に膀胱腫瘍重量が増加していた．Type A の ADM, CDDP, 膀注群は type B の同群より膀胱腫瘍重量が増加していた．ADM, CDDP, 群は2つの type でコントロール群に比較し膀胱腫重瘍量の減少した例と増加した例が見られた．組織学的に各群間に差はなかったが，type A の ADM 群に high stage 例が多く見られた．

以上より膀胱腫瘍発生に対し，生食水は promote 作用を，ADM および CDDP は抑制および promote 作用を有することが示唆された．また再発予防のための膀胱内注入療法はやみくもに施行すべきでないと思われた．

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