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Effects of intravesical instillation of antitumor drugs on the induction of preneoplastic bladder lesions in rats

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Summary. *The effects of adriamycin (ADR) and mitomycin C (MMC) as inhibitors of the development of bladder tumors in rats were studied. Six-week-old female F344 rats were divided into nine groups, five of which received 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) in their drinking water for the first 4 weeks, no treatment for 1 week, and then intravesical instillation once a week of ADR, MMC, or physiological saline or no instillation (no catheterization) for 12 weeks. The other four groups received no BBN for the first 5 weeks of the experiment and then received ADR, MMC, or physiological saline as above for 12 weeks. The bladders were examined by light microscopy 17 weeks after the beginning of the experiment. Results showed that development of preneoplastic lesions induced in the bladders of the rats by BBN was stimulated by subsequent instillation of ADR or MMC. This result suggests that ADR and MMC have promoting activities in bladder carcinogenesis of rats.*

Introduction

Superficial bladder tumors frequently recur [7, 8, 10] and transurethral resection often fails to control their recurrence. Therefore, intravesical instillation of various chemotherapeutic drugs has become a common treatment aimed at inhibition of recurrence [4, 12].

In the present study, the effects of intravesical instillation of adriamycin (ADR) and mitomycin (MMC), which are widely used clinically, on the development of bladder tumors induced in rats by 0.05% *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN) were examined.

Materials and methods

Chemicals. BBN (Izumi Chemical Co., Yokohama) was administered in drinking water at a dosage of 0.05%. ADR and MMC were kindly supplied by Farmitalia Carlo Erba Co., Ltd, Tokyo and Kyowa Hakko Co., Ltd, Tokyo, Japan, respectively, and were administered intravesically through a catheter.

Animals and experimental design. One hundred twenty-four female Fischer 344 rats (Charles River Japan, Inc., Tokyo, Japan) were divided into nine groups (Fig. 1). Five groups received 0.05% BBN in their drinking water for 4 weeks, no treatment for 1 week, and then an intravesical instillation once a week of 0.3 or 0.15 mg/0.3 ml of ADR, 0.15 mg/0.3 ml of MMC, 0.3 ml physiological saline, or no instaillation (no catheterization) for 12 weeks. The other four groups (groups 6–9) received no BBN for the first 5 weeks and then ADR, MMC, or physiological saline as above for 12 weeks. Before and 1 h after instillation, the bladder contents were emptied by light abdominal massage under nembutal anesthesia, so that the instilled compounds were retained for 1 h. Rats were killed at the beginning of instillation or 1 week after the end of instillation, namely in week 5 or 17 of the experiment.

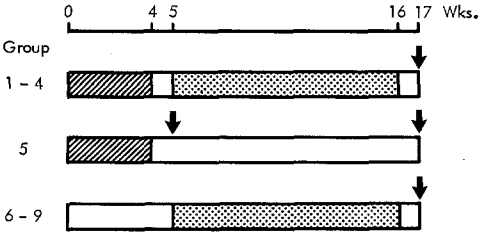

Fig. 1. Experimental design for examining the effects of intravesical instillation of antitumor agents on bladder carcinogenesis in rats. (▨) 0.05% BBN in drinking water; (⊡) intravesical instillation, once a week for 12 weeks, of ADR (1 mg or 0.5 mg/ml), MMC (0.5 mg/ml), or saline (↓) sacrifice

Histological observation. A solution of formalin in 10% phosphate buffer (pH 7.4) was injected into the urinary bladder through the urethra. Then the bladder was removed and fixed in the same solution. Each bladder was cut into 12 serial sections, which were stained with hematoxylin and eosin. Bladder lesions were classified as described previously [3, 6, 9]. The number of these bladder lesions was counted in each section and the length of bladder basement membrane was measured with a color video image processor, model IVP-21C, Olympus-Ikegami Tsushin Co., Tokyo, Japan. The numbers of different types of bladder lesions (papillary or nodular hyperplasia, hereinafter referred to as PN, or papilloma) (Fig. 2) were counted by light microscopy, and the number per 10 cm of basement membrane in each group was calculated.

Results

All groups receiving instillation showed growth retardation during the instillation period.

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Table 1. Findings in urinary bladder at 17 weeks

Group	Treatment	No. of rats	Papillary or nodular hyperplasia		Papilloma		Cancer
			Incidence (%)	No./10 cm BM[a]	Incidence (%)	No./10 cm BM	Incidence (%)
1	BBN → ADR (1 mg/ml)	12	12 (100)[c]	9.4 ± 8.1[b, d]	3 (25.0)	0.4 ± 0.7	0 –
2	BBN → ADR (0.5 mg/ml)	12	10 (83.3)[e]	5.0 ± 6.4[f]	1 (8.3)	0.3 ± 1.3	0 –
3	BBN → MMC (0.5 mg/ml)	12	6 (50)[g]	1.5 ± 2.2	1 (8.3)	0.2 ± 0.7	1 (8.3)
4	BBN → Saline	12	1 (8.3)	0.3 ± 0.9	0 –	0	0 –
5	BBN → –	12	0 –	0	0 –	0	0 –
6	– → ADR (1 mg/ml)	12	1 (8.3)	0.3 ± 1.0	0 –	0	0 –
7	– → ADR (0.5 mg/ml)	10	0 –	0	0 –	0	0 –
8	– → MMC (0.5 mg/ml)	10	0 –	0	0 –	0	0 –
9	– → Saline	12	0 –	0	0 –	0	0 –

[a] BM, basement membrane
[b] Means ± SD
Values for the following groups were significantly different: groups 1 and 4, [c]$P < 0.001$, [d]$P < 0.01$; groups 2 and 4, [e]$P < 0.001$, [f]$P < 0.05$; groups 3 and 4, [g]$P < 0.05$

a

b

Fig. 2a and b. Bladder lesions: **a** papillary or nodular hyperplasia; **b** papilloma

In week 5, that is just before instillation of chemotherapeutic agents into the bladder, 20 rats that had received BBN were examined. Simple hyperplasia was seen in all and PN hyperplasia in three rats, but no rat had papilloma or cancer.

The findings in the urinary bladder in each group at week 17, that is 1 week after the end of instillation of drugs, are summarized in Table 1. Microscopically simple hyperplasia was found in all rats in the BBN-treated groups. In each of groups 4 and 5 only one rat had PN hyperplasia, and none had papilloma or cancer. Both the incidence and number of PN hyperplastic lesions were significantly higher in rats given ADR at either dose than in rats given saline only (group 4). The incidence of PN hyperplasia was significantly higher in rats given MMC (group 3) than in those given saline (group 4), but the number of PN hyperplasia/10 cm of basement membrane was not significantly different in groups 3 and 4. In BBN-pretreated groups, instillation of ADR or MMC caused papilloma or cancer in a few rats, but no rat had papilloma or cancer in groups 4 or 5, which were given saline by instillation or no instillation. A few rats given ADR or MMC without BBN pretreatment (groups 6, 7, and 8) had simple hyperplasia, and instillation of a high dose of ADR alone induced PN hyperplasia in only one rat. No rats in any of the groups not treated with BBN had papilloma or cancer.

Discussion

Many antitumor drugs have been used clinically for intravesical instillation to eradicate superficial bladder tumors or inhibit their recurrence [4, 12]. Intravesical instillation of ADR or MMC has been reported to reduce the recurrence rate of tumors after transurethral resection [1, 5, 11, 13]. However, in animal experiments, the prophylactic effects of intravesical instillation of ADR, MMC, or thio-TEPA was not clear [14].

PN hyperplasia of the bladder in rats treated with carcinogens develops before the induction of papilloma and cancer, and there is a good correlation between the induction of PN hyperplasia and cancer. It is clear that PN hyperplasia is a preneoplastic lesion of the rat bladder [2, 3, 6]. The present short-term experiment was performed to evaluate the effects of intravesical instillation of ADR and MMC on bladder carcinogenesis in rats: the effects of these chemicals on the induction of the preneoplastic lesions rather than on advanced stages of bladder carcinogenesis were examined.

We anticipated that ADR and MMC would inhibit bladder carcinogenesis. Unexpectedly, however, we found that intravesical instillation of ADR at a high dose or of MMC significantly increased the incidence and the number of preneoplastic lesions of the bladder. Moreover, treatment with

ADR at a lower dose significantly increased the incidence of preneoplastic lesions. These results indicated that under these conditions ADR and MMC were promoters of bladder carcinogenesis. These results were not consistent with those in humans. Further studies on this discrepancy are required and long-term follow-up studies are required to clarify the effects of intravesical instillation of these chemotherapeutic agents in humans and animals. An experiment in rats with longer observation periods after the end of instillations of ADR and MMC is in progress.

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