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Promoting Effect of Unilateral Nephrectomy on Urinary Bladder Carcinogenesis in Rats

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The effect of unilateral nephrectomy on the growth of bladder tumors was investigated in male F344 rats treated with 0.05% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine(BBN) for 6 weeks by means of macroscopical and microscopical examinations. Unilateral nephrectomy 4 weeks after the initiation of BBN administration significantly increased both the number and the volume of visible tumors per rat as compared with the sham-operated group. Histological examination also revealed that the numbers of papillonodular hyperplasia and cancer per basement membrane were significantly increased by unilateral nephrectomy. The results imply a growth-enhancing effect of unilateral nephrectomy on carcinogenesis of the rat bladder.

Key words: Unilateral nephrectomy — Bladder cancer — Chemical carcinogenesis — Promoter

Proliferation of the bladder epithelium in rats is enhanced after unilateral ureteric ligation[1] or renal ischemia.[2] Although the exact mechanism of this proliferative response is unknown, we considered that unilateral nephrectomy might enhance bladder carcinogenesis.

Unilateral nephrectomy is one of the treatments clinically performed for transitional cell carcinoma of the upper urinary tract. Tumor development in the bladder after such treatment is frequently observed.[3] But there have been surprisingly few definitive investigations on the effect of unilateral nephrectomy on bladder carcinogenesis. In the present study, we evaluated the effect of unilateral nephrectomy on chemical carcinogenesis of rat urinary bladder.

MATERIALS AND METHODS

Chemicals and animals BBN was obtained from Nacalai Tesque Inc., Kyoto, and was given as a 0.05% solution in the drinking water. A total of 60 male six-week-old F344 rats (CLEA Japan, Inc.,Tokyo) was used. Two or three rats were housed in each plastic disposable cage. Rats were given conventional diet and water *ad libitum* throughout the experiment. They were kept under standard laboratory conditions at 24°C and 60% relative humidity, with 12 h of artificial light during the daytime and in the dark for the remaining time in the experimental animal laboratory of Fukui Medical School.

Experimental protocol The rats were divided into 4 groups as shown in Fig. 1. Groups 1 (22 rats) and 2 (20 rats) were given drinking water containing 0.05% BBN for the first 6 weeks. Groups 3 (6 rats) and 4 (8 rats) were given no carcinogen. At 4 weeks after the start of the experiment, the animals in groups 1 and 3 were anesthetized with ether. A flank incision was made, and the left kidney was exposed. The left renal pedicle was ligated with silk, and the kidney was removed. The animals in group 2 underwent sham operation with similar anesthesia and incision. No surgical procedure was done for the rats in group 4. The total observation period was 24 weeks. At the end of the experiment all the animals were killed and the bladders were removed. The urinary bladder was inflated by intraluminal injection of 10% phosphate-buffered formalin solution and divided along the midline in the sagital direction after fixation. For macroscopic quantitative analysis, the number and size of visible tumors were evaluated under a stereo microscope. Tumor volume was calculated from the lengths on the longest and shortest axes, on the assumption that a tumor is an elliptical mass. The bladder was then cut into 8 longitudinal strips and stained with hematoxylin and eosin for histological examination. For microscopic quantitative analysis, urinary bladder lesions were counted by light microscopy, the total length of the basement membrane was measured with a color video image processor, and the numbers of lesions were expressed per 10 cm of basement membrane.

Data evaluation Data are expressed as the mean±SD. Statistical comparisons were performed using Welch's *t* test.

RESULTS

Data on water consumption and *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) intake are given in Table I. There was no difference in average water consumption

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or BBN intake between groups 1 and 2 before or after operation.

Visible tumors were noted in the bladder of the rats in groups 1 and 2 (Fig. 2), while no macroscopic change was observed in groups 3 and 4. The number and volume of tumors per one bladder were significantly higher in group 1 (2-fold and approximately 10-fold respectively) than in group 2 ($P<0.005$, Table II).

Microscopical examination confirmed that all the visible tumors in groups 1 and 2 were low-grade, non-invasive, transitional cell carcinomas. Preneoplastic lesions, simple hyperplasia and papillonodular hyperplasia (PN hyperplasia) were also seen. Although the incidence of tumor was not statistically different between groups 1 and 2, the number of lesions of PN hyperplasia and cancer per 10 cm of basement membrane was significantly increased in group 1 ($P<0.005$, Table III). No significant pathological change was observed in groups 3 and 4.

DISCUSSION

In the present study, unilateral nephrectomy significantly increased the number and volume of bladder tumors as compared with those of sham-operated rats. These findings raise the possibility that unilateral nephrectomy itself enhances proliferation of tumor cells in the bladder.

Cell division in the epithelium of the urinary tract is comparatively infrequent, but bladder epithelial cells are

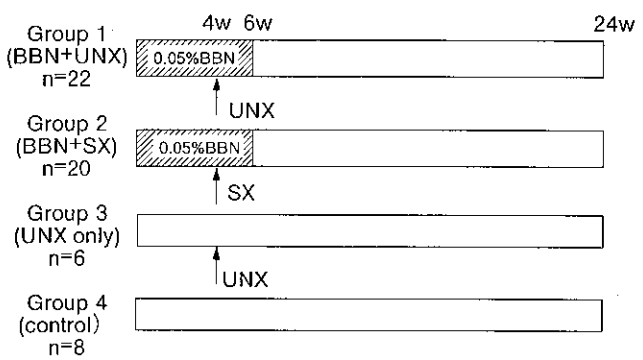

Fig. 1. Experimental design. UNX, unilateral nephrectomy; SX, sham operation.

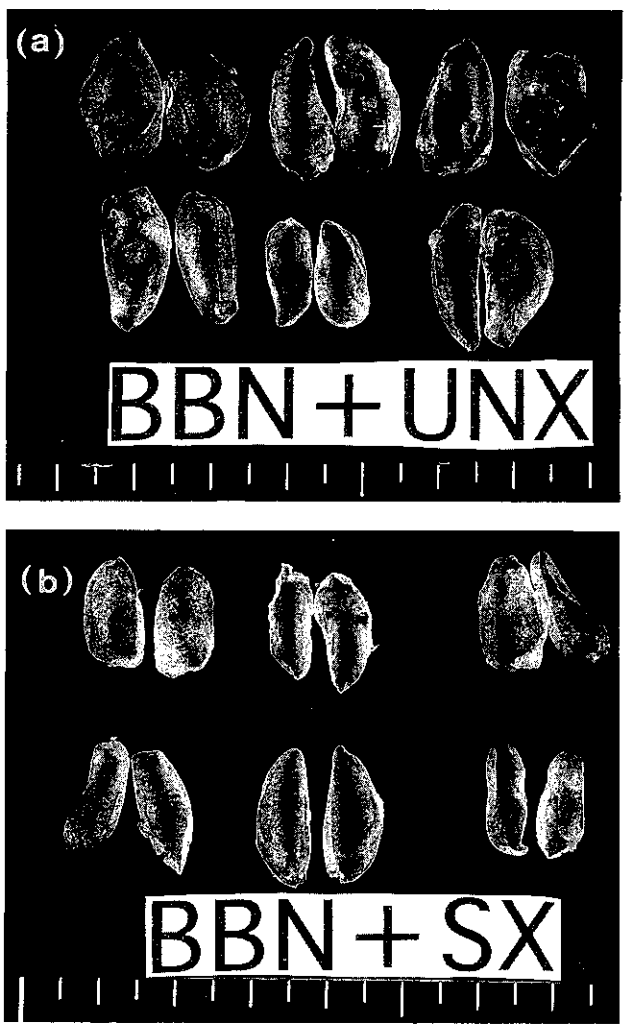

Fig. 2. Macroscopic appearance of bladder tumors in group 1(a) and group 2(b).

Table I. Water Consumption and BBN Intake

Group	Treatment	No. of rats	Water consumption (ml/rat/day)		Total BBN intake (mg/rat)
			preoperation (0–4 weeks)	postoperation (4–6 weeks)	
1	BBN+NX	22	24.8	23.1	508.9
2	BBN+SX	20	25.0	23.8	516.6
3	NX only	6	24.8	23.0	0
4	No treatment	8	24.5	24.6	0

Table II. Macroscopic Changes in the Urinary Bladder

Group	Treatment	No. of rats	No. of rats with visible tumor	No. of visible tumors per one bladder	Tumor volume per one bladder (mm^3)
1	BBN+NX	22	22 (100)[a]	7.3±3.2[b,c]	29.0±29.1[c]
2	BBN+SX	20	18 (90)	3.8±2.3	2.4±2.6
3	NX only	6	0 (0)	0	0
4	No treatment	8	0 (0)	0	0

a) Numbers in parentheses, percentage.
b) Mean±SD.
c) Significantly different from group 2 at $P<0.005$.

Table III. Microscopic Changes in the Urinary Bladder

Group	Treatment	No. of rats	Simple hyperplasia incidence	PN hyperplasia		Cancer	
				Incidence	No./10 cm of BM[a]	Incidence	No./10 cm of BM
1	BBN+NX	22	22 (100)[b]	22 (100)	5.6±4.2[c,d]	22 (100)	3.5±1.8[d]
2	BBN+SX	20	20 (100)	20 (100)	1.9±1.4	20 (95)	1.5±0.8
3	NX only	6	0 (0)	0 (0)	0	0 (0)	0
4	No treatment	8	0 (0)	0 (0)	0	0 (0)	0

a) BM, basement membrane.
b) Numbers in parentheses, percentage.
c) Mean±SD.
d) Significantly different from group 2 at $P<0.005$.

known to have potential for rapid and substantial proliferation after various stimuli.[1,2,4] We have conducted a short experiment to examine the proliferative effects of unilateral nephrectomy on the bladder urothelium using proliferative cell nuclear antigen (PCNA) immunohistochemical staining. Sequential PCNA labeling indices increased to a peak 2 days following unilateral nephrectomy and decreased gradually until loss of PCNA staining after 56 days (manuscript submitted). Although the mechanism of this proliferative response in the bladder epithelium is unknown, it is considered that unilateral nephrectomy enhances bladder carcinogenesis.

One possible explanation is that some factor(s) with growth-promoting activity were produced after renal damage and acted as mitogens on bladder epithelial cells. Recently, the presence and role of renotropic factors after unilateral nephrectomy or acute renal failure have been extensively studied.[5,6] Unilateral nephrectomy usually results in tubular cell proliferation of the contralateral kidney.[7] Growth factors, such as EGF,[8] IGF-1,[9] TGF-β[10] and HGF[11] have been considered to be renotropic factors which play an important role in the compensatory hypertrophy of the kidney. One or more of these renotropic factors may also act as a proliferative stimulus on bladder epithelium after unilateral nephrectomy. In addition to these possible renotropic factors, KGF directly enhances the growth of urothelium.[12] It is also reported that unilateral nephrectomy causes rapid and striking proliferation of xenografted Wistar/Furth Wilms' tumor.[13]

Clinically, the development of bladder cancer is frequently observed after unilateral nephrectomy for transitional cell carcinoma of the upper urinary tract.[3] It is tempting to speculate that nephrectomy itself has some enhancing effect on bladder carcinogenesis in human, as well as in rats. Further study is needed to evaluate the mechanism of the influence of nephrectomy on bladder carcinogenesis in both experimental and clinical settings.

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