

Effects of Foreign Bodies on Development of Urinary Bladder Tumors in Rats Treated with N-Butyl-N-(4-Hydroxybutyl) Nitrosamine

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Summary. The effects of chronic mechanical stimulation induced by glass balls or paraffin balls on precursor lesions of the urinary bladder of male Wistar strain rats induced by N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) in the drinking water were studied.

When foreign bodies were present in the bladder, proliferative lesions of the urinary bladder epithelium were found even without administration of carcinogen. When the foreign bodies were implanted into the urinary bladder and then rats were given BBN in the drinking water for the first 4 weeks of the experimental period, a high in-

cidence of tumors of the urinary bladder was observed. The incidence of tumors induced by BBN was increased by the presence of glass balls or paraffin balls in the urinary bladder.

These results clearly show that foreign bodies in the urinary bladder have a significant effect in promoting carcinogenesis of the urinary bladder in rats.

Key words: Rat urinary bladder cancer, promoting effect, foreign bodies, N-butyl-N-(4-hydroxybutyl) nitrosamine.

In 1964, Druckrey et al. reported that when rats were given N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) continuously in the drinking water for about 40 weeks, they all developed urinary bladder tumors (6). Recently, we reported on the histogenesis of urinary bladder tumors in rats induced by 0.05% BBN in the drinking water (9). We found that when rats were given 0.05% BBN in the drinking water for 4 weeks and then water without BBN for 16 weeks, hyperplasia of the bladder epithelium developed in 6 of 11 rats (54.6%) and papilloma in only one (9.1%). Control rats did not develop papilloma or cancer of the urinary bladder.

It is well known that when foreign bodies are implanted in the urinary bladder of rodents, the mucosa of the bladder develops proliferative lesions, such as hyperplasia or neoplasia of the epithelium (1, 3, 4, 5, 11, 12).

Therefore, it is of interest to examine how vesicular foreign bodies influence precursor lesions of the urinary bladder in rats treated with BBN.

This paper reports results on the effects of vesicular foreign bodies implanted in the urinary

bladder of rats on hyperplasia of the bladder epithelium induced by BBN.

Material and Methods

Wistar strain male rats (Fuji Animal Farm, Tokyo) weighing 140-170 g, were divided into six groups as shown below. The carcinogen, BBN (Izumi Chemical Co., Yokohama) was given as a 0.05% solution in the drinking water. All rats received commercial stock diet (Oriental MF, Oriental Yeast Co., Osaka) and water ad libitum.

The foreign bodies used were paraffin balls and glass balls. The glass balls were smooth and were 3 mm in diameter and 50 mg \pm 3 mg in weight. The paraffin balls were made by dropping hot liquid paraffin (m.p. 56.5°C, Fisher Scientific Co., USA) into cold water and selecting those which were smooth balls of 3 mm diameter and 15 mg \pm 2 mg weighing. The foreign bodies were sterilized by immersion for 60 min in 0.3% Hibitane solution. After sterilization they were washed with sterilized saline before use. The

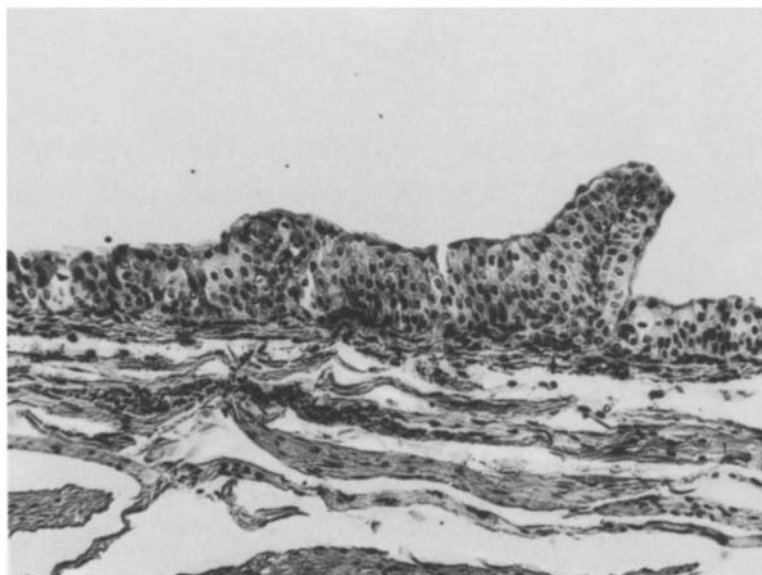


Fig. 1. Hyperplasia and slight papillomatous growth of bladder epithelium in a rat after glass ball implantation and BBN treatment for 4 weeks. H-E. X100

technique for implantation of foreign bodies was as described originally by Jull (11). A cystotomy was made through a low abdominal midline incision under ether anaesthesia. A foreign body was inserted through a small incision in the fundus and the bladder was closed by a single silk ligature. The abdominal wall was then closed in two layers with interrupted silk suture.

Group 1. Twelve rats were given drinking water supplemented with BBN for 4 weeks and then water without BBN for 16 weeks.

Group 2. Twelve rats were implanted with glass balls. Then, they were given drinking water without BBN for 20 weeks.

Group 3. Twelve rats were implanted with paraffin balls. Then, they were given drinking water without BBN for 20 weeks.

Group 4. Twelve rats were implanted with glass balls. They were given drinking water with BBN for 4 weeks and then water without BBN for 16 weeks.

Group 5. Twelve rats were implanted with paraffin balls and given drinking water with BBN for 4 weeks and then water without BBN for 16 weeks.

Group 6. Twelve rats were given drinking water without BBN for 20 weeks.

Rats were housed three to a cage in wire cages in an air conditioned room at 24°C, and weighed once a week. Animals that died within 18 weeks of the start of the experiment were excluded.

At the end of the experiment animals were sacrificed with ether and carefully examined. The carcass, liver, both kidneys and spleen were weighed and samples were taken for histological studies. The urinary bladder was punctured at the



Fig. 2. Transitional cell carcinoma of bladder epithelium in a rat after glass ball implantation and BBN treatment for 4 weeks. H-E. X40

Table 1. Effect of glass balls and paraffin balls in the urinary bladder of rats given 0.05% BBN in the drinking water for 4 weeks and then water only for 16 weeks

Group	Treatment	No. of Rats	Incidence of changes in urinary bladder (%)						
			Calculi	Hyperplasia	Papilloma	Cancer	Total Tumors	Invasion	Metaplasia
1	BBN	12	0 -	7 (58.3)	2 (16.7)	0 -	2 (16.7)	0 -	0 -
2	G.B.	10	3 (30.0)	6 (60.0)	2 (20.0)	1 (10.0)	3 (30.0)	0 -	1 (10.0)
3	P.B.	11	7 (63.6)	8 (72.7)	6 (54.5)	2 (18.2)	8 (72.7)	2 (18.2)	4 (36.4)
4	BBN + G.B.	8	4 (50.0)	8 (100.0)	4 (50.0)	3 (37.5)	7 (87.5)	3 (37.5)	1 (12.5)
5	BBN + P.B.	12	7 (58.3)	12 (100.0)	3 (25.0)	9 (75.0)	12 (100.0)	8 (66.7)	6 (50.0)
6	Control	12	0 -	0 -	0 -	0 -	0 -	0 -	0 -

G.B.: Glass ball implanted into the urinary bladder

P.B.: Paraffin ball implanted into the urinary bladder



Fig. 3. Well differentiated squamous cell carcinoma in bladder of a rat, showing many cancer pearls. H-E. X100

urethrovesical junction and 0.5 ml of 10% buffered formaldehyde solution was injected into the bladder. After fixation for 1 day, the bladder was carefully examined for the presence of calculi and tumors. All tissues were routinely stained with Hematoxylin and Eosin, van Gieson, PAS and Mallory stains. All animals which showed epithelial changes in the dome of the urinary bladder where the single silk ligature was inserted, were excluded from the study.

Results

The average body weight of all groups increased throughout the experiment and the body weight of test groups was similar to that of controls.

Findings in the Urinary Bladder

The incidence of calculi and tumors of the urinary bladder and histopathological findings in the urinary bladder are shown in Table 1.

The epithelial pattern was classified as follows: (1): normal, (2): diffuse or nodular hyperplasia, (3): papilloma, and (4): cancer. Microscopically, hyperplasia in most cases appeared as localized thickening of the bladder epithelium (Fig. 1). Papilloma consisted of extensive epithelial proliferation, with slight cellular irregularity and a few mitotic figures. Cancer was usually of the transitional cell type (Fig. 2). Squamous metaplasia (Fig. 3), mitotic figures and muscular invasion were noticed (Fig. 4). Hemorrhage and in-



Fig. 4. Infiltrative growth of cancer cells in muscular layer of the bladder wall of a rat after glass ball implantation and BBN treatment for 4 weeks. H-E. X100

H-E. = Hematoxylin and Eosin stain

flammatory changes in the stromal tissues were also seen in this type.

Group 1. Hyperplasia of the bladder epithelium in this group developed in 7 of 12 rats (58.3%). Papillomas developed in 2 of 12 rats (16.7%). No rats developed cancer of the urinary bladder. No concretions in the urinary bladder were found in this group.

Group 2. Concretions on the glass balls or free calculi in the urinary bladder were found in 3 of 10 rats (30.0%).

Hyperplasia of the bladder epithelium was seen in 6 of the 10 rats (60.0%) and papilloma in 2 (20.0%). Cancer was seen in only one (10.0%). No invasion of the bladder wall was seen.

Group 3. Concretions on the paraffin balls or free calculi in the urinary bladder were found in 7 of 11 rats (63.6%).

Hyperplasia of the bladder epithelium was seen in 8 of the 11 rats (72.7%), papilloma in 6 (54.5%) and cancer in 2 (18.2%). Two of the 11 rats (18.2%) showed muscular invasion of the urinary bladder wall by tumor while squamous metaplasia developed in 4 rats (36.4%).

Group 4. Concretions on the glass balls or free calculi in the urinary bladder were found in 4 of 8 rats (50.0%).

All the rats in this group developed hyperplasia of the bladder epithelium. Papilloma was found

in 4 rats (50.0%), and cancer in 3 (37.5%). Two of the rats (18.2%) showed muscular invasion of the bladder wall by tumor and squamous metaplasia developed in one rat (12.5%).

Group 5. Concretions on the paraffin balls or free calculi in the urinary bladder were seen in 7 of 12 rats (58.3%).

All the rats developed hyperplasia and tumors: 3 developed papilloma (25.0%) and 9 developed cancer (75.0%). Eight of the 12 rats (66.7%) showed muscular invasion of the bladder wall by tumor and 6 showed squamous metaplasia (50.0%).

Group 6. No rats developed hyperplasia or tumors of the bladder epithelium. No histological abnormalities were observed.

Other Organs

No marked gross or microscopic changes were seen in the liver, Kidney, or lung.

Discussion

The present experiments show that foreign bodies, such as glass balls and paraffin balls, when implanted into the urinary bladder of rats stimulate the epithelium. Paraffin balls were more stimulatory than glass balls, and more frequently induced concretions in the urinary bladder. After

implantation of paraffin balls into the urinary bladder, the incidence of proliferative or metaplastic lesions of the bladder epithelium was significantly higher than after implantation of glass balls. The incidence of proliferative epithelial lesions is known to increase in the presence of a foreign body in the urinary bladder of rats or mice (1, 3, 4, 5, 12). Bonser et al. (4) concluded that squamous metaplasia was more likely to be present, in the presence of a concretion but that there was no relation between the presence of concretions and development of malignant tumors. Previously we reported that from Japanese literature, it seemed that patients with squamous cell carcinoma of the renal pelvis show a very high incidence of calculi: namely calculi were present in thirty of 64 cases of squamous cell carcinoma of the renal pelvis (8). Many authors reported a similar high incidence of calculi in cases of squamous cell carcinoma of the renal pelvis (7, 13, 14). Thus it seems that proliferative and metaplastic lesions of urothelium may be induced by prolonged stimulation from calculi or associated infection.

The present work also showed that the incidence of precursor lesions was very low in the absence of concretions and high in the presence of concretions. These results suggest a positive relationship between concretions and proliferative epithelial lesions.

The carcinogenic action of BBN has been reported by many investigators in various animals (2, 6, 9, 10). Druckrey et al. reported that BBN has a specific, organotropic, carcinogenic action on the urinary bladder and induces a high incidence of cancer in the urinary bladder of rats (6). We previously reported that when rats were given 0.05% BBN for 16 weeks, precursor lesions of the epithelium developed in the urinary bladder (9). In this study, we obtained similar results. Namely, in group 1, treated with BBN only, hyperplasia of the epithelium developed in 7 of 12 rats (58.3%) and papilloma in 2 (16.7%) but no cancer was seen.

However, after implantation of foreign bodies and treatment with BBN for the first 4 weeks of the experimental period, a high incidence of tumors was observed. Furthermore the incidence of cancer was increased by the presence of either paraffin balls or glass balls in the urinary bladder.

These results clearly show that foreign bodies in the urinary bladder promote carcinogenesis of the bladder induced in rats by BBN. Thus, it seems likely that in man long-standing calculi of the urinary bladder may promote development of bladder tumors.

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