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Renal Pelvic Carcinoma which Shows Metastatic Potential to Distant Organs, Induced by N-Butyl-N-(4-hydroxybutyl)nitrosamine in NON/Shi Mice

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Renal pelvic carcinoma was induced in mice by giving N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN). Initially, differences in renal pelvic carcinogenesis by BBN were examined in three male mouse strains: NON/Shi, which demonstrate spontaneous hydronephrosis with incidences of 10–30%, and DS/Shi and B6C3F1, which do not exhibit hydronephrosis. When mice of these strains were given 0.05% BBN in the drinking water for 12 weeks followed by water without BBN for 8 weeks, renal pelvic carcinoma morphologically similar to human carcinomas developed in 8 of 23 NON/Shi mice (35%). Metastasis to the lung was found in one of them (13%). B6C3F1 and DS/Shi mice had no pelvic tumors, but the response to urinary bladder carcinogenesis in NON/Shi mice was nearly equal to that in DS/Shi and B6C3F1 mice. These results suggest that renal pelvic carcinogenesis is related to the presence of stagnant urine containing carcinogen in the renal pelvis. In a second experiment, we examined renal pelvic carcinogenesis in NON/Shi mice given BBN for 4 weeks followed by water without BBN for 32 weeks. The incidence of renal pelvic carcinoma (28%) was similar to that in the first experiment, but the incidence of metastasis was markedly elevated to 60%. These results indicate that BBN treatment can induce renal pelvic carcinoma which often metastasizes to the lung in NON/Shi mice.

Key words: NON/Shi mouse — Renal pelvic carcinoma — Metastasis

Of all kidney malignancies, renal pelvic tumors account for only a minority of cases.[1] Although there has been a growing number of reports of renal pelvic and ureteral neoplasms occurring in patients, possibly due to improved diagnosis, the difficulties in diagnosing the lesion at an early stage without metastases to multiple sites make these neoplasms still particularly pernicious.[2,3] However, an appropriate animal model of malignant renal pelvic cancer to investigate the process of tumor development, diagnosis, prognosis, and therapy, has not been available.

Attempts to induce neoplasms of the renal pelvis in rats and mice with carcinogens have met with little success.[4] Ito *et al.*[5] suggested that stagnation of urine containing a proximate carcinogen in the renal pelvis may be important in induction of tumors. Long contact of target cells with the carcinogen is probably necessary to induce tumors in the renal pelvis through the urine in experimental animals, and this could occur in certain conditions such as hydronephrosis. SD/cShi rats, maintained in our laboratories, have been observed to have hereditary hydronephrosis.[6] We successfully induced neoplasms of the renal pelvis in SD/cShi rats with oral administration of N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) in high incidence,[7] but these tumors did not metastasize.

We have observed spontaneous hydronephrosis in the right kidney in inbred NON/Shi (non-obese non-diabetic) mice in life- span studies, with incidences of 10–30% and a higher occurrence in males than in females (unpublished data). This mouse strain was derived simultaneously with the NOD mouse from a cataract-developing substrain of the outbred Jcl-ICR mouse by selective breeding from 1974 to 1980 in our laboratory,[8] and is now maintained in our laboratory. Mature male NON/Shi mice show impaired glucose tolerance but do not display diabetes, which NOD/Shi mice have.[9]

It was reported that urinary bladder carcinomas in all strains of rats induced by various carcinogens were of the papillary, pedunculated type and were usually multiple and superficial.[10–16] In contrast, the mouse urinary bladder epithelium shows flat growth in response to urinary bladder carcinogens, which becomes invasive cancer.[17–20] These observations suggest that urinary epithelial tumors induced by chemical carcinogens in mice behave more malignantly than do those in rats.

We, therefore, hypothesized that use of NON/Shi mice might make it possible to induce malignantly behaving carcinoma in the renal pelvis following oral adminis-

Abbreviations: BBN, N-buty-N-(4-hydroxybutyl)nitrosamine; PN hyperplasia, papillary and nodular hyperplasia.

tration of BBN. In this paper, we report the induction of renal pelvic carcinoma, which resembles malignant renal pelvic tumors in humans, by means of oral administration of BBN to NON/Shi mice. Since DS/Shi mice, which are an inbred strain of mice maintained in our laboratory, and B6C3F1 mice, which are usually utilized in long-term carcinogenicity tests, do not show spontaneous hydronephrosis, we used these mice as controls.

MATERIALS AND METHODS

Animals and chemicals Fifty-six NON/Shi (Aburahi Lab. of Shionogi Co., Shiga), 17 DS/Shi (Aburahi Lab. of Shionogi Co.), and 19 B6C3F1 (Charles River Japan, Inc., Atsugi) male mice, 5 weeks old, were obtained and given CA-1 diet (Clea Japan Inc., Tokyo) and water *ad libitum*. They were housed 5 mice per cage on dry chip bedding in an air-conditioned room at 25±1°C and 50±10% humidity with a 12-h light/dark cycle. BBN was obtained from Tokyo Kasei Kogyo Co., Ltd., Tokyo, and used at a concentration of 0.05% in the drinking water.

Experimental design Exp. 1: After 1 week of acclimatization, 23 NON/Shi, 17 DS/Shi, and 19 B6C3F1 mice were designated as Group 1, Group 2, and Group 3, respectively, and given drinking water with 0.05% BBN for 12 weeks and then water without BBN for 8 weeks, through experimental week 20.

Exp. 2: After 1 week of acclimatization, 33 NON/Shi mice were randomly divided into 2 groups of 18 and 15 mice, Group 1 and Group 2, respectively. In the first 4 weeks, mice in Group 1 were given drinking water with 0.05% BBN, and then they were given water without carcinogen for 32 weeks. Mice in Group 2 were used as intact controls, which were given water without BBN for the 36 weeks of the experiment.

Survivors in each group were anesthesized and killed at the end of the experimental period, and animals found dead or killed when moribund were dissected and macroscopically inspected. The kidneys and urinary bladders were fixed with 10% phosphate-buffered (pH 7.4) formalin. Tissues were embedded in paraffin and stained with hematoxylin and eosin for histological examination.

The epithelial lesions in the renal pelvis and urinary bladder were classified into four categories, simple hyperplasia, papillary and nodular (PN) hyperplasia, papilloma, and carcinoma. Classification of carcinomas was done according to Fukushima *et al.*[21] To distinguish carcinoma multicentricity, carcinoma-bearing mice were classified into categories according to a modification of the system described by Akaza *et al.*[22] Carcinomas arising only in one kidney are categorized as A, those in one kidney and in the urinary bladder are B, those in both kidneys with or without urinary bladder carcinoma are C, and those only in the urinary bladder are D.

Statistical analysis Statistical analysis of histopathological lesion incidences was done by using Fisher's exact probability test.

RESULTS

Exp. 1 The survival rates, average changes in body weights, and average BBN intakes of mice are summarized in Table I. In general, no remarkable changes were seen in the body weight or BBN intakes. Some mice in all groups which showed weight loss and piloerection died or were killed between experimental weeks 11 and 19, mainly due to the development of urinary tract tumors. The numbers of mice surviving through the experimental period of 20 weeks in groups 1–3 were 12/23 (52%), 11/17 (65%), and 16/19 (84%), respectively.

Yields of renal pelvic lesions are summarized in Table II. These lesions were seen only in NON/Shi mice. Eight mice (35%) developed carcinoma in the right kidney between 11 and 20 weeks after the experiment started. The mean induction time was 15 weeks. Five carcinomas (62%) were classified as transitional cell carcinomas (Fig. 1) and three (38%) as squamous cell carcinomas. The growth pattern of these carcinomas was of the non-papillary, invasive type. One of these (13%)

Table I. Survival Rates, BBN Intakes and Average Body Weights

Exp. No.	Group No.	Mouse strain	BBN treatment (weeks)		No. of mice		BBN intake (mg/kg/day)	Body weight (g)	
			+	−	start	final		start	final
1	1	NON/Shi	12	8	23	12	100	30±2	37±3
	2	DS/Shi	12	8	17	11	86	28±1	39±3
	3	B6C3F1	12	8	19	16	105	25±1	36±5
2	1	NON/Shi	4	32	18	13	121	30±2	39±3
	2	NON/Shi	0	36	15	15	−	29±2	42±3

Table II. Incidences of Renal Pelvic Lesions

Exp. No.	Group No.	Mouse strain	BBN	No. of mice examined	No. of mice with lesions (%)		
					Papilloma	Carcinoma	Metastasis[a]
1	1	NON/Shi	+	23	0	8 (35)	1 (13)
	2	DS/Shi	+	17	0	0	−
	3	B6C3F1	+	19	0	0	−
2	1	NON/Shi	+	18	1(6)	5 (28)	3 (60)
	2	NON/Shi	−	14	0	0	−

a) Number of mice with metastasis derived from renal pelvic carcinoma per number of mice with that carcinoma.

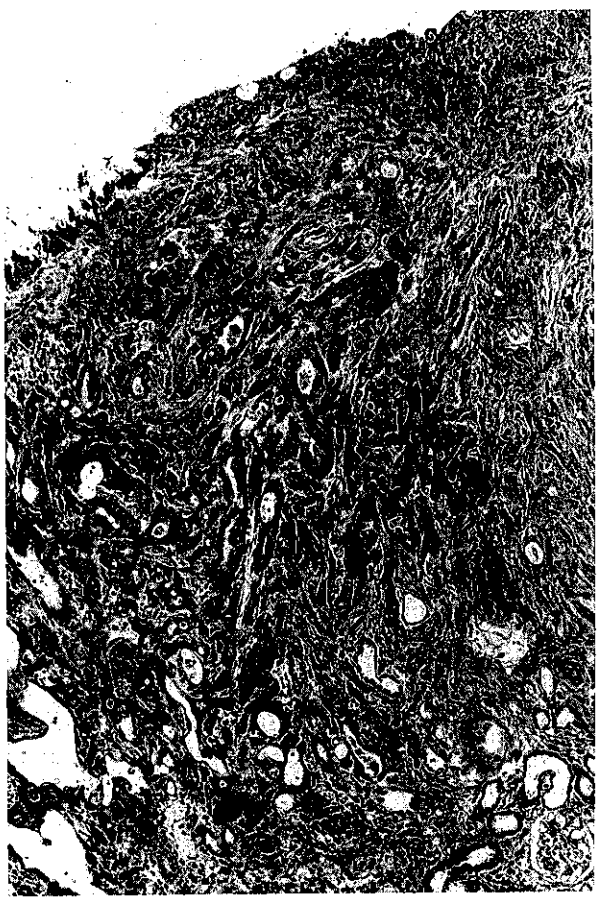

Fig. 1. Transitional cell carcinoma developing in renal pelvis. H&E, ×200.

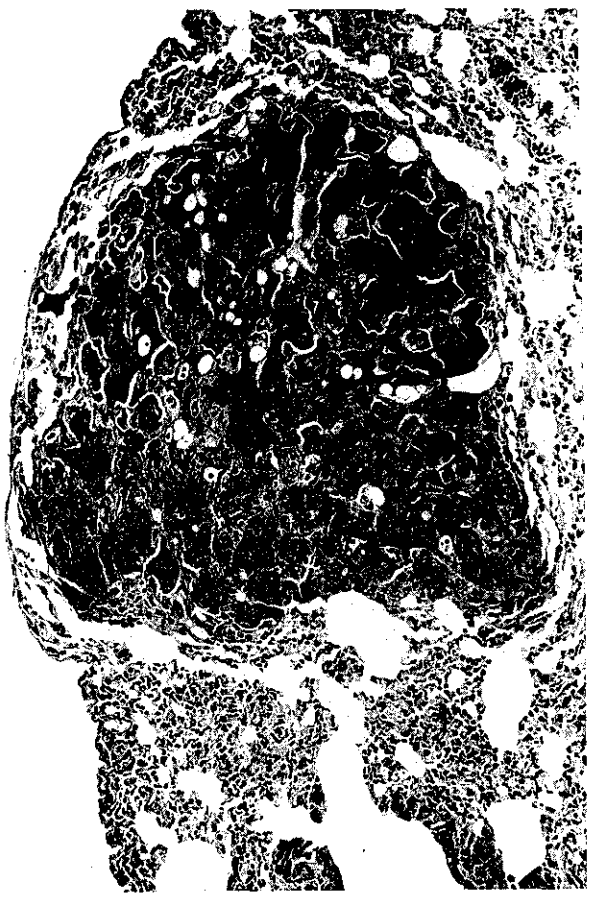

Fig. 2. Lung metastasis of a pelvic carcinoma. H&E, ×200.

metastasized to the lung (Fig. 2). Neither papilloma nor PN hyperplasia was observed. Simple hyperplasia was observed in one mouse (4%) at 20 weeks after the experiment started.

Urinary bladder lesions are shown in Table III. Carcinoma was found in 19 mice (83%) in Group 1, 16 (94%) in Group 2, and 18 (95%) in Group 3. The first carcinoma was found after 15 weeks in two NON/Shi mice, after 15 weeks in one DS/Shi mouse, and after 17 weeks in three B6C3F1 mice. The average induction time of urinary bladder carcinoma was 19 weeks in Groups 1 and 2, and 20 weeks in Group 3. Histologically, transitional cell carcinomas were dominant in Groups 1 and 2, and squamous cell carcinomas were dominant in Group 3. In

Table III. Incidences of Urinary Bladder Tumors in Mice

Exp. No.	Group No.	Mouse strain	BBN	No. of mice examined	No. of mice with lesions (%)		
					Papilloma	Carcinoma	Metastasis[a]
1	1	NON/Shi	+	23	0	19 (83)	1 (5)
	2	DS/Shi	+	17	0	16 (94)	1 (6)
	3	B6C3F1	+	19	0	18 (95)	2 (11)
2	1	NON/Shi	+	15	0	3 (20)	0
	2	NON/Shi	−	14	0	0	−

a) Number of mice with metastasis derived from urinary bladder carcinoma per number of mice with that carcinoma.

Table IV. Distributions by Category in NON/Shi Mouse

Exp. No.	No. of mice with carcinomas	A	B	C	D
1	22	3	5[a]	0	14[a]
2	7	4[b]	1	0	2

A: unilateral renal pelvic carcinoma alone; B: unilateral renal pelvic carcinoma and bladder carcinoma; C: bilateral renal pelvic carcinomas with or without bladder carcinoma; D: bladder carcinoma alone.
a) one and *b*) three of these metastasized to other organs.

all groups the urinary bladder carcinomas induced were of the non-papillary or papillary invasive type. Metastasis was found in one NON/Shi mouse (5%, Group 1), one DS/Shi mouse (6%, Group 2), and two B6C3F1 mice (11%, Group 3). Neither papilloma nor PN hyperplasia was seen in any mouse. Simple hyperplasia was found in 2, 1 and 1 mice, respectively. Thus, the incidences of urinary bladder lesions were not significantly different among the three strains.

Table IV indicates the distribution of renal pelvic and urinary bladder tumors of NON/Shi mice by category. Of 22 carcinoma-bearing mice, three had renal pelvic carcinoma alone (category A), five had multicentric carcinomas (category B) and fourteen had urinary bladder carcinoma alone (category D). No mouse was observed in category C. One mouse in category B had a metastasis which was thought to derive from the renal pelvic carcinoma because of the histopathological similarities between the primary and metastatic tumors.

The incidences of hydronephrosis are summarized in Table V. Hydronephrosis was found in 19 mice (83%) in Group 1, 13 (77%) in group 2, and 12 (63%) in Group 3. Left hydronephrosis was found only in 2 mice in Group 1, and these mice had urinary bladder carcinomas alone (category D). Right hydronephrosis was found in 9 mice (39%) in Group 1, 2 (12%) in Group 2, and 3 (16%) in Group 3. Three of these 9 mice in Group 1 had only renal pelvic carcinoma (category A) and one mouse in Group 1 had no carcinoma in the urinary tract (category N). Mice in Groups 2 and 3 had tumors only in the urinary bladder (category D). Bilateral hydronephrosis was found in 8 mice (35%) in Group 1, 11 (65%) in Group 2, and 9 (47%) in Group 3. Three of these 8 mice in Group 1 had both renal pelvic and urinary bladder carcinomas. Mice in Groups 2 and 3 had only urinary bladder carcinomas (category D). Hydronephrosis in mice in category B or D could be related to ureteral

Table V. Incidences of Hydronephrosis

Exp. No.	Group No.	Mouse strain	BBN	No. of mice examined	No. of mice with hydronephrosis (%)											
					total	left				right				bilateral			
						A	B	D	N	A	B	D	N	A	B	D	N
1	1	NON/Shi	+	23	19 (83)	0	1[a]	1	0	3	0	5	1	0	3	5	0
	2	DS/Shi	+	17	13 (77)	0	0	0	0	0	0	2	0	0	0	11	0
	3	B6C3F1	+	19	12 (63)	0	0	0	0	0	0	3	0	0	0	9	0
2	1	NON/Shi	+	18	2 (11)	0	0	0	0	0	0	0	1	0	0	1	0
	2	NON/Shi	−	14	2 (14)	0	0	0	0	0	0	0	2	0	0	0	0

A: unilateral renal pelvic carcinoma alone; B: unilateral renal pelvic carcinoma and bladder carcinoma; D: bladder carcinoma alone; N: no carcinoma.
a) Renal pelvic carcinoma was found in the right kidney.

obstruction by urinary bladder carcinomas, but hydronephrosis in mice in category A or N appeared to have arisen spontaneously.

Exp. 2 The survival rates, average changes in body weights, and average BBN intakes of mice are summarized in Table I. In general, no remarkable changes were seen in body weights and BBN intakes. Some mice in Group 1 died or were killed between 15 and 35 weeks due to the development of urinary tract tumors. The numbers of mice surviving through the experimental period of 36 weeks in groups 1 and 2 were 13/18 (72%) and 15/15 (100%), respectively.

The renal pelvic lesions observed are shown in Table II. Carcinoma was observed in five mice of Group 1. Four of these were observed after 15 to 21 weeks and one, after 35 weeks. The mean induction period was 21 weeks. Histologically all carcinomas were transitional cell carcinomas, and the growth pattern of these carcinomas was always of the non-papillary, invasive type. Three mice (60%) killed after 15 to 21 weeks developed metastasis to distant organs, including lung, vena cava, lymph nodes and abdominal organs such as liver and adrenal (Figs. 3 and 4). No carcinoma was observed in Group 2. Papilloma was observed in one mouse of Group 1. Simple hyperplasia was observed in two mice in each of Groups 1 and 2. PN hyperplasia was not observed in either group.

Urinary bladder lesions are listed in Table III. Carcinoma was observed in three mice (20%) in Group 1. Based on histologic type and growth pattern, these were all non-papillary, invasive transitional cell carcinomas. Neither papilloma nor PN hyperplasia was observed in Groups 1 or 2. Simple hyperplasia was observed in thirteen mice (87%) in Group 1 and two mice (14%) in Group 2.

Table IV indicates the distribution of tumors in NON/Shi mice by category. Of the 7 carcinoma-bearing mice, four were classified into category A, one into B, and two into D. No mouse was classified as C. Three mice in category A had metastases. Although the urinary bladder

Fig. 3. Liver metastasis of a pelvic carcinoma. H&E, ×200.

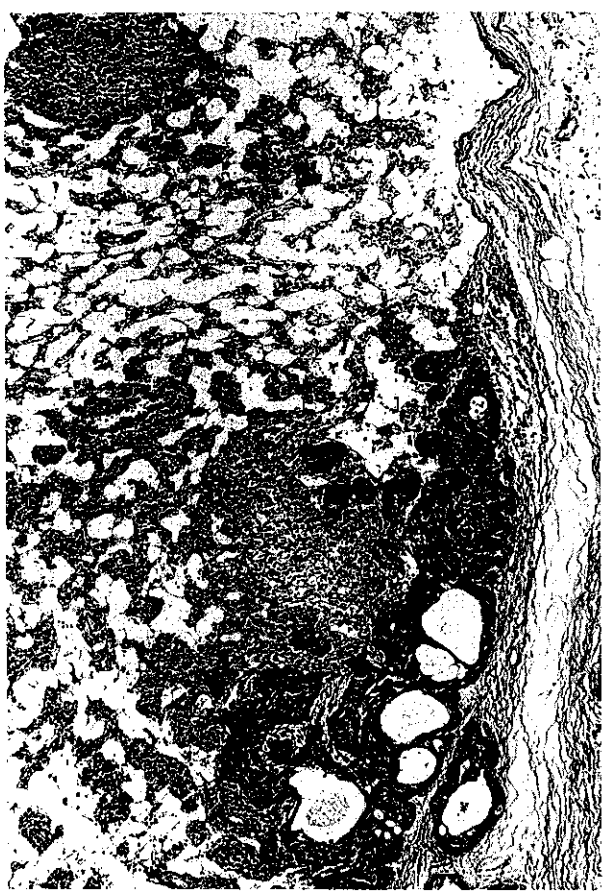

Fig. 4. Lymph node metastasis of a pelvic carcinoma. H&E, ×250.

was lost in these three mice due to extensive cannibalization, precluding examination, these metastases were thought to have been derived from the renal pelvic carcinomas because the first urinary bladder carcinoma did not occur until 15 weeks after the last renal pelvic carcinoma with metastasis was found.

The incidences of hydronephrosis are summarized in Table V. Hydronephrosis was found in 2 mice (11%) in Group 1 and 2 (14%) in Group 2. Left hydronephrosis was not found in either group. Right hydronephrosis was found in one mouse (6%) in Group 1 and two (14%) in Group 2, and these were thought to have occurred spontaneously because of the absence of urinary tract carcinoma. Bilateral hydronephrosis accompanied with urinary bladder carcinoma was found in one mouse in Group 1.

DISCUSSION

Ertürk *et al.*[23] suggested that renal pelvic tumors seemed to be related to a high degree of partial urinary obstruction which caused a significant incidence of hydronephrosis. Some investigators have suggested that prolonged exposure of the urothelium to a carcinogen might be related to renal pelvic tumorigenesis.[5, 24] Such a condition may be caused by hydronephrosis. We have presented evidence that hereditary hydronephrosis in SD/cShi rats may correlate with the induction of renal pelvic tumors by a chemical carcinogen, BBN.[6, 7] This was confirmed in the present study with mice. Of three strains of mice with similar susceptibility of the urinary bladder epithelium to BBN, only NON/Shi mice, which have hereditary hydronephrosis, developed renal pelvic carcinoma, while B6C3F1 and DS/Shi mice did not.

There is no significant difference in the incidences of renal pelvic carcinomas between experiments 1 and 2, while urinary bladder carcinomas showed lower incidences in experiment 2 than in experiment 1. These observations indicated that latent hydronephrosis in NON/Shi mice might induce continuous stagnation of urine containing the chemical carcinogen, while urination would prevent stagnation of urine in the urinary bladder, in contrast to the case of hereditary hydronephrosis. Considering this, in the urinary bladder or normal kidney total exposure time of the epithelium to a carcinogen may depend directly upon the total period of carcinogen administration.

Histopathologically, transitional cell carcinoma induced by BBN in the renal pelvis was observed in 75–100% of carcinomas of NON/Shi mice. These tumors were morphologically similar to the lesion most commonly seen in humans; 82% of renal pelvic tumors have been reported as transitional cell carcinomas, with 17% being squamous cell.[25] Their growth pattern was of the non-papillary, invasive type. The nature of this type of carcinoma in NON/Shi mice was similar to that in humans. Sixty per cent were metastatic tumors, and the prognosis is less favorable in patients with this type of tumor.[26] Therefore, NON/Shi mice may serve as a good experimental model for the study of renal pelvic carcinomas in humans.

One of the striking findings in experiment 2 was that renal pelvic carcinomas often metastasized to distant organs, especially to the lung, although the incidence of development of renal pelvic carcinomas was not high. In the case of mice with urinary bladder tumor, direct spread of cancerous tissue occurred predominantly within the abdominal cavity at lower incidence than in our renal pelvic carcinoma model, and a solitary metastatic focus to lung was only rarely seen.[18, 27] On the contrary, renal pelvic cancer seems to be apt to metastasize and also to form metastatic foci in distant organs such as lung. In addition, a recent study showed that metastasis was induced in 80% of NON/Shi mice with renal pelvic carcinoma by phenacetin treatment prior to BBN treatment (unpublished data). If a treatment regimen that yields metastatic renal pelvic carcinoma in nearly 100% of treated mice were to be established, this would become a good model not only for the experimental study of renal pelvic tumors in humans but also for the experimental study of metastasis.

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