Urinary Bladder Tumors Induced by \( N\)-Butyl-\( N\)-(4-hydroxybutyl)nitrosamine in Dogs\(^1\)

Eigoro Okajima,\(^2\) Tadashi Hiramatsu, Kazuya Hirao, Masumi Ijuin, Yoshihiko Hirao, Katsuhiro Babaya, Shoichiro Ikuma, Sochi Ohara, Tsutomu Shiomii, Takashi Hijioka, and Hajime Ohishi


ABSTRACT

Clinicopathological, radiological, and histological studies were performed on urinary bladder neoplasia induced by \( N\)-butyl-\( N\)-(4-hydroxybutyl)nitrosamine (BBN) in five adult beagle dogs and in ten adult mongrel dogs. Tumors of the urinary bladder developed in dogs given various daily doses of BBN p.o. for different periods. The latent period of tumor induction was 4 years in dogs receiving a daily dose of 80 mg of BBN, 2 to 2.5 years in dogs receiving a daily dose of 160 mg of BBN, and 1.5 years in dogs receiving a daily dose of 240 mg of BBN. The total dose of BBN ingested by the dogs until the first tumors were observed by urological examinations was nearly the same in all groups, 100 to 140 g. These results suggest that there is a correlation between dose and induction time, but further dose-response studies are required. Histologically, tumors of the urinary bladder were transitional cell papillomas or transitional cell carcinomas resembling morphologically those found in human cases. It is possible to observe the process of development of urinary bladder tumors from initial lesions to invasive tumors using routine urological examinations. We believe that this experimental model is valuable for clinicopathological studies of urinary bladder tumors.

INTRODUCTION

There have been many reports on experimental studies of urinary bladder cancer induced by various carcinogens in animals. However, most experiments in this field have mainly been carcinogenic testing of suspicious chemicals or biochemical, histological, or histochemical studies (2, 3, 5, 7, 8, 11, 16, 17, 19, 25, 28, 32–34, 36, 37), but clinicopathological studies of urinary bladder tumors in experimental animals were frequently insufficient.

In 1964, Druckrey et al. (13) reported the carcinogenicity of BBN with organ specificity for the urinary bladder only and in inducing a high incidence of urinary bladder cancer in rats. Recently, Ito et al. (20, 21) and others (1, 4) have demonstrated that BBN-induced bladder tumors provide a useful model in rats and mice for morphological and histogenetic studies, but no sufficient experimental model for the study of bladder tumors is available in dogs. The present experiments were performed to study the induction of primary bladder tumors in dogs treated with BBN and compare cystoscopic, cytological, and histological examinations as well as histopathology.

MATERIALS AND METHODS

Adult beagle dogs (Fuji Animal Farm, Tokyo, Japan) weighing an average of 7 to 10 kg and adult mongrel dogs weighing 10 to 23 kg were used for the experiment. BBN was purchased from Izumi Chemical Laboratory Co., Yokohama, Japan, and it was packed in hard gelatin capsules containing 80, 160, or 240 mg, respectively, and stored in a refrigerator at 4°. The capsules were administered p.o. 6 days/week during the experiment. The dogs were divided into 6 groups: in Group 1, one female and 2 male mongrel dogs received capsules containing 80 mg of BBN; in Group 2, 3 male beagle dogs received the same dose of BBN; in Group 3, 4 female mongrel dogs received a daily dose of 160 mg of BBN; in Group 4, 2 female beagle dogs received a daily dose of 160 mg of BBN; in Group 5, one male and 2 female mongrel dogs received a daily dose of 240 mg of BBN; and in Group 6, one female and 2 male mongrel dogs were used for the control.

All animals were fed commercial stock diet (Dog Chow-DB; Oriental Yeast Co., Ltd., Tokyo, Japan) each morning, 35 g per kg body weight per day, and were given water ad libitum. All dogs were housed in individual steel cages in an air-conditioned room, and beagle dogs were separated completely from mongrel dogs. All dogs were weighed weekly before being fed.

Laboratory Examinations. Once a month, blood samples from each animal were taken to examine the erythrocyte and leukocyte counts, hematocrit, and hemoglobin content. These were determined with a microcell counter (TOA Medical Electronics, Tokyo, Japan). SGPT, SGOT, ALP, lactate dehydrogenase, blood urea nitrogen, and creatinine content were measured with a Hitachi type 400 automatic analyzer. Electrophoresis of serum protein was performed in a Beckman Model R-101 microzone cell with a cellulose acetate membrane.

Clinical Examinations. Photocystoscopic examinations of the female dogs were made under general anesthesia for the first 25 weeks of the experimental period at intervals of 8 to 12 weeks. After lesions in the urinary bladder epithelium were recognized cystoscopically, whenever possible, visualized lesions were biopsied, and the intervals were shortened to obtain more details of the developmental phase of the epithelial changes. It was impossible to do transurethral cystoscopic examinations due to the anatomical difficulty of inserting the cystoscope into the male dog urethra.

After administration of BBN for 25 weeks, excretory urographies at an interval of 20 weeks were performed with 2 ml of a contrast medium per kg body weight, sodium amidotrizoate meglumine (Schering AG, Berlin, West Germany), injected i.v.
were performed when malignant lesions in the urinary bladder after injection in the anteroposterior position. Double-contrast cystograms under i.v. general anesthesia were performed when malignant lesions in the urinary bladder were detected by cystoscopic examinations in female dogs or by urinary cytological examinations in male dogs. A 12 or 14 French-sized straight rubber catheter was inserted into the urinary bladder, and the urine in the urinary bladder was extracted. From 60 to 80 ml volume of air and from 10 to 20 ml of a contrast medium, sodium amidotrizoate meglumine, were instilled in the urinary bladder through the catheter. The film was exposed in the anteroposterior position and in the lateral position.

In selected dogs, angiograms were performed for detection of tumor development under i.v. general anesthesia when tumors in the urinary bladder were recognized by cystoscopic examination or by double-contrast cystograms. A single pre-shaped radiopaque polyethylene catheter (length, 60 cm; external diameter, 2.1 mm; internal diameter, 1.3 mm) was introduced into the aorta via the femoral artery, which is the same method used in human cases. The tip of the catheter was placed in the aorta over the bifurcation using an image intensifier and X-ray television equipment. Then, 10 to 20 ml of amidotrizoate meglumine were injected as fast as possible by hand pressure. The average speed of injection was 3.3 ml/sec, which was comparable to the speed obtained using an automatic injector. Angiograms were taken with an Elma-Schoenander cut-film changer programmed at 2 frames/sec for 13 sec in the lateral position. Air (100 ml volume) was placed inside the urinary bladder through a rubber catheter for providing a negative contrast medium before angiographies were performed. The exposure values were 0.08 to 0.10 sec, 400 ml, and 80 to 85 kV. A grid with 100 lines/inch, Simens Diamant intensifying screens, and Kodak R.P. 54 film were used. The film focal distance was 100 cm, and the focal spot of the tube was 1.2 x 1.2 mm.

Cytological Study. Urinalysis and urinary exfoliative cytological examinations were performed monthly throughout the experimental period. Both catheterized urine and irrigation specimens were collected from each dog in the early morning by using a rubber catheter (12 or 14 French-sized). Normal 0.9% NaCl solution was agitated vigorously inside the bladder with a Toomy syringe, and about 50 ml of irrigants were then withdrawn for cytological study. At least 50 ml of each specimen were utilized in preparing the cellular material for microscopic examination. These fluids were processed on glass slides by spreading slides with the product of centrifugation at 1500 rpm for 15 min. To decrease the number of cells which might wash off during fixation and staining, slides were precoated with albumin. Prepared slides were fixed in 95% ethyl alcohol and stained by the Papanicolaou method. All excess product of centrifugation and any tissue fragments were made into cell blocks which were fixed in 10% buffered formalin and embedded in paraffin and sectioned. Then cell block sections were stained with hematoxylin and eosin. The numerical Papanicolaou classification was used for reporting cellular findings.

Histopathological Study. Tissue specimens were taken transurethrally by using cystoscopic biopsy forceps when visualized epithelial lesions or tumorous changes of the urinary bladder epithelium were recognized by cystoscopic examination. Postmortem examinations were carried out on all dogs that died or were killed when urinary bladder tumors developed or when they became moribund. Tissue specimens of urinary bladder, kidney, ureter, urethra, and other organs such as lung, liver, spleen, and pelvic and paraaortic lymph nodes were fixed in 10% buffered formalin solution; embedded in paraffin; and sectioned 5 mm thick for light microscopic studies.

Sections were routinely stained with hematoxylin and eosin, and selected tissue was stained with Mallory’s, Van Gieson’s, the periodic acid-Schiff reaction, and silver impregnation. For histological definition of lesions of urothelium, 4 basic criteria described by Mostofi (27) were used: the patterns of growth; the type of cells and tissues affected; the grade of anaplasia; and the pathological stage of the tumor.

RESULTS

The control animals and the animals that received BBN grew well during the experiment and remained in generally good condition. However, Dog 21 in Group 1 which received a daily dose of 80 mg of BBN and Dog 26 in Group 5 which received a daily dose of 240 mg of BBN died of suffocation during anesthesia prior to radiological examination and cystoscopic examination. The changes in body weight, total intake of BBN, period of first observed tumors, and histopathological diagnosis of each dog are shown in Table 1.

The results of hematological and serum analysis for all groups showed no change during the experimental period. Although microscopic hematuria had been recognized in all dogs given BBN from the early period of the experiment, RBC remained in the normal range. Several dogs were affected by anemia shortly before death or at the end of the experimental period. Their condition was characterized by a decrease in RBC, hematocrit value, and hemoglobin content. At the same time, no evidence of hepatic or renal toxicity of BBN was found because SGOT, SGPT, ALP, blood urea nitrogen, and creatine contents were always well within normal limits. However, SGOT, SGPT, and ALP increased slightly to moderately shortly before death or at the end of the experimental period.

During the early period of the experiment, microscopic hematuria was observed in urinary sediment of all dogs fed BBN. This progressed to intermittent macrohematuria that became progressively more severe and frequent. Changes in the urinary bladder epithelium were observed cystoscopically as petechiae, followed by hemorrhage with ulceration as a result of moderate to severe inflammation. After 25 weeks or more of BBN treatment, simple elevation and, in some cases, velvety elevation of the bladder mucosa with hemorrhage were recognized by cystoscopic examination in female dogs (Fig. 1). These lesions, when biopsied, showed denuded cystitis and simple hyperplasia. Histologically, mucous velvety elevation also showed slight to moderate atypical hyperplasia. From these lesions, the development of carcinoma in situ and papillary or sessile tumors of the urinary bladder were observed by periodic cystoscopic examinations (Fig. 2).

Excretory urographies showed no evidence of upper urinary tract abnormalities, but Dog 12 at 108 weeks and Dog 23 at 150 weeks demonstrated abnormalities in which hydronephrosis caused by development of tumor in the renal pelvis and at the orifice was observed. Double-contrast cystograms also revealed tumor of the bladder.
When tumors were recognized by previous examinations, pelvic angiographs were performed which revealed abnormal vessels, such as corkscrew vessels or giant capillaries, or the presence of arteriovenous shunts within tumors and faint prolonged capillary perfusion when the tumor grew to large size with invasion (Figs. 3 and 4). Gross findings of the urinary bladder after BBN treatment were observed in 13 dogs when tumors were subsequently observed by periodic examination (Fig. 11). Transitional cell carcinoma of the urinary bladder appeared in 5 dogs which died by accident at 122, 108, and 54 weeks (Fig. 11). Transitional cell carcinoma of the urinary bladder epithelium appeared in 3 dogs which were biopsied at 132 and 142 weeks (Fig. 12 to 14). Dogs B-4 and B-5 showed Grade II tumors which were biopsied at 132 and 142 weeks (Fig. 15). These dogs are still alive with bladder tumor. Renal pelvic tumors were present in 2 dogs (Dogs 12 and 23) and showed noninvasive papillary transitional cell carcinoma; in these dogs, inflammatory cell infiltration in the underlining tissue of the renal pelvic tumors was observed. There were no changes in the renal and hepatic parenchymal cell. No tumors were recognized in the other organs of the dogs in this experimental group.

**DISCUSSION**

It is well known that urinary bladder tumors have been produced by various chemical carcinogens in animals. Some nitroso compounds exhibit carcinogenic activity toward the urinary bladder, but BBN seems to affect only the urinary bladder in mice (1, 4) and rats (13, 20). Ito et al. (21) observed precursor lesions such as focal hyperplasia of the epithelium in the urinary bladder of hamsters and guinea pigs, when these animals were treated with only 0.025% BBN in drinking water for 20 weeks. The urinary concentration of BBN metabolites may be important in the induction of bladder cancer in animals. Okada et al. (30) reported that the principal urinary metabolite of BBN and of \(\beta\)-butyl-\(\beta\)-(3-carbomethoxy-4-methylphenyl)nitrosamine was \(\beta\)-butyl-\(\beta\)-(3-carbomethoxy-4-methylphenyl)nitrosamine.
boxypropyl)nitrosamine in several animal species. The carcinogenic activity of N-butyl-N-(3-carboxypropyl)nitrosamine toward the urinary bladder of rats was demonstrated by Hashimoto et al. (18). Although, in the present experiment, development of urinary bladder tumors induced in dogs by BBN p.o. required longer periods than that in rats or mice, the incidence and development of bladder tumors and morphological patterns were essentially the same. Moreover, BBN induced transitional cell carcinoma in dog urinary bladder, and its process of tumor development was very similar to that observed in human cases by the same methods of examination.

There have been several reports on experimental induction of bladder tumors in dogs treated with various chemical carcinogens including 2-naphthylamine, 4-aminoazobenzene, 4-aminofluorene, 4-amino-3:2'-azotoluene, and N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (5, 6, 10, 12, 15, 16, 19, 26, 28, 33, 34, 36). Most of these compounds, except 4-aminoazobenzene, affect not only the urinary tract but also other organs. The tumor induction time is also long. In previous work (31, 33, 34, 36), most of these compounds, except 4-aminofluorene, 4-amino-3:2'-azotoluene, and A/-[4-(5-nitro-2-

ditions of bladder tumors induced by BBN in dogs as well as in human cases (23, 24, 29). In previous studies (5, 9, 14, 16, 22), urinary exfoliative cytology, cystoscopic, or cystographic observations were made on animals with experimentally induced bladder tumors.

The present investigation has shown that the periodic routine study of exfoliative urethelial cells is useful for the early detection of bladder tumors induced by BBN in dogs as well as in human cases, since atypical urothelial cells are detectable in urine prior to the detectable appearance of tumors in the urinary bladder. Epithelial atypia were detected as early as 55 weeks after treatment and tumor-positive cells were detected as early as 72 weeks after treatment by bladder washing and cell block. Further studies are in progress to determine the correlation between the detection of atypical or malignant cells and their process of development into tumors in the bladder epithelium.

The first cystoscopically observed lesions of the urinary bladder in dogs after ingestion of BBN showed petechiae and elevation of the urinary bladder mucosa. Velvety elevations of the mucosa were also observed. These lesions gradually evolved into multiple papillary or sessile tumors observed during subsequent cystoscopic examinations. These findings were essentially the same as those observed in previous studies (9, 16, 32) and in human cases.

In general, double-contrast cystograms and pelvic angiograms are also important diagnostic procedures for the determination of staging of urinary bladder tumors in human cases. In the present experiment, tumors of the urinary bladder were detected by double-contrast cystograms when tumors had reached a diameter of at least 2 to 3 mm. Typical tumor characteristics such as vascular dilatation, appearance of new tumor vessels, and tumor staining when tumors had reached

Table 2

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Dog</th>
<th>Sex</th>
<th>BBN (mg/day)</th>
<th>Cytological findings at following times</th>
<th>Period of first observed tumors (wk)</th>
<th>Histological diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50 wk</td>
<td>100 wk</td>
<td>150 wk</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td>F</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>M</td>
<td>80</td>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>F</td>
<td>80</td>
<td>II</td>
<td>II</td>
<td>II</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>F</td>
<td>160</td>
<td>II</td>
<td>II</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>F</td>
<td>160</td>
<td>II</td>
<td>II</td>
<td>III(85)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>F</td>
<td>160</td>
<td>II</td>
<td>II</td>
<td>III(104)IV(120)</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>F</td>
<td>160</td>
<td>II</td>
<td>II</td>
<td>III(110)IV(134)</td>
</tr>
<tr>
<td>4</td>
<td>26</td>
<td>F</td>
<td>240</td>
<td>II</td>
<td>III</td>
<td>III(55)IV(72)</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>F</td>
<td>240</td>
<td>II</td>
<td>III</td>
<td>III(60)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>M</td>
<td>240</td>
<td>II</td>
<td>III</td>
<td>III(60)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of histology</th>
<th>Grade</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperplasia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC papilloma</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TC carcinoma</td>
<td>I</td>
<td>0</td>
</tr>
<tr>
<td>TC carcinoma</td>
<td>II</td>
<td>A</td>
</tr>
<tr>
<td>TC carcinoma</td>
<td>II</td>
<td>A</td>
</tr>
<tr>
<td>TC carcinoma</td>
<td>II</td>
<td>C</td>
</tr>
<tr>
<td>TC carcinoma</td>
<td>II</td>
<td>B</td>
</tr>
<tr>
<td>TC carcinoma</td>
<td>II</td>
<td>A</td>
</tr>
<tr>
<td>TC carcinoma</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>TC carcinoma</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>TC carcinoma</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>TC carcinoma</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>Hyperplasia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC carcinoma</td>
<td>II</td>
<td>A</td>
</tr>
<tr>
<td>TC carcinoma</td>
<td>II</td>
<td>A</td>
</tr>
<tr>
<td>TC carcinoma</td>
<td>II</td>
<td>0</td>
</tr>
</tbody>
</table>

a Cytological classification according to Papanicolaou.
b Numbers in parentheses, weeks.
c TC, transitional cell.
d Died by accident.

The tumor induction time relationship for the induction of tumors of the urinary bladder of rats was demonstrated by Hashimoto et al. (18). Although, in the present experiment, development of urinary bladder tumors induced in dogs by BBN p.o. required longer periods than that in rats or mice, the incidence and development of bladder tumors and morphological patterns were essentially the same. Moreover, BBN induced transitional cell carcinoma in dog urinary bladder, and its process of tumor development was very similar to that observed in human cases by the same methods of examination.

There have been several reports on experimental induction of bladder tumors in dogs treated with various chemical carcinogens including 2-naphthylamine, 4-aminoazobenzene, 4-aminofluorene, 4-amino-3:2'-azotoluene, and N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (5, 6, 10, 12, 15, 16, 19, 26, 28, 33, 34, 36). Most of these compounds, except 4-aminoazobenzene, affect not only the urinary tract but also other organs. The tumor induction time is also long. In previous work (31, 32), N,N-dibutylamine showed disturbances of blood chemistry which may be due to hepatotoxicity such as fatty changes and hepatic fibrosis. However, the data obtained during the experimental period did not show BBN to be toxic except in the urinary tract, particularly in the urinary bladder. Histologically, there are no changes in the hepatic and renal parenchymal cell.

Conzelman and Moulton (10) reported a dose-response and time relationship for the induction of tumors of the urinary bladder in dogs given 2-naphthylamine p.o. Tumor induction time in the present experiment was estimated to be about 4 years in Groups 1 and 2, given the lowest daily dose (80 mg) of BBN, and about 1.5 years in Group 5, given 240 mg of BBN. The total dosage of BBN ingested by the dogs during the experiment until the time of the first detection of tumors was nearly the same in all groups, at least 100 g or more of BBN. Thus, it appears that tumor induction time is markedly shorter in dogs given sufficient amounts of BBN than in dogs given other chemical carcinogens. Best results were achieved with a daily dose of 240 mg BBN as shown in Table 1.

Several diagnostic procedures such as urinary exfoliative cytology, cystoscopy, urography, angiography, and transurethral biopsy are used for the early detection of grading and staging of urinary bladder tumors in human cases (23, 24, 29). In previous studies (5, 9, 14, 16, 22), urinary exfoliative cytological, cystoscopic, or cystographic observations were made on animals with experimentally induced bladder tumors.

The present investigation has shown that the periodic routine study of exfoliative urethelial cells is useful for the early detection of bladder tumors induced by BBN in dogs as well as in human cases, since atypical urothelial cells are detectable in urine prior to the detectable appearance of tumors in the urinary bladder. Epithelial atypia were detected as early as 55 weeks after treatment and tumor-positive cells were detected as early as 72 weeks after treatment by bladder washing and cell block. Further studies are in progress to determine the correlation between the detection of atypical or malignant cells and their process of development into tumors in the bladder epithelium.

The first cystoscopically observed lesions of the urinary bladder in dogs after ingestion of BBN showed petechiae and elevation of the urinary bladder mucosa. Velvety elevations of the mucosa were also observed. These lesions gradually evolved into multiple papillary or sessile tumors observed during subsequent cystoscopic examinations. These findings were essentially the same as those observed in previous studies (9, 16, 32) and in human cases.

In general, double-contrast cystograms and pelvic angiograms are also important diagnostic procedures for the determination of staging of urinary bladder tumors in human cases. In the present experiment, tumors of the urinary bladder were detected by double-contrast cystograms when tumors had reached a diameter of at least 2 to 3 mm. Typical tumor characteristics such as vascular dilatation, appearance of new tumor vessels, and tumor staining when tumors had reached
The study of the pathology of tumors of the lower urinary tract. We believe that an ideal experimental model for clinical-pathological studies of urinary bladder tumors has certain basic requirements: (a) the tumors induced should be morphologically similar to human bladder tumors; (b) a simple and natural method for the administration of carcinogen is required; (c) the carcinogen should not be toxic and should affect only the epithelium of the urinary tract and, furthermore, the incidence of tumors should be reliable and high; (d) the tumor induction time should be relatively short; and (e) changes in the urinary bladder during the experiment should be observable by routine urological examination. In the present experiment, the tumors resemble those found in human cases, the method of administration of carcinogen is natural (p.o.), the carcinogen is not toxic, and it induces tumors only in the urinary bladder. Except 3 dogs which died by accident and showed epithelial hyperplasia of the urinary bladder, all animals treated with a total dose of about 100 g of BBN developed carcinomas within 1.5 to 4 years. The process of development of bladder tumors from initial lesions to invasive tumors was observed by routine urological examination. We believe, therefore, that experimental bladder tumors induced by BBN in dogs, as in the present experiment, have the necessary requirements for experimental model for bladder cancer and that this model may be useful in studying not only the pathology but also the biochemistry, diagnosis, immunology, and treatment of urinary bladder tumors.

REFERENCES

Fig. 1. Cystoscopic appearance of Dog 24 at 80 weeks of treatment with a daily dose of 160 mg of BBN. Small bleeding areas are seen in the left posterior wall of the urinary bladder.

Fig. 2. Cystoscopic appearance of Dog 24 at 104 weeks of treatment with a daily dose of 160 mg of BBN. Two pea-sized papillary tumors are seen in the posterolateral wall near the vault.

Fig. 3. Double-contrast cystogram in the lateral position of Dog 24 at 104 weeks of treatment with BBN. A few small filling defects are seen in the posterolateral wall near the vault of the urinary bladder.

Fig. 4. Double-contrast cystogram in the lateral position of Dog 24 at 120 weeks of treatment with BBN. Multiple round filling defects are seen in the posterolateral wall, and irregular outline coated with contrast medium is also seen in the vault of the urinary bladder.
Fig. 5. Pelvic angiogram, early arteriographic phase, of Dog 24 at 120 weeks of treatment with BBN. Many abnormal vessels within the tumor, such as corkscrew vessels, are seen in the vault (arrow). A small area deeply dyed with contrast medium is also seen in the posterolateral wall (double arrow), and early filling of large striate-appearing venous structures is also seen.

Fig. 6. Multiple tumor stainings involving the vault and disappearance of arteriovenous shunt within the tumor in the posterolateral wall are seen in a late phase of the pelvic angiogram of Fig. 5.

Fig. 7. Gross appearance of bladder lumen opened along the ventral surface of Dog 24 when sacrificed just after the pelvic angiography at 120 weeks after BBN treatment. Multiple variously sized papillary or sessile tumors are seen in all areas except part of the trigone of the urinary bladder.
Fig. 8. Moderately enlarged transitional cells with slightly enlarged nuclei in urine of Dog 26 after treatment with 240 mg of BBN per day at 54 weeks. Papanicolaou, × 1000 (original magnification).

Fig. 9. Severely atypical transitional cells with enlarged nuclei and coarsely granular chromatin in urine of Dog 25 after treatment with 160 mg of BBN per day at 130 weeks. Papanicolaou, × 1000 (original magnification).

Fig. 10. Definitely malignant transitional cells in cell block of urine sediment of Dog B-4 after treatment with 160 mg of BBN per day at 120 weeks. H & E, × 400 (original magnification).

Fig. 11. Focal hyperplasia of the transitional epithelium of urinary bladder in Dog 26 after treatment with 240 mg of BBN per day autopsied at 54 weeks. H & E, × 200 (original magnification).
Fig. 12. A papillary transitional cell carcinoma, Grade II, Stage 0, of urinary bladder in Dog 24 after treatment with 160 mg of BBN per day autopsied at 120 weeks. Carcinoma has become organized into separate papillae with connective tissue stalk. H & E, × 40 (original magnification).

Fig. 13. A sessile tumor of urinary bladder in Dog 24 showing outward and invasive growth of transitional cell carcinoma, Grade II, Stage A, with some squamous metaplasia. H & E, × 40 (original magnification).

Fig. 14. Deeply invasive area throughout muscle layer to serosa of transitional cell carcinoma, Grade II, Stage C, in urinary bladder of Dog 23 after treatment with 160 mg of BBN per day autopsied at 180 weeks. H & E, × 100 (original magnification).

Fig. 15. Transitional cell carcinoma in situ of urinary bladder in Dog B-4 biopsy at 132 weeks after treatment with 160 mg of BBN per day. H & E, × 400 (original magnification).
Urinary Bladder Tumors Induced by \textit{N}-Butyl-\textit{N}-(4-hydroxybutyl)nitrosamine in Dogs

Eigoro Okajima, Tadashi Hiramatsu, Kazuya Hirao, et al.


\textbf{Updated version} Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/41/5/1958

\textbf{E-mail alerts} Sign up to receive free email-alerts related to this article or journal.

\textbf{Reprints and Subscriptions} To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

\textbf{Permissions} To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.