Urol Res (1997) 25:315–323

ORIGINAL PAPER

Eigoro Okajima · Seiichiro Ozono · Katsunori Yoshida
Shoji Samma · Hitoshi Momose · Akio Iwai
Hirotsugu Uemura · Shoichi Tabata · Kenichi
Tsumatani · Yoshihiko Hirao · Kunihiko Tsunemi

A histopathological mapping study of the urinary bladder tumors induced by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine in dogs

Received: 6 March 1997 / Accepted: 11 March 1997

Abstract Bladder tumors were induced by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) in five Beagles and four mongrel dogs. The tumors were observed for long periods and the tumor progression was traced using histopathological mapping. The results indicated (1) that low-dose BBN over a long period induced multiple low-grade (G1–2) and low-stage (pTa-1) papillary tumors, resembling superficial bladder cancer in humans; (2) that high-dose BBN over a short period induced high-grade (G2–3) and high-stage (pT3b) nonpapillary tumors and carcinoma in situ (CIS) resembling invasive cancer and CIS in humans; (3) that beagle dogs required longer periods and higher total doses of BBN as compared with mongrel dogs; (4) that the tumors induced by low-dose BBN in beagles were observed without BBN as long as the animals lived, and neither increasing numbers of tumors nor malignant features such as deep infiltration and metastasis was observed; and (5) that low-dose BBN seems to induce mild dysplasia, which is followed by Brunn's nest-like proliferation in the lamina propria and nodular change, eventually leading to the development of papillary noninvasive transitional cell carcinoma (TCC); and that high-dose BBN seems to induce severe dysplasia which leads to CIS and nonpapillary invasive TCC. These results may contribute to clarifying the natural history of human bladder cancer.

Key words Bladder tumors · *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine · Mapping study · Natural history · Dog

E. Okajima (✉) · S. Ozono · K. Yoshida · S. Samma
H. Momose · A. Iwai · H. Uemura · S. Tabata · K. Tsumatani
Y. Hirao
Department of Urology, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634, Japan, Fax: 0744-22-9282

K. Tsunemi
Pharmaceutical Research Laboratory,
Chugai Pharmaceutical Co. Ltd.,
1-135 Komakado, Gotemba, Shizuoka 412, Japan

Introduction

About two-thirds of human urinary bladder cancers are of a papillary, noninvasive type, while the remaining one-third are nonpapillary and invasive. The former type shows lesser degrees of cell atypism and has a better prognosis. The latter type shows greater degrees of cell atypism and has a higher incidence of metastasis and a poor prognosis.

To clarify the natural history of human bladder cancer, it is indispensable to establish valid animal models of human bladder cancer. This is one of the important issues pertaining to the study of chemical-induced cancer.

Druckrey et al. [2] synthesized *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) and reported that BBN is a carcinogen whose only target of action in rats is the urinary bladder. Thereafter, BBN-induced experimental urinary bladder tumors in rats [6] and mice [12] were established as models of human bladder cancer. These models have been used to study various factors related to the development and progression of bladder cancer in detail. BBN induced papillary, noninvasive tumor in rats, while in mice nonpapillary, invasive tumor was induced. It has therefore been desirable to develop a technique by which both types of urinary bladder tumor, resembling human bladder cancer, can be experimentally induced.

When we induced bladder tumor in dogs by oral treatment with BBN, papillary, noninvasive tumors were induced at a low dose of BBN, while nonpapillary, invasive tumors were induced at a higher dose [13]. Histopathological examination revealed that the BBN-induced bladder tumor was very similar to human bladder cancer, and that the development of tumor correlated with the dose of the carcinogen. We also reported that oral treatment with BBN following instillation of *N*-methyl-*N*-nitrosourea (MNU) into the urinary bladder resulted in the development of carcinoma in situ (CIS) short periods after the start of BBN treatment even when the dose of BBN was low [14]. It has thus

been demonstrated that bladder tumor, induced experimentally in dogs, shows a natural history similar to that of human bladder cancer and is therefore useful as a model of human bladder cancer.

In the present study we carried out (1) histopathological examination of the papillary, noninvasive urinary bladder tumors induced by low-dose BBN in mongrel dogs as well as the nonpapillary, invasive urinary bladder tumor induced by high-dose BBN in mongrel dogs, (2) histopathological observation of the course of tumors over long periods after tumor development in beagle dogs, and (3) investigation of the manner of onset and progression of urinary bladder tumors in dogs.

Materials and methods

Experimental animals

Four adult female mongrel dogs (weight 7.8–10.5 kg) and five, approximately 8-week-old beagle dogs (one male and four females; Fuji Animal Farm) were used in this study. They were allocated individually to air-conditioned kennels, fed with a solid dog diet (DB, Oriental Yeast Co., Osaka, Japan) and allowed free access to water.

Experiments were performed according to the "Principles of laboratory animal care" of the NIH on the care and use of laboratory animals.

Carcinogen

BBN (Izumi Chemical, Yokohama, Japan) was used as a carcinogen. Animals were orally administered a gelatin capsule once a day for 6 days a week. Each capsule contained either 80, 160 or 500 mg of BBN.

Protocol

The animals were divided into three groups on the basis of BBN dosage: (1) BBN 80 mg/day group (a male beagle, no.B-3), (2) BBN 160 mg/day group (two females mongrels, nos. 24 and 25, and two female beagles, nos. B-4 and B-5), and (3) BBN 500 mg/day group (two female mongrels, nos. 32 and 37, and two female beagles, nos. B-12 and B-13). The length of the BBN dosing period and the total duration of observation were determined separately for each group.

For the male dog treated with 80 mg BBN, cystoscopy was carried out using transabdominal bladder puncture on radiographical detection of the urinary bladder tumor. This dog was killed when urinary bladder tumor was confirmed in biopsy samples collected using endoscopic biopsy forceps. The female mongrel dogs treated with 160 or 500 mg BBN were killed when urinary bladder tumor was confirmed by cystoscopy and histopathological examination. Observation of the beagles treated with 160 or 500 mg BBN was continued for as long as they lived, while discontinuing BBN administration to the two beagles (nos. B-12 and B-13) from the 500 mg treatment group upon confirmation of urinary bladder tumor.

Animals were killed by exsanguination under Isozol (thiamylal sodium) anesthesia. The whole urinary bladder was removed from each animal, and immediately inflated by transurethral injection of 10% formalin. The urethral portion of the urinary bladder was then ligated, and was immersed in the fixative. After fixation, the urethral stump and the anterior wall of both the urethra and the urinary bladder were incised along the median line up to the vertex of the urinary bladder. Subsequently, the bladder wall was incised along the ureter on both sides up to the ureteral orifice. In this way, serial sections of the urinary bladder, parallel to the line connecting the right and left ureteral orifices, were prepared. The sections were embedded in paraffin and cut into thin slices, which were then stained with hematoxylin and eosin for histopathological examination. Histopathological changes of the bladder were assessed, according to the *General Rules for Urological and Pathological Studies on Bladder Cancer* [8] and the *Atlas for Tumor Pathology* (AFIP) [11].

Results

Table 1 summarizes the histopathological features of bladder tumors induced by oral BBN treatment.

BBN 80 mg group (no. B-3)

In animal B-3, bladder tumor was detected by cystoscopy and biopsy 205 weeks after the beginning of BBN treatment. This animal was killed immediately and numerous papillary tumors of various sizes (4–12 mm in diameter) were seen in almost the entire mucosa of the bladder (Fig. 1). It was noted that papillary, broad-based transitional cell carcinoma (TCC) grade 2, pT1b (Fig. 2) as well as nonpapillary, noninvasive grade 2 TCC and proliferation of Brunn's nests in the lamina propria. In addition, epithelial dysplasia and local squamous metaplasia were seen. The mucosa was not normal in any area.

BBN 160 mg group

Tumors were detected 104 weeks after the beginning of BBN treatment in dog 24, and after 134 weeks in dog 25. Dog 24 was killed after 120 weeks of BBN treatment, and no. 25 was killed after 150 weeks of treatment. The bladder of dog 25 showed a number of papillary tumors

Table 1 Urinary bladder tumors induced by *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine (BBN) in dogs. *TCC* transitional cell caranoma

Dose (mg/day)	Dog	*n*	Mean time elapsed before first observed tumors (weeks)	Mean observation period without BBN treatment (weeks)	Mean total experimental period (weeks)	Mean total dose at first observed tumors (g)	Histopathological finding		
							Histology	Grade	Stage
80	Beagle	1	205	0	205	98.4	TCC	II	pT1b
160	Mongrel	2	119	16	135	114.22	TCC	II	pT1b,pT2
	Beagle	2	137	447	584	131.48	TCC	II	pTis,pT1b
500	Mongrel	2	58.5	0.5	59	175.5	TCC	III	pTis,pT3b
	Beagle	2	123	0	220	369.0	TCC	III	pTis,pT3b

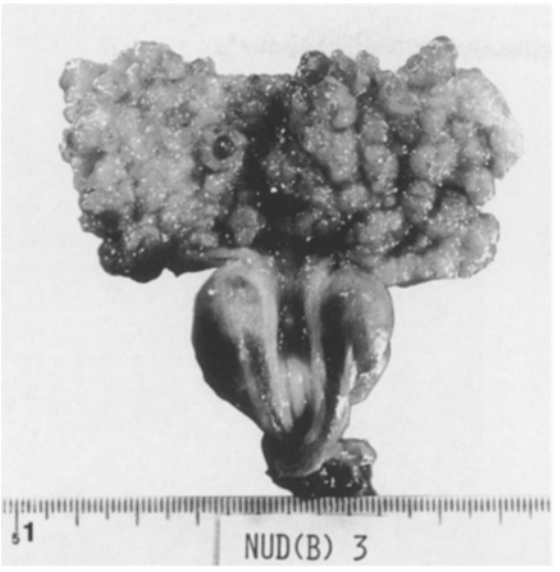
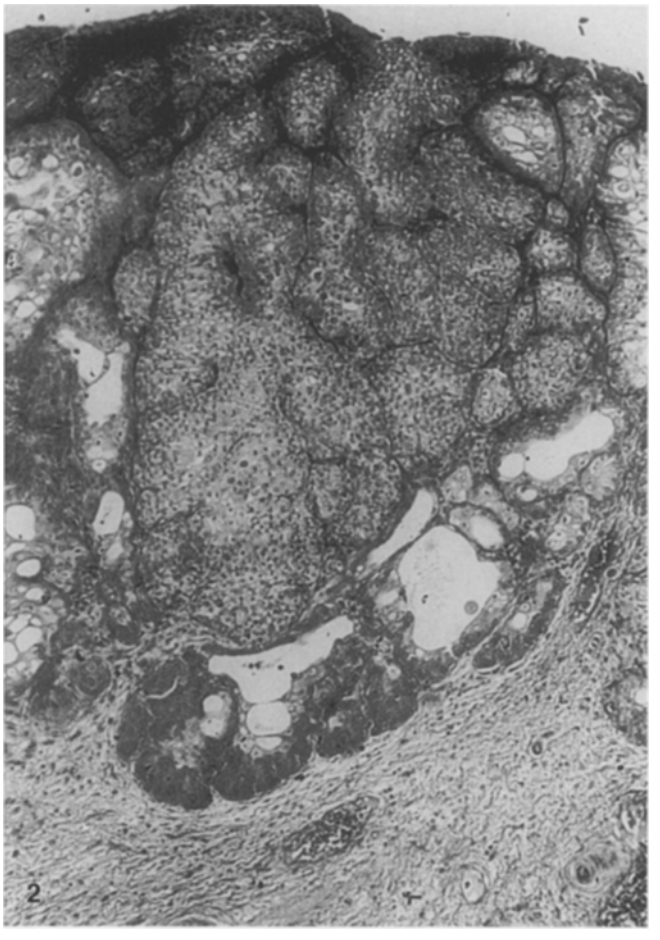


Fig. 1 Gross finding of the urinary bladder in beagle dog B-3 treated with 80 mg/day of *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) for 205 weeks. Multiple variously sized papillary tumors are seen in all areas of the urinary bladder

Fig. 2 Papillary, noninvasive transitional cell carcinoma (TCC), grade 2 in beagle dog B-3. H & E, ×40

Fig. 3 Gross finding of the urinary bladder in dog 25 treated with 160 mg/day of BBN for 134 weeks. Multiple variously sized papillary tumors are seen in all areas of the urinary bladder



of various sizes (2–10 mm in diameter) on almost the entire mucosa and papillary, nonpeduncular, noninvasive, grade 2 TCC was seen in almost the whole mucosa of the bladder (Fig. 3). The pathological stage of the tumor was pT2 in dog 24 and pT1b in dog 25 (Fig. 4). When the bladder of dog 25 was mapped, nonpapillary, noninvasive TCC, accompanied by proliferation of Brunn's nests, was also seen in the lamina propria around the papillary, noninvasive tumor. The tumor-free regions of the mucosa showed epithelial dysplasia. There was little normal mucosa on the map.

Multiple, papillary grade 1 or 2 TCC was detected by cystoscopy and biopsy after 132 weeks of treatment in the no. 4 female beagle (Figs. 5, 6) and after 142 weeks of treatment in the no. 5 female beagle. Upon detection of the tumors, BBN was discontinued and the two dogs were then observed without any treatment. Beagle B-4 survived 11 years and beagle B-5 survived 11 years and 6 months after the experiment started. Cystoscopy of beagle B-5 in the 350th week revealed no signs of tumor progression, although the number of tumors had increased slightly (Fig. 7). Because both beagles showed a tendency to weaken due to aging, they were finally killed.

When the bladders of these two beagles were observed macroscopically, the posterior wall, the right wall and the anterior wall showed papillary tumors of 20 mm in diameter and the entire mucosa showed small papillary or nodular changes (Fig. 8). In these two beagles, the tumors were papillary, nonpeduncular and noninvasive, TCC grade 2 or 3, pT1b. Figs. 9 and 10 are histopathological pictures of dogs B-4 and B-5. The lamina propria of dog B-5 showed nonpapillary, noninvasive TCC grade 2 or 3, pT1b, accompanied by nodular change. CIS also accompanied the tumors in the posterior wall, the right wall, the left wall and the trigone. The other changes observed in the mucosa included a number of papilloma, simple hyperplasia (SH) and dysplasia. There was little normal mucosa.

BBN 500 mg group

Of the two female adult mongrel dogs treated with 500 mg BBN, dog 32 was found to have bladder tumor by cystoscopy and biopsy 72 weeks after the beginning of treatment. This dog was killed in the 73rd week. Histopathological examination of this dog revealed

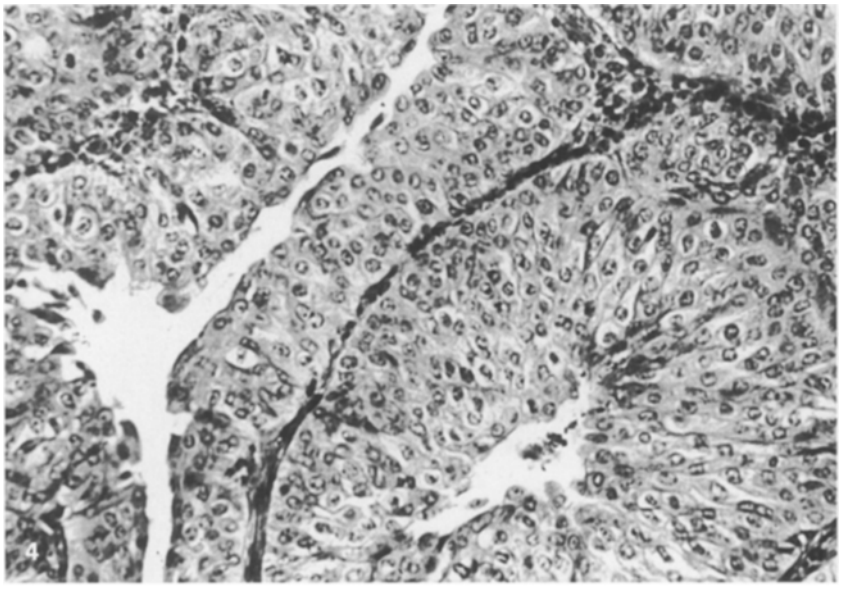


Fig. 4 Papillary, noninvasive transitional cell carcinoma, grade 2 in dog 24 treated with 160 mg/day of BBN for 104 weeks. H & E, ×100

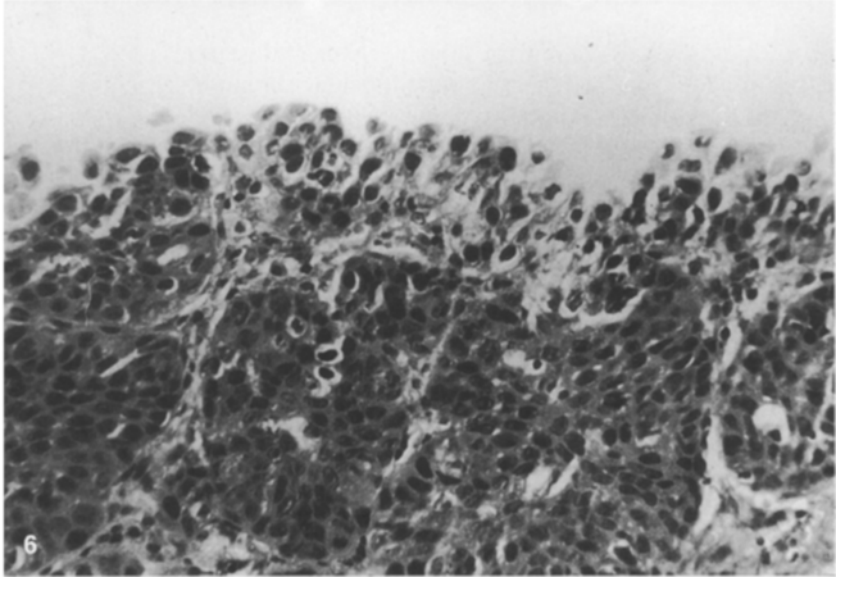
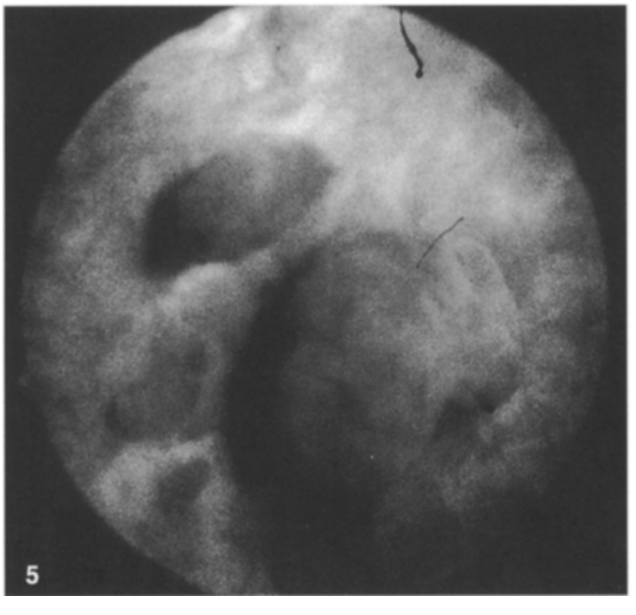


Fig. 5 Cystoscopic appearance of beagle dog B-5 at 142 weeks of treatment with 160 mg/day of BBN. Broad bean- and soybean-sized papillary tumors are seen

Fig. 6 Histopathological finding of the biopsied specimen of beagle dog B-5 at 142 weeks. Papillary TCC grade 2 can be seen. H & E., ×200



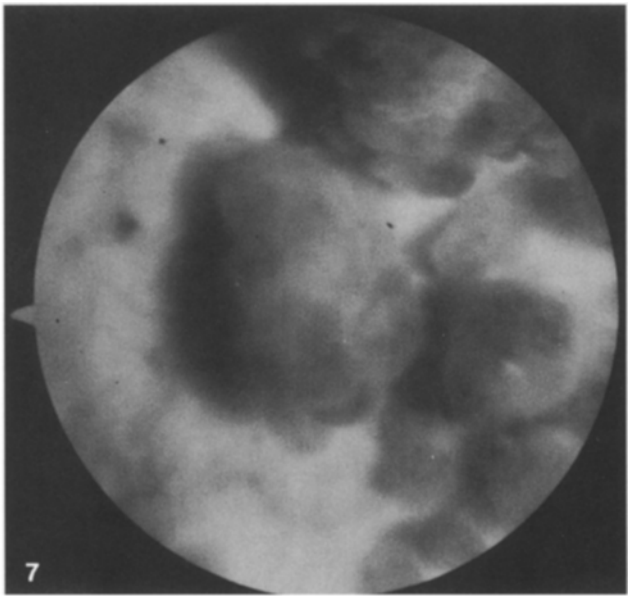


Fig. 7 Cystoscopic appearance of beagle dog B-5 at 350 weeks. Papillary tumors are not seen enlarged or developed

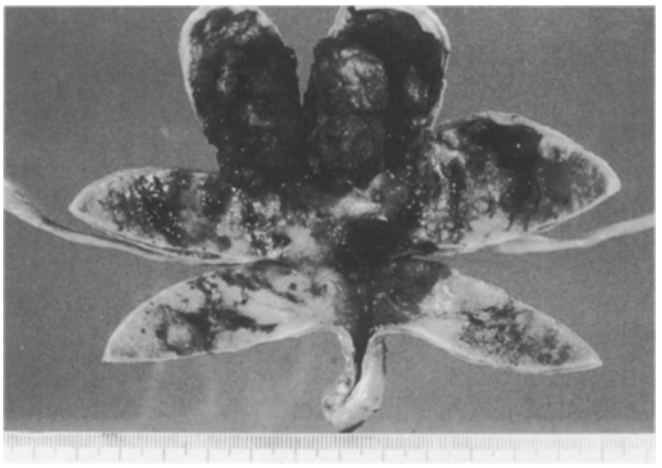


Fig. 8 Gross finding of the urinary bladder in beagle dog B-5 treated with 160 mg/day of BBN for 142 weeks. Variously sized papillary or nodular tumors are seen in all areas of the urinary bladder

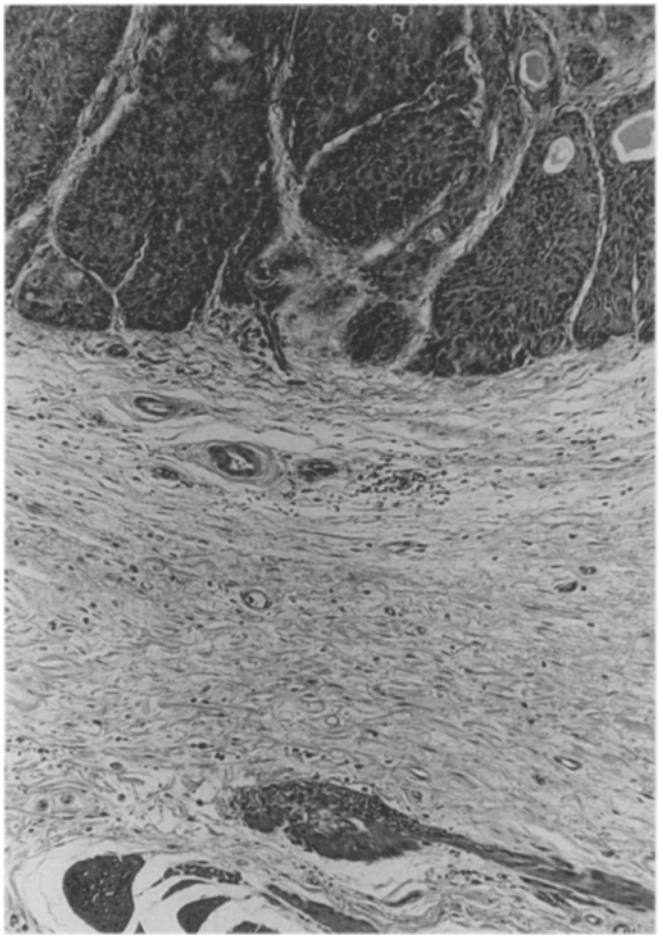


Fig. 9 Papillary, noninvasive TCC grade 2 in beagle dog B-4. H & E, ×200

nonpapillary, TCC grade 3, pT3b, excluding the trigone, as well as nonpapillary, invasive TCC in the lamina propria of the bladder, excluding the dome and right wall (Fig. 11). SH and mild to severe dysplasia were noted around the tumors. Little normal mucosa was visible. The other dog (no. 37) died of an accident in the 45th week during anesthesia for cystoscopy. No tumor was visible in the bladder mucosa, and SH and severe dysplasia over the entire mucosa as well as primary CIS in a part of the mucosa were observed histopathologically (Fig. 12).

The two female beagles treated with 500 mg BBN developed macrohematuria about 1 year after the beginning of BBN treatment. Subsequent cystoscopy revealed diffuse congestion, hemorrhagic lesions and ulcers in the mucosal epithelium of the bladder. The cystoscopy and biopsy, carried out 123 weeks after the beginning of BBN treatment, disclosed multiple, nonpapillary, nodular TCC. Thereafter, food ingestion in the dogs decreased and their condition weakened, so BBN treatment was suspended for one month. Thereafter, administration of BBN was stopped, while observing the condition of these dogs; hematuria continued to be seen. The dogs were killed about 4 years after the beginning of BBN treatment because their general condition had deteriorated markedly by that time.

When the bladder of beagle B-12 was observed macroscopically in the 220th week, small nodular lesions were visible on almost the entire mucosa (Fig. 13). The nodular lesions were rated as representing nonpapillary, TCC grade 3, pT3 (Fig. 14). In addition, the lamina propria of the bladder was found to have nonpapillary, noninvasive TCC, accompanied by nodular growth. Papillary, noninvasive TCC and associated CIS were noted in a part of the lamina propria. No tumor metastasis to any other organ was visible. There was little normal mucosa in the bladder.

In the 500 mg/day group, all dogs except mongrel dog 37, which died in the 45th week, were found to have a sporadic distribution of lymph follicles in the lamina propria. Infiltration by numerous plasma cells was visible in the lamina propria immediately below the epithelium and in the stroma of the tumor-affected area, although it was almost absent in the areas showing SH or dysplasia.

Discussion

Human bladder cancer can be divided into two types: a papillary, noninvasive type and a nonpapillary, invasive type. We experimentally induced bladder tumor in dogs by oral administration of BBN and reported that papillary, noninvasive tumors developed following treatment with low-dose BBN, while nonpapillary, invasive tumors were induced by high-dose BBN [13]. The results suggest that the histopathological features of urinary bladder tumor induced by BBN are quite similar to those of human bladder cancer. We orally treated dogs with BBN after instillation of MNU into the bladders, and found that CIS developed short periods after the start of BBN treatment [14]. In addition, using a scanning electron microscope, we chronologically traced

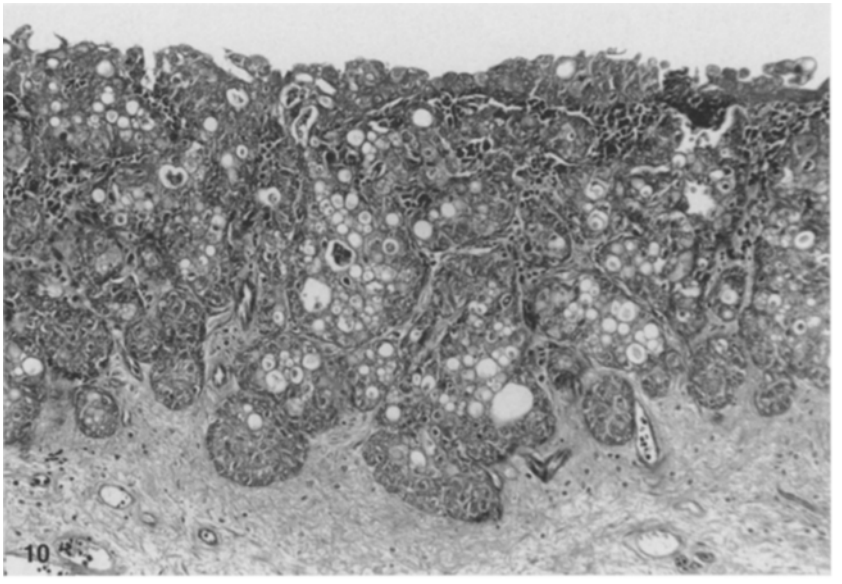


Fig. 10 Nonpapillary, noninvasive TCC grade 3 in beagle dog B-5. Proliferation of Brunn's nests-like lesions is seen. H & E, ×100

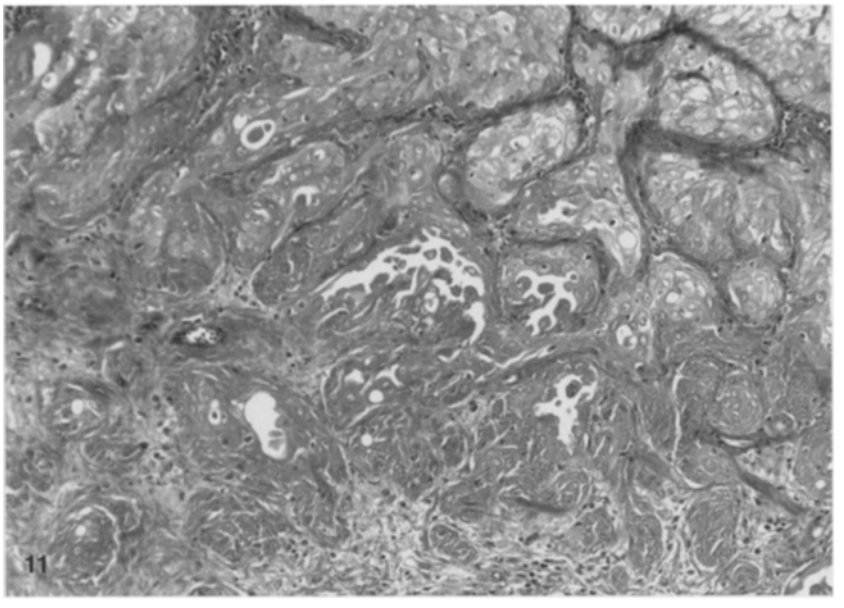


Fig. 11 Nonpapillary, invasive transitional cell carcinoma grade 3 in dog 32 treated with 500 mg/day of BBN for 72 weeks. Note infiltrative growth of cancer cells in the submucosa. H & E, ×100

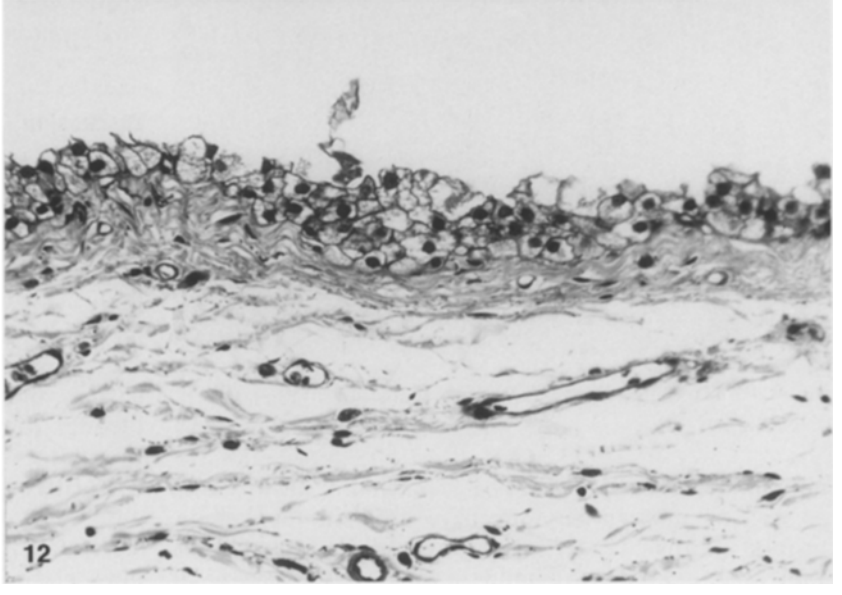
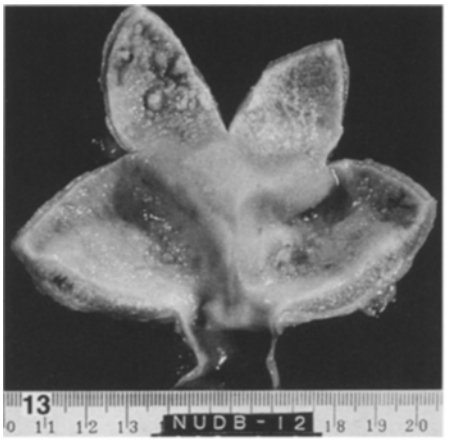
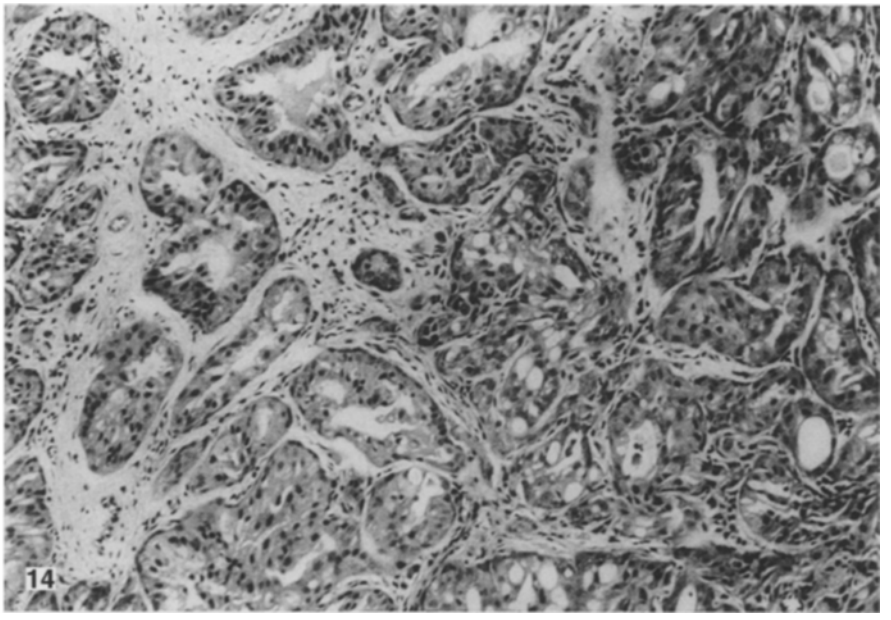


Fig. 12 Carcinoma in situ in dog 37. H & E, ×240





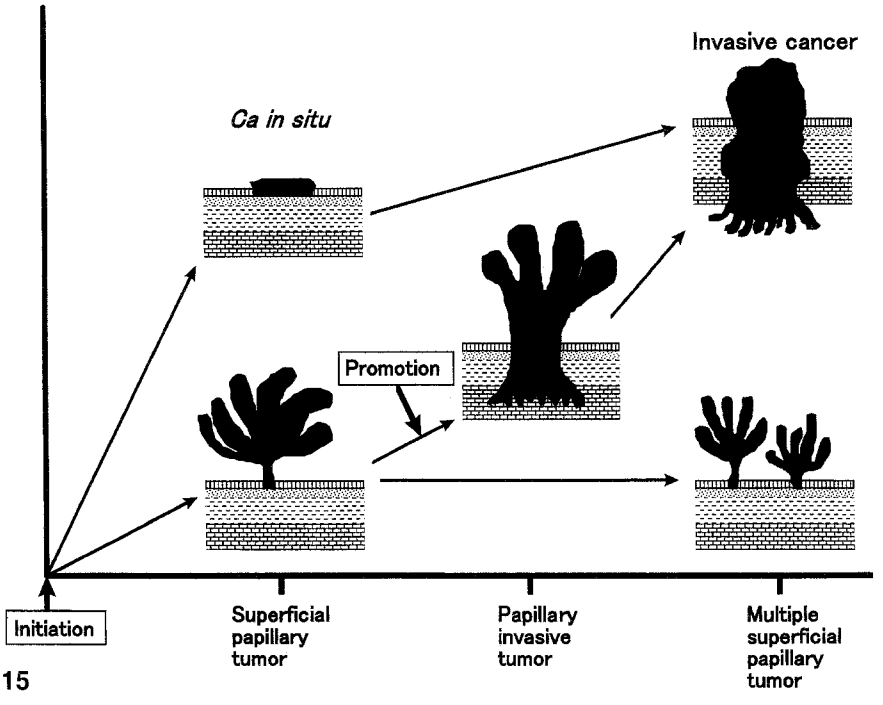


Fig. 13 Gross finding of the urinary bladder in beagle dog B-12 treated with 500 mg/day of BBN for 220 weeks. Small nodular tumors are seen in all areas of the urinary bladder

Fig. 14 Nonpapillary, invasive transitional cell carcinoma grade 3 in beagle dog B-12. H & E, ×100

Fig. 15 Natural history of bladder carcinoma in dogs

changes in the superficial microstructure of the bladder epithelium of dogs during the course of tumor development and found that changes resembling those in human bladders occurred in dog bladders [5]. These previous studies indicated that dog bladder tumor shows patterns of histogenesis and progression similar to the natural history of human bladder cancer, and that it is highly useful as a model of human bladder cancer.

As described in our previous report [16], our histopathological examination of changes in the bladder epithelium of 50 elderly beagle dogs suggested the high utility of beagles for experiments involving bladder carcinogenesis. The present study was undertaken to study histopathologically two types of bladder tumor induced in mongrel dogs. The study was also intended to trace histopathologically the course of tumor progression after tumor induction in beagle dogs, for the purpose of investigating the natural history of bladder tumors in dogs.

In the present study, oral treatment of beagle dogs with low-dose BBN (80 or 160 mg/day) resulted in the development of multiple, papillary, noninvasive TCC and nonpapillary, noninvasive tumors showing Brunn's nest-like growth. Papillary, invasive tumor was also seen in part of the bladder of these dogs. In the two beagle dogs in which BBN treatment (160 mg/day) was discontinued upon detection of tumors at 2.5 years, and whose course was traced for more than 8 years there-

after, the papillary, noninvasive tumors of grade 1 or 2 atypism showed very slow progression, and TCC of grade 2 or 3 and pT1b, which resembled the initial tumors, was seen when these animals were finally killed.

The lifespan of beagles reared on animal farms with a favorable environment has been reported as 13–14 years. In the present experiment, the beagles survived for 11 years in the air-conditioned experiment room. These beagles seem to have completed almost the natural span of their lives. They reached high ages which correspond to over 70 years of age in humans. However, hematological and biochemical parameters showed no abnormalities, and cystoscopy, which was carried out at several points, revealed no marked growth or spread of tumors after BBN was discontinued. It is known that the development of experimental low-grade papillary, noninvasive tumor of the bladder is accelerated by promoters [1, 3, 7]. In the present experiment, however, long-term observation of dogs in which treatment with the carcinogen was discontinued upon detection of tumor revealed no further growth or spread of the tumors when the animals were kept controlled from the point of view of feeding and other living conditions. This suggests that urinary bladder tumors once developed are unlikely to progress much if no promoters are present after the development of tumors. It has been reported that about 10% of all cases of human papillary, noninvasive TCC become invasive carcinomas after sequences of recurrence [4, 9]. This is probably because the exposure of humans to various carcinogens and promoters induces an elevation in the initially low grade of atypism of the superficial cancer, resulting in progression of the atypism-intensified area into invasive cancer.

The two beagles (B-4 and B-5) were found to have many papillomas. In the papilloma-affected areas of these dogs, plasma cell invasion was seen immediately below the epithelium, but no epithelial cell proliferation was seen in the lamina propria of these dogs.

In the high-dose BBN treatment group (500 mg/day), nonpapillary, invasive TCC developed in the bladder, accompanied by papillary, noninvasive tumors and primary CIS in part of the bladder. Other than these changes, severe dysplasia was noted as well. In the two beagles in which BBN treatment (500 mg/day) was temporarily suspended after tumor detection in the 123rd week but subsequently recommenced although intermittently, the initial nonpapillary, invasive tumors of TCC grade 3 pT3b did not increase in severity or metastasize to any other organ.

When the urinary bladder mucosa of elderly beagles was examined, the lamina propria of every region of the mucosa in all dogs had Brunn's nests, and the same finding was obtained in 66.7% of younger beagles [16]. Brunn's nests are regarded as representing proliferative changes [8]. In the present experiment, the bladder tumor induced in all dogs was multiple in nature. In both the low-dose and high-dose BBN groups, it seems likely that many of the cancerous epithelial cells progress via Brunn's nest-like proliferation into papillary, noninvasive carcinoma, accompanied by stroma and vessel formation, or, in areas with more intense atypism, they progress into nonpapillary, invasive carcinoma. Kishi [10] reported on Brunn's nest-type early cancer as a morphological type of early bladder cancer. Regarding the pathogenesis of papillary cancer of the urinary bladder, Kishi speculates that some cases of CIS, which initially show Brunn's nest-like growth, gradually assume a papillary form during the course of its growth. It seems likely that Brunn's nest formation is related to the development and progression of bladder tumors in dogs. Brunn's nests have been detected in various regions at high incidences in both humans and dogs [15]. It seems likely that the formation of Brunn's nests is related to the development and progression of bladder cancer in humans also.

In the present study, the papillary, noninvasive tumors of the bladder, induced by low-dose BBN treatment of dogs, continued to have superficial cells. However, in the areas of the bladder where CIS or severe dysplasia was induced by high-dose BBN treatment, extensive defects of superficial cells were seen, and the tumors in these areas tended to progress into invasive tumors.

It seems likely that intense damage of the superficial cells due to exposure to potent carcinogens or large amounts of carcinogens results in loss of the barrier function, and that long-term exposure to carcinogens after the loss of the barrier can induce severe changes in the epithelial cells of the urinary bladder, e.g., CIS or severe dysplasia. It is also likely that in the areas where CIS or dysplasia has developed, highly atypical cells invade the submucosa tissue and muscle layers before epithelial cells proliferate in the lamina propria.

The length of time from the start of BBN treatment to the development of bladder carcinoma was 137–205 weeks for beagles treated with low-dose BBN, 123 weeks for beagles treated with a high-dose BBN, 119 weeks for mongrel dogs treated with low-dose BBN and 58.5 weeks for mongrel dogs treated with a high-dose BBN. The period was thus slightly longer in beagle dogs. The total BBN dose required for urinary bladder tumor to develop was 131.48 g for the low-dose beagle group, 369.0 g for the high-dose beagle group, 114.22 g for the low-dose mongrel group, and 175.5 g for the high-dose mongrel group. Thus, a higher total dose of BBN was required for carcinoma to develop in beagles. These differences between mongrel and beagle dogs seem to represent differences in the environment to which the dogs had been exposed before the start of the present study. That is, the beagles had been raised under known conditions, while the age and the rearing conditions for mongrels were unknown.

The urinary bladder tumors induced by oral treatment of dogs with low-dose BBN were histopathologically similar to human papillary TCC of the bladder of low grade and low stage, while the bladder tumors induced by oral treatment of dogs with high-dose BBN resembled human nonpapillary transitional cell carci-

noma of high grade and high stage. The manner of progression of these two types of urinary bladder tumor induced in dogs also resembled those in humans. It is known that the expression of oncogenes and their variations or defects are major factors related to the development of urinary bladder cancer in humans. The results of the present experiment suggest that the malignant potential of transitional cells of the bladder is determined to some extent by the quality and quantity of initiators involved in the development of urinary bladder cancer. Regarding the manner of progression of bladder cancer, we may say therefore that papillary, superficial bladder cancer of low grade has low potentials for malignancy and remains superficial cancer unless it becomes invasive due to the effects of some promoters, while high-grade bladder cancer soon begins invasive growth, without undergoing papillary growth, and becomes nonpapillary, invasive cancer. The malignancy potentials and the extent of the spread of bladder cancer seem to be determined by many factors, including promoters and host factors (Fig. 15). Studies of the pathogenesis of experimental bladder tumors in dogs seem to be very useful in clarifying the development and progression of human bladder cancer.

Acknowledgements This work was supported by grants-in-aid for Cancer Research from the Ministry of Health and Welfare and from the Ministry of Education, Science and Culture, Japan.

References

1. Cohen SM, Arai M, Jacobs JB, Friedell GH (1979) Promoting effect of saccharin and DL-tryptophan in urinary bladder carcinogenesis. Cancer Res 39:1207
2. Druckrey H, Preussmann R, Ivankovic S, Schmidt CH, Mennel HD, Stahl KW (1964) Selektive Erzeugung von Blasenkrebs an Ratten durch Dibutyl und *N*-Butyl-*N*-butanol(4)-nitrosamin. Z Krebsforsch 66:280
3. Fukushima S, Thamavit W, Kurata Y, Ito N (1986) Sodium citrate: a promoter of bladder carcinogenesis. Jpn J Cancer Res 77:1
4. Greene LF, Hanash KA, Farrow GM (1973) Benign papilloma or papillary carcinoma of the bladder? J Urol 110:205
5. Hirao Y, Sanma S, Ozono S, Babaya K, Okajima E (1987) Scanning electron microscopy of changes in the urinary bladder in dogs treated with *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine(BBN), Urol Res 15:25
6. Ito N, Hiasa Y, Tamai A, Okajima E, Kitamura H (1969) Histogenesis of urinary bladder tumors induced by *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine in rats. Jpn J Cancer Res 60:401
7. Ito N, Fukushima S, Shirai T, Nakanishi K (1983) Effects of promoters on *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine-induced urinary bladder carcinogenesis in the rat. Environ Health Perspect 50:61
8. Japanese Urological Association and The Japanese Pathological Society (1993) General rules for clinical and pathological studies on bladder cancer, 2nd edn. (in Japanese) Kanehara Press, Tokyo
9. Kakizoe T, Matsumoto K, Tobisu K, Takai K (1987) Growth and progression patterns of papillary superficial bladder cancer. Jpn J Urol 78:1065
10. Kishi K (1986) Pathology Progress in cancer clinics 4, Bladder cancer. Medicalview, Tokyo, pp 108–116
11. Koss LG (1975) Tumors of the urinary bladder, Atlas of tumor pathology, fascicle 11, Armed Forces Institute of Pathology, Washington, DC
12. Ohtani M, Kakizoe T, Nishio Y, Sato S, Sugimura T, Fukushima S, Niijima T (1986) Sequential changes of mouse bladder epithelium during induction of invasive carcinomas by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine. Cancer Res 46:2001
13. Okajima E, Hiramatsu T, Hirao K, Ijuin M, Hirao Y, Babaya K, Ikuma S, Ohara S, Shiomi T, Hijioka T, Ohishi H (1981) Urinary bladder tumors induced by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine in dogs. Cancer Res 41:1958
14. Samma S, Uemura H, Tabata S, Iwai A, Nakatsuji F, Matsuki H, Babaya K, Hirao Y, Okajima E (1984) Rapid induction of carcinoma in situ in dog urinary bladder by sequential treatment with *N*-methyl-*N'*-nitroso-urea and *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine. Jpn J Cancer Res 75:385
15. Shirai T, Fukushima S, Hirose M, Ohshima M, Ito N (1987) Epithelial lesions of the urinary bladder in three hundred and thirteen autopsy cases. Jpn J Cancer Res 78:1073
16. Tsunemi K, Ozono S, Yamaguchi H, Hayashi Y, Babaya K, Hirao Y, Okajima E (1994) Histopathological findings of the urinary bladder epithelium in aged dogs. J Toxicol Pathol 7:73