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Endoscopic observation for detection and monitoring of *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine - induced bladder tumor in rats

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Abstract Recent advances in tumor carbohydrate biochemistry have demonstrated antitumor effects of locally administered G_{M3} ganglioside on mouse MBT-2 tumor. When intravesical therapy in *N*-butyl-*N*(4-hydroxybutyl)nitrosamine (BBN)-induced rat bladder tumor is attempted, it is essential to identify the tumor, to classify its size before therapy and to monitor the effect of the therapy. To establish a more reliable experimental therapeutic system, we assessed the development of BBN-induced rat bladder tumor by endoscopic observation. BBN-induced bladder tumors in rats were observed serially using a 4.2-F flexible fiberscope. The endoscopic findings were compared with the histopathological findings. Intravesical tumor growth varied greatly between individual rats. The smallest change detected by endoscopy was a small edematous lesion histologically proved to be papilloma. The largest nodular lesion was determined to be a papillary, transitional cell carcinoma. This noninvasive method makes the BBN rat experimental system more reliable by allowing confirmation of tumor formation and classification of the tumor volume prior to therapy.

Key words BBN · Bladder tumor · Rat · Endoscopy

Introduction

In 1964, Druckrey et al.[4] reported that when *N*-butyl-*N*(4-hydroxybutyl)nitrosamine (BBN) was given continuously in drinking water, urinary bladder tumors were induced. Since then, there have been numerous reports describing the histopathological findings in BBN-induced rat bladder tumor. This system has been established as an important model for human bladder tumor [2, 5, 7] . Since almost all BBN-induced bladder tumors are papillary and noninvasive transitional cell carcinomas, they provide a useful experimental therapeutic model for the study of human superficial bladder tumor [2, 5, 7].

Recently, the important role of carbohydrate chains as the adhesion molecule in tumor invasion and metastasis has been emphasized [17, 19]. We have previously reported that the glycolipid composition in some human urological tumors is of vital importance, not only in differentiating benign from malignant lesions, but also in predicting the metastatic potential [9–11, 16]. Furthermore, several attempts have already been made to apply recent advances in tumor carbohydrate biochemistry to cancer treatment, so called anti-adhesion therapy [15].

In bladder tumor, we have demonstrated the antitumor effect of locally administered G_{M3} ganglioside on mouse MBT-2 tumor as a model for human invasive bladder tumor [12]. As a candidate for anti-adhesion therapy for papillary and superficial bladder tumor, we selected the intravesical instillation system in the BBN-induced rat bladder tumor model.

In conventional orthotopic models of murine bladder tumors, we cannot confirm tumor formation or evaluate tumor volume before therapy. To overcome this problem, we have assessed the feasibility of endoscopic observation of BBN-induced bladder tumors in rats to classify the tumor volume by a noninvasive method.

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Materials and methods

Animals

Female Fischer 344 strain rats weighing 100–150 g were purchased from SLC Japan (Hamamatsu, Japan). All animals received a commercial stock diet and water ad libitum.

Carcinogen

BBN (Iwai Chemical, Tokyo, Japan) was given as a 0.05% solution in the drinking water for 12 weeks as described elsewhere [2, 5].

Endoscopic observation of BBN-induced bladder tumor

After BBN treatment, rats were anesthetized with pentobarbital (0.05 mg/g body weight, intraperitoneally), and their urethras were catheterized with a 4-F indwelling feeding tube (Atom, Tokyo, Japan). After 0.2 ml air was instilled into the bladders, and a 4.2-F PF-14 fiberscope: (Olympus, Tokyo, Japan) was inserted, the bladder was examined endoscopically. After observation, the largest and most demonstrable lesions were selected, and the lesions including bladder neck were photographed. Endoscopic observations were performed at the 12th, 14th, 17th and 20th weeks after initiation of BBN treatment.

Histopathology

Following endoscopic observation, three rats were sacrificed at the 12th, 14th, and 17th weeks, respectively. The remaining 13 rats were sacrificed at the 20th week for histological examinations. After death, rats were catheterized and 0.2 ml buffered formalin was instilled into the bladder. The urethras were then ligated and the bladders were removed. Fixed specimens were embedded in paraffin and five 3 μm thick horizontal slices in each rat were prepared at 2mm intervals followed by routine hematoxylin and eosin staining.

Results

Prior to the endoscopic observation with air instillation into bladder, we initially attempted water instillation normally used in conventional clinical examination, but failed to obtain a clear visual field because of cloudy intravesical water. Accordingly, we decided to apply the air instillation method. The smallest endoscopic finding in BBN-induced tumorigenesis was a small whitish and edematous change, which was 200–300 μm in diameter. By sequential observation, the small mucosal lesion was found to be actually larger and multiple.

The endoscopic findings were classified as follows:

Type I: small whitish spot without mucosal swelling (Fig. 1a)
Type II: flat mucosal swelling (Fig. 1b)
Type III: hemisphere or bullet-shaped nodular lesion (Fig. 1c)

At the 12th week, type I or type II findings were observed in each animal. At the 14th week, these lesions tended to increase in number and become larger, and great differences in size were noted among the rats. Of the 19 rats receiving endoscopy at the 14th week, 5 rats had definitely larger tumors (type III) than the others. Figure 2 shows the smallest and the largest lesions, respectively, at the 14th week. Table 1 shows the variations of endoscopic findings in each week. Table 2 shows the relationships between endoscopic and pathological findings.

Discussion

Because almost all BBN-induced bladder tumors in rats are papillary and superficial, unless the tumors become huge [7], application of this model can be used not only as a therapeutic model but also as an important method of studying the etiology of human superficial bladder tumor [2, 5]. There have been many reports which have mentioned the effect of anticancer agents using this model.

Histogenesis of bladder tumors in Wistar strain rats after administration of 0.05% BBN has been precisely investigated [7]. After 12 weeks of BBN administration, 80% of the rats had papilloma and 20% had cancer, and after a sequential 8-week treatment without BBN all rats had papilloma and 72.7% had cancer. Although 4-week treatment with 0.05% BBN did not produce tumors in Fisher 344 strain rats, 12-week treatment induced cancer in 70–80% of rats by the 20th week [13]. The experimental therapeutic system of 8-week BBN treatment is appropriate for the study on promoters [2], and 12-week BBN treatment is suitable for the study of the antitumor effect [5]. Accordingly, we selected a system ending at the 20th week after 12 weeks of BBN treatment. One major defect of the BBN-induced rat bladder tumor procedure is that the presence, localization and volume of the tumors cannot be confirmed since anti-cancer procedures are initiated before confirming the presence of the tumor. Moreover, we cannot monitor the changes in tumors after treatment by the noninvasive procedure.

Generally, at the 12th week of BBN treatment, three different histological types of lesions can be seen, i.e., hyperplasia, papilloma and cancer [7]. Without doubt, tumor size at the initiation of therapy will influence the results. A more reliable experimental system is essential to confirm the tumor presence and classify the tumor size to some extent before initiating therapy.

One recent breakthrough concerning this problem has been the utilization of larger animals such as dogs [14]. This system, however, requires considerable time to obtain tumor-bearing dogs and has the problem of obtaining a sufficient number of animals.

Along with the recent advances in diagnostic methods, some noninvasive techniques, for example, magnetic resonance imaging (MRI) [3] or transrectal ultrasonography [1], have been attempted. Alexander et al. [1] demonstrated the advantages of transrectal ultrasound over MRI because of its lower cost and time consumption.

Even though our endoscopic method has the disadvantage compared to ultrasound in that precise quantitation of tumor response is more difficult, endoscopy has a capability equal to or greater than that of ultrasound and has the advantage of being able to detect smaller lesions. In fact, the endoscopic method we used detected a minimal mucosal change which proved histologically to be a small papilloma less than 0.5 mm in radius. The

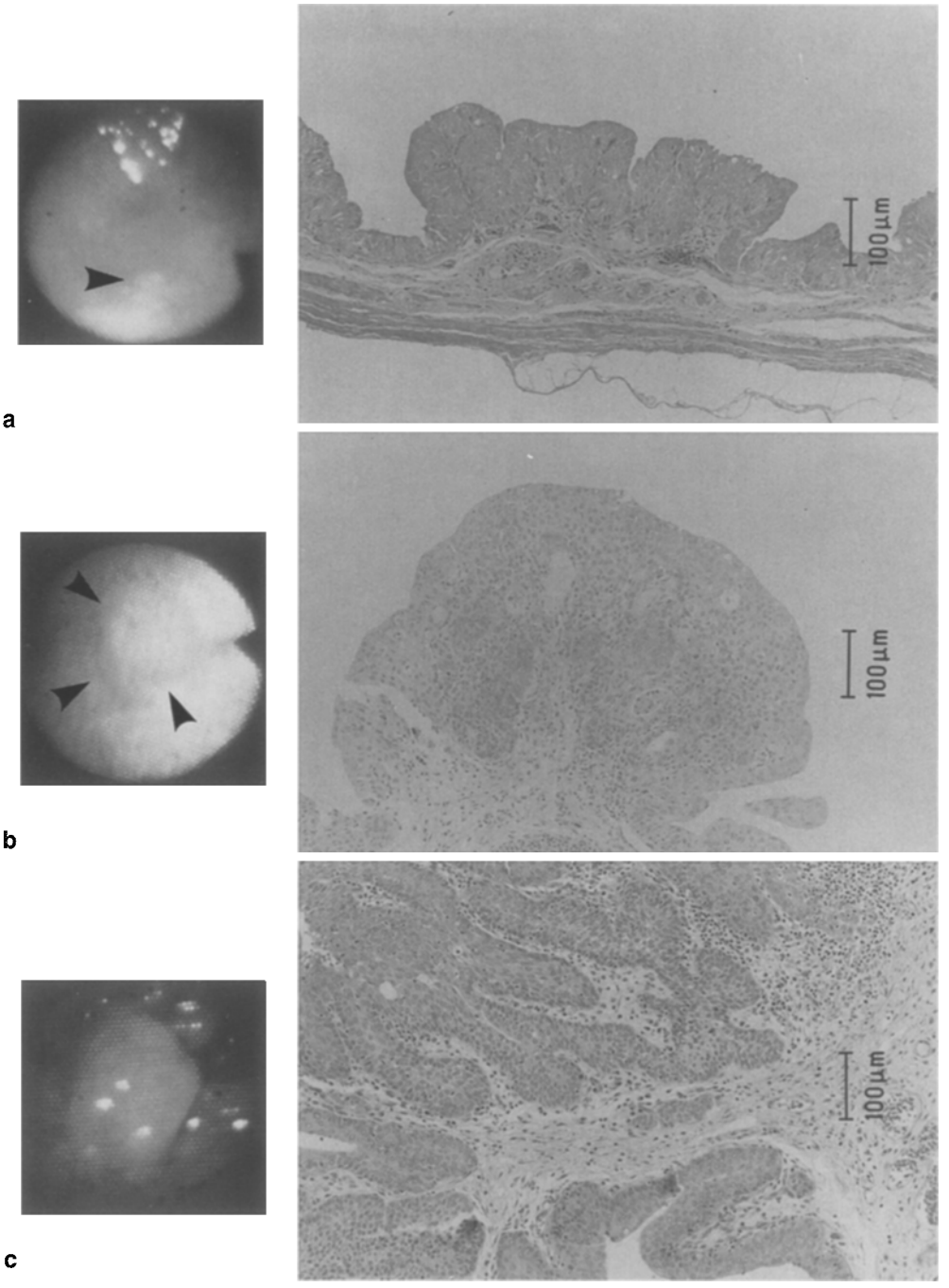

Fig. 1a–c Endoscopic classification and histology. **a** Type I change (*arrowhead*) histologically proved to be small papilloma, × 100. **b** Type II change (*arrowheads*). This lesion was composed of papilloma and low-grade transitional cell carcinoma, × 100. **c** Type III change. This lesion was identical to the grade 2 transitional cell carcinoma, × 100

major aims of endoscopy in the animal experimental system are to confirm irreversible tumoral change and to exclude extreme variations in tumor size before initiation of therapy.

At the 12th week of the present study, mucosal changes were seen in all the rats examined. The smallest change detected by endoscopy was a small whitish mucosal lesion without protrusion which was histologically proven to be papilloma. Hyperplasia was not detected by endoscopy on this occasion. At the 14th week, these mucosal lesions increased in both number and in size. Some animals had large, nodular, protruding tumors forming into hemispheric or bullet shapes. Such large tumors proved histologically to be carcinoma. All type I changes in endoscopy were papilloma, but type II tumors were composed of papillomas and carcinomas. All type III tumors were carcinomas. These results suggest that this endoscopic method may provide a histological diagnosis to some extent.

Our ultimate aim is to control growth, invasion and metastatic potential of tumor cells by altering their surface glycosylation phenotype. We have already demonstrated the antitumor effect of locally administered G_{M3} on murine MBT-2 tumor [12].

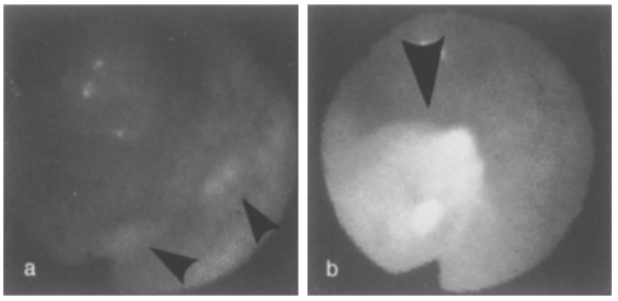

Fig. 2a, b The smallest (**a**) (*arrowheads*) and the largest (**b**) tumors at the 14th week

Table 1 Serial changes in endoscopic findings

Endoscopic finding	Weeks			
	12	14	17	20
Type I	10	0	0	0
Type II	12	14	7	3
Type III	0	5	9	10

Table 2 Relationship between endoscopic and histological findings

Endoscopic finding	Histological finding		
	Hyperplasia	Papilloma	Cancer
Type I	0	3	0
Type II	0	4	3
Type III	0	0	12

As an intravesical instillation model, we chose BBN-induced rat bladder tumor. The anti-G_{M3} monoclonal antibody [6] or blocking agents of the glycosylation pathway such as brefeldin A [18] and fumonisins [8] may serve as promising drugs for intravesical instillation. To assess the antitumor effect, we will use the experimental protocol of 12 weeks treatment with BBN followed by 7 or 8 weeks of therapy. From the results of the present small study, tumoral lesions were found in all rats at the 14th week, but there was a great variation in tumor size. Five animals had definitely larger tumors than the others. Such cases should be excluded in an actual therapeutic trial.

In conclusion, to establish a more reliable BBN rat experimental system, endoscopic classification of the tumor size before therapy is considered essential. In future studies, we hope to elucidate the feasibility of intravesical anti-adhesion therapy on BBN-induced rat bladder tumor.

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