Sequential Changes of Mouse Bladder Epithelium during Induction of Invasive Carcinomas by N-Butyl-N-(4-hydroxybutyl)nitrosamine

Mikinobu Ohtani, Tadao Kakizoe, Yasunori Nishio, Shigenori Sato, Takashi Sugimura, Shoji Fukushima, and Tadao Niijima

ABSTRACT

Invasive carcinoma of the bladder in humans shows aggressive growth with poor prognosis. Little is known about its preceding lesions. Sequential changes of the bladder epithelium following administration of N-butyl-N-(4-hydroxybutyl)nitrosamine (BHBN) were studied in mice. Female C3H/He mice were divided into 4 groups. Three groups were given 0.05, 0.01, and 0.005% concentrations of BHBN, respectively, in their drinking water, and the control group was given tap water. The mice were killed at regular intervals over a period of 26 weeks, and their bladder epithelium was examined histologically. Dysplasia, carcinoma in situ, and invasive carcinoma were observed sequentially in the groups treated with BHBN, and the incidences of dysplasia, carcinoma in situ, and invasive carcinoma were dependent on the dose of BHBN. The data indicate that bladder carcinoma in mice is a good model of invasive bladder carcinoma in humans, although it is not fully compatible with the human model because of the complete absence of metastases.

INTRODUCTION

Human bladder carcinoma can be classified into at least two types: papillary superficial bladder carcinoma and non-papillary invasive bladder carcinoma. The former frequently recurs after treatment such as transurethral resection or coagulation, but its prognosis is good. The latter frequently shows metastasis and has a very poor prognosis. The reason why two different types of bladder carcinoma can develop in the urinary bladder is unknown.

Detailed studies on the development of bladder carcinoma in rats have been performed using various bladder carcinogens, such as BHBN, N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide, N-2-fluorenylacetamide, and N-methyl-N-nitrosourea (1-4). In their extensive studies on bladder carcinogenesis in rats, Ito et al. (5-8) examined the dose dependence, strain difference in susceptibility, sequential changes in the bladder, and differences in susceptibility of bladder epithelium in various animals during carcinogenesis induced by BHBN. They found that regardless of the type or dose of carcinogen, bladder carcinomas induced in all strains of rats are of a papillary pedunculated type and are usually multiple and superficial, but they found that when these carcinomas become very large, invasion of deeper tissues is sometimes observed. They concluded that bladder carcinomas in rats are a satisfactory model of papillary superficial bladder carcinoma in humans.

Bladder carcinogenesis in mice has not been studied as extensively as that in rats. Hirose et al. (8) reported that bladder carcinomas in mice were almost always of a non-papillary invasive type. Thus since detailed analysis of bladder carcinogenesis in mice should provide information on the histogenesis and progression of invasive bladder carcinomas in humans, in our work we examined sequential changes in the bladder of mice during development of invasive bladder carcinoma.

MATERIALS AND METHODS

Animals. A total of 123, 5-week-old female C3H/He mice were obtained from Charles River Japan (Kanagawa, Japan) and housed, 5-6 per plastic cage, in an air conditioned room at 23°C with 50% humidity with a 12 h-12 h light-dark cycle. At 8 weeks of age, they were divided at random into 4 groups of 30-31 mice each.

Experimental Design. BHBN was purchased from Tokyo Kasei Co. (Tokyo, Japan), dissolved in tap water to concentrations of 0.05, 0.01, and 0.005% and supplied to groups 1 to 3, respectively, ad libitum. Mice from each group were killed in weeks 4, 8, 12, 16, 20, and 26 of the experiment (Fig. 1). Group 4 was given tap water throughout. Mice were weighed every 2 weeks. When the animals were killed, the bladder was fixed by an injection of about 0.10 to 0.15 ml of 10% buffered formalin (pH 7.4) into the lumen. Each bladder was then cut into 8 serial sections and stained with hematoxylin and eosin for light microscopic examination.

Light Microscopic Classification. The lesions found in the urinary bladder epithelium were classified into 4 types: simple hyperplasia; dysplasia; carcinoma in situ; and invasive carcinoma. These lesions were defined as follows: simple hyperplasia, diffuse or focal thickening of the epithelium with 4 or more layers of transitional epithelial cells; dysplasia, focal thickening of the epithelium with mild to severe cellular anaplasia; carcinoma in situ, severe cellular anaplasia with loss of polarity and presence of mitotic figures; and invasive carcinoma, carcinoma infiltrating the submucosa or muscle layer with transitional cell or partly squamous cell features (Figs. 2-6).

RESULTS

Weight gain was similar in all groups until week 20, when subsequently growth of all mice given BHBN became retarded. At the end of the experiment, the mean body weight of the mice in group 1 was markedly less than those of the other three groups (Fig. 7), probably owing to severe hydronephrosis resulting from ureter obstruction by invasive bladder carcinomas.

Light microscopic findings in the urinary bladder epithelium are shown in Table 1. The sequential changes of the bladder epithelium in groups 1-3 were simple hyperplasia, dysplasia, carcinoma in situ, and invasive carcinoma (Table 1 and Fig. 8). The incidences of dysplasia, carcinoma in situ, and invasive carcinoma seemed dependent on the dose of BHBN. On gross observation, induced carcinomas always showed unifocal non-papillary growth, although the margins of the tumors were indistinct because of gradual transition of the tumor to the diffusely-thickened surrounding tissue. No gross papillary lesions were observed, but microscopic papillary lesions such as papillary hyperplasia, papillary carcinoma, and invasive papillary carcinoma were observed in groups 1 and 2 (Fig. 9, Table

2001
shown that bladder carcinoma induced by BHBN and other related nitroso compounds in C3H/He mice is highly malignant and invasive. However, there have been no detailed reports on the relationship between preneoplastic changes and the development of invasive bladder carcinoma in mice, although Bertram and Craig (10) and Akagi et al. (11) suggested that bladder carcinomas in mice might develop directly from hyperplastic or dysplastic areas. The present data confirm their suggestion.

2). All of these lesions developed unifocally. Development of microscopic invasive papillary carcinoma was a rather exceptional finding. There were no papillary lesions in groups 3 and 4. No bladder calculi were observed in any of the mice, and no distant metastases were detected by gross examination. In week 26, hydronephrosis due to bladder carcinoma was seen in all mice in group 1 but in none in groups 2 or 3.

DISCUSSION

The present results show that simple hyperplasia, dysplasia, carcinoma in situ, and invasive carcinoma were induced sequentially by BHBN and that the incidences of these lesions depended on the dose of BHBN. Several studies (8–12) have
Invasive carcinoma in the bladder epithelium of mice probably develops from carcinoma in situ or dysplasia.

Okajima et al. (13) reported that BHBN induced both superficial papillary and sessile invasive carcinomas in the urinary bladder of dogs; they found that a short period of treatment with a high dose of BHBN induced carcinoma in situ and sessile invasive carcinomas, whereas a long period of treatment with a low dose of BHBN induced papillary non-invasive bladder carcinoma. Their findings are not consistent with those in rats, in which both high and low doses of BHBN induce papillary bladder carcinomas (14). In the present experiment, both high and low doses of BHBN induced non-papillary invasive bladder carcinomas in mice. Microscopic papillary lesions of the bladder were also observed in groups 1 and 2; these lesions were small but usually invasive (Fig. 9), and they were observed in the late stage of carcinogenesis. In contrast, the papillary lesions observed in rats, such as papillary hyperplasia and papilloma, seemed to be precursors of papillary carcinoma, since later papillary carcinomas developed from these precursor lesions.

When they become large, the carcinomas invaded the submucosa, muscle layer, or occasionally even deeper layers.

Kaye and Lange (15) analyzed the medical histories of 166 patients with invasive bladder cancer and found that only 16% had prior noninvasive bladder tumors. Greene et al. (16) analyzed 100 consecutive patients with grade 1 transitional cell carcinoma who were followed up for 15 years or more and found that 73% of the patients had recurrent tumors, but only 10 patients developed invasion later. Brawn (17) studied 104 cases of invasive bladder carcinoma to identify their precursor

<table>
<thead>
<tr>
<th>Period (wk.)</th>
<th>Effective no. of mice</th>
<th>Simple hyperplasia</th>
<th>Dysplasia</th>
<th>Carcinoma in situ</th>
<th>Invasive carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>5 (100)*</td>
<td>1 (20)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>5 (100)</td>
<td>1 (20)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>5</td>
<td>5 (100)</td>
<td>1 (20)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>5 (100)</td>
<td>1 (20)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>26</td>
<td>6</td>
<td>6 (100)</td>
<td>1 (20)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

---

**Table 1. Light microscopical findings in urinary bladder**

---

**Fig. 7. Body weight changes of mice.**

**Fig. 8. A, incidences of dysplasia; B, incidences of carcinoma in situ; C, incidences of invasive carcinoma.**

2003
lesions and concluded that papillary neoplasms of the bladder may not be common precursors of invasive carcinoma but that other less visible non-papillary lesions may be the most frequent precursors of invasive bladder carcinoma. We also analyzed 90 cystectomized specimens of bladder carcinoma by step-sectioning (18) and suggested that high grade invasive carcinoma developed mainly from carcinoma in situ and occasionally from original high grade papillary carcinoma. However, on careful examination of the mucosa, no concomitant dysplasia or carcinoma in situ in the mucosa of the bladder was found in 15 cases of solitary invasive carcinoma.

The difference in the tumor types observed in rats and mice might be attributable to the different genetic backgrounds of the animals, resulting in different responses to carcinogens. The different tumor types observed in humans might be explained in a similar way.

Detailed studies on the relationship between papillary superficial lesions and non-papillary invasive lesions of the urinary bladder are clearly required. Further studies on mice should provide information on the mechanism of development of invasive carcinoma.

REFERENCES


Table 2 Incidences of papillary hyperplasia, papillary carcinoma, and invasive papillary carcinoma

<table>
<thead>
<tr>
<th>Group</th>
<th>Period (wk.)</th>
<th>Effective no. of mice</th>
<th>Papillary hyperplasia or papillary carcinoma</th>
<th>Invasive papillary carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>5</td>
<td>2 (40)*</td>
<td>1 (20)</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>5</td>
<td>5 (100)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>5</td>
<td>4 (80)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>4</td>
<td>4 (100)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>5</td>
<td>3 (60)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>5</td>
<td>5 (100)</td>
<td>1 (20)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.
Sequential Changes of Mouse Bladder Epithelium during Induction of Invasive Carcinomas by \( N \)-Butyl-\( N \)-(4-hydroxybutyl)nitrosamine

Mikinobu Ohtani, Tadao Kakizoe, Yasunori Nishio, et al.