Enhancement of $N$-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamidine-induced Carcinogenesis by Urinary Tract Infection in Rats

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ABSTRACT

Epidemiological studies suggest that urinary tract infection is an important risk factor in the development of bladder cancer. Chronic urinary tract infection in rats is associated with urothelial hyperplasia and papillomatosis. In the Sprague-Dawley strain, exposure to the 5-nitrofuran, $N$-[4-(5-nitro-2-furyl)-2-thiazolyl]formamidine (FANFT), is associated in particular with the development of renal pelvic tumors. The present study was designed to evaluate whether chronic urinary tract infection could enhance tumor development in FANFT-induced urinary tract carcinogenesis. One hundred forty-four female Sprague-Dawley rats were divided into the following groups. Group 1 received 0.2% FANFT in the diet for 7 wk followed by control diet. Group 2 received 0.2% FANFT in the diet for 7 wk followed by control diet. One wk after completion of FANFT administration, the suspension of 0.5 ml of Escherichia coli (06K13H1) was injected into the bladder through the urethra. Group 3 received 0.2% FANFT in the diet for 7 wk followed by control diet. One wk after completion of FANFT administration, a suspension of heat-killed E. coli (06K13H1) was injected into the bladder through the urethra. Group 4 received a suspension of 0.5 ml of E. coli (06K13H1) through the urethra and received control diet throughout the experiment. Group 5 was fed control diet only. The experiment continued for 104 wk. A significantly higher number of urinary tract tumors, particularly of the renal pelvis, was recorded in Group 2 compared to Groups 1, 3, 4, and 5. The majority of the rats in Groups 2 and 4 had morphological signs of urinary tract infections, particularly pyelitis and/or pyelonephritis. Thus, a single injection of E. coli (06K13H1) into the bladder resulted in an enhancement of FANFT-induced urinary tract carcinogenesis in the Sprague-Dawley rat, especially for renal pelvic tumors. The formation of dimethylnitrosamine or other nitroso compounds from nitrates in the urine or increased cell proliferation due to chronic inflammation or both may be important pathogenetic factors in the tumor development.

INTRODUCTION

Data from urinary tract cancer patients and animal models support the hypothesis that inflammation or infection of the urinary tract may be important risk factors for the development of bladder cancer (1–6). Patients who are particularly susceptible to chronic cystitis, such as spinal cord injury patients, have been reported to have an excess of bladder cancer (7, 8). The majority of the tumors in these patients have been squamous cell carcinomas or carcinomas with a mixed squamous and urothelial pattern (8). Patients with squamous metaplasia, i.e., due to schistosomiasis or squamous metaplasia per se, have a markedly increased risk of developing squamous cell carcinoma (7, 9).

In rats, long-term urinary bladder infections both with and without bladder implants have resulted in urothelial hyperplasia, dysplasia, squamous metaplasia, and even bladder papillomata (6).

Administration of 0.2% FANFT in the diet for 10 wk to Fischer 344 rats is associated with 100% development of urinary bladder tumors (10). When FANFT was discontinued after 6 wk the urothelial lining, although hyperplastic, regressed to normal within 44 wk (10, 11). In male Sprague-Dawley rats, administration of 0.2% FANFT in the diet for 11 wk resulted in a high incidence of urinary tract tumors, particularly of the renal pelvis (12).

The present study was designed to investigate whether injection of Escherichia coli into the bladder enhances the incidence of urinary tract tumors in FANFT-induced urinary tract carcinogenesis.

MATERIALS AND METHODS

Six-wk-old female specific-pathogen-free Sprague-Dawley rats supplied by Anticimex AB, Stockholm, Sweden, weighing approximately 60 g were used. FANFT was purchased from Saber Laboratories, Morton Grove, IL. Semisynthetic, pelleted diet containing 0.2% FANFT was made by Astra Ewos AB, Sodertalje, Sweden. The rats received the diet for 7 wk.

The rats were exposed to cycles of 12 h of light and 12 h of darkness. They were kept at a temperature of 22–23°C, and the relative humidity was kept at 55 to 60%. They received food and tap water ad libitum.

One hundred forty-four rats were randomly divided into five groups (see below). One rat in Group 2 died within 1 wk after the injection of E. coli and has been excluded from the study. Group 1 (30 rats) received 0.2% FANFT in the diet for 7 wk followed by control diet. Group 2 (25 rats) received 0.2% FANFT in the diet for 7 wk followed by control diet. One wk after completion of FANFT administration, a suspension of 0.5 ml of E. coli (06K13H1) (10⁷ bacteria/ml) was injected into the bladder through the urethra (13). Group 3 (30 rats) received 0.2% FANFT for 7 wk followed by control diet. One wk after completion of FANFT administration, a suspension of 0.5 ml of E. coli (06K13H1) (10⁷ bacteria/ml) was injected into the bladder through the urethra (13). Group 4 (30 rats) received, at the start of the experiment, a suspension of 0.5 ml of E. coli (06K13H1) (10⁷ bacteria/ml) into the bladder, and the rats were maintained on control diet throughout the experiment. Group 5 (30 rats) were fed control diet only.

The E. coli bacteria used in the present experiments were taken from log cultures, and the injection was done within minutes after the cultures were taken out of the incubator, which means that almost all of the bacteria should be alive.

Urine cultures were taken at 20 wk of the experiment (12 wk after the injection of the E. coli). The animals were kept in metabolism cages for 4 h and the urine was collected on ice.

The rats were sacrificed when persistent hematuria occurred, when their general condition deteriorated, or after 2 yr. Complete postmortem examination was performed. The bladder was inflated with 4% buffered formaldehyde. The kidneys were bisected transversely, and the renal pelvis was examined for tumors. Histopathological examination also included liver, lungs, and heart as well as all grossly abnormal tissue. Five-μm sections were stained with hematoxylin-eosin and according to the Weigert van Gieson method. Comparison of the number of animals with tumors was performed by the χ² test and Fisher's exact test (14).

The abbreviation used is: FANFT, $N$-[4-(5-nitro-2-furyl)-2-thiazolyl]formamidine.

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RESULTS

The dietary intake and body weight did not significantly differ between the various groups of rats throughout the study. The cumulative intake of FANFT in g/rat was 1.51 g in Group 1, 1.43 g in Group 2, and 1.49 g in Group 3.

Positive urine cultures for E. coli (06K13H1) were found only in Groups 2 and 4. Fifteen rats (60%) in Group 2 and 19 rats in Group 4 (65%) had positive cultures 12 wk after the E. coli injection.

In Table 1 are given the mean survival time, tumor incidence, and tumor location within the urinary tract as well as type and frequency of inflammatory lesions in the urinary tract.

Three rats in Group 1 and 4 rats in Group 3 had, each, 6 urinary tract tumors (Table 1). Four and five of these tumors, respectively, were located in the renal pelvis, and two and one in the bladder, respectively. Eleven rats in Group 2 which were treated with 0.2% FANFT in the diet for 7 wk followed by injection of E. coli had a total of 16 urinary tract tumors. Thirteen of these tumors appeared in the renal pelvis and three in the bladder. The size of the renal pelvic tumors varied from 0.4 to 1.8 cm in largest diameter. Two rats in Group 4, comprising rats treated with a single injection of E. coli into the bladder, had urinary tract tumors. One of these was a papillary well-differentiated noninvasive urothelial tumor of the bladder detected at 2 yr. The other was a poorly differentiated invasive squamous cell carcinoma of the right renal pelvis found at 60 wk. Urinary tract tumors were not detected in Group 5 rats. None of the rats in any of the experimental groups had urinary tract calculi.

The majority of the tumors were well-differentiated noninvasive urothelial tumors (Fig. 1). This was the case for all tumors in rats in Groups 1 and 3. In Group 2, 10 tumors were well differentiated and noninvasive, and 6 were invasive. Three of the latter ones were squamous cell carcinomas.

Statistical evaluation showed that the number of tumor-bearing rats in Group 2 was significantly higher than in Groups 1 and 3 (P < 0.01 and 0.02, respectively) and in Groups 4 and 5 (P < 0.01 and 0.001, respectively).

The frequency of histological signs of urinary tract infection in the various groups is presented in Table 1. Signs of urinary tract infection were recorded, especially in the kidneys and renal pelvis of rats in Groups 2 and 4 exposed to E. coli, and almost all of the rats with urinary tract tumors had signs of marked chronic inflammation (Table 1). The lesions included cystitis, pyelitis (Figs. 2 and 3), and pyelonephritis (Fig. 4) with and without scarring. The inflammation of the renal pelvis was almost invariably associated with urothelial hyperplasia (Figs. 2 and 3). Two rats in Group 2 and three in Group 4 had unilateral renal papillary necrosis.

DISCUSSION

Female Sprague-Dawley rats exposed to 0.2% FANFT in the diet for 7 wk followed by an injection of E. coli (06K13H1) 1 wk later developed a significantly higher incidence of urinary tract tumors than rats exposed to FANFT alone or FANFT followed by intravesical injection of heat-killed E. coli or rats receiving E. coli alone. Urine cultures were found to be positive for E. coli in 60% and 65%, respectively, of the rats in Groups 2 and 4 at 20 wk. Although cultures were not performed at sacrifice there was a marked inflammatory response present which almost invariably was associated with urothelial hyperplasia of the renal papilla and/or urinary tract tumors. Thus, the injection of E. coli into the urinary bladder, which has been shown to produce acute as well as chronic nonlethal urinary tract infection in rats (13, 15), enhanced the carcinogenic effect of FANFT. This finding is in accordance with Cohen et al. (6), who observed development of bladder tumors after 2 wk of 0.2% FANFT followed by regenerative hyperplasia caused by freeze ulceration. Furthermore, in a rat model reported by Davis et al. (6), repeated intravesical bacterial infections were found to be associated with hyperplastic alterations of the urothelium, dysplasia, and early lesions consistent with neoplasia. Our results are in accordance with those of Davis et al. (6) and indicate urinary tract infection to be of importance for urinary tract carcinogenesis. Epidemiological studies in humans also support urinary tract infection to be an important risk factor for development of bladder carcinoma (5).

The majority of the tumors in the present study were located in the renal pelvis which previously has been shown to be the main target tissue in FANFT-induced urinary tract carcinogenesis using the Sprague-Dawley strain of rats (12). In our previous study the right-sided tumors in rats exposed to FANFT only were more common than left-sided ones, which possibly could be explained by the fact that ureteral reflux is particularly common on the right side of these rats. In the present study right-sided renal pelvic tumors were also slightly more frequent, especially in the rats receiving FANFT followed by injection of E. coli. Administration of E. coli enhances the carcinogenic effect of FANFT for the urinary tract and especially the renal pelvis. This correlation is further strengthened by the fact that all tumors in rats exposed to FANFT only were urothelial, well differentiated, and noninvasive, while 6 of 16 tumors in the group of rats exposed to FANFT and E. coli were moderately to poorly differentiated invasive tumors. This is in accordance with the results by Higgy et al. (17) who found that N-(4-hydroxybutyl)nitrosamine-exposed rats with E. coli infection developed more malignant lesions than the rats exposed to N-(4-hydroxybutyl)nitrosamine only. Three of our six poorly differentiated tumors and one renal pelvic tumor in the group of

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean survival time (wk)</th>
<th>No. of rats with urinary tract tumors</th>
<th>Tumor location</th>
<th>Type of inflammatory lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Right renal pelvis</td>
<td>Left renal pelvis</td>
</tr>
<tr>
<td>I (n = 30)*</td>
<td>88 ± 14.9 (48-102)*</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>III (n = 29)</td>
<td>87 ± 14.5 (52-97)</td>
<td>4</td>
<td>4 [1]</td>
<td>1</td>
</tr>
<tr>
<td>IV (n = 29)</td>
<td>83 ± 15.1 (52-101)</td>
<td>2</td>
<td>1 [1]</td>
<td>1 [1]</td>
</tr>
<tr>
<td>V (n = 30)</td>
<td>89 ± 14.2 (53-102)</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

* Number effective of rats.

** Mean ± SD.

† Numbers in parentheses, range.

‡ Numbers in brackets, tumor-bearing animals with histopathological signs of urinary tract infection.

* Significant compared to Groups I (P < 0.01), III (P < 0.02), IV (P < 0.01), and V (P < 0.001) with x² test and Fisher’s exact test.
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Fig. 1. Well-differentiated papillary urothelial carcinoma of the renal papilla. Group 2 at 96 wk. H & E, x 126.

Fig. 2. Renal pelvic mucosa exhibiting marked chronic inflammation, fibrosis, and urothelial hyperplasia. Group 2 at 84 wk. H & E, x 126.

Fig. 3. Renal papilla exhibiting marked chronic inflammation, fibrosis, and urothelial hyperplasia. Group 2 at 100 wk. H & E, x 126.

Fig. 4. Renal cortex with signs of acute and chronic pyelonephritis. Note tubular atrophy, interstitial fibrosis, interstitial lymphocytic infiltrates, and neutrophils in several tubules. Group 2 at 94 wk. H & E, x 126.

dimethylnitrosamine can occur, especially in the acidic urine usually present with bacterial infection. Thus, dimethylnitro-
samine may be responsible for the increased tumor incidence. However, the inflammation may also be involved, since inflam-
mation results in an increased proliferation and cell turnover in the urothelium which may also enhance tumor formation (16).

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REFERENCES


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