Aspirin Inhibition of N-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide-induced Lesions of the Urinary Bladder Correlated with Inhibition of Metabolism by Bladder Prostaglandin Endoperoxide Synthetase

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ABSTRACT

The effects of aspirin on N-[4-(5-nitro-2-furyl)-2-thiazolyl]-formamide (FANFT) -induced urinary bladder lesions, endogenous bladder prostaglandin E2 synthesis, and the metabolism of FANFT by bladder epithelial microsomes were examined. Rats were fed 0.5% aspirin and/or a diet containing 0.1% or 0.2% FANFT. Bladder lesions were observed with light and scanning electron microscopy, and the prostaglandin E2 content of rat bladder was measured by radioimmunoassay. Metabolism of FANFT was measured by decreased absorbance at 400 nm. Aspirin inhibited the appearance of hyperplastic lesions induced by feeding 0.1% or 0.2% FANFT for 6 or 12 weeks. Aspirin reduced bladder prostaglandin E2 content at 1, 2, 6, and 13 weeks compared to corresponding control values. Rat and rabbit microsomal metabolism of FANFT were dependent upon specific fatty acid substrates and prevented by specific inhibitors (including aspirin) of prostaglandin endoperoxide synthetase. Other inhibitor and substrate specificity studies suggest that FANFT was not metabolized by xanthine oxidase, lipoxygenase, lipid peroxidation, or mixed-function oxidases. These results suggest that the metabolism of FANFT by prostaglandin endoperoxide synthetase may be involved in the metabolic activation of FANFT necessary for the induction of bladder cancer in rats.

INTRODUCTION

Urinary bladder carcinogens benzidine (21), 2-amino-4-(5-nitro-2-furyl)thiazole (22), and FANFT (23) are metabolized in vitro by PES. Metabolism has been demonstrated with both intact tissue and microsomes (14, 21) to involve a radical mechanism which results in the covalent binding of metabolic products to tissue nucleophiles such as proteins, glutathione, tRNA, and DNA. PES has been localized subcellularly on the endoplasmic reticulum and nuclear membrane (15). This subcellular localization would make nucleic acids readily accessible to reactive products generated during cooxidative metabolism. Rat and rabbit urinary bladder epithelium contain substantial prostaglandin synthetic capacity (1). We have proposed that metabolic activation by bladder PES could be involved in the genesis of chemically induced urinary bladder cancer. Consistent with that hypothesis would be the concomitant inhibition of PES-mediated metabolism of carcinogens and reduction in chemically induced bladder cancer. Nonsteroidal antiinflammatory agents such as aspirin inhibit PES (5). To test this hypothesis, rats were fed aspirin in conjunction with the urinary bladder carcinogen FANFT. Aspirin effects on bladder microsomal PES-mediated FANFT metabolism, urinary bladder PGE2 content, and FANFT-induced hyperplastic bladder lesions were examined. The results are consistent with the hypothesis that metabolic activation by PES could be involved in the genesis of chemically induced urinary bladder cancer.

MATERIALS AND METHODS

FANFT was obtained from Saber Laboratories (Morton Grove, Ill.), and aspirin was from Sigma Chemical Co. (St. Louis, Mo.). They were mixed in Charles River powdered laboratory chow (Charles River, Inc., Wilmington, Mass.) and fed to male F344 rats (Charles River, Inc.) 5 weeks old at the beginning of the experiment according to the schedule indicated in Chart 1. Rats that would receive aspirin with or without FANFT (Groups 2, 4, and 5) were fed 0.5% aspirin in the diet for 2 days, and then FANFT was begun in appropriate groups (Groups 1 to 4) at doses of either 0.2% or 0.1% of the diet (Day 0 was counted as the time FANFT was started). Rats were weighed and food consumption was determined periodically. Rats were killed from each group at the end of Days 42 and 84. The rats were anesthetized with i.p. Nembutal (Abbott Laboratories, Chicago, Ill.), and the bladders were inflated with glutaraldehyde in cacodylate buffer. Half of the bladder was processed for light microscopic examination and half was processed for scanning electron microscopic examination as described previously (2, 6, 9, 10). Any rats with pleomorphic microvilli were tabulated since we have never seen them in normal rats. Rats were tabulated as having uniform microvilli or ropy microridges if they had significantly more and larger foci than the few small foci seen in control rats (13). Statistical comparisons between groups were made by the exact method for 2 x 2 contingency tables (17).

Male New Zealand rabbits (1.5 to 2.0 kg; Eldridge Laboratory Animals, Barnard, Mo.) and male F344 rats (5 weeks old at the beginning of the experiment) were anesthetized, and their bladders were removed. Pieces of rat bladder (45 to 70 mg) were placed in ice-cold 0.003 N HCl, immediately homogenized, and centrifuged at 2,000 x g for 10 min, and the supernatant was saved for radioimmunoassay of PGE2 content. Rabbit bladders were opened and stretched flat on a cork board, and the transitional epithelium was carefully separated from the rest of the bladder with forceps. A dissecting microscope was used to remove rat bladder transitional epithelium. Microsomes from rabbit and rat bladder epithelium were prepared as described previously (1). Protein content was determined by the method of Lowry et
al. (12), using bovine serum albumin as the standard. Radioimmunoas-
say of PGE₂ was performed as described previously (1). FANFT me-
tabolism was measured by the rate of decrease in absorbance at 400
nm, at 25°C. An extinction coefficient of 8.1 mw⁻¹ cm⁻¹ for FANFT was
used (23).

RESULTS

Rats fed FANFT and/or aspirin consumed food and grew at
rates comparable to the control group. The number of rats in
each group with various bladder lesions after 6 and 12 weeks
are shown in Table 1. As seen in previous studies (6, 9, 10),
scanning electron microscopy was more sensitive than light
microscopy in detecting bladder lesions. Aspirin (0.5% of diet)
showed inhibition of bladder hyperplasia induced by 0.2%
FANFT after 6 weeks as viewed by light microscopy. Although
the number of rats in which the various bladder lesions were
detected was not significantly different between the groups at
6 weeks, the number of lesions in each affected rat fed aspirin
plus 0.2% FANFT was considerably less, and the lesions were
smaller in size than those in rats fed 0.2% FANFT (Figs. 1 and
2). After 12 weeks, there was again some inhibition by aspirin
of the lesions induced by 0.2% FANFT, but complete inhibition
of the lesions induced by 0.1% FANFT (Figs. 3 and 4). No
lesions were seen in rats fed aspirin without FANFT or in
control rats.

With rabbit transitional epithelium microsomes, FANFT me-
tabolism was not observed prior to addition of arachidonic acid
(Table 2). Arachidonic acid elicited a dose-dependent metab-
olism of FANFT with 19.4 ± 1.3 (S.E.) and 12.5 ± 1.3 S.E.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Chemicals</th>
<th>6 wk</th>
<th>12 wk</th>
<th>6 wk</th>
<th>12 wk</th>
<th>6 wk</th>
<th>12 wk</th>
<th>6 wk</th>
<th>12 wk</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.2% FANFT</td>
<td>8</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>0.2% FANFT</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>0.1% FANFT</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>0.1% FANFT</td>
<td>5</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Aspirin</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>3</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Hyperplasia was assessed by light microscopy, and the other lesions were assessed by scanning electron microscopy.

Table 2

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Inhibitor (mM)</th>
<th>Specific activity (nmol/mg protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>ND*</td>
<td>ND</td>
</tr>
<tr>
<td>5,8,11,14-Eicosatetraenoic acid (arachidonic acid)</td>
<td>Aspirin (1)</td>
<td>19.4 ± 1.3 b</td>
</tr>
<tr>
<td>5,8,11,14-Eicosatetraenoic acid</td>
<td>Aspirin (0.1)</td>
<td>3.1 ± 0.6</td>
</tr>
<tr>
<td>5,8,11,14-Eicosatetraenoic acid</td>
<td>Indomethacin (0.02)</td>
<td>ND</td>
</tr>
<tr>
<td>5,8,11,14-Eicosatetraenoic acid</td>
<td>Allopurinol (0.13)</td>
<td>19.7 ± 1.4</td>
</tr>
<tr>
<td>5,8,11,14-Eicosatetraenoic acid</td>
<td>SKF-525A (0.05)</td>
<td>19.1 ± 0.9</td>
</tr>
<tr>
<td>5,8,11,14-Eicosatetraenoic acid</td>
<td>α-Naphthoflavone (0.1)</td>
<td>ND</td>
</tr>
<tr>
<td>11,14,17-Eicosatetraenoic acid</td>
<td>SKMF-525A</td>
<td>ND</td>
</tr>
<tr>
<td>NADPH</td>
<td></td>
<td>ND</td>
</tr>
</tbody>
</table>

* ND, not detectable; below sensitivity of the assay.

b Mean ± S.E.
Aspirin Inhibition of FANFT-induced Lesions and Metabolism

nmol per mg protein per min observed with 0.104 and 0.06 mm arachidonic acid, respectively. The PES inhibitors aspirin (1.0 mm) and indomethacin (0.02 mm) completely prevented FANFT metabolism. By contrast, allopurinol, SKF-525A, and α-naphthoflavone did not inhibit arachidonic acid-mediated metabolism of FANFT. FANFT metabolism was not initiated by arachidonic acid, mediated by PES. Aspirin at 0.1 and 1.0 mm caused 60 and 100% inhibition, respectively, of rat microsomal FANFT metabolism.

The effect of addition of 0.5% aspirin to the diet on rat bladder prostaglandin synthesis was examined. Rats were fed either a control diet or a diet with 0.5% aspirin. After 1, 2, 6, and 13 weeks, the urinary bladder PGE2 content was 0.46 ± 0.15, 0.54 ± 0.2, 0.23 ± 0.03, and 0.43 ± 0.08 ng/mg wet weight, respectively. In aspirin-fed rats, the PGE2 content of urinary bladder was 0.1 ± 0.06, 0.06 ± 0.03, 0.04 ± 0.01, and 0.22 ± 0.02 ng/mg wet weight at 1, 2, 6, and 13 weeks, respectively.

DISCUSSION

These data indicate that aspirin (a) inhibited the early morphological changes in the urinary bladder induced by FANFT, (b) inhibited PES-mediated FANFT metabolism by transitional epithelial microsomes, and (c) inhibited PGE2 synthesis by the bladder.

It has been demonstrated previously that the morphological changes induced by 0.2% FANFT at 12 weeks are irreversible and progress to urinary bladder cancer even if the feeding of FANFT is stopped at 12 weeks (2, 9, 10). The present study demonstrates that the changes induced by 0.2% or 0.1% FANFT at 6 and 12 weeks were inhibited by the coadministration of aspirin in the diet. Although the number of rats examined was small, statistically significant differences were found at the lower dose at 12 weeks. In addition, the number of abnormal foci in an affected bladder and the size of the lesions were considerably decreased in rats fed aspirin with FANFT (Figs. 1 to 4). There also appeared to be a dose effect; the lesions induced by 0.1% FANFT through 12 weeks were inhibited to a greater extent by aspirin than the lesions induced by 0.2% FANFT. However, the present study shows inhibition only of these early lesions. A long-term study is under way to determine if the formation of carcinoma is also inhibited.

Several enzyme systems have been shown to metabolize FANFT. These include nitroreductase (18), xanthine oxidase (20), and aryliiformamidase (19). These enzyme systems have not been shown to be inhibited by aspirin or indomethacin or initiated by arachidonic acid. Nitroreductase is inhibited by oxygen (18). In contrast, the PES-mediated metabolism of FANFT requires oxygen (23). ANFT, the deformedyl product of FANFT, was not identified as a reaction product. Metabolism of FANFT by these enzymes has also been difficult to relate to the genesis of FANFT-induced bladder cancer. For example, when allopurinol was administered to rats in conjunction with FANFT, there appeared to be enhancement of the carcinogenic potential of FANFT to induce bladder cancer rather than the expected inhibition (20). The metabolism of FANFT by bladder microsomes did not appear to be due to lipoxygenase enzymes (8) or lipid peroxidation since 11,14,17-eicosatrienoic acid did not initiate cooxidation. In addition, the lack of effect of SKF-525A (16), α-naphthoflavone (7), and NADPH suggests that mixed-function oxidase enzymes were not involved in FANFT cooxidation.

The most likely mechanism for aspirin inhibition of the morphological changes induced by FANFT is that aspirin inhibited the metabolism of FANFT mediated by PES. Aspirin is a known inhibitor of this enzyme (5), and PES has been shown to metabolize urinary bladder carcinogens such as FANFT (23) and benzidine (21). This metabolism has been shown to involve a radical mechanism and results in the covalent binding of metabolic products to tissue nucleophiles such as nucleic acids (21). Indirect evidence suggests that ANFT may be involved in FANFT carcinogenesis. However, both ANFT and FANFT are metabolized by PES. In addition, rats fed a diet containing 0.5% aspirin had significantly lower bladder PGE2 content. Rats were also fed a diet containing indomethacin. However, the dose of indomethacin required to inhibit bladder prostaglandin synthesis proved toxic due to the chronic basis with which indomethacin administration was required for this study. The in vivo data complement the in vitro results and suggest bladder transitional epithelial PES is involved in the pathogenesis of FANFT-induced urinary bladder cancer.

Aspirin also has other pharmacological effects. Studies in the mouse 2-stage skin cancer model have shown an inhibiting effect by aspirin and other nonsteroidal antiinflammatory agents of various aspects of the carcinogenic process. These effects are particularly on the promotion phase (11) and are independent of effects on carcinogen metabolism. In addition, aspirin is transported by the organic acid transport system in the renal cortex (3). It is possible that part of the effect of aspirin was an inhibition of the entry of FANFT into the urine. Alloxanthine, the major metabolite of allopurinol, accumulates during chronic administration of allopurinol and also is transported by the organic acid transport system (4). However, as mentioned previously, coadministration of allopurinol and FANFT resulted in an increased incidence of bladder tumors (20). Thus, the role of the renal organic acid transport system in FANFT bladder carcinogenesis remains unclear at the present.

ACKNOWLEDGMENTS

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REFERENCES


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Fig. 1. Scanning electron micrograph of a hyperplastic area of the urinary bladder of a rat fed 0.2% FANFT for 6 weeks showing pleomorphism of the cells. The luminal surface is covered with ropy microridges, small uniform microvilli, and pleomorphic microvilli in contrast to the peaked microridge system of the normal urinary bladder. × 1200.

Fig. 2. The luminal surface of the bladder of a rat fed 0.2% FANFT plus 0.5% aspirin for 6 weeks showing slight pleomorphism of the cells and covered predominantly with peaked and ropy microridges with a few small uniform microvilli. × 1200.

Fig. 3. The bladder of a rat fed 0.1% FANFT for 12 weeks showing cellular pleomorphism and abnormal surface features. × 1200.

Fig. 4. The surface of the bladder of a rat fed 0.1% FANFT plus 0.5% aspirin for 12 weeks with a normal luminal surface except for a small area having slight variation in cell size. × 1200.
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