Effect of Dose of Sodium Saccharin on the Induction of Rat Urinary Bladder Proliferation

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ABSTRACT

The effect of sodium saccharin fed to male F344 rats at levels of 5.0, 2.5, 1.0, 0.5, and 0.1% of the diet for 10 weeks was evaluated by autoradiography and scanning electron microscopy. A dose response was evident with both techniques. The labeling index was statistically significantly elevated at all doses above 0.1%. As observed by scanning electron microscopy, the number of foci containing ropy microridges or uniform microvilli was elevated at doses of 1.0, 2.5, and 5.0% of the diet, and pleomorphic microvilli were observed at the two highest doses.

INTRODUCTION

The 2-stage model of carcinogenesis, initiation and promotion, was initially demonstrated in mouse skin (1, 2, 13) and has subsequently been demonstrated in several tissues including the urinary bladder (3, 4, 6-8). Although sodium saccharin has been reported to be a weak carcinogen (12), increasing evidence suggests that its greatest effect in urinary bladder carcinogenesis is as a tumor promoter (4, 6). Promoting activity was initially demonstrated in mouse skin (1, 2, 13) and it has been previously demonstrated in this laboratory, using the sensitive techniques of autoradiography and scanning electron microscopy, that mild bladder epithelial hyperplasia is induced by sodium saccharin feeding (5). In the present study, we demonstrate a dose-response effect of sodium saccharin for the induction of bladder hyperplasia in male Fischer rats.

MATERIALS AND METHODS

Five-week-old male Fischer F344 rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were divided into 6 groups of 10 rats each. Five groups were continuously fed sodium saccharin (Lot 59C-0122; Sigma Chemical Co., St. Louis, Mo.), synthesized by the Maumee procedure, at levels of 5.0, 2.5, 1.0, 0.5, and 0.1% of the powdered diet (Charles River rat chow). Control rats received the same basal diet but without sodium saccharin. Rats were weighed and food consumption was determined periodically. Ten weeks after the beginning of the experiment, all rats were killed. A previous study demonstrated the presence of pleomorphic microvilli and elevated labeling index in the bladders of rats fed 5% sodium saccharin for 9 weeks (5). In each group, 5 rats were processed for scanning electron microscopic examination of the bladder, and 5 rats were processed for light microscopic and autoradiographic examination by the methods described previously (5, 9, 10).

For autoradiography, each rat received a single i.p. injection of [methyl-3H]thymidine (New England Nuclear; 20 Ci/mmol) at a dose of 1 µCi/g body weight 1 hr before sacrifice. All rats were killed at the same time of day to avoid variations in the mitotic rate due to the circadian rhythm of the rat bladder. The bladder was inflated with 10% buffered formalin, cut into longitudinal strips, and processed as described previously (5). Several serial sections of all strips of each bladder were examined, and at least 5000 epithelial cells were counted per bladder. Slides processed for autoradiography were used for light microscopic evaluation of mucosal hyperplasia.

For scanning electron microscopy, each bladder was inflated with 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for scanning electron microscopic examination of the bladder, and 5 rats were processed for light microscopic and autoradiographic examination by the methods described previously (5, 9, 10).

All rats receiving sodium saccharin grew at the same rate as did control rats, and no evidence of toxicity was observed. Food consumption was similar for all groups. Macroscopically, the urinary bladder showed no abnormalities on the luminal surface, and no calculi were observed in the urine or in the bladder when examined at autopsy.

The bladder mucosa with mild simple hyperplasia (Fig. 1) by light microscopy was 4 to 7 cells thick in contrast to 2 to 3 cells thick in normal rat bladder. Its incidence at the different doses is shown in Table 1. Papillary or nodular hyperplasia, papilloma, and cancer were not observed. Autoradiographically, most of the labeled nuclei were observed in basal cells; superficial cells were rarely labeled. The labeling index showed a close relationship with dose, and it was significantly increased compared to the control group at all doses examined except the 0.1% dose (Table 1).

Scanning electron microscopy was more sensitive than light microscopy in detecting bladder lesions, and quantitation was possible by counting the number and size of foci with cells having ropy microridges, uniform microvilli, and pleomorphic microvilli on their luminal surface (Fig. 2). In the normal rat bladder, the large polygonal, superficial cells occasionally ex-
Dose Effect of Sodium Saccharin

Fig. 1. Simple hyperplasia in urinary bladder of rat treated with 5% sodium saccharin. The bladder was inflated with 10% buffered formalin. H & E, x 100.

Table 1
Effect of dose of sodium saccharin on rat urinary bladder epithelium examined by light microscopy and autoradiography

<table>
<thead>
<tr>
<th>Level of sodium saccharin in the diet (%)</th>
<th>No. of rats</th>
<th>No. of rats with simple hyperplasia</th>
<th>Labeling index</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>5</td>
<td>4 (80)*</td>
<td>0.36 ± 0.13e</td>
</tr>
<tr>
<td>2.5</td>
<td>5</td>
<td>3 (60)</td>
<td>0.19 ± 0.07d</td>
</tr>
<tr>
<td>1.0</td>
<td>5</td>
<td>1 (20)</td>
<td>0.12 ± 0.05e</td>
</tr>
<tr>
<td>0.5</td>
<td>5</td>
<td>0</td>
<td>0.12 ± 0.03d</td>
</tr>
<tr>
<td>0.1</td>
<td>4</td>
<td>0</td>
<td>0.06 ± 0.03f</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0.04 ± 0.03</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

b Mean ± S.D.
c p < 0.005.
d p < 0.01.
e p < 0.05.
f p < 0.4.

DISCUSSION

The data presented provide additional, more quantitative evidence that sodium saccharin induces proliferation of the urinary bladder mucosa, and the response detected by light microscopy, autoradiography, and scanning microscopy is related to dose. Although no significant response was detected at the lowest dose, this study examined only one time interval and relatively few rats. Previously, it was observed in this laboratory that feeding 5% sodium saccharin increased the labeling index within 1 week, and the labeling index remained the same through 18 weeks of feeding (5).

Pleomorphic microvilli appear as a surface feature in the urinary bladder epithelium only, with hyperplasia, either with prolonged stimulation (5, 9, 10) or with a marked nodular and

foliate exposing the underlying smaller, round intermediate cells with uniform short microvilli on their surface. As the intermediate cells mature to replace the superficial cell, the uniform microvilli gradually merge to form rounded, ropy microvilli, and the cell enlarges and becomes polygonal. The appearance of peaked microvilli on the luminal surface represents terminal differentiation of the transitional cell epithelium, and it results from the presence of the characteristic asymmetrical membrane of the urothelium. The rate of exfoliation and replacement in the urothelium of a normal bladder is estimated by the number of foci with ropy microridges and uniform microvilli present on the luminal surface of the cells (Table 2). All rats at all doses in the control group had cells with ropy microridges and uniform microvilli. Sodium saccharin administration at doses of 1.0, 2.5, and 5.0% increased the number of these foci, and it also increased the size of these foci as determined by counting the number of cells in each focus with these features. In addition, at the 2 highest doses, pleomorphic microvilli were observed, a surface feature not seen in normal Fischer rats. Pleomorphic microvilli were observed in 3 of 3 rats fed 5% sodium saccharin and in 3 of 5 rats fed 2.5%.
papillary proliferation. Pleomorphic microvilli were first observed after 9 weeks in rats fed sodium saccharin as 5% of the diet (5), and they continued to be present through 18 weeks with no apparent progression of the lesions. Recently, we observed by scanning electron microscopy the bladders of rats continuously fed 5% sodium saccharin for 2 years. Pleomorphic microvilli were present, but again there was no progression of the lesion first observed after 9 weeks and no evidence of nodular hyperplasia, papilloma, or cancer of the bladder. A property of the hyperplasia induced by promoting agents is reversibility if the agent is discontinued. Although we have not yet systematically evaluated the reversibility of sodium saccharin-induced hyperplasia, rats fed 5% sodium saccharin from 11 to 88 weeks of age (total of 77 weeks) followed by control diet to 109 weeks of age (21 weeks of control diet) had normal-appearing bladder mucosa by light and scanning electron microscopy (4).

ACKNOWLEDGMENTS

We gratefully acknowledge the comments and advice of Drs. Jerome B. Jacobs, Gilbert H. Frieden, and Robert E. Greenfield; the technical assistance of Margaret St. John; and the assistance of Dorothy Morrissey in the preparation of the manuscript.

REFERENCES


Table 2

Effect of dose of sodium saccharin on rat urinary bladder epithelium examined by scanning electron microscopy

<table>
<thead>
<tr>
<th>Level of sodium saccharin in the diet (%)</th>
<th>No. of rats</th>
<th>No. of foci in the bladder</th>
<th>No. of cells in the bladder with surface feature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of rats</td>
<td>No. of foci with pleomorphic microvilli</td>
</tr>
<tr>
<td>5.0</td>
<td>3</td>
<td>3</td>
<td>148 ± 39b, b</td>
</tr>
<tr>
<td>2.5</td>
<td>5</td>
<td>3</td>
<td>112 ± 32b</td>
</tr>
<tr>
<td>1.0</td>
<td>3</td>
<td>0</td>
<td>75 ± 40c</td>
</tr>
<tr>
<td>0.5</td>
<td>4</td>
<td>0</td>
<td>40 ± 27</td>
</tr>
<tr>
<td>0.1</td>
<td>4</td>
<td>0</td>
<td>23 ± 18</td>
</tr>
<tr>
<td>0</td>
<td>4</td>
<td>0</td>
<td>36 ± 28</td>
</tr>
</tbody>
</table>

* Mean ± S.D.
, b p < 0.01.
, b p < 0.2.
, b p < 0.025.
, b p < 0.05.

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