Uracil-induced Urolithiasis and the Development of Reversible Papillomatosis in the Urinary Bladder of F344 Rats

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ABSTRACT

Male F344 rats were given a diet supplemented with uracil at concentrations of 1 or 3% for 15 or 30 wk. In the group given 3% uracil, numerous calculi of uracil were observed in the urinary tract with marked hyperplasia and papillomas of the urinary bladder mucosa in Wk 15 and 30. In Wk 30, dysplasia of the ureteral mucosa and one transitional cell carcinoma of the bladder were also found. Neither these marked proliferative lesions nor calculi except for one papilloma were observed in rats maintained on normal basal diet for 15 wk after a diet containing 3% uracil for 15 wk. In the group given 1% uracil, no calculi or hyperplasia was seen in Wk 15 (five rats), and only one of ten rats examined in Wk 30 had a few stones and mild epithelial hyperplasia of the bladder. Scanning electron microscopy showed that most surface cells of papillomas had numerous short uniform microvilli and ropy rounded microridges. By transmission electron microscopy, epithelial cells of papillomas showed essentially normal differentiation. The present findings suggested that most hyperplasias and papillomas induced by bladder stones were reversible.

INTRODUCTION

In rats and mice, proliferative responses of the urinary bladder epithelium have been observed in response to various types of mechanical irritation, such as foreign bodies inserted into the bladder and calculi formed experimentally or spontaneously within the vesicle (1–5). Regenerative papillary hyperplasias after formation of bladder ulcers in rats have also been reported (6). Proliferation of the transitional epithelium in response to stones in rats was usually extensive, and in some cases the entire bladder mucosa was covered with finger-shaped papillomas, a condition named papillomatosis (2, 5). When administration of chemicals to rodents results in both tumor formation and stones in the bladder, the question arises of whether the chemical itself or the bladder stones are responsible for tumor formation. Chemicals that result in both tumors and stones include 2,3'-azotoluene (7), 4-ethylsulfonylnaphthalene-1-sulfonamide (8), terephthalic acid (9), and melamine (10). Bladder calculi are also induced by repeated intravesical administration of physiological saline solution (5). Therefore, it seems very important to clarify the biological characteristics of bladder tumors induced by stones. If these stones could be removed during the experiment, the fate of tumors related to stones, that is, their regression or progression, could be determined. However, if bladder stones contain a certain amount of calcium, they rarely disappear. In 1966, Lalich (11) reported a high frequency of induction of urolithiasis in rats given uracil p.o. We found that addition of uracil to the diet induced bladder stones in rats and that these disappeared when uracil was withdrawn from the diet.

RESULTS

No reduction in body weight gain was observed in rats given 1% uracil, judging from our previous data on the growth of male F344 rats, but growth of rats given 3% uracil was retarded, and their body weights were about 80% and 77% of those of rats given 1% uracil in Wk 15 and 30, respectively. Withdrawal of uracil from the diet in Wk 15 resulted in recovery of growth and nearly normal body weights in Wk 30.

Macroscopic Findings. The incidences of urinary tract lesions, including microscopic changes, in the different groups are summarized in Tables 1 and 2. In Wk 15, the urinary bladders of all 5 rats given 3% uracil were almost completely filled with numerous yellowish-white calculi, and the ureters were slightly dilated. The wall of the urinary bladder was irregularly thickened, especially at the dome (Fig. 1). The calculi were irregular in shape with a rather smooth surface, and they varied from 1 to 3 mm in diameter (Fig. 2). Some small stones were found in the ureter and kidney pelvis. In rats given the lower dose (1%) of uracil, there were no calculi or marked changes of the urinary tract. Continuous administration of 3% uracil resulted in more marked formation of calculi in Wk 30, than in Wk 15, with hydroureter and hydronephrosis. In contrast, only 1 of 5 animals given 3% uracil for 15 wk and then uracil-free diet for another 15 wk had a very few small stones, and none of these rats had a thickened bladder mucosa. There were no distinct changes in the urinary tract of any of the 10 rats given a low dose of uracil for 30 wk, except in one rat, which had a few bladder stones. During fixation in buffered formalin, many

MATERIALS AND METHODS

Male F344 rats (purchased from Charles River Japan, Inc., Kana- gawa, Japan) were 6 wk old and weighed about 123 g at the beginning of the experiment. Animals were housed 5 to a plastic cage on hard wood chips in an air-conditioned room with a 12-h light, 12-h dark cycle and given food (Oriental MF; Oriental Yeast Co., Tokyo, Japan) and water ad libitum. Uracil was purchased from Wako Pure Chemical Co., Osaka, Japan, and added to the basal diet at concentrations of 1.0 and 3.0%. Two groups of 15 rats each were given these levels of uracil for up to 30 wk. In Wk 15, 5 animals in each group were killed, and 5 rats that had been given 3.0% uracil, which were all found by laparotomy to have bladder stones, were placed on basal diet not containing uracil for another 15 wk to investigate whether the bladder stones would disappear.

Rats were killed in Wk 15 or 30. The urinary bladder, ureter, and renal pelvis were inflated and fixed with 11.8% phosphate-buffered formalin (pH 7.2) at 4°C and routinely processed for histological examination. Sections were stained with hematoxylin-eosin. Some samples of the urinary bladder fixed in phosphate-buffered formalin were processed for scanning and transmission electron microscopic examination after postfixation in 1.0% osmic acid (12).
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Table 1 Incidences of calculi and proliferative lesions in the urinary bladder of rats given uracil

<table>
<thead>
<tr>
<th>Treatment with uracil (%)</th>
<th>No. of rats</th>
<th>Calculi</th>
<th>Simple hyperplasia</th>
<th>PN hyperplasia</th>
<th>Papilloma</th>
<th>Papillomatosis</th>
<th>Carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (15)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>3 (15) → 0 (15)</td>
<td>5</td>
<td>1</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>0</td>
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<td>3 (30)</td>
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<td>5</td>
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<td>1</td>
</tr>
<tr>
<td>1 (15)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 (30)</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* PN hyperplasia, papillary or nodular hyperplasia.
* Numbers in parentheses, number of weeks of treatment.
* A very few small stones remained.

Table 2 Incidences of urothelial lesions of the kidney and ureter of rats given uracil

<table>
<thead>
<tr>
<th>Treatment with uracil (%)</th>
<th>No. of rats</th>
<th>Kidney</th>
<th>Ureteral mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hydro-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nephrosis</td>
<td>Hyperplasia</td>
</tr>
<tr>
<td>3 (15)</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>3 (15) → 0 (15)</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>3 (30)</td>
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<td>5</td>
</tr>
<tr>
<td>1 (15)</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 (30)</td>
<td>10</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number of weeks of treatment.

Fig. 1. Macroscopic appearance of the urinary bladder of a rat given 3% uracil and killed in Wk 15. Note irregularly thickened mucosa and many stones.

Fig. 2. Urinary bladder calculi collected from 3 rats after treatment with 3% uracil for 15 wk.

Fig. 3. Marked papillary tumors of the urinary bladder of a rat exposed to 3% uracil for 15 wk. H & E, × 40.

Microscopic Findings. The mucosal lesions found in the urinary bladder including the ureter and kidney pelvis, which were classified as described previously (13), are summarized in Tables 1 and 2. Marked urothelial proliferations of the urinary bladder, such as papillomas and papillary hyperplasias of the ureter, were found in all rats examined in Wk 15 and 30 after continuous administration of 3% uracil. These proliferations were all related to the presence of calculi and involved the entire mucosa. These lesions, simple hyperplasia, papillary or nodular hyperplasia, and papillomas, were connected with each other. Papillomas were multiple, a condition named papillomatosis. The papillomatosis was characterized histologically by elongated slender papillary processes fusing with one another to produce an elaborate branching pattern (Fig. 3). These tumors were composed of hyperplastic epithelium of 5 to 10 cells in thickness and thin but distinct vascular stromal cores. The luminal surface of the thickened urothelium was covered by a monolayer of flattened umbrella cells (Fig. 4). Generally slender projections of thickened epithelium in the urinary bladder were shorter in Wk 30 than in Wk 15, probably because of compression of the mucosa by more numerous stones within the vesicle. Infiltration of polymuclear leukocytes into the submucosa of lesions was occasionally seen. In a rat given 3% uracil for 30 wk, the hyperplastic transitional epithelium extended into the muscular wall giving the appearance of a diverticulum.

One transitional cell carcinoma was found in a rat given 3% uracil for 30 wk (Fig. 5). This was a tumor mass protruding into the lumen without any invasion into the underlying connective tissue. Papillary hyperplasias of the urothelium were stones dissolved in the fixative and disappeared.
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Fig. 4. Higher magnification of Fig. 3. Papillomatosis of the urinary bladder consists of hyperplastic epithelium without cytological atypia supported by thin fibrovascular cores. H & E, x 200.

Fig. 6. Mucosal dysplasia of the ureter of a rat given 3% uracil for 30 wk. Marked cell atypia and mitoses are noted. H & E, x 250.

Fig. 5. Transitional cell carcinoma seen in the urinary bladder of a rat given 3% uracil for 30 wk. Cell atypia and mitoses are prominent. H & E, x 200.

Fig. 7. Urinary bladder epithelium of a rat given 3% uracil for 15 wk and uracil-free diet for another 15 wk. Mild hyperplasia but no papillary proliferation is observed (nodular hyperplasia). H & E, x 200.

Fig. 8. Urinary bladder of a rat treated in the same way as that for Fig. 7. Mild epithelial hyperplasia with slight irregularity of nuclear arrangement is seen with a dilated vein and hyperplastic arteries in the submucosa. H & E, x 200.

also seen in the kidney pelvis of all 5 rats given 3% uracil for 30 wk and in 1 of 5 rats given 3% uracil for 15 wk. Dysplasias were found in the ureteral mucosa of 3 rats given 3% uracil for 30 wk (Fig. 6). In these areas of the mucosa, the nuclei were hyperchromatic and varied in size, and many mitoses were seen.

In contrast to these findings in rats given 3% uracil continuously, no distinct proliferative mucosal lesions were seen in animals given basal diet for 15 wk after 3% uracil for 15 wk, except occasional simple hyperplasia and papillary or nodular hyperplasia (Figs. 7 and 8). In these animals, dilated blood vessels were occasionally seen in the submucosa of the bladder (Fig. 8). One rat in this group had a papilloma, but no stones. However, mild hyperplasia of the ureter was observed in some rats.

No squamous cell metaplasia of the urothelium and no mineralized foci in the urinary tract, including the kidney parenchyma, were observed in any rat.

Electron Microscopic Findings. Scanning electron microscopy showed that the numerous mucosal projections were mucosal folds connected with each other and forming an irregular mesh-like pattern (Fig. 9). Superficial cells in these mucosal folds were somewhat round and varied in size. Most of the cells were covered with short uniform microvilli and had ropy rounded...
The urinary bladder of a rat given 3% uracil for 15 wk. Mucosal folds connected with each other are prominent. × 100.

Fig. 9. Scanning electron microscopic appearance of the luminal surface of the urinary bladder of a rat given 3% uracil for 15 wk. Mucosal folds connected with each other are prominent. × 100.

mitochondria were also seen in their cytoplasm. Cell membranes were symmetrical, and cells were joined by numerous desmosomes also contained numerous small rounded vesicles and a number of these vesicles were observed by transmission electron microscopy, was not a marker of early malignant changes, and they are considered to be irreversible and to progress to noninvasive or invasive carcinomas in weeks or months (13, 17, 18, 24). During urinary bladder carcinogenesis, the epithelium passes through several stages, beginning with simple hyperplasia and progressing to nodular and papillary hyperplasia and then papilloma and carcinoma (13, 17). Fukushima et al. (25) reported that the appearance of pleomorphic microvilli on the surface cells of urothelial tumors, and papillary hyperplasia and then papilloma and carcinoma beginning with simple hyperplasia and progressing to nodular

Fig. 10. Detail of surface cells of papillomatosis shown in Fig. 9. Short uniform microvilli and ropy rounded microridges are seen. × 10,000.

DISCUSSION

Uracil, a component of RNA, was first found to induce numerous calculi in the urinary tract of rats by Lalich (11); he fed Sprague-Dawley rats a diet containing 2, 2.5, and 3% uracil for 29 to 54 days and found that, on all these treatments, uracil-induced bladder stones at high frequency.

In the present study, administration of 3% uracil in the diet induced urinary bladder stones and urothelial hyperplasias and papillomas involving the entire mucosa of the bladder in all male F344 rats examined. The bladder stones were found to be composed of uracil. Longer treatment with uracil for 30 wk resulted in development of transitional cell carcinoma of the bladder and dysplastic lesions of the ureter, but when administration of uracil was stopped after 15 wk, the stones and almost all the marked proliferative mucosal lesions disappeared.

Unexpectedly, the uracil stones were found to be soluble in the fixative. Uracil is poorly soluble in water, especially in acidic conditions. A preliminary experiment showed that its solubility in water depended on the pH and that a turbid saturated solution became clear on raising the pH from 7.76 to 8.32 with 0.1 N NaOH. From these findings and the composition of the stones, we conclude that the stones were formed by precipitation of uracil in the bladder from the urine, which has a neutral or slightly acidic pH. Therefore, once bladder stones have been formed, they probably remain for as long as the concentration of uracil in the urine is sufficiently high. But when uracil treatment is discontinued, they gradually dissolve in the urine and disappear.

The hyperplasias and papillomas observed in the present experiment were associated with uracil stones and probably resulted from cell division of the epithelium of the urinary tract induced by the irritant effect of the stones. The changes were probably not due to a carcinogenic effect of uracil itself, because histologically the tumors appeared very similar to those associated with bladder calculi induced by intravesicular insertion of chalk powder (2) or feeding of chemicals (10, 14), but they differed from tumors induced by chemical carcinogens such as N-butyl-N-(4-hydroxybutyl)nitrosamine (13, 15, 16) and N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (17) on cellular arrangement and nuclear atypism. Papillary lesions associated with stones are characterized by finger-like mucosal projections which consisted of hyperplastic epithelium having nearly constant cell thickness and thin vascular cores (Fig. 4). The presence of prominent relatively large umbrella cells covering epithelium is also characteristic of uracil-induced papillary lesions.

A striking finding in the present study was the reversibility of papillomas. Although the present data were based on groups of only 5 rats, in our recent work, disappearance of papillomatosis was also observed in all 15 rats given 3% uracil for 15 wk. The reversibility of hyperplasias of the urinary bladder epithelium induced by mechanical damage (6, 18, 19) or administration of various chemicals (20, 21) has often been observed.

There is much controversy about whether human papillomas of the bladder are benign or malignant (22, 23). In rats treated with a bladder carcinogen, however, papillomas appear to be early malignant changes, and they are considered to be irreversible and to progress to noninvasive or invasive carcinomas in weeks or months (13, 17, 18, 24). During urinary bladder carcinogenesis, the epithelium passes through several stages, beginning with simple hyperplasia and progressing to nodular and papillary hyperplasia and then papilloma and carcinoma (13, 17). Fukushima et al. (25) reported that the appearance of pleomorphic microvilli on the surface cells of urothelial tumors, observed by scanning electron microscopy, was not a marker of irreversibility. The present experiment supports their findings. Thus, there may be biological differences between uracil-induced papillomas and carcinogen-induced ones.

3 T. Shirai, S. Fukushima, A. Masuda, and N. Ito, unpublished observations.

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Fig. 11. Transmission electron microscopic appearance of uracil-induced papillomatosis in the urinary bladder of a rat in Wk 15. Superficial cells are darker than intermediate cells. Cells contain numerous mitochondria, tonofilaments, ribosomes, and fusiform vesicles. × 3,500.

Hasegawa et al. (27) recently reported that freeze ulceration of the bladder, which produces morphologically reversible epithelial hyperplasia, induced irreversible changes of epithelial cells related to bladder cancer initiation and that sodium saccharin promoted the tumorigenic expression of these freeze ulceration-induced cellular changes. Therefore, stimulation by bladder calculi may be able to initiate bladder carcinogenesis. We are now carrying out experiments on the initiation and promotion potentials of uracil stones.

The present method of producing bladder calculi with reversible hyperplasias and papillomas in the rat by feeding uracil provides a useful method for studying various biological phenomena in the urinary bladder, such as regulation of epithelial growth, cell differentiation, comparison of processes with irreversible carcinogenic processes, and the role of mechanical irritation in urothelial tumorigenesis.

REFERENCES

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