Effects of Urine and Continued Exposure to Carcinogen on Progression of Early Neoplastic Urinary Bladder Lesions

Randall G. Rowland, Michael O. Henneberry, Ryoichi Oyasu, and John T. Grayhack

ABSTRACT

Based on reports of regression of superficial bladder tumors after urinary diversion, a study was designed to measure the effects of urine and continued exposure to carcinogen on the incidence of progression of N-(4-(5-nitro-2-furyl)-2-thiazolyl)formamide-induced early urinary bladder lesions to invasive tumor. After being fed 0.2% N-(4-(5-nitro-2-furyl)-2-thiazolyl)formamide diet for 14 weeks, one-half of the male Fischer rats had urinary diversion by ureterosigmoidostomy, and the remainder were sham operated. One-half of each of these two groups was continued on the N-(4-(5-nitro-2-furyl)-2-thiazolyl)formamide diet while the remaining animals were fed regular chow postoperatively. One-half of each of the four groups was sacrificed at 3 months, and the remainder were sacrificed at 6 months after ureterosigmoidostomy or sham-operation. The incidence and mean number of tumors as well as the incidence of invasive tumor were tabulated.

The combined 3- and 6-month data indicate that excreted carcinogen in the urine influences progression of the preinvasive lesions more than urine alone or systemic carcinogen alone. However, urine alone had a significant effect (p < 0.025) on tumor incidence (8 of 19 sham-operated animals with tumor versus 1 of 18 diverted animals with tumor). Urine acts as a promoter in this experimental system. These findings may have clinical applications in the treatment of early transitional cell carcinoma.

INTRODUCTION

In treating carcinoma of the bladder, most urologists utilize urinary diversion either as a palliative procedure or as a necessary adjunct to their primary mode of therapy, cystectomy. Isolated reports can be found in which urinary diversion was used as the primary method of therapy. Abeshouse and Scherlis (1) noted regression of 6 cases of bladder tumor after urinary diversion, and Davis (4) reported the disappearance of superficial bladder tumors (but persistence of invasive tumors) in 2 patients who had undergone ureterosigmoidostomies. In addition, McDonald and Lund (6) and Scott and Boyd (8) have studied the effects of urinary diversion on β-naphthyamine-induced bladder tumors in dogs. These experiments showed that tumors will not develop in a bladder which has never been in contact with urine during the course of carcinogen exposure. However, simply diverting the urine from the bladder epithelium after the formation of bladder tumors is ineffective as a means of preventing further tumor progression.

A murine model utilizing the carcinogen FANFT has been extensively studied both by others (3, 5) and in our laboratory. It has been well documented in this model that hyperplastic and severely dysplastic surface changes progress to papillary and eventually invasive tumors after a minimum of 10 to 14 weeks of carcinogen exposure, even when no further carcinogen is given. This particular murine model closely parallels human bladders which progress from carcinoma in situ to invasive tumors.

The development of ureterosigmoidostomy in rats (7) has made it practical to study the effects of urinary diversion in experimental tumor systems. By this method, 80 to 90% of diverted rats survived 6 months, and no differences in the serum creatinine or electrolytes were observed when compared to sham-operated controls.

This study combines the FANFT tumor model and urinary diversion to examine the effect of urine alone, systemic carcinogen, and urine with excreted carcinogen on the progression of early preinvasive transitional cell carcinoma.

MATERIALS AND METHODS

Ninety male Fischer rats (Charles River Breeding Laboratory, Wilmington, Mass.) weighing 100 to 125 g were housed in group cages. They were given FANFT (0.2% by weight; Saber Laboratories, Inc., Morton Grove, Ill.) pelleted in Charles River rat chow and water ad libitum.

After 14 weeks, 10 animals were killed to document the degree of mucosal change at that time. They were reported as the "Control Group." The remaining animals were divided into 2 sets of 40 designated groups, A and B (Chart 1). One-half of each group had a urinary diversion, and the remainder had a sham procedure leaving the urinary tract intact. Group A received no further carcinogen after the surgical procedure while Group B resumed the FANFT diet postoperatively. One-half of each set of animals were sacrificed at 3 months, and the remainder were sacrificed at 6 months after urinary diversion or sham operation.

A modification of the Coffey method of ureterosigmoidostomy (2) was utilized as a method of urinary diversion (Chart 2). This procedure has been described in detail in a previous report (7). The sham procedure consisted of the same lower midline incision and sigmoid colotomy. Both procedures were performed under Ketamine (Bristol Laboratories, Syracuse, N. Y.) anesthesia. In all animals, the colotomy was closed with 6-0 chronic catgut as were the rectus muscles with 4-0 chronic catgut and the skin with metal clips. Rats received tobramycin (7.5 mg/kg) Eli Lilly and Co., Indianapolis, Ind.) i.m. 1 hr before the procedure and i.p. at the end of the operation. All animals

1 This work was supported by USPHS Grant CA 14649 through the National Bladder Cancer Project and by Cancer Center Support Grant CA 15145 through the National Cancer Institute and the Northwestern University Cancer Center.

2 To whom requests for reprints should be addressed, at Department of Urology, 1100 West Michigan, Indianapolis, Ind. 46223.

Received January 4, 1980; accepted August 29, 1980.

4524 CANCER RESEARCH VOL. 40

Downloaded from cancerres.aacrjournals.org on November 13, 2017. © 1980 American Association for Cancer Research.
The recorded index of hyperplasia was based on the degree of cells thick; 2+, 6 to 8 cells thick; or 3+, 8 to 10 cells thick.

Hyperplasia. This was defined as an increase in the number of epithelial cell layers without definite exophytic lesions. Hyperplasia was graded as 0 (normal), 1+, 2 cells thick; 2+, 3 to 5 cells thick; 3+, 6 to 8 cells thick; or 4+, 8 to 10 cells thick. The recorded index of hyperplasia was based on the degree of hyperplasia present in at least two-thirds of the mucosal surface.

Exophytic Tumors. These lesions were identified as bladder tumors which were polypoid, nodular, or papillary in form (10). The total number of tumors seen grossly per bladder was recorded.

Invasion. The presence of tumor cells below the lamina propria or within the connective tissue stalk of an exophytic tumor was considered to be evidence of invasion.

Statistical Analyses. The indices of hyperplasia and atypia and the number of tumors per bladder were compared by Student's t test. The incidence of gross tumor and tumor invasion were compared by the \( \chi^2 \) method, combining the 3- and 6-month data.

RESULTS

After 14 weeks of exposure to FANFT (control group), the mean index of hyperplasia and the mean index of atypia were increased. However, no tumors were observed either grossly or microscopically (Table 1).

In both Group A and Group B, 1 sham-operated and 2 diverted animals died at surgery or within 2 days after surgery. No other animal deaths occurred prior to the sacrifice of the animals.

Hyperplasia. The degree of hyperplasia did not vary significantly among the ureterosigmoidostomy groups regardless of the length of carcinogen exposure. Likewise, the sham-operated rats returned to a normal diet had no significant difference in their hyperplasia indices at 3 months and at 6 months after their operations. However, the sham-operated rats maintained on a FANFT diet showed a significant \( (p < 0.01) \) decrease in the degree of hyperplasia at 6 months as compared to 3 months postoperatively. The degree of hyperplasia seen in the diverted bladders was consistently greater than the sham-operated bladders on similar diets for corresponding lengths of time.

Atypia. Within each of the various treatment groups, there was no statistically significant difference between the degree of atypia seen at 3 months and that seen at 6 months after the operations. Nor was there a significant difference between the diverted and nondiverted bladders of rats which were returned to a normal diet (Group A). Having been exposed to 14 weeks

and the presence of mitotic figures. The mildest degree of atypia corresponded to 1+, whereas 5+ heralded the most anaplastic changes. The average of all 24 sections was recorded as the atypia index for each bladder.

Chart 1. Experimental design. --- ---, FANFT; - - - - , normal diet; \( \bullet \), ureterosigmoidostomy; \( \bigcirc \), sham operation; \( \bigtriangledown \), sacrificed; numbers in parentheses, number surviving surgery; numbers in brackets, number of animals. Posi-op, postoperatively.

Chart 2. Modified Coffey 1 ureterosigmoidostomy used in rats.

also received 10 ml of sterile 0.9% NaCl solution i.p. at the end of the procedure.

All animals were maintained on half-strength Vivonex (Eaton Laboratories, Norwich, N. Y.) for 2 days preoperatively and postoperatively.

Rats were sacrificed by decapitation. Urethral catherization was performed with polyethylene 10 tubing, and the bladder was filled with 0.5 to 1.0 ml of 10% formalin. After ligating the bladder neck, the entire bladder was excised and placed in formalin. After fixation, the bladder was bisected longitudinally, and each half of the bladder was viewed through a dissecting microscope. The number of bladder tumors was recorded. Each hemibladder was cut into thirds. All 6 pieces were embedded in the same paraffin block. Four step sections were cut from each block and stained with hematoxylin and eosin. Thus, 24 sections were obtained for histological examination in each animal.

The slides were examined independently by 2 observers. Grading essentially followed the criteria set forth by Squire et al. (9).

Hyperplasia. This was defined as an increase in the number of epithelial cell layers without definite exophytic lesions. Hyperplasia was graded as 0 (normal), 1+, 2 cells thick; 2+, 3 to 5 cells thick; 3+, 6 to 8 cells thick; or 4+, 8 to 10 cells thick. The recorded index of hyperplasia was based on the degree of hyperplasia present in at least two-thirds of the mucosal surface examined.

Atypia. Increasing atypia of the bladder epithelium was indicated by greater pleomorphism, loss of cell polarity, hyperchromasia, prominent nucleoli, giant cells, chromatin clumping,
of FANFT, the atypia was no worse in sham-operated rats maintained on FANFT compared to those which were returned to a normal diet. There was, however, a difference of atypia between the diverted and nondverted bladders maintained on a FANFT diet. This difference was statistically significant in this group (B) at both 3 months \((p < 0.005)\) and 6 months \((p < 0.001)\) postoperatively.

**Gross Tumors.** In Group A (normal diet after operation), 8 of 19 sham-operated rats developed grossly visible bladder tumors compared with only 1 of 18 rats with urinary diversion (Chart 3), a difference which is statistically significant \((p < 0.025)\). A typical bladder from the diverted animals in Group A is shown in Fig. 1. More than one tumor was present in some animals (Table 1). When exposed to continued carcinogen (Group B), both diverted and sham-operated rats had a slightly higher, although statistically insignificant, incidence of bladder tumors than did their respective mates which were switched to a normal diet after their surgical procedures. An example of a bladder from a sham-operated animal is shown in Fig. 2. There was a highly significant increase in the incidence of tumors \((p < 0.01)\) seen in the sham-operated animals which continued to receive a FANFT diet when compared to those which were still exposed to FANFT but had had urinary diversion (Table 1; Chart 3).

**Invasion.** In the rats maintained on a normal diet (Group A), only 2 of 8 sham-operated rats with tumors showed microscopic evidence of invasion. There was no invasion seen in the only diverted rat of this group which developed bladder tumors. In contrast to this, all of the 17 nondverted rats which developed tumors after continued carcinogen exposure had evidence of superficial invasion. One additional rat in this group without gross tumor showed invasion in a nonpapillary area of frank carcinoma. Also, in Group B, 3 of 4 diverted rats with tumor had invasion (Chart 4).

**DISCUSSION**

By combining the technique of ureterosigmoidostomy in rats with the FANFT tumor model, a system has evolved to study the effect of urinary diversion, with and without systemic carcinogen exposure, on the progression of preinvasive bladder lesions. The control animals given 14 weeks of FANFT diet demonstrated marked epithelial atypia without gross tumor and could be compared to carcinoma in situ of the human urinary bladder, which is now well recognized as a potential preinvasive lesion.

Hyperplasia in diverted rats was greater than in nondverted rats and did not vary with the length of carcinogen exposure. This suggests that the hyperplasia seen in bladders no longer exposed to urine was a result of diversion itself and was independent of the carcinogen effect. The degree of atypia, on the other hand, was the same in diverted and nondverted bladders when a normal diet was resumed postoperatively. The diverted bladders, however, appeared to be protected from the more severe atypia seen in nondverted bladders exposed to the continued carcinogen. This could be explained by not having continued exposure to the excreted carcinogen and urine. By extension, one would expect to see fewer gross...
bladder tumors or a lower incidence of invasive tumors in rats maintained on a FANFT diet which have undergone ureterosigmoidostomy. In fact, such differences were noted and were highly significant (p < 0.01 and p < 0.005, respectively) (Charts 3 and 4). Difference was also seen in the number of gross tumors between the nondverted and diverted bladders when the diet was changed to normal chow postoperatively (p < 0.025). Two of 19 sham-operated rats developed invasive tumors, while none of 18 did after the diet was switched to the normal. The difference, however, was not statistically significant. The above data indicate that the presence of carcinogen in urine influences the progression of preinvasive neoplastic lesions to invasive tumors more than does the presence of systemic carcinogen or urine alone. Urine does, however, appear to be a cocarcinogen or promotor at least in the environment of already atypical or initiated transitional cell epithelium.

REFERENCES

Effects of Urine and Continued Exposure to Carcinogen on Progression of Early Neoplastic Urinary Bladder Lesions


**Updated version**

Access the most recent version of this article at:

http://cancerres.aacrjournals.org/content/40/12/4524

**E-mail alerts**

Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**

To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/40/12/4524. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.