Early Lesions in Experimental Bladder Cancer: Experimental Design and Light Microscopic Findings

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Summary

N-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide (FANFT) fed to male and female Fischer rats at a dose of 0.2% of the diet induces lesions of the urinary bladder which progress from mild hyperplasia at 2 to 4 weeks, to moderate hyperplasia at 6 to 8 weeks, severe nodular and papillary hyperplasia at 10 to 14 weeks, and microinvasive carcinomas by 25 weeks as observed by light microscopy. Male Fischer rats fed FANFT for 2 or 4 weeks and then maintained on control diet show regression of the bladder lesions within 2 weeks to normal-appearing mucosa which persists through 50 weeks. Rats fed FANFT for 6 weeks show regression of the moderately hyperplastic epithelium to normal within 4 weeks after being placed on control diet which persists through 50 weeks. Rats fed FANFT for 8 or 10 weeks show regression of the hyperplastic bladder epithelium within 2 weeks of receiving control diet, but focal areas of mild hyperplasia are detectable through 50 weeks of FANFT administration. Rats fed FANFT for 12, 14, or 20 weeks continued to progress to invasive tumors (microinvasive or invasion of muscle) after the rats had been maintained on control diet through 50 weeks. Thus, the hyperplastic lesions developing through 6 weeks of FANFT administration appear to be reversible if FANFT is discontinued, but later lesions appear to be irreversible.

Introduction

Transitional cell or squamous cell carcinomas of the human urinary bladder, like carcinomas of the uterine cervix, appear to be preceded by noninvasive neoplastic epithelial proliferation. Unlike carcinoma of the cervix, the majority of noninvasive bladder tumors are papillary rather than flat, and the relationship between the papillary and the flat varieties is yet to be defined clearly, as is the pattern of progression from noninvasive to invasive carcinoma. Even less is known about the existence and character of any preneoplastic lesions that might eventuate in carcinoma.

Several investigators have therefore undertaken the development of in vivo experimental models of human bladder cancer. We have focused our attention on developing a model in Fischer rats with bladder tumors induced by the inclusion of 0.2% FANFT in the diet. Since it is an inbred strain, the Fischer rat is a good animal for a tumor-host model in which to study immunological changes associated with the development of tumors. The commercial availability of histocompatible Fischer rats from different sources also permits laboratories using this strain to exchange and compare data and materials with other investigators. In particular we have been interested in the reversibility of some of the lesions produced by FANFT and in the development of a variety of means of detecting those structural or functional markers that might signal the entry of epithelial proliferation into the irreversible phase. Previous studies in this (8) and other laboratories (2–6) utilizing the carcinogenic nitrofuran FANFT have demonstrated in various species and strains the sequence of recognizable epithelial changes that precedes the development of invasive tumors. What has not been shown well, however, is the neoplastic potential of these proliferative epithelial lesions, i.e., the likelihood with which each type of lesion will, if untreated, progress to carcinoma. This study was designed to provide this type of information. Various morphological markers were identified and followed through the sequence of changes from reversible to irreversible. This paper will describe primarily the methodology and the light microscopic findings. The results of electron microscopic studies will be detailed by Jacobs et al. (7).

Materials and Methods

Eight groups of 4-week-old Fischer male rats were fed FANFT in the diet at a dose of 0.2% by weight for 2, 4, 6, 8, 10, 12, 14, or 20 weeks, respectively. Following the period of FANFT feeding, each group of test animals was maintained on the carcinogen-free control diet (Charles River rat chow) until the end of the experiment. At the end of each test period, 6 rats from the group in which FANFT feeding was being discontinued and 5 rats from each group that had previously had FANFT discontinued were each anesthetized.

1 Presented at the Conference “Early Lesions and the Development of Epithelial Cancer,” October 21 to 23, 1975, Bethesda, Md. This study was supported in part by USPHS Grant CA 15945 from the National Cancer Institute through the National Bladder Cancer Project.

2 Presenter.

3 On leave of absence from the Department of Pathology II, University of Göteborg, Göteborg, Sweden.

4 The abbreviation used is: FANFT, N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide.
Reversibility of Early Bladder Lesions

Results

The amount of FANFT consumed by the rats in the various groups is summarized in Table 1. There was no evidence of acute or subacute toxicity, and no growth retardation was evident in rats receiving FANFT.

Mild, focal transitional cell hyperplasia of the urinary bladder was present in the rats fed FANFT for 2 weeks and became more diffuse after 4 weeks (Fig. 1). These lesions reverted to normal after 2 weeks on the control diet, and at the end of the experiment the light microscopic appearance of the mucosa was normal.

Moderate focal transitional cell hyperplasia, with the epithelium up to 10 cells in thickness in hyperplastic areas, was present after 6 weeks of FANFT feeding (Fig. 2). After 8 weeks of FANFT the hyperplasia was moderate to marked (Fig. 3), and there was suggestion of nodular or papillary lesions. Within 2 weeks after FANFT was discontinued the hyperplasia had regressed, but focal areas of mild hyperplasia were still evident when multiple sections of the bladder were examined. These residual lesions appeared similar to the mildly hyperplastic lesions induced by FANFT after 2 or 4 weeks of feeding. Focal mildly hyperplastic lesions were present in both groups by the 20th week, but 3 of 4 rats fed FANFT for 6 weeks were normal at Week 50. The single exception was a small papillary lesion with a delicate connective tissue core covered with slightly hyperplastic but otherwise normal-appearing epithelium. The four 8-week-FANFT-fed rats, however, had moderate to marked epithelial hyperplasia at the 50th week of the experiment with increased vascularity of subepithelial tissue.

Rats fed FANFT for 10, 12, or 14 weeks had marked hyperplasia of the bladder epithelium with multiple distinct nodular and papillary tumors which were predominantly located in the dome of the bladder. After 4 to 6 weeks on the control diet, the rats fed FANFT for 10 weeks had regression of their lesions to mild to moderate diffuse or focal hyperplasia, without distinct nodular or papillary lesions. In the rats fed FANFT for 12 and 14 weeks, on the other hand, there was no regression of lesions so that by 20 weeks the bladders looked like those from rats fed FANFT the entire time. By the 50th week all rats fed FANFT for 10 or more weeks had bladder tumors. One rat fed FANFT for 10 weeks had transitional cell carcinoma extending through the entire thickness of the bladder wall. Some of the tumors in rats

with a lethal dose of 1.0 ml of Nembutal (50 mg/ml; Abbott Laboratories, North Chicago, Ill.) i.p., and the bladders were inflated and removed. For example, 6 weeks after the onset of the experiment 6 rats from each of the following groups were sacrificed: the group fed FANFT for 6 weeks; the group fed FANFT for 4 weeks followed by 2 weeks of control diet; and the group fed FANFT for 2 weeks followed by 4 weeks of control diet. Six untreated control rats of the same age as the test rats were also sacrificed at each time period (see Chart 1). At 50 weeks, 30 weeks after FANFT feeding had been discontinued in all groups, 2 to 4 rats were sacrificed from each of the 8 experimental groups and from the noncarcinogen-fed group.

Inflation of the urinary bladders was accomplished while the rat was anesthetized by transurethral injection through a 25-gauge needle of 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, under a constant pressure of 50 mm of mercury to a volume of 0.4 to 0.5 ml, depending on the age of the rat. The proximal urethra was ligated after inflation, and the bladder was promptly removed and immediately placed in the same fixative. After 15 min the inflated bladders were bisected and the mucosal surface was examined with a dissecting microscope. Representative portions were taken for transmission and scanning electron microscopy and for light microscopy. All visible lesions were bisected with portions being processed for both light and electron microscopy. Approximately one-half of each bladder was placed in 10% buffered formalin and processed for paraffin embedding, sectioning, and staining with hematoxylin and eosin. When no lesions were evident, one-half of the bisected bladder was placed directly in formalin for ultimate light microscopy and the other half was processed for electron microscopic examination. Each paraffin block was step-sectioned and multiple sections of each bladder were examined.

Specimens for transmission electron microscopy were rinsed briefly in 0.1 M cacodylate buffer, pH 7.4; postfixed in 1% OsO4 in 0.1 M cacodylate buffer, pH 7.4; and rapidly dehydrated through an ascending alcohol series. Specimens were rinsed in propylene oxide, embedded in Epon 812, and examined in a Zeiss EM9S2 electron microscope. The preparation for scanning electron microscopy is described elsewhere in this issue by Jacobs et al. (7).

Table 1

Cumulative consumption of FANFT

<table>
<thead>
<tr>
<th>Group</th>
<th>Total wk fed</th>
<th>Cumulative dose of FANFT (g/rat)</th>
</tr>
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<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
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<tr>
<td>3</td>
<td>4</td>
<td>0.7</td>
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<tr>
<td>5</td>
<td>8</td>
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<td>14</td>
<td>3.2</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Chart 1. Experimental design. Shaded areas, time periods in which FANFT was administered; clear areas, time periods in which control diet (without FANFT) was fed. Rats from each group were also examined after 50 weeks of the experiment.
fed FANFT for 10 or more weeks were noninvasive, while
transmission electron microscopy demonstrated that in
other animals, although there were multifocal breaks in the
basement membrane, there was no invasion into muscle
except in the 1 lesion. It has been demonstrated previously
that rats fed FANFT for 20 or 25 weeks eventually develop
large bladder tumors which, if the rats survive long enough,
invade the muscular layers and occasionally metastasize.

Discussion

FANFT has demonstrated potent carcinogenic activity
with specificity toward the urinary bladder in several spe-
cies (1–6) including the inbred strain of Fischer rats (8). It
induces epithelial lesions in the bladder reproducibly in
this strain when fed at a dose of 0.2% of the diet. The lesions
progress from mild, focal hyperplasia after 2 weeks of
FANFT feeding to microinvasive carcinomas after 25 weeks.
If animals are taken off the FANFT-containing diet after 25
weeks and maintained on the carcinogen-free diet, the
tumors continue to grow, and the rats eventually die of severe
hematuria and wasting. Invasion of the muscular layers of
the bladder is seen in some animals and rarely distant
metastases are present. The present study was performed
to determine at which time interval the lesions in the blad-
der become irreversible, reaching the point where given
adequate time they will become invasive carcinomas. Les-
ions that appear through 6 weeks of FANFT feeding such
as mild to moderate hyperplasia are completely reversible to
normal with the exception of the single lesion described
above; lesions after 8 weeks of FANFT feeding regress
toward normal but remain mildly hyperplastic at 20 weeks
and progress to moderate to markedly hyperplastic lesions
by 50 weeks. Lesions present after 10 weeks of FANFT
feeding initially regress somewhat after FANFT is removed
from the diet but eventually progress to invasive carcino-
mas. Lesions present after 12 weeks or more of FANFT
feeding do not regress and the animals develop invasive
carcinoma by 50 weeks.

The lesion that is present at 10 weeks consists of marked
nodular and papillary hyperplasia with an intact basement
membrane as seen by light and transmission electron mi-
croscopy. At this stage it is by light microscopy a noninva-
sive lesion, but one which we have demonstrated will even-
tually progress to the development of carcinoma in a high
percentage of cases. Several morphological alterations
have occurred in the bladder by 10 weeks of FANFT admin-
istration including thickening of the basement membrane,
increased microvascularity, increased numbers of mast
cells in the subepithelium and, in some instances, loss of
superficial transitional cells. None of these findings taken
alone, or as a group, would permit us to make a diagnosis of
a cancer, although in this particular model they are certainly
suggestive that the bladder epithelium has become neo-
plastic. However, the results of our studies with scanning
electron microscopy have been of more help in providing
morphological markers of this change. These findings will
be discussed in another presentation in this symposium (7).

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Fig. 1. Mild hyperplasia of the bladder epithelium after 4 weeks of FANFT feeding (left half) as compared to normal bladder epithelium (right half) by use of a comparison microscope. H & E, × 400 (original magnification).

Fig. 2. Moderate hyperplasia of the bladder epithelium after 6 weeks of FANFT feeding. H & E, × 400 (original magnification).

Fig. 3. Severe hyperplasia of the bladder epithelium after 8 weeks of FANFT feeding. H & E, × 400 (original magnification).
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