Carcinogenicity of $N$-[4-(5-Nitro-2-furyl)-2-thiazolyl]acetamide in Female Rats

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SUMMARY

$N$-[4-(5-Nitro-2-furyl)-2-thiazolyl]acetamide was fed to 70 female Sprague-Dawley weanling rats at a dosage of 0.199% by weight for 46 weeks, followed by 20 weeks of the control diet. Forty control rats received only ground diet for the entire 66 weeks. Rats were weighed and food consumption was determined biweekly at first and then monthly. With weekly palpation the first mammary tumor was detected at 16 weeks, and tumor incidences are based on the number of rats alive at this time. Every 1 or 2 weeks, 1 rat was sacrificed to determine any early pathological changes of the urinary tract or other internal organs.

Of the 56 rats that survived 16 or more weeks, 52 had tumors with the following organ distribution: 47 mammary tumors (24 fibroadenomas and 23 adenocarcinomas), 6 salivary gland adenocarcinomas, 7 alveolar cell carcinomas of the lung, 2 transitional cell carcinomas of the kidney, 3 skin tumors (squamous cell carcinoma, fibrosarcoma, mastocytoma), 1 small intestinal adenocarcinoma, and 1 splenic lymphosarcoma with hepatic metastases. A systemic lymphoreticular response involving the spleen and lymph nodes was seen in a majority of the rats, and several rats had edema, hemorrhage, and partial destruction of the adrenal gland. No tumors were found in the control rats.

A fibroadenoma, an adenofibroma, and an adenocarcinoma of mammary origin and an adenocarcinoma of salivary gland origin were transplanted to the subcutaneous tissue of female Sprague-Dawley weanling rats with the following incidences of transplantable tumors: 7/24, 4/12, 9/12, and 14/24, respectively. Two of the 9 mammary adenocarcinomas that grew in recipient rats metastasized: one through the abdominal wall and into the liver, the other to the spleen.

INTRODUCTION

$N$-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide was shown to be an extremely potent bladder carcinogen in Sprague-

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Temporal sequence of appearance of mammary or salivary gland tumors in rats fed NFTA compared with untreated control rats

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Time of palpation of mammary or salivary gland tumors at Week</th>
<th>No. of rats with tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mammary gland</td>
<td>16 20 24 28 32 36 40 44 48 52 56 60 64 66</td>
<td>0</td>
</tr>
<tr>
<td>Salivary gland</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>NFTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mammary gland</td>
<td>3a 6 4 3 6 3 8 5 2 2 3 2</td>
<td>47</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>1 1 1 1 1 1 1 1 1 1</td>
<td>6</td>
</tr>
</tbody>
</table>

*aEach number in the table represents the number of rats in the period of a given week that had a mammary or salivary gland tumor palpated for the first time.

One salivary gland and 3 mammary gland tumors were transplanted to female Sprague-Dawley weanling rats. The tumor was removed aseptically from the donor rat (anesthetized with ether) and placed in sterile 0.9% NaCl solution. Prior to transplantation, a representative tissue sample was prepared for histological examination. The tumor was washed, transferred to another dish of NaCl solution, and cut into very small pieces, making a tumor cell suspension. A 0.5-ml inoculum was injected s.c. into both sides of the recipient rat with a 16-gauge needle attached to a 10-ml syringe. These rats were weighed as above and palpated weekly. A single Bicillin injection (0.1 ml) was given i.m. to the rats about 7 weeks after tumor inoculation.

When a rat died or was sacrificed, an autopsy was performed and the urinary bladder, heart, lung, liver, spleen, adrenal, kidney, gastrointestinal tract, uterus and ovary, mammary tissue, chest wall, lumbar vertebrae, and any tumors were preserved. Two to 10 tissue samples from each organ or gross tumor were obtained and prepared for histological study. The bladder was inflated with Bouin’s solution and then bleached with 70% ethanol saturated with Li₂CO₃. The other tissues were fixed in a mixture of 95% ethanol (2 liters), glacial acetic acid (0.1 liter), formaldehyde (0.6 liter), and water (4 liters). All tissues were sectioned and stained with hematoxylin and eosin. Some tissues were stained with PAS.

RESULTS

No difference between the growth rate of rats receiving NFTA and that of the controls was observed (Chart 2). The rats readily consumed the diet containing NFTA, and the approximate maximal cumulative consumption of chemical was 8.55 g/rat for the 46 weeks. The dosage of NFTA was approximately 220 mg/kg/day at the beginning of the experiment, but as the rats grew and food consumption increased only slightly, the dosage of NFTA approached 100 mg/kg/day by the 46th week. Fifty-six rats receiving NFTA survived 16 weeks or more, and 52 of these rats had tumors. The distribution of tumors that were found is listed in Table 2. None of the 14 rats sacrificed prior to the 16th week had neoplastic changes in any organ. 3 rats sacrificed at the 16th, 17th, or 18th week and 1 rat sacrificed at the 66th week had no tumors. Twenty-eight control rats survived 16 weeks or more and 25 of these survived 66 weeks and had no tumors. However, with other comparable nitrofuran feeding experiments recently conducted in these laboratories with Sprague-Dawley female rats, the incidence of mammary
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Table 2

Distribution of tumors by histological type
induced by N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide

<table>
<thead>
<tr>
<th>Organ of origin</th>
<th>Histological type</th>
<th>No. of rats with tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammary gland</td>
<td>Adenocarcinoma</td>
<td>23</td>
</tr>
<tr>
<td>Mammary gland</td>
<td>Fibroadenoma</td>
<td>16</td>
</tr>
<tr>
<td>Mammary gland</td>
<td>Adenofibroma</td>
<td>6</td>
</tr>
<tr>
<td>Mammary gland</td>
<td>Fibroma</td>
<td>1</td>
</tr>
<tr>
<td>Mammary gland</td>
<td>Adenoma</td>
<td>1</td>
</tr>
<tr>
<td>Renal pelvis</td>
<td>Transitional cell carcinoma</td>
<td>2</td>
</tr>
<tr>
<td>Lungs</td>
<td>Alveolar carcinoma</td>
<td>7</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>Adenocarcinoma</td>
<td>6</td>
</tr>
<tr>
<td>Skin</td>
<td>Squamous cell carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>Fibrosarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>Mastocytoma</td>
<td>1</td>
</tr>
<tr>
<td>Small intestine</td>
<td>Adenocarcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Spleen</td>
<td>Lymphosarcoma</td>
<td>1</td>
</tr>
</tbody>
</table>

*Same animal may have more than 1 type of tumor.*

fibroadenoma detected has been 4/35 (1) and 2/29 (6). No other tumors have been found in these control rats (1, 6).

Mammary Tumors. Forty-seven of the 56 rats (84%) had tumors originating from the mammary gland (Tables 1, 2). The first mammary tumor palpated occurred at 16 weeks, and several of the rats had multiple mammary tumors. The tumors were hard masses located along the mammary line on the chest and abdomen, and had a variety of histological appearances.

Some tumors contained connective tissue and epithelial elements and others were typical adenomas or atypical fibroadenomas resembling scirrhous adenocarcinomas with frequent mitotic figures. These latter tumors resembled those classified by Hadidian et al. (7) as "scirrhous-type of mammary adenocarcinoma." In spite of the malignant characteristics of these atypical mammary tumors, we have classified them as fibroadenomas (Table 2). When atypical tumors were observed adjacent to adenocarcinomas within the same tumor mass, these neoplasms were classified as adenocarcinoma in Table 2.

Adenocarcinomas originated from glandular elements, contained very little or no connective tissue, and exhibited several mitoses (from 5 to 15) per high-power field. These adenocarcinomas showed several types of histological architecture [fibrotic, cystic, or cystic papillary (Fig. 1)], and a few tumors contained cells with PAS-positive granules in the cytoplasm.

Other Malignant Tumors. Adenocarcinomas arising from the submandibular, submaxillary, or parotid glands were found in 6 rats (Tables 1, 2). These tumors originated from either serous or mucous parts or both portions of the glands (Fig. 2) and grew rapidly into medullary, gray tumors located on the neck or just below the jaw. These tumors were invasive and spread into nearby connective and muscular tissue of the neck. Frequent mitoses were seen with 7 to 10 mitotic figures per high-power field. Mitotic activity was greater in mucous adenocarcinomas than in serous adenocarcinomas. Three different skin tumors were found: a squamous cell carcinoma, a fibrosarcoma, and a PAS-positive mastocytoma (Table 2). Pulmonary carcinomas arising from the alveolar respiratory epithelium were found in 7 rats (Table 2). One lymphosarcoma arising in the spleen demonstrated hepatic vascular metastasis. Two transitional cell carcinomas, originating in the renal pelvis, were identical with those described earlier (2, 3, 6). One small intestinal adenocarcinoma was found.

Nonneoplastic Changes. Mild hyperplasia of the transitional epithelial cells of the renal pelvis was observed 2 weeks following initiation of NFTA feeding. All rats sacrificed or dying after 6 weeks had moderate hyperplasia in the renal pelvis, which progressed to severe hyperplasia by 60 weeks. In spite of these changes, only 2 transitional cell carcinomas arising in the renal pelvis were detected (Table 2).

Mild to severe hyperplasia of the spleen was seen in a majority of the rats receiving NFTA and was also often seen in the regional lymph nodes. Edema, hemorrhage, and partial loss of architectural integrity were commonly observed in the adrenals.

Transplantations. A fibroadenoma, an adenofibroma, and an adenocarcinoma of mammary origin and an adenocarcinoma of salivary gland origin were transplantable to female Sprague-Dawley weanling rats (Table 3). The latent period and incidence were related to the histological degree of malignancy of the tumor transplanted. The mammary adenocarcinoma had a latent period of only 2 weeks and showed an increase in epithelial content (Fig. 3). The transplanted mammary adenocarcinoma grew to a mass 4 cm in diameter within 6 weeks after initial palpation. Conversely, the growth rate of the fibroadenoma and adenofibroma was less rapid with an average mass of 2 cm in diameter at 6 weeks after initial palpation. The mammary adenocarcinoma was found to be capable of metastasizing in the recipient rats, metastases being found in 2 of the 9 recipient animals in which the tumor was transplantable. One rat showed metastases through the abdominal wall and into the liver (Figs. 4, 5). Another rat showed metastasis to the spleen (Fig. 6).

The salivary gland carcinoma had a latent period of 9 weeks. These transplantable tumors showed increased glandular areas and usually were solid carcinomas (Fig. 7) with cellular pleomorphism and high mitotic activity (Fig. 8).

DISCUSSION

NFTA is not the first nitrofuran derivative shown to have carcinogenic activity in rats (1–6, 8). N-[4-(5-Nitro-2-furyl)-2-thiazolyl] formamide induced urinary bladder carcinomas at a 100% incidence and gave rise to some renal transitional cell carcinomas in male and female rats (3, 5, 6). In view of its very close structural resemblance to N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide, it was surprising to find that NFTA demonstrated only hyperplasia and rare transitional epithelial cell carcinomas of the renal pelvis with no lesions in the urinary bladder. The seemingly minor structural difference between these 2 compounds apparently plays an important role in determining the response of the urothelium to these chemicals. However, the organ specificity of carcinoogenic nitrofurans is not determined solely by formyl-amino or...
Carcinogenicity of NFTA

Table 3
Transplantability into female recipient rats of mammary and salivary gland tumors induced by N-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide

<table>
<thead>
<tr>
<th>Group</th>
<th>Histology of chemically induced tumors</th>
<th>No. of recipients</th>
<th>Time of palpation of first tumor (wk)</th>
<th>No. of rats with palpable viable transplants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mammary fibroadenoma</td>
<td>24</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>Mammary adenofibroma</td>
<td>12</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Mammary adenocarcinoma</td>
<td>12</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>Salivary gland adenocarcinoma</td>
<td>24</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>5a</td>
<td></td>
<td>18</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

*aControl rats were sacrificed after 37 weeks of observation.

Acetylamino substituents. When 5-acetamido-3(5-nitro-2-furyl)-6H-1,2,4-oxadiazine was administered to rats, a 100% incidence of hemangioendothelial sarcomas arising from the mesentery, liver, and lungs was observed (4). This latter compound differed from NFTA in that the thiazolyl portion of the molecule was replaced with an oxadiazine ring (4).

However, not all carcinogenic nitrofuran derivatives have such organ specificity. Formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide induced tumors arising in the breast, kidney, intestines, auditory canal, and liver (2, 8). Mammary and renal carcinomas induced by this compound were transplantable (E. Ertürk, S. M. Cohen, and G. T. Bryan, unpublished observations). NFTA also appears to induce tumors in a variety of organs, and in this respect it resembles formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide.

The organ most susceptible to tumor induction by NFTA was the mammary gland (Tables 1, 2). A wide variety of neoplastic alterations were observed in this tissue, but nearly 50% of the rats had 1 or more adenocarcinomas. The time of appearance of these adenocarcinomas was not related to the duration of feeding of NFTA, as the earliest one appeared after 16 weeks of feeding. Cessation of administration of NFTA at the 46th week did not prevent the continuous progression of established mammary tumors or the appearance of new, palpable mammary tumors. Thus, it does appear that the development of these neoplasms was directly related to the administration of NFTA.

The classification of chemically induced rat mammary tumors has not been clearly defined. The classical criteria of malignancy includes anaplasia, metaplasia, invasiveness, distant metastases, and transplantability. The NFTA-induced mammary tumors that were recorded as adenocarcinomas met all of these criteria and were classified as malignant. Those lesions designated as adenofibromas demonstrated low mitotic activity and no metastases, but did show disruption of the basement membranes and were transplantable. Although these latter tumors have been classified as benign, the possibility that they are malignant is not excluded since they do possess some of the criteria of malignancy.

Several, but not all, of the nitrofurans tested have shown carcinogenic activity in the rat (1–6, 8). All of the carcinogenic nitrofuran derivatives have potent antibacterial activity and have received attention as possible antibiotics. Indeed, one of these derivatives, NFTA, has been used clinically as a urinary tract antibacterial agent. In view of the carcinogenic activity of NFTA for a wide variety of rat tissues including the mammary gland, it would seem that the usage of this chemical as a drug for humans should be reevaluated. It is also suggested that all nitrofuran analogs under serious consideration as possible antimicrobial drugs or food additives be tested for carcinogenic activity in rats prior to their usage in humans.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 1. Cystic papillary adenocarcinoma of rat mammary gland. X 80.
Fig. 2. Salivary gland adenocarcinoma consisting of both serous and mucous elements. X 100.
Fig. 3. Transplanted mammary adenocarcinoma observed in recipient rat. X 80.
Fig. 4. Striated muscular invasion of transplanted mammary adenocarcinoma. X 125.
Fig. 5. Hepatic metastases appearing in recipient rat receiving transplantable rat mammary adenocarcinoma. X 130.
Fig. 6. Metastases to spleen of transplanted rat mammary adenocarcinoma. X 110.
Fig. 7. Transplanted salivary gland adenocarcinoma of predominantly mucous type. X 30.
Fig. 8. Salivary gland adenocarcinoma transplanted into recipient rat demonstrating high mitotic activity. X 125.
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