Urinary Bladder Carcinogenicity of \(N\)-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide in Female Swiss Mice

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SUMMARY

\(N\)-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide was fed to 50 female 5-week-old Swiss mice at a dose of 0.094% by weight of diet for 46 weeks, followed by 7 weeks of the control diet. Fifty-six mice received only the control diet. Thirty-three test mice survived 40 weeks or longer and had the following tumor incidences: urinary bladder, 31/33; generalized leukemia, 23/33; lung, 6/33; and 1 uterine tumor. In the controls, the following tumor incidences were found: generalized leukemia, 15/44; and lung, 1/44.

INTRODUCTION

FANFT was shown to be a powerful urinary bladder carcinogen in rats (7-9). To determine the possible bladder carcinogenicity of FANFT in a different animal species, it was fed to female Swiss mice for 46 weeks.

MATERIALS AND METHODS

FANFT, obtained as a gift from Abbott Laboratories (North Chicago, Ill.), was fed to 50 female 5-week-old Swiss mice (Rolfsmeyer Company, Madison, Wis.) at a dose of 0.094% by weight of diet for 46 weeks, followed by 7 weeks of the control diet. Fifty-six mice received only the control diet. The diet was mixed in ground Wayne Lab-Blox (Allied Mills, Inc., Chicago, Ill.) as described earlier (7). The dose of FANFT fed the mice was 50% of the dose which had been used for rats (7-9).

Two male mice were accidentally included with the female mice when shipped to us, and as a result we obtained 4 litters of mice. The mothers were fed FANFT, as above, while pregnant and while nursing. The young mice were weaned at the end of 3 weeks and divided into male and female groups of test and control mice, as above. The observations from these mice are only preliminary, as only a few mice were alive at weaning.

Food consumption, weighing, and palpation of the mice were performed at the end of the 1st, 3rd, and 6th weeks, and then monthly until the end of the experiment. Injections of 0.1 ml Bicillin LA (Wyeth Laboratories, Philadelphia, Pa.) i.m. were given to each mouse during the 13th and 28th weeks after feeding of FANFT began in order to control any possible infection in the mouse colony. Autopsy procedures and tissue preparation were as described earlier (7). All histological sections were stained with hematoxylin and eosin.

RESULTS

Thirty-three mice that received FANFT survived 28 weeks or more and 31 of them had multiple tumors of the urinary bladder varying in size from 1 mm to masses that filled the entire bladder lumen. In the latter cases, urinary flow was partially obstructed and the resulting hydronephrosis was accompanied by pericarditis and skin irritations with a foul odor. Gross hematuria was seen occasionally in mice with large bladder tumors. The tumors were grayish white with red areas seen occasionally; bleeding tumors had a red-brown color. The small tumor masses arose from the transitional epithelium either as projecting carcinomas or growths into the submucosa causing thickening of the bladder wall. Invasion into the subserosal connective and fat tissues and through the serosa was frequently found.

The first indication of tumor formation was an increase in the cell layers of the normally 1- to 2-cell thick transitional cell epithelium (2, 12, 13). The proliferative lesions eventually developed into carcinomas projecting into the lumen or invasive carcinomas showing solid ingrowths and nests of epithelial derivation that penetrated through the submucosa, all 3 muscular layers, and through the serosa. Numerous mitoses were present along with metaplastic transformations such as squamous metaplasia and bizarre cells with giant dense nuclei containing several nucleoli. Connective tissues and blood vessels were also involved in some instances of tumor formation, leading to scirrhous carcinomas, hemangiomas, or hemangiendothelial sarcomas. The histological changes observed

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4 The abbreviations used are: FANFT, \(N\)-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide; FNT, formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide.
were similar to those seen in mice subjected to bladder carcinogen testing by the pellet implantation technique (2, 3, 12, 13) and rats fed FANFT (7, 9).

Apart from these neoplastic responses, most of the urinary bladders showed aggregates of lymphocytes in the submucosa that may be the result of a generalized leukemia that simultaneously developed.

The generalized leukemia was present in 23 of the mice that received FANFT and involved either the thymus, spleen, lymph nodes, or any combination of these. The incidence of leukemia was significantly greater \((p < 0.02)\) than that observed in untreated control mice when the probability of statistical significance was computed by the exact method for \(2 \times 2\) tables (10). The pathology of these leukemias was described earlier and was identical with that induced by FNT (6) in mice. Leukemic infiltration of other organs occurred, including the kidneys, adrenals, lung, and urinary bladder.

Six of the 33 FANFT mice had solitary or multiple nodules under the pleura or deep in the lung tissue. The incidence of these pulmonary tumors was significantly greater \((p < 0.02)\) than that found in untreated control mice. These tumors were grayish white in color and ranged in size from less than 1 mm to 1 cm in diameter. They arose from the alveolar epithelium and developed into typical lung carcinomas with 2 to 4 mitoses/high-power field and anaplasia, and were similar to those induced by FNT (6). One adenoma of the uterus was found in a FANFT-fed mouse. In the control mice, no urinary bladder lesions were detected, but an incidence of 15/44 generalized leukemias and 1/44 lung carcinoma was found.

Eleven male and 7 female mice born to mothers fed FANFT and which were fed this compound after weaning survived 20 weeks or more and all had urinary bladder carcinomas. Tumors of the forestomach were also found in 2 of 11 males. Most of these mice had hyperkeratosis and acanthoses of the squamous epithelium of the forestomach and degenerative and necrotic lesions were seen in the liver. The forestomach and hepatic lesions were not seen in the mice which received FANFT and which were fed this compound after weaning.

Their offspring were used as control mice and did not receive FANFT in their diet after weaning. No lesions were found in these mice except for 1 female mouse with bladder hyperplasia.

**DISCUSSION**

Numerous attempts have been made to induce urinary bladder carcinomas in mice by the systemic route of chemical administration, but to date only two chemicals, 2-acetylaminofluorene (1) and 4-ethylsulfonylnaphthalene-1-sulfonamide (4), have induced a moderate incidence of about 50% bladder carcinomas. Additionally, 2-aminofluorene (11) and 4-aminodiphenyl (5) induced a low incidence of bladder carcinomas in mice. FANFT appears to be the most potent systemic urinary bladder carcinogen yet discovered for male and female rats (7, 9) and mice and, as such, may provide a useful model for studying many aspects of urinary bladder cancer.

FANFT was also found to be leukemogenic in mice, in a similar fashion as another nitrofuran compound, FNT (6). The appearance of stomach and liver lesions in mice raised from conception by mothers that received FANFT suggests that the mother forms an active carcinogenic metabolite which is passed through the milk. This possibility is being studied further.

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**REFERENCES**

Carcinogenicity of FANFT in Mice

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