Production of Leukemia and Stomach Neoplasms in Swiss, RF, BALB/c, and C3H Female Mice by Feeding N-[4-(5-Nitro-2-furyl)-2-thiazolyl]acetamide

S. M. Cohen, E. Ertürk, and George T. Bryan

Division of Clinical Oncology, University of Wisconsin Medical School, Madison, Wisconsin 53706

SUMMARY

N-[4-(5-Nitro-2-furyl)-2-thiazolyl]acetamide was fed to female 5-week-old Swiss mice at a dose of 0.1% by weight of diet for 13 weeks, with a cumulative dose of 490 mg/mouse/13 weeks. The mice were observed for an additional 14-week period while being fed control diet. Because of the sudden appearance of leukemia during the 13th week and cannibalization of moribund mice by other mice, only 16 mice survived at least 17 weeks to be grossly and microscopically evaluated. Of these, 15 had generalized lymphocytic leukemia and 3 had squamous cell tumors of the stomach. In the control group of 56 mice, no cancers were detected during the 27-week period of observation.

For determination of the effect of dosage, groups of 30 female 5-week-old Swiss mice were fed NFTA at dietary levels of 0.1, 0.05, 0.025, and 0.01% for 14 weeks. The respective leukemia incidences were 8/9, 9/13, 8/16, and 7/14. The latent period was 12 weeks for the 0.1 and 0.05% groups and 18 weeks for the 0.025 and 0.01% groups. Stomach tumors were present in 12/52 mice. In the control group of 35 mice, 1 mouse developed a pulmonary adenoma.

RF, BALB/c, and C3H female mice, regarded as having a low incidence of spontaneous leukemia, were as susceptible to the leukemogenic effect of NFTA as were Swiss mice. The following incidences of leukemia were observed after 14 weeks of feeding a diet composed of 0.1% NFTA: RF, 12/16 (controls, 3/16); BALB/c, 21/29; C3H, 12/24; and Swiss, 22/22. A low incidence of stomach tumors was found in all NFTA-treated groups.

INTRODUCTION

NFTA (Chart 1), an antibiotic drug currently used in the therapy of human infectious disease, was supported in part by Grant CA-10341 from the National Cancer Institute, USPHS, and by a grant from the Wisconsin Division of the American Cancer Society.

MATERIALS AND METHODS

NFTA was obtained as a gift from U. Ravizza (Milan, Italy), and its purity was checked by ultraviolet absorption measurements. It was fed to 50 female 5-week-old Swiss mice (Rolfsmeier Company, Madison, Wis.) at a dosage of 0.1% by weight of ground Wayne Lab-Blox (Allied Mills, Inc., Chicago, Ill.) for 13 weeks. They received control diet for an additional 14 weeks. The diet was mixed mechanically as described (7, 13), and water and food were supplied ad libitum. Fifty-six control mice were fed only ground Wayne Lab-Blox for the duration of the experiment. Food consumption estimations and weighing of the mice were performed at the end of the 1st, 3rd, and 6th weeks, and then monthly. Injections of 0.1 ml of Bicillin Long-Acting (Wyeth, Philadelphia, Pa.) i.m. were given to each mouse during the 13th week of feeding. Autopsy procedures and tissue preparation were as described (6, 13). All histological sections were stained with hematoxylin and eosin.

In order to test the effect of dose variations, groups of 30 female 5-week-old Swiss mice were fed NFTA at doses of 0.01, 0.025, 0.05, and 0.1% by weight of diet for 14 weeks, followed by control diet for an additional 14 weeks. A group of 35 mice served as a negative control group. One mouse from each group was sacrificed at the end of each week to ascertain the latent period of leukemia formation. At the time of autopsy, leukocyte counts, hematocrits, and splenic weights were determined. No Bicillin or any other therapy was given to these mice.

For investigation of mouse strain susceptibility to the leukemogenic effect of NFTA, groups of RF, BALB/c, C3H (Jackson Laboratory, Bar Harbor, Maine), and Swiss female mice were fed NFTA (0.1%) for 14 weeks, followed by control diet for 14 weeks. The ages of the mice at the start of the experiment were: RF, 7 weeks; BALB/c 6 weeks; C3H, 8 weeks; and Swiss, 5 weeks. Control groups for each strain composed of about the same number of mice as those fed NFTA received only ground Wayne Lab-Blox. Leukocyte counts, hematocrits, and splenic weights were determined at
autopsy. No Bicillin or any other therapy was given to these mice.

RESULTS

Initial Test of NFTA in Swiss Mice. The daily dose of NFTA ingested by mice was 4.5 to 5.7 mg/mouse with a mean cumulative dose of 490 mg/mouse/13 weeks. The mice tolerated the feeding of NFTA well during the first 10 weeks, but then began to develop a progressively more severe weight loss that continued even with the deletion of NFTA from the diet after the 13th week. Only 16 test mice were inspected at autopsy could be done. Thus, only 16 test mice were inspected each week, and many of them had been cannibalized before an autopsy could be done. Thus, only 16 test mice were inspected grossly and microscopically, and 15 of them had generalized lymphocytic leukemia. None of the 51 control mice that survived 14 to 29 weeks developed leukemia or any other tumors.

The leukemia produced by administration of NFTA was grossly characterized by marked enlargement of the thymus, spleen, and/or lymph nodes (Figs. 1 and 2). In 11 of 15 mice the leukemia involved the lymphoreticular tissues of the thymus, spleen, and regional and peripheral lymph nodes. In these mice, the thymus nearly completely filled the thoracic cavity (Figs. 1 and 3), surrounding the heart and compressing the pulmonary tissue inferiorly and posteriorly, resulting in severe atelectasis (Fig. 3). Mediastinal, tonsillar, cervical, axillary, inguinal, iliac, supramammary, renal, mesenteric, and intestinal nodes were enlarged up to 2 cm in diameter and were frequently confluent. In 2 mice, the leukemia involved only the thymus; in 2 other mice, only the spleen and lymph nodes were involved. The involved spleens were 5 to 10 times the mass of those present in control mice (normal mouse spleen weight, 120 to 200 mg). The kidneys, lungs, livers, urinary bladders, and other organs from leukemic mice were pale and exhibited areas of white or gray nodules.

Microscopically, the cortical and medullary portions of the thymus were indistinguishable and were characterized by an actively proliferating cell population with numerous mitoses (Fig. 4). The size and shape of the leukemic cells varied from ones with small, but prominent, nuclei and scant cytoplasm to ones with large nuclei containing enlarged nucleoli (Figs. 4 and 5). The spleens (Fig. 6), lymph nodes, and bone marrow (Fig. 7) were occupied by actively proliferating cells demonstrating numerous mitoses and a loss of normal cellular architecture. Surrounding tissues were frequently infiltrated by leukemic cells, and the osseous portions of vertebral bones were partially destroyed. Examples of lymphocytic leukemia cell infiltration into the pectoral muscles of the chest wall (Fig. 8), liver (Fig. 9), kidneys (Fig. 10), perivertebral muscles (Fig. 11), and the spinal canal and leptomeninges (Fig. 12) were frequently seen. Less frequent were leukemic infiltrates in the adrenals, uterus, urinary bladder, pericardium, and gastrointestinal tract. The morphology of the lymphocytic leukemia observed was identical to that described by Dunn (4).

In 3 of the test mice fed NFTA, small, white to gray papillary tumors were seen as projecting nodules from the forestomach. These tumors demonstrated cellular characteristics identical to those described for the completely invasive and metastatic squamous cell forestomach carcinomas produced in mice by feeding formic acid 2-[4-(5-nitro-2-furyl)-2-thiazoyl]-hydrazide (1). However, the forestomach tumors seen in mice fed NFTA demonstrated only either basement membrane penetration and submucosal infiltration or invasion into the stomach muscular wall. No serosal invasion or metastases to distant organs were found. Since these papillary tumors did not penetrate the serosal surface, they were not classified as carcinomas, in accordance with the criterion of Stewart et al. (17) proposed for rat stomach tumors. Whether this stringent criterion would be fulfilled if the animals were kept alive for a longer period of time is a question for further study.

Effect of Dose Level. The survival rate, latent period, and incidence of lymphocytic leukemia in Swiss mice fed varying doses of NFTA for 14 weeks are presented in Table 1. At doses of 0.1 and 0.05%, most of the mice that lived 13 or more weeks developed leukemia with a latent period of 12 weeks. The leukemic mice had markedly enlarged thymus glands, spleens, and lymph nodes in addition to having a peripheral leukocytosis as high as 82,000 cells/cu mm. At doses of 0.025 and 0.01%, the latent period was 18 weeks, and the incidence of leukemia was only 50%. These mice demonstrated a lesser degree of organomegaly, and peripheral leukocytosis occurred with less frequency than seen in mice fed the 2 higher doses of NFTA. The histological characteristics of the lymphocytic leukemia were identical to those described above. None of the control mice developed leukemia within this rather brief experiment period. Twelve of 52 mice that were fed NFTA for 14 weeks developed forestomach squamous cell tumors (Table 1). The incidence of these stomach tumors was highest in the group of mice fed the largest dose of NFTA. No other tumors were detected in the test or control groups of mice in a significant incidence.

Effect of Strain. The survival rate, latent period, and incidence of lymphocytic leukemia associated with thymomegaly

| Dose of NFTA fed, survival rate, incidence and latent period of leukemia, and incidence of squamous cell tumors of stomach in Swiss female mice |
|---|---|---|---|---|---|
| Dose % by weight (mg/mouse) | Total No. of mice alive (wk) | No. of mice with leukemia | No. of mice with leukemia found (wk) |
| Mean wt | Start | 14 | 18 | 28 | 18 wk | 29 wk |
| 0.00 | 0 | 35 | 18 | 15 | 8 | 0 | 0 |
| 0.010 | 60 | 30 | 14 | 10 | 6 | 0 | 7 | 18 | 3 |
| 0.025 | 160 | 30 | 16 | 12 | 2 | 0 | 8 | 18 | 3 |
| 0.050 | 350 | 30 | 13 | 3 | 0 | 7 | 9 | 12 | 0 |
| 0.100 | 630 | 30 | 9 | 1 | 0 | 8 | 8 | 12 | 6 |

*One mouse had pulmonary adenoma.

*One mouse had pulmonary adenoma, and one mouse had perianal gland carcinoma.
apart in Swiss, RF, BALB/c, and C3H female mice fed 0.1% of NFTA for 14 weeks are presented in Table 2. A high incidence of leukemia occurred in all strains of mice with a latent period of 13 to 15 weeks. The morphology of the leukemia was identical to that described above, and the leukemic mice frequently had a peripheral leukocytosis. Several of the mice also developed forestomach squamous cell tumors (Table 2). No other tumors were detected in the test or control groups of mice in a significant incidence.

### DISCUSSION

NFTA induced carcinomas of the breast, salivary glands, lungs, and renal pelvis when fed to female Sprague-Dawley rats (5). Responsiveness of the lymphoreticular tissue of animals to NFTA was suggested by the appearance of splenic and lymph node hyperplasia (5). This suggested lymphoreticular tissue reaction to NFTA (5) has been clarified by the production of lymphocytic leukemia in several strains of mice by feeding NFTA. The latent period of development and the leukemic incidence appeared to be related to the dose of NFTA administered, i.e., the larger the dose fed, the shorter the latent period and the higher the incidence of lymphocytic leukemia. The precocious and explosive appearance of this chemically induced disease occurred in several strains of mice with a low incidence (11, 12, 16) of spontaneous leukemia. The mice tested were all 5 weeks of age or older at the time of initial exposure to NFTA, and perhaps were not at a most sensitive age for chemical induction of leukemia. Young mice, especially those given injections of chemicals such as dimethylbenzanthracene or 3-methylcholanthrene at birth, appear most susceptible to the subsequent formation of leukemia (9, 11, 12, 15, 18). Despite the greater age of mice fed NFTA, the 3-month latent period of leukemogenesis was comparable to that found with hydrocarbon injection into neonates (9, 12, 15, 18) or viral-induced murine leukemias (11). The morphological appearance of NFTA-induced leukemia was identical to that described (4, 11, 12) for chemical-, viral-, or radiation-induced lymphocytic leukemia. The thymus was prominently involved in the majority of the mice, as were the spleen and lymph nodes. Leukemic infiltrates were identified in many organs, and peripheral leukocytosis, often with the presence of anemia, was demonstrable. The mechanism of action of NFTA as a mouse leukemogen is not clear at present. Deserving of consideration are direct action as a chemical leukemogen (12), activation of latent, vertically transmitted viruses (11, 12), or immunosuppression with clonal selection of preexisting neoplastic cells (14).

A low incidence of squamous cell forestomach tumors was found in all strains of mice fed NFTA. The production of these tumors was dose related in Swiss mice with a latent period of 15 weeks. Although the histological characteristics of these tumors were identical to the completely invasive and metastatic forestomach squamous cell carcinomas induced in mice by another 5-nitrofuran analog, formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide (1), the stomach tumors induced by NFTA did not completely penetrate the muscle layers of the stomach. In accordance with the restrictive criterion of Stewart et al. (17), these tumors could not be classified as carcinomas. The stomach carcinomas produced by formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide did not become large, invasive, or metastatic in mice living less than 30 weeks of experimental observation (1). In the present study of NFTA carcinogenicity, none of the treated mice lived beyond 29 weeks. It appears possible that the stomach tumors observed might have developed into carcinoma (1) had the mice lived for a longer period of time.

The molecular basis of the carcinogenicity of NFTA in mice and rats (5) is not known. The possibilities that the 5-nitro group of the furan ring may be metabolically converted to a hydroxylamine "proximate" carcinogen, or that metabolic alteration of the acetamide substituent of the thiazolyl ring may occur, were discussed (1, 2, 5). Metabolic studies in progress may clarify the mode of carcinogenic action of NFTA.

The demonstration of the oncogenic activity of NFTA in the mouse and rat (5) suggests that the further use of this clinically effective antimicrobial agent (3, 10) should be reevaluated (5). Although not all 5-nitrofuran analogs studied have demonstrated carcinogenic activity (1, 2, 5–8, 13), many of these compounds have been carcinogenic for the rat (2, 5, 7, 8, 13) or the mouse (1, 6). Carcinogenic testing of 5-nitrofuran analogs under consideration as candidate drugs for human or veterinary use, or as food additives or preservatives, should be adequately conducted in mice (1, 6) or rats (2, 5, 7, 8, 13) prior to the generalized introduction of these compounds into the environment.

### ACKNOWLEDGMENTS

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**Table 2**

<table>
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<th>Strain</th>
<th>No. of mice alive (wk)</th>
<th>Time first leukemia by Squamous mes</th>
<th>Thymus</th>
<th>By Total</th>
<th>Enlarged stomach</th>
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</tbody>
</table>

*Two mice had pulmonary adenoma.
*One mouse had mammary adenocarcinoma, and one mouse had pulmonary adenoma.
*One mouse had salivary gland adenocarcinoma.
REFERENCES


Leukemogenicity of NFTA in Mice

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