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# The Effect of Adult Thymectomy and Adult Splenectomy on the Production of Leukemia and Stomach Neoplasms in Mice by *N*-[4-(5-Nitro-2-furyl)-2-thiazolyl]acetamide[1]

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## SUMMARY

The effect of thymectomy and splenectomy at 3 to 4 weeks of age on the oncogenicity of *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide was tested in female Swiss mice. The drug was administered at 0.1% for 14 weeks beginning when the mice were 5 weeks old; the mice were then placed on control diet until their death or until 35 or 45 weeks of age. Without thymectomy the incidence of lymphocytic leukemia was 26 of 27; in partial thymectomized mice, it was 7 of 7; and in totally thymectomized mice, it was 0 of 15. The incidences of forestomach papillomas in these three groups were 1 of 27, 0 of 7, and 12 of 15, respectively, with infiltrative and metastatic squamous cell carcinoma of the forestomach in three of the latter group. Thus, thymectomy appears to prevent the appearance of lymphocytic leukemia in mice fed *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide but allows for a greater incidence and degree of malignancy of the forestomach neoplasms. Splenectomy and sham operations had no effect on the incidence of leukemia or forestomach neoplasms.

## INTRODUCTION

NFTA[4] (Chart 1) is an antibacterial drug currently used in the therapy of human infectious disease (7, 16). It is oncogenic in female Sprague-Dawley rats, inducing mammary, salivary gland, pulmonary, and renal pelvic carcinomas (8); and in several strains of mice, inducing lymphocytic leukemia and forestomach tumors (6). The murine lymphocytic leukemia was characterized by large thymus, spleen, and lymph nodes; by lymphocytic infiltration of most other tissues; and by the usual bone marrow cells replaced largely by leukemic cells. The leukemia was induced in 12 to 14 weeks and most of the mice fed at a dose of 0.1% of NFTA did not survive beyond 20 weeks (6). Although most lymphosarcomas or lymphocytic leukemias in mice have been demonstrated to be thymic dependent (12), others (1, 23) have occurred without thymic involvement. The lymphatic leukemia induced by bracken fern (23) was histologically very similar to that induced by NFTA with regard to spleen, lymph nodes, and involvement of other tissues, but the thymus did not demonstrate any morphological abnormalities. Thymectomy and splenectomy were thus performed in an attempt to determine the pathogenesis of the lymphocytic leukemia induced by NFTA. Small forestomach papillomas, never penetrating deeper than the muscular layer of the stomach, also were induced in mice by NFTA, and the effect of thymectomy and splenectomy on these tumors was also determined.

## MATERIALS AND METHODS

NFTA was received as a gift from U. Ravizza (Milan, Italy), and its identity and purity were checked by melting point, infrared, and uv absorption measurements and by paper chromatography in a solvent system of methanol:1-butanol:benzene:water (2:1:1:1) and the same solvent system plus 1% glacial acetic acid (3, 17). Swiss female mice (Rolfsmeyer Company, Madison, Wis.) were used, and the surgical procedures designated in Table 1 were performed when the mice were 22 to 26 days old. They were fed the control diet, ground Wayne Lab-Blox (Allied Mills, Inc., Chicago, Ill.), until age 35 days when they were fed either control diet or control diet with NFTA added at a dose of 0.1% by weight (Table 1). This was considered Time 0 of the experiment. The diet was mixed mechanically as described (22), and food and water were supplied *ad libitum*. Food consumption estimations and weighing of the mice were performed at the end of Weeks 1, 3, 6, and 10 and monthly thereafter. At the end of the 14th week of feeding NFTA (age 133 days), all groups were placed on control diet until the end of the experiment. Mice remaining in Groups 5, 7, 9, and 11 were killed 30 weeks after Time 0 (age 245 days) since the mice in Groups 6, 8, 10, and 12 had all died by that time. Those remaining in Groups 1 to 4 were killed 40 weeks after Time 0 (age 315 days). Autopsy procedures and tissue preparation were as described (9, 22), and all histological sections were stained with hematoxylin and eosin.

Thymectomy (10) and splenectomy (21) were performed under ether anesthesia. Sham thymectomy or sham splen-

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[4] The abbreviation used is: NFTA, *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide.

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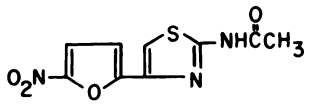
ectomy were performed on other groups of mice with all steps of the thymectomy or splenectomy operation performed except actual removal of the tissue (see Table 1). Success of the operative procedures was evaluated at autopsy and by histological examination. The mice in Groups 3 and 4 that underwent subtotal thymectomy had thymic remnants confirmed at autopsy and are referred to as partially thymectomized mice.

## RESULTS

All 12 groups had essentially the same growth rates, and the 6 groups receiving NFTA had similar maximal cumulative doses of the chemical (average, 0.65 g/mouse) (Table 1). The incidence of leukemia and stomach tumors observed in the various groups is listed in Table 1 and is based on the number of mice alive in each group at 10 weeks. The diagnosis of leukemia was based on microscopic examination of tissues as described (6) and in all cases was characterized by a large thymus with or without splenic or nodal involvement. The mean latent period for the development of leukemia was calculated on the basis of time elapsed from Time 0 (age 35 days) to the date of death. Stomach tumors were classified by the criteria of Stewart *et al.* (26), in which penetration of all layers of the stomach is required for the tumor to be classified as carcinoma. The forestomach tumors were all squamous cell and were similar to those induced by formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl] hydrazide (5).



N-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide

Chart 1. Structure of mouse oncogen, NFTA.

**Lymphocytic Leukemia.** The group without a surgical procedure performed (Group 12) and the groups with either sham thymectomy, splenectomy, or sham splenectomy (Groups 6, 8, and 10, respectively) had essentially the same incidences of lymphocytic leukemia, and the average latent periods were statistically similar. Mice began dying in these groups 10 weeks after Time 0 with the typical gross picture of leukemia, *i.e.*, enlarged thymus, spleen (except in Group 8), and lymph nodes, seen with this chemical. The mean latent period was approximately 14 weeks for all 4 groups, and none of the mice in these groups survived beyond the 20th week from Time 0. The incidences of leukemia in Groups 6, 8, 10, and 12 were 13 of 13, 14 of 16, 10 of 12, and 26 of 27, respectively. The corresponding control groups (Groups 5, 7, 9, and 11) had low incidences of leukemia, and most of the mice in these groups survived the entire 30 weeks from Time 0 at which time they were killed. The mouse with leukemia in Group 5 had a large thymus, but leukemias in Groups 9 and 11 were discovered microscopically.

Groups 3 and 4 had thymectomies performed but had thymic tissue present at autopsy and were thus considered as partial thymectomies. All 7 such mice in Group 4 developed lymphocytic leukemia, although with a prolonged latent period ($p < 0.01$ when comparing Group 4 to Group 6). One mouse of 4 in Group 3 had leukemia, which was discovered by microscopic examination. The totally thymectomized mice (Groups 1 and 2) did not develop leukemia whether receiving NFTA or not, thus demonstrating that thymectomy totally prevents the induction of lymphocytic leukemia by NFTA.

**Forestomach Neoplasms.** The incidences of forestomach tumors in the unoperated, sham thymectomized, splenectomized, and sham splenectomized groups (Groups 6, 8, 10, and 12, respectively) were low, and all were classified as forestomach squamous cell papillomas with invasion as deep as the submucosa or the muscularis. None were carcinomas as classified by Stewart *et al.* (26). There were no forestomach

Table 1

*Experimental design and resulting incidences of leukemia and forestomach tumors*

| Group | Operative procedure | Dose of NFTA (% by wt) | Mean total cumulative dose (g/mouse) | Mice alive at end of week | | | | | | Leukemia | | Forestomach neoplasms[a] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 0 | 10 | 15 | 20 | 30 | 40 | No. | Av. latent period (wks) | Papillomas | Carcinomas |
| 1 | Thymectomy | 0.0 | 0.0 | 24 | 18 | 17 | 16 | 16 | 16 | 0 | | 0 | 0 |
| 2 | Thymectomy | 0.1 | 0.68 | 19 | 15 | 15 | 13 | 9 | 5 | 0 | | 9 | 3 |
| 3 | Partial thymectomy | 0.0 | 0.0 | 4 | 4 | 4 | 3 | 2 | 2 | 1 | 17.4 | 0 | 0 |
| 4 | Partial thymectomy | 0.1 | 0.68 | 7 | 7 | 6 | 4 | | | 7 | 19.8 ± 4.7[b,c] | 0 | 0 |
| 5 | Sham thymectomy | 0.0 | 0.0 | 27 | 27 | 27 | 26 | 24 | | 1 | 30.0 | 0 | 0 |
| 6 | Sham thymectomy | 0.1 | 0.60 | 14 | 13 | 3 | | | | 13 | 13.8 ± 2.1 | 2 | 0 |
| 7 | Splenectomy | 0.0 | 0.0 | 25 | 23 | 22 | 21 | 20 | | 0 | | 0 | 0 |
| 8 | Splenectomy | 0.1 | 0.71 | 22 | 16 | 5 | | | | 14 | 14.5 ± 2.1 | 5 | 0 |
| 9 | Sham splenectomy | 0.0 | 0.0 | 21 | 20 | 18 | 17 | 15 | | 2 | 15.0, 26.0[d] | 0 | 0 |
| 10 | Sham splenectomy | 0.1 | 0.66 | 17 | 12 | 4 | | | | 10 | 14.1 ± 1.5 | 2 | 0 |
| 11 | None | 0.0 | 0.0 | 30 | 27 | 26 | 24 | 23 | | 1 | 30.0 | 0 | 0 |
| 12 | None | 0.1 | 0.59 | 30 | 27 | 12 | | | | 26 | 15.0 ± 2.4 | 1 | 0 |

[a] If a mouse had both a forestomach papilloma and a forestomach carcinoma, the mouse was counted as having a forestomach carcinoma.

[b] Latent period of Group 4 was statistically significantly different from Group 6; $p < 0.01$ as determined by Student's $t$ test (25). Groups 6, 8, 10, and 12 were statistically similar; $p > 0.5$.

[c] Mean ± S.D.

[d] Since only 2 mice in this group had leukemia, the latent period of each is given.

changes either in any of the control mice or in the partially thymectomized mice.

Of the 15 completely thymectomized mice receiving NFTA (Group 2), 12 had forestomach neoplasms. Nine of the forestomach tumors were classified as squamous cell papillomas with invasion of submucosa and muscularis, numerous mitotic figures were present, and 5 of these 9 mice with forestomach papillomas died before 30 weeks, with the earliest death occurring at 18 weeks. Three mice in Group 2 had forestomach squamous cell carcinoma with invasion through the serosa and into the surrounding tissues including diaphragm, esophagus, glandular stomach, spleen, mesentery, lymph nodes, pancreas, and liver. Metastatic nodules were also found in the right lobe of the liver not in continuity with the primary tumor, and several metastatic nodules were found in the lungs of 1 mouse. The 3 mice with forestomach carcinomas died during Weeks 28, 32, and 40 after Time 0.

## DISCUSSION

In several strains of mice, NFTA induced a high incidence of lymphocytic leukemia that was characterized by a large thymus, large spleen, large lymph nodes, bone marrow infiltration, and invasiveness of other tissues by leukemic cells (6). It has been shown now that complete thymectomy at 3 to 4 weeks of age completely inhibits the induction of leukemia by NFTA and that splenectomy has no effect on leukemia induction. Similarly, lymphocytic leukemia or lymphosarcoma induced by viruses (18), radiation (11), or other chemical carcinogens such as the polycyclic hydrocarbons (13) were prevented by thymectomy but not by splenectomy. The requirement of complete thymectomy for leukemia prevention was shown in the 7 mice in Group 4 that had partial thymectomies and subsequently developed leukemia. It was not clear from the experiments with thymic-dependent leukemias (Refs. 11, 13, and 18; this report) whether thymectomy simply removed the cells that would eventually give rise to leukemia or whether thymectomy removed a substance produced by the thymus which was necessary for leukemia induction. The latter explanation was suggested by the results of Law and Potter (14) and Carnes *et al.* (4) who demonstrated that, in thymectomized animals with genetically identifiable thymic grafts, the leukemias that developed often originated in the host rather than from the thymic graft.

The forestomach tumors induced by NFTA in previous experiments (6) were classified as squamous cell papillomas by the criteria of Stewart *et al.* (26), and the incidence was less than 50%. Although these forestomach tumors (6) were never invasive through the serosa and never metastasized, they had many of the cellular characteristics of the completely invasive and metastatic forestomach squamous cell carcinomas induced by formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl] hydrazide (5), such as numerous mitotic figures per high-power field and basement membrane and forestomach muscular invasion. In the completely thymectomized mice reported above (Group 2), NFTA induced forestomach tumors in 12 of 15 mice with 3 of these tumors being classified as squamous cell carcinomas, with invasion through the serosa into surrounding tissues, and with distant metastases. That this effect may be due to the thymectomy and not simply to longer survival is suggested by the occurrence of these carcinomas as early as 28 weeks. None of the mice in previous experiments (6) had forestomach carcinomas, even if they survived to 30 weeks. Also, the forestomach carcinomas induced by formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl] hydrazide in mice (5) were not metastatic or invasive into surrounding tissues until after 30 weeks. These results suggest that the squamous cell papillomas induced previously by NFTA were probably malignant, but in an early stage of development. Extension of the time for development or production of a depressed immune system in the animal allows these tumors to express their total malignant potential with invasiveness into surrounding tissues and distant metastases. Thymectomy in 3- to 4-week-old mice depresses the immune system as early as 4 weeks postthymectomy as recently demonstrated with the sensitive mixed leukocyte culture technique (24). Adult thymectomy had been demonstrated previously (19, 20, 27) to depress the immune system after longer periods of time, but less sensitive monitoring techniques had been used. Adult thymectomy has also been demonstrated to have an effect on transplanted tumor growth (15). Unlike the few studies reported of the effects of adult thymectomy on tumors, there are numerous reports of the effects of neonatal thymectomy on tumors. It has been shown to have very dramatic effects on the immune system and on tumors, either increasing the incidence of tumors induced by viruses or carcinogens, shortening latent periods, or increasing the rate of metastases in several instances (12), but not in all cases (2).

As with the leukemia induced by NFTA, splenectomy did not have an effect on forestomach tumors induced by NFTA.

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