Lymphatic Leukemia and Pulmonary Tumors in Female Swiss Mice Fed Bracken Fern (Pteris aquilina)¹

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SUMMARY

Bracken fern (Pteris aquilina) mixed with a grain mixture (1:3 by weight) was fed to a group of 40 female 6-week-old Swiss mice intermittently every other week for a total experimental period of 60 weeks. Thirty-three mice survived 30 or more weeks and all developed lymphatic leukemia with multiple organ involvement. Additionally, 5 mice developed multiple pulmonary tumors. No urinary bladder or intestinal tumors, common in rats fed a similar bracken fern diet mixture, were found. No tumors were detected in 38 control mice that survived for 30 to 60 weeks while ingesting the grain mixture.

INTRODUCTION

The potent carcinogenic activity of bracken fern has been demonstrated for many species of animals. Several factors influence the pattern, location, and incidence of neoplastic lesions induced by bracken fern; i.e., species and age of animals, duration of bracken fern feeding, thiamine supplementation, and induced microsomal enzyme activity. The tumor organs vary with the species but include the small intestine, colon, and urinary bladder in rats and quail (5, 6, 8, 13, 19); the urinary bladder and hemopoietic system in cows (12, 19); the urinary bladder in guinea pigs; and the lung in mice (22). Age dependence at the time of initial exposure to the fern has been shown for rats (5), i.e., young rats appear to be more susceptible than older ones, but not for cows (12, 19). The duration of bracken fern feeding determined the occurrence of simultaneous tumors at various sites in rats. Intermittent feeding to young rats produced only intestinal tumors (6), while continuous administration for 12 months resulted in the development of coexisting intestinal and urinary bladder tumors (13, 17, 19). Thiamine supplementation increased the incidence of urinary bladder carcinomas in rats fed bracken fern (15, 17). Induction of microsomal activity by chronic phenothiazine administration reduced the incidence of intestinal and urinary bladder neoplasms by more than 50% in bracken fern-fed rats (16).

Bracken fern has an inhibitory effect on myeloid tissue in cows (12, 19), causing a progressive diminution in the number of white blood cells and platelets in the peripheral blood. These alterations maximally coincide with evidence of clinical toxicity due to bracken fern. The leukocytic depression is associated with a drastic diminution in the number of polymorphonuclear leukocytes. This inversion of the ratio of neutrophils to lymphocytes suggests that bracken fern severely damages the bone marrow. However, the effect, if any, of bracken fern on the lymphoreticular tissue of rodents is not known. The objective of this study was to investigate the possible murine leukemogenic and carcinogenic effects of chronic p.o. administration of bracken fern.

MATERIALS AND METHODS

Bracken fern (P. aquilina) was collected in June 1969 from farms in the Bolu Province, Turkey, where the incidence of bovine urinary bladder cancer, associated with a diet composed of a substantial portion of bracken fern, is high (10–12, 19). The bracken was dried in the shade to preserve its natural dark green color and was then milled and mixed with a basic grain mixture, the composition of which was described previously (17), in the ratio of 1 part of powdered bracken to 2 parts of grain diet. By the use of steam and compression, the basic diet and bracken fern-containing diet were molded into pellets that were immediately dried to avoid mold growth. These pellets were fed to mice during the experiment.

Female 6-week-old Swiss mice (Institute of Bacteriology, Elazig, Turkey), free from “spontaneous” leukemia, were housed in screen-bottomed metal cages, 6 mice/cage, and were fed their diets and water ad libitum. A total of 80 mice were divided equally in 2 groups. Group 1 received the bracken fern-containing diet. Because of the acute toxicity of this diet, manifested by failure of the mice to gain weight, it was fed intermittently every other week. On alternate weeks, the animals received the basic diet. This feeding schedule was carried out for 60 weeks. Group 2, a negative control group, was fed only the basic grain diet. No thiamine supplement was administered to either group of animals as was done in previous studies with rats (17).

Mice that died or were killed were subjected to necropsy. The urinary bladders were distended with Bouin’s fixative injected through the urethra. Representative histological sections of intestine, stomach, liver, spleen, kidneys, adrenals,
lungs, heart, thymus, lymph nodes, and urinary bladder were prepared and stained with hematoxylin and eosin.

RESULTS

The daily dose of bracken fern ingested by the mice was 1.5 g/mouse with a mean maximal cumulative dose of 315 g/mouse/60 weeks. The mice tolerated the bracken fern very well; only 7 test mice died during the period of the experiment. Two control mice died during the 11th and 18th experimental weeks. The bracken fern administration was related to the development of lymphatic leukemia in all 33 test animals that survived more than 30 weeks (Table 1). The leukemia produced was grossly characterized by marked enlargement of the spleen and lymph nodes but not of the thymus. The spleen was increased to about 5 times its normal size and on section had an accentuated follicular pattern. The lymph nodes were enlarged 2- to 3-fold and were soft and white. In addition, 5 of 33 mice had multiple primary lung tumors ranging in size from microscopic to 1.5 cm in diameter. The cut surface showed a pinkish-gray, medullary tumor with white. In addition, 5 of 33 mice had multiple primary lung tumors ranging in size from microscopic to 1.5 cm in diameter. The cut surface showed a pinkish-gray, medullary tumor with white.

Microscopic examination of the organs revealed that the principal changes were in the spleen, lymph nodes, liver, kidney, and lungs. The incidence of lymphatic leukemia and a list of organs that were infiltrated with lymphoid cells are given in Table 1. Enlargement of lymph nodes and spleen was due to a marked proliferation of lymphoid or reticular cells. The usual leukemic cells were of the large lymphocyte variety. Invasion of the capsule by lymphoid cells was constant. The leukemic cells varied in size. They might closely resemble a normal lymphocyte and have a deeply basophilic, small, round nucleus with a scant rim of clear basophilic cytoplasm, or they might be much larger than the normal lymphocyte and have a round or slightly indented vesicular nucleus that was less deeply basophilic, with one or more prominent nucleoli. The cytoplasm might be clear or distinctly basophilic.

In the spleen, leukemic cells were present in both follicles and pulp (Fig. 1). In many instances of marked involvement, the demarcation of the follicles and pulp was lost and almost the entire spleen was replaced by leukemic cells. The spleen was rich in megakaryocytes. In all cases (33/33), the lymph nodes showed a marked infiltration by lymphoblasts. In 27/33 instances, perivascular and intracapillary collections of leukemic cells were found in the kidneys (Fig. 2). In 17/33 cases, the liver was involved in a similar manner. The sinusoids were widely distended by large numbers of lymphoblasts, and there was marked atrophy of the hepatic cords (Fig. 3). In the lung (9/33 cases), there was a widespread infiltration of the alveolar walls with characteristic lymphoblasts. The alveolar spaces were diminished in size, and occasional tumor cells could be seen lying free in the blood vessels. In addition, in 5/33 instances, there were pulmonary epithelial tumors with histological features of adenoma (3 cases) and adenocarcinoma (2 cases) (Fig. 4). Surprisingly, no urinary bladder or intestinal tumors were found. No neoplastic lesions were present in the control mice.

DISCUSSION

Bracken fern contains a potent leukemogen for mice, and when chronically fed to them, results in a 100% incidence of lymphatic leukemia in this species. The lymphatic leukemia was characterized by splenomegaly, accompanied by enlargement of lymph nodes and leukemic infiltration of the kidneys, liver, and lungs. Noteworthy was the absence of enlargement of the thymus, and apparently the involvement of the thymus was not essential for induction of murine lymphocytic leukemia with bracken fern. Studies in the mouse indicate that the thymus may be the primary site of development of lymphatic leukemia in certain chemical (1, 2, 4, 7, 20) and viral-induced (3, 9, 21) murine leukemias. The fundamental histological changes in these murine lymphatic leukemias begin in the thymus. The histological patterns of lymphatic leukemia do not appear to differ in their essential nature. Further studies on the pathogenesis of bracken fern-induced leukemia in Swiss mice are needed to clarify the role of the thymus in this system.

Pulmonary adenoma and adenocarcinoma occurred in 5 animals with lymphatic leukemia, with an incidence of 15%. Lung tumors associated with lymphatic leukemia were demonstrated in 5-week-old Swiss mice fed formic acid 2-[4-(5-nitro-2-furyl)-2-thiazolyl]hydrazide (1) or N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (2). A similar association was noted in newborn Swiss mice administered 7, 12-dimethylbenz(a)anthracene, benz(a)pyrene, 3-methylcholanthrene, or urethan (9, 18, 20). The incidence of pulmonary adenomas and their associated malignant hemopoietic counterpart ranged from 59 to 96% depending upon the carcinogen used (18).

The duration of bracken fern feeding determined the occurrence of simultaneous lymphatic leukemia and pulmonary tumors. Continuous feeding appears necessary for the induction of these associated neoplasms in mice. Widdop (22) produced only lung adenomas in 3-month-old mice by feeding them an ethanol extract of bracken fern for a total of 12 months.

The results of the experiment are presented in Table 1. The percentage of mice with leukemia in the following organ sites is given in Table 1 (Table 1). The percentage of mice with leukemia in the following organ sites is given in Table 1 (Table 1).

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>No. of mice alive at</th>
<th>No. of mice with leukemia in the following organ sites</th>
<th>No. of other tumors of the lung</th>
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<tbody>
<tr>
<td></td>
<td>Start</td>
<td>Week 30</td>
<td>Week 60</td>
</tr>
<tr>
<td>Control</td>
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<td>Bracken fern</td>
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Table 1: Survival, lymphatic leukemia, and pulmonary tumors in female Swiss mice fed bracken fern.
period of 44 days. He did not observe leukemic changes in animals sacrificed 10 months after the start of feeding.

The absence of intestinal or urinary bladder carcinomas, neoplasms that are commonly found in rats fed a similar bracken fern diet mixture (13), may be due to differences in metabolism of the bracken fern carcinogen in the 2 species of animal. Conversely, it may be due to differences in the binding sites of the carcinogen in the 2 species of rodents. It appears that the target organs in rats are the intestine and urinary bladder, depending upon the amount of bracken fern administered. In mice, lymphoreticular tissue is primarily involved in cancer. However, this does not necessarily rule out the possibility of excretion of the carcinogen in murine bile or urine, but the amount excreted may be insufficient to induce neoplasms in the intestine or urinary bladder or both. Continuation of the feeding of the fern to mice beyond 60 weeks might also alter the sites of neoplasm formation. It was shown that topical application of bracken fern extracts to the mouse urinary bladder epithelium by the pellet implantation technique produced a high incidence of bladder carcinoma (14). These data suggest that mouse urinary bladder epithelium is responsive to the carcinogenic activity of certain fern extracts. The absence of urinary bladder tumors in mice in the present experiment may suggest that the carcinogen present in bracken fern was destroyed, perhaps in the small intestine or liver, suggesting that only a small amount of active material reached the intestine or urinary bladder. This amount could not initiate cancer in either organ, but it was enough to cause lymphatic leukemia. Further investigation concerning the interesting differences in species susceptibility to the bracken fern carcinogen must await chemical identification of this substance.

REFERENCES


Fig. 1. Massive splenic replacement by primitive neoplastic cells. H & E, X 260.
Fig. 2. Neoplastic lymphoblasts diffusely infiltrating between the renal tubules. H & E, X 260.
Fig. 3. Neoplastic lymphoblasts infiltrated around the central vein of the liver. H & E, X 240.
Fig. 4. Adenocarcinoma of the lung. H & E, X 105.
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