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INDUCTION OF URINARY BLADDER AND INTESTINAL TUMORS IN MICE BY FEEDING BRACKEN FERN¹

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Eğreltiotu yedirilmiş Farelerde Görülen Sidik Kesesi ve İncebarsak Tümörleri.

Bracken fern-induced murine bladder and intestinal tumors

Summary: Simultaneous urinary bladder and intestinal neoplasms were induced in male and female mice by feeding them bracken fern (*Pteridium aquilinum*) for their lifetimes. Bladder and intestinal tumors were first detected in animals dying 9 months after the start of the feeding experiment. Bladder tumors were present in 14 and intestinal neoplasia in 8 of 32 mice surviving more than 25 weeks of the experiment. Lymphatic leukemia appeared in all of these 32 mice. In 27 untreated control mice surviving for 25 months, 2 lung adenomas and 4 lymphatic leukemias were detected.

Özet: Ömürleri süresince Eğrelti otu (*Pteridium aquilinum*) yedirilmek suretiyle erkek ve dişi farelerde sidik kesesi ve incebarsak tümörleri meydana getirilebilmiştir. Tümörler denemenin 9. ayının sonundan itibaren görülmüştür. Denemede 25 haftadan fazla yaşayabilen 32 farenin 14 tanesinde sidik kesesi, 8 tanesinde de intestinal tümör bulunmuştur. Yaşayan 32 farenin tamamında ise lenfatik lösemi şekillendiği saptanmıştır. Normal yem ile beslenen ve 25 aydan fazla yaşayan 27 kontrol faresinden ikisinde akciğer adenomları, 4 tanesinde ise lenfatik lösemi şekillendiği görülmüştür.

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Intraduction

Bracken fern (*Pteridium aquilinum*) (BF), fed to Swiss mice resulted in a high incidence of lymphatic leukemia and a lower incidence of lung tumors (1). Evans (2) also reported the development of lung adenomas in mice fed BF, but did not report the presence of other tumors. Hirono et al (3) produced intestinal tumors in mice of the C₁ BL/6 strain by feeding a diet containing the young BF for 4 months. On the other hand, intestinal tumors were not induced in mice of dd strain, whereas lung adenomas were encountered in 7 of 10 mice that survived 3 months or more, after the start of the experiment (3).

The absence of urinary bladder neoplasms in mice did not necessarily exclude the possibility of excretion of the BF carcinogen in murine urine. Recently, the induction of urinary bladder tumors was accomplished in female mice by feeding a BF-containing diet after surgical implantation of glass beads into the urinary bladder lumen (4), suggesting that the mouse urinary bladder epithelium is more responsive to the carcinogenic metabolites of BF if an intravesicular foreign body is present. It seemed to us that the amount of carcinogen excreted in the urine might be insufficient to induce neoplasms in the mouse urinary bladder free of a foreign body. The duration of BF feeding might be a determining factor in the genesis of bladder tumors, and continuous feeding for a longer time could be necessary for the induction of murine bladder neoplasms. Thus, the objective of this study was to investigate the carcinogenic effect of lifetime oral administration of BF to Swiss mice.

Materials And Methods

BF was collected in July 1974 from farms in the Bolu province of Turkey. It was dried in the shade to preserve its natural dark green color and then milled and mixed with a basic grain mixture, the composition of which was described previously (5) as the ratio of 1 part powdered BF to 2 parts grain diet. By the use of steam and compression, the basic diet and BF-containing diet were molded into pellets which were immediately dried to avoid mold growth. These pellets were fed to mice during the experiment.

Six-week-old Swiss mice (Institute of Bacteriology, Etlik, Ankara, Turkey) were housed in screen-bottomed metal cages, 6 mice/cage, and were fed their diets and water *ad libitum*. A total of 66 mice were divided into 2 groups. Group 1 consisted of 36 mice (12 males and

24 females) and received the BF-containing diet. Because of the diet's acute toxicity, manifested by failure of the mice to gain weight, it was fed intermittently every other week for the first month, and on alternate weeks, the mice from Group 1 received the basic diet. The animals then received the BF diet continuously until they died or were killed. Group 2, a negative control group, consisted of 30 mice (15 males and 15 females) and was fed only the basic grain diet until the termination of the experiment at 25 months. Mice that died or were killed were subjected to necropsy. The urinary bladders were distended with formalin solution injected through the urethra.

Representative histological sections of intestine, stomach, liver, spleen, kidneys, adrenals, heart, thymus, lymph nodes and urinary bladder were prepared and stained with hematoxylin and eosin. Urinary bladders were evaluated as described previously (7).

Results

The daily dose of BF ingested by the mice was from diet consumption data estimated to be 1,5 g/mouse. After the first month, the mice tolerated the BF diet well. During the first 6 months of the experiment, the average weights of the experimental and control groups were comparable. After this time, the animals of the experimental group gained weight at a comparable rate but grew more slowly than the mice in the control group.

The interval and survival times of mice of the experimental group are presented (Table 1). The majority of the mice in the test group lived more than 60 weeks. Four animals from Group 1 died during the first 6 months prior to the discovery of the first tumor. Three mice from the control group died during 3 and 5 months of the experiment. The remaining 27 control mice survived for 25 months and were killed at the termination of the experiment.

The number of urinary bladder and intestinal tumors, lymphatic leukemia and miscellaneous neoplasms found in mice fed the BF-containing diet for their lifetimes is shown in Table 1. The initial detection of tumors in the experimental mice group was: lymphoreticular, 6 months; bladder and intestinal, male and female, 9 months. These time periods are only approximate, as mice were not sacrificed at periodic intervals to determine the earliest period for tumor formation.

TABLE 1. Time at which mice fed BF-containing diet died or were killed, and the corresponding number of animals with neoplasia.

Time after initiation of feeding (weeks)	M	F	No. of deaths Total	No. of mice with tumors of:													
				Bladder		Ileum		Stomach		Leukemia		Breast		Lung		Uterus	
				M	F	M	F	M	F	M	F	M	F	M	F	F	
0-25	3	1	4														
26-30		1	1								1	2					
31-40	1	2	3	1			1			1	2				1		
41-50	2		2		1					2	2						
51-60	1	2	3		1					1	2						
61-70	2	4	6	2	1	1	1			2	4		1	1	1		
71-80	2	7	9	2	3	1	1			2	7		2		1		
81-90	1	6	7		3	1	2			1	6		1				
91-104		1	1						1		1						1
TOTAL	12	24	36	5	9	3	5		1	9	23		4	1	3		1

M = male, F = female.

Urinary Bladder Tumors

Fourteen of 32 mice surviving more than 25 weeks of Group 1 developed urinary bladder tumors. The first urinary bladder tumor was found approximately 31 weeks after the start of experiment. In most cases, bladder tumors were grossly detectable, and the majority were papillary or sessile transitional cell carcinomas. Bladder carcinomas were usually multifocal in development and in one animal the tumor nearly occluded the bladder lumen. Infiltration of the carcinoma into submucosa (11 cases), muscular wall (2 cases), and subserosal tissue (1 case) was observed. No local pelvic or distant metastasis was found. Induced carcinomas were predominantly transitional cell type. Squamous cell carcinoma occurred occasionally. In most infiltrating transitional carcinomas, areas of extensive squamous cell metaplasia were observed, and it was sometimes so extensive as to warrant the diagnosis of squamous cell carcinoma. Bladder tumors occurring later appeared more anaplastic and invasive. The morphologic features of the BF-induced murine bladder tumors resembled those present in rats (5-7).

Intestinal Tumors

Eight mice from Group 1 had intestinal tumors with nearly equal sex distribution. Postmortem examination revealed multiple tumors protruding into the lumen of the small intestine. The tumors, predominantly in the ileum, were 2-8 mm in diameter. Grossly, the small intestinal polyps (5) and carcinomas (3) were nearly all broad based. None were ulcerated and some tumors were dark red. In 2 cases, the tumors, appeared to penetrate the intestinal wall and protrude through the serosa. Adenomatous polyps could not be distinguished from adenocarcinomas by gross examination. The feces in the colon were soft but no diarrhea was noticed.

Histologically, the tumors of the small intestine were either adenomatous polyps or adenocarcinomas. Adenomatous polyps exhibited a moderate to marked glandular and cellular atypia. At the edge of the polyps and carcinomas, a transition from normal to abnormal epithelium was sharply distinguishable. The diagnosis of adenocarcinoma was not made unless invasiveness, varying from invasion into or through the muscularis mucosa, into the submucosa and occasionally into the subserosa, was demonstrated. Adenocarcinoma tended to produce excessive mucin. No metastasis was detected. One mouse developed adenocarcinoma of the stomach.

Leukemia

Lymphatic leukemia was present in all 32 test mice that survived more than 25 weeks. The leukemia was grossly characterized by marked enlargement of the spleen, lymph nodes and thymus. Other organs appeared normal in gross appearance. Microscopic examination of the organs revealed that the principal changes were in the spleen (25/32), lymph nodes (12/32), kidneys (17/32), lung (16/32), liver (8/32) and thymus (8/32). The gross and microscopic characteristics of BF-induced lymphatic leukemia in mice were described (1), and the lesions found in the present experiment were identical to those.

Miscellaneous Tumors

Mammary adenocarcinoma (4/32), lung carcinoma (4/32) and uterine adenoma (1/32) were detected in mice of Group 1.

In the control mice that survived until the termination of the experiment, only 2 lung adenomas and 4 lymphatic leukemias were detected.

Discussion

A high incidence of simultaneous urinary bladder and intestinal neoplasms were induced in male and female Swiss mice fed BF diet for their lifetimes. Carcinogenic activity of BF was expressed also in lung and lymphoid tissues (Table 1). Earlier studies demonstrated that a low level of dietary BF produced tumors either in lung (1,2) or in lymphoid tissue (1), and in lung (2) or in intestine alone (3) in mice. Recently, urinary bladder tumors were induced in mice with BF feeding, following intravesical glass bead instillation into the urinary bladder (4). Our data suggests that the duration of BF feeding determined the occurrence of simultaneous tumors in the different organs of mice. Continuous and lifetime BF feeding to mice appears necessary for the induction of these associated bladder and intestinal neoplasms. In earlier experiments, the feeding of BF was terminated prior to 60 weeks (1-3). These data also suggest that the small amount of active material in BF reaching the urinary bladder in a short exposure period is not sufficient itself to trigger the neoplastic changes in the mouse urinary bladder epithelium.

The induction period of urinary bladder and intestinal tumors was longer in mice than in rats (5,6). The majority of the urinary bladder and intestinal tumors occurred in mice between 69-90 weeks

after the start of feeding, whereas in rats most of the urinary bladder and intestinal tumors appeared between 35-50 weeks (5,6). The difference in response of mice and rats to BF carcinogenicity was striking in the relative incidence of the intestinal and bladder tumors. In rats, the incidence of intestinal tumors and urinary bladder tumors was 100 % and 81 %, respectively (6). These new data indicate that the target organ response of mice to BF is somewhat different than that observed for rats. Mice developed urinary bladder tumors more than intestinal tumors, whereas with rats intestinal tumors occurred more frequently than urinary bladder tumors. The reason for this difference between mice and rats is not known.

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