Histopathogenesis of Bracken Fern-induced Experimental Tumor of Urinary Bladder

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Abstract. Experiments were performed to investigate the carcinogenic potential of Bracken fern (*Pteris aquilina*) using 211 rats, with pathomorphological assessment of vesical tumors. Groups of rats receiving bracken in feed developed neoplastic growths in the small intestines, subcutis, kidney and palate as well as in the urinary bladder, thus providing evidence for marked carcinogenicity of the plant. Histological observation revealed the development in the urinary bladder of papilloma (60 cases), transitional cell carcinoma (9 cases), squamous cell carcinoma (17 cases), adenocarcinoma (2 cases), fibroma (6 cases), leiomyosarcoma (2 cases) and hemangioma (1 case) showing benign to malignant properties. The tumors were more frequent and had greater degrees of malignancy in the groups receiving higher concentrations of bracken in feed. Of particular note in the development of epithelial tumors were hyperplasia of the epithelial layer and behavior of mast cells with associated edema of the mucosal epithelium and appearance of acidophilic spindle cells.

There have been numerous studies published concerning experimental induction of tumor of the urinary bladder and a wide variety of chemical substances have been demonstrated to possess carcinogenic potential for this organ. Meanwhile, spontaneous bladder tumors are generally thought to be relatively rare in various species of domestic or laboratory animals but cattle. Spontaneous vesical tumors of cattle have long been recognized in various parts of the world in close connection with bovine enzootic hematuria (haematuria vesicalis bovis) [2, 4, 9, 11, 14, 16–18, 26, 30]. The bracken fern (*Pteris aquilina*) is accounted much of in the etiology of the vesical tumor [6, 7, 25]. Evans et al. [6] described in their detailed review on the toxicity of brackens that the manifestation of toxic effects of this plant varies with the length of time, amount of the poison ingested and is subject to various influence by such factors as species, sex and age of animals. Their report further describes that chronic toxicity of the fern produces clinical effects compared to those of ionizing radiation characterized by induction of genetic mutations and oncogenicity. However, the toxic substance in bracken etiologically responsible for so-called bracken poisoning is not clearly identified yet whilst it has been reported to contain a carcinogenic substance which can be converted to shikimic acid [8] and a carcinogenic tannin [28]. Experimental studies on the oncogenicity of bracken in guinea pigs [7, 29], in rats [7, 11, 22, 24],
in mice [6, 20, 29] and in cattle [5, 21, 24, 25, 27] have been reported by many investigators, with the results providing evidence for its oncogenic property. Most of such studies, nevertheless, pertained to experimental induction of tumor by administration of the plant, but reports of detailed pathomorphological investigation of the tumor appear to be few as yet. Previous paper from this laboratory had described histopathological study of spontaneous vesical tumor of cattle [30], and the present investigation was undertaken to assess the oncogenicity of the bracken fern thriving in a natural grassland of the enzootic area, using rats.

### Materials and Methods

Specimens of the urinary bladder from a total of 211 rats were examined in this study. They comprised 174 rats given bracken, 84 female and 90 male, and 37 controls, 19 female and 18 male (Table 1). The specimens were obtained upon sacrifice by exsanguination from all rats except 18 which were found dead before tremination.

Animals: Four-week-old rats of the JCL-Wistar strain were used. Males and females were housed separately in aluminum cages and supplied with a commercial rat powder feed. As can be seen in Table 1, bracken powder was mixed in the feed at weight ratios of 1:2, 1:5 and 1:16. The bracken powder was prepared as follows: curled and unfurled bracken fronds grown in a grassland were collected in June to August, shredded, dried thoroughly direct in the sun and ground into powder by means of a powder pulverizer.

Experimental procedure: Groups of rats received bracken powder at the four graded concentrations (none, 1:2, 1:5, or 1:6) in feed ad libitum for an average period of 20 weeks. The bracken-feed mixtures were then replaced with normal powder feed which was continued till the time of sacrifice for necropsy. Three animals were bled to death at 10 weeks of dosing and 5 animals at 20 weeks from Group A1, and examined for developing lesions. All groups of animals had free access to tap water all the time and accommodated in the same environment of the laboratory. Throughout the period of experiment all rats were observed daily for signs and for mortality and their weights determined at weekly intervals.

All rats which died or sacrificed were examined systemically at autopsy, which also included gross observation for development of tumors. The urinary bladder was opened by ventral midline incision as to permit examination of the entire area of vesical mucosa for neoplastic changes and changes of other nature. Immediately after necropsy, the bladder tissue and other organs from each rat were fixed in Bouin’s solution or in 10% formalin. Representative sections of these tissues were then embedded in paraffin and cut by the usual method, and examined microscopically with hematoxylin and eosin stain (HE) or, where deemed necessary, with toluidine blue, periodic acid-Schiff or elastica Van Gieson stains.

### Results

Deterioration in general status such as decreased feed intake, depression, ruffled hair and emaciation were seen in the groups receiving high concentrations of bracken in feed. These groups, however, showed recovery in general condition with gain in weight following replacement with normal feed after 20 weeks of dosing. During the observation period the animals developed cancerous cachexia (17 rats), subcutaneous

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**Table 1. Experimental design**

<table>
<thead>
<tr>
<th>Group</th>
<th>Bracken fern consumed (per diet)</th>
<th>Number of animals</th>
<th>Period of administration (days)</th>
<th>Survival days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>males</td>
<td>females</td>
<td></td>
</tr>
<tr>
<td>A 1</td>
<td>1:2 unfurled bracken</td>
<td>66</td>
<td>59</td>
<td>133–145</td>
</tr>
<tr>
<td>A 2</td>
<td>1:2 curled bracken</td>
<td>11</td>
<td>13</td>
<td>125–145</td>
</tr>
<tr>
<td>B 1</td>
<td>1:5 unfurled bracken</td>
<td>3</td>
<td>3</td>
<td>127</td>
</tr>
<tr>
<td>B 2</td>
<td>1:5 curled bracken</td>
<td>7</td>
<td>6</td>
<td>145</td>
</tr>
<tr>
<td>C</td>
<td>1:16 unfurled bracken</td>
<td>3</td>
<td>3</td>
<td>228</td>
</tr>
<tr>
<td>Control</td>
<td>normal diet only</td>
<td>18</td>
<td>19</td>
<td>0</td>
</tr>
</tbody>
</table>
Experimental Vesical Carcinoma

Table 2. Histological types of tumors in the urinary bladder

<table>
<thead>
<tr>
<th>Histological type</th>
<th>Number of rats with tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A 1</td>
</tr>
<tr>
<td></td>
<td>male</td>
</tr>
<tr>
<td>Papilloma</td>
<td>22</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>5</td>
</tr>
<tr>
<td>Transitional cell carcinoma</td>
<td>4</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Fibroma</td>
<td>2</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>1</td>
</tr>
<tr>
<td>Hemangioma</td>
<td>0</td>
</tr>
</tbody>
</table>

masses (13 rats) and diarrhea (34 rats) and were sacrificed one after another for necropsy. Three rats became to have intermittent hematuria about 31 weeks of observation.

Incidence of tumor: Apparently neoplastic changes were noted in the small intestine (116 rats), urinary bladder (57) (Fig. 1), kidneys (3), subcutis (13) and palate (1) of rats dosed orally with bracken powder in feed. Within the small intestine, the ileum was the most frequently involved site and was unfailingly involved in all animals surviving for 22 weeks or longer. The tumorous lesions showed higher incidence in the groups receiving high concentrations of bracken in feed.

The neoplastic changes in the urinary bladder were seen grossly in the forms of papilloma, papillary carcinoma, white tumorous nodules or mucosal hyperplasia. Papillomas were encountered in 26 rats; the lesions were solitary to sporadic, ranged in size from pinhead to rice grain and, in two cases, were hemorrhagic. Papillary carcinomatous proliferation of epithelium was observed in 19 animals; the lesions were diverse, representing rice grain to India bean-sized polypoid growths and in some instances a papillomatous growth characterized by numerous stringy papillations reminding us of a cauliflower, occupying almost the entire vesical cavity. Virtually all these tumors projecting into the vesical lumen from the mucosal surface with broad-based stems and, in two cases, with hemorrhage. There were eight rats in which white nodules ranging up to the size of a rice grain were seen. Diffuse thickening of the mucous membrane were found in six animals. Petechial hemorrhages were noted in two cases. The vesical tumors appeared to be more frequent in females than in males. No tumorous changes were noted in any of rats from the control group.

Histological findings: There was microscopic evidence of overt tumorous or cancerous changes in the urinary bladder of rats dosed orally with bracken powder in feed (Table 2). The tumors were usually papillomatous or carcinomatous proliferation of epithelium and, in a few instances, non-epithelial tumors also occurred. Papillomas were present in sixty animals; this type of tumor was characterized by growth of stratified transitional epithelium surrounding a very thin connective tissue stalk (Fig. 2). Intermixed among the epithelial cells constituting the papillomatous proliferation were acidophilic spindle cells with varying degrees of atypia. Localized proliferation of epithelium with metaplasia to stratified squamous type (in 1 case) and solid ingrowths into subepithelial tissue (in 19 cases) were also observed. There were
nineteen rats with papillary carcinoma of the urinary bladder, of which fifteen had also papillomas. Microscopically, markedly malignant changes were observed. Most of the epithelial cells had abundant cytoplasm or were undifferentiated, presenting an extremely irregular arrangement (Fig. 3). Mitotic figures were frequently seen. An another characteristic change, a marked infiltrative growth was found. The lesion of infiltrative proliferation mostly consisted of cord-forming growths reaching the deeper layer, which were similar to those of transitional cell carcinoma (in 9 cases) (Fig. 4). Besides, squamous cell carcinoma with typical cancriform formation (in 17 cases) and adenocarcinoma (in 2 cases) were also concomitantly pointed out (Figs. 5 and 6). In cases of squamous cell carcinoma and adenocarcinoma, metaplasia of the transitional epithelium was always found. The stroma in the cancerous proliferation consisted of marked inflammatory changes and fibrosis. Six rats were found to have fibromatous changes which, grossly, had been seen as white nodules. These were characterized microscopically by circumscribed proliferation of fibroblasts, histiocytic cells and collagen fibers in the lamina propria, with invasion into the muscularis in one case. Leiomyosarcoma, or sarcomatous proliferation of remarkably atypical smooth muscle fibers, was observed in the muscular layer to serosa. Hemangiomatous changes were seen coexisting with papillary carcinoma in one case; they were noted as a localized lesion consisting of angioblastic proliferation with irregular canalizations of various sizes.

These tumorous changes with diverse morphologic features tend to show higher incidence and greater variety in the groups receiving higher concentrations of bracken powder in feed.

The observation also revealed many other findings of profound interest for the vesical mucosa of bracken-dosed rats, namely, stratified proliferation (144 cases) and downward growth (61 cases) of the mucosal epithelium, besides the above-described tumorous changes. Eosinophilic spindle-shaped cells with varying degrees of atypia were seen among these mucosal epithelial cells. They had a striking cytomorphological resemblance to those cells constituting the foregoing papillomas or cancerous tissues. In all instances, these acidophilic fusiform cells were aligned basally in the epithelial layer and often had taste-bud-like structures (Fig. 8). Inter-epithelial edema was noted with high frequencies in addition to the atypical proliferative changes of the mucosa (Fig. 7). Furthermore, there was a tendency for degranulated mast cells to be increased in number, in association with these pathologic changes. In the meantime, edema and circumscribed or diffuse fibrosis were frequently seen in the lamina propria. Dilatation of the blood vessels in the vesical wall, edematous loosening of the vascular wall and hydropic degeneration of smooth muscle fibers were also noted with high frequencies in the group given bracken in feed.

Discussion

As evident from the data herein reported, the bracken fern has a significant carcinogenic potential. The findings that cancerous changes were demonstrable with higher frequencies and of greater severity particularly in those groups receiving higher concentrations of bracken powder in feed indicates the possible occurrence of an unidentified carcinogenic factor in the plant. However, chemical nature of the toxic component in the bracken as yet is scarcely known. Experimental induction of ma-
lignant neoplasms in the urinary bladder by administration of bracken fern has been demonstrated in a wide variety of species of animals such as cattle, guinea pig, rat, and mouse, where various types of epithelial and nonepithelial tumors were observed concurrently in the bladder [7, 21, 23, 24, 27, 29], along with carcinomatous or sarcomatous proliferation in the small intestines [11, 22, 24], leukemia and pulmonary fibrosis [20]. In the present experimental study in rats, high incidence of tumors in the small intestines and urinary bladder were noted, thus consistent with the reports of many other investigators. The demonstration of development of tumors in the subcutis, kidneys and palate of the rats besides these organs would also provide evidence for carcinogenicity of the bracken and strongly suggest carcinogenic potential of the plant causing multicentral neoplastic growths. In addition, the fact that the treated animals developed benign and malignant tumors of epithelial or nonepithelial origin presenting diverse morphologic features in the urinary bladder bespeaks intricacy of the carcinogenic bracken poison [6]. The higher incidence or tumors in female rats would seem to indicate sex-related predisposition in the pathogenesis of these neoplasms. The experimentally induced tumors of the urinary bladder in rats given bracken in feed bore remarkable resemblance in many respects to spontaneous vesical tumors in cattle. Yoshikawa and Oyamada [30] pointed out a relationship of the morphologically diverse papillomatous proliferation and cancerous growth in their pathomorphological study of bovine spontaneous vesical tumors. Pamukcu and Price [22] described the fact that the vesical papilloma of cattle often acquire malignant neoplastic properties, developing into a carcinoma. The pathologic states of mucosal epithelium and stroma observed in the present series were of considerable interest as viewed in relation to morphogenesis of tumor. Namely, the hyperplastic changes of the epithelium noted in practically all cases were characteristics and reminded us of morphologic features of an early developing tumor. Pamukcu et al. [23] interpreted epithelial hyperplasia as representing an initial change of vesical tumor in bracken-treated rats and postulated the possible alteration from the epithelial hyperplasia into papilloma and further into various types of cancerous proliferation. Such proliferative changes of the mucous membrane have been frequently demonstrated in experimental studies of vesical tumor with aniline dyes or other chemical agents and are regarded as early carcinomatous changes or as carcinoma in situ [1, 3, 13, 15]. Besides the proliferative changes of the mucosal epithelium, the appearance of atypical acidophilic spindle-shaped cells noted in the present study is considered to be a morphologic findings that should not be overlooked as an early change. The edematous and necrotic changes of epithelial cells seen concomitantly with it would suggest a possible involvement of some deteriorative stimuli. Furthermore, the increase in number of mast cells and their degenerative changes (degranulation) in the epithelial layer are assumed to have some important significance to the epithelial pathogenesis. It is of profound interest that the toxic substance in bracken was shown to have histamine-liberating activity by Evans [6] and Ishii et al. [12] in their chemical studies of bracken poison. Further pursuit of studies directed toward chemical analysis and elucidation of the carcinogenic mechanism of bracken poison is anticipated.
References


要約

ワラピによる実験的膀胱腫瘍の組織発生：吉川 壽・小山田 隆・吉川博康・坂口直紀子（北里大学歯医療学部歯帝王学教室）——ワラピ（pteris aquilina）の腫瘍原性について，ラット（211例）を用いて実験的研究を行った。そして特に膀胱腫瘍について組織形態学的検討を試みた。ワラピ給与例では膀胱の他，小腸，肺下，腎臓および口蓋に腫瘍の発生を認め，ワラピの著しい腫瘍原性が確認された。組織学的に膀胱には良性から悪性性を示す乳頭腫（60例），移行上皮癌（9例），腺癌（2例），腺癌腫（6例），平滑筋肉腫（2例），血管腫（1例）が認められた。腫瘍の発生はワラピ多給群において，より高率かつ悪性であった。上皮性腫瘍の発生に対して粘膜上皮層の水腫および好酸性縦糸形細胞の出現を伴う上皮層の増生並びに肥厚細胞の動態が注目された。

Explanation of Figures

Fig. 1. Urinary bladder from Group A (Case No. 31) showing through the sessile nodules.

Fig. 2. Urinary bladder from Group A (Case No. 117) showing the papillary proliferation of transitional epithelium. HE. ×165.

Fig. 3. Group A (Case No. 55). Showing the downward growth of epithelial cells. Epithelial cells have abundant cytoplasm or are undifferentiated, with an intense atypia. HE. ×330.

Fig. 4. Group C (Case No. 170). Transitional cell carcinoma. Marked infiltrative proliferatoin consisting mostly of cord-forming growth extending into the deeper layer. HE. ×165.

Fig. 5. Group A (Case No. 31). Adenocarcinoma. HE. ×330.

Fig. 6. Group A (Case No. 31). Squamous cell carcinoma with typical cancroid formation. HE. ×330.

Fig. 7. Group A (Case No. 103). Epithelial cells showing the eosinophilic spindle-shape with varying degrees of atypia. Inter-epithelial edema are also noted. HE. ×330.

Fig. 8. Group A (Case No. 13). Acidophilic fusiform cells are aligned basally in the epithelial layer and showing the taste-bud-like structures. There are a tendency for degranulated mast cells to be increased in number (arrows). HE. ×330.