NON-UROTOXIC INDUCTION OF HEMORRHAGIC CYSTITIS BY BRAXIN C, A BRACKEN TOXIN, IN GUINEA PIGS

Masaru YOSHIDA and Toshiyuki SAITO

Department of Veterinary Pharmacology, Faculty of Agriculture,
Tottori University, Koyama-cho, Tottori 680, Japan

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ABSTRACT—Single subcutaneous injections of 5 and 10 mg/kg of braxin C (BC), ptaquiloside, a toxin isolated from bracken fern, have been shown to induce hemorrhagic cystitis in guinea pigs. In order to determine whether the hemorrhagic cystitis induced by BC is due to the urotoxic effects or not, BC was administered to the guinea pigs in which the flow of urine to the urinary bladder had been intercepted by cannulation of the ureters under anesthesia. In the animals treated ureteral cannulation, edema and hemorrhage were observed in the urinary bladder following BC administration (10 mg/kg s.c.). These findings suggest that BC induced hemorrhagic cystitis is mediated by hematogenous and not urotoxic factors.

KEY WORDS : Bracken, Cystitis, Toxin, Ptaquiloside.

INTRODUCTION

Bracken fern, *Pteridium aquilinum* (L.) Kuhn, is known to induce serious poisoning in cattle and guinea pigs. In guinea pigs, the characteristic findings in the bracken poisoning are edema and hemorrhagic change in the urinary bladder with hematuria (Maeda, 1975 ; Morita *et al.*, 1976, 1977 ; Saito *et al.*, 1982 ; Ushijima *et al.*, 1983). Recently, Saito *et al.* isolated 4 glucosides from the rhizomes or fronds of the bracken, named braxin A1, A2, B, and braxin C (BC), and demonstrated that these toxins induced hemorrhagic cystitis in guinea pigs (Saito and Mochizuki, 1986 ; Saito *et al.*, 1987, 1989b). On analysis by HPLC, BC was found to have the same retention time as ptaquiloside (Saito *et al.*, 1989b), which was isolated from bracken fern as a carcinogen (Hirono *et al.*, 1984). In a previous paper (Yoshida and Saito, 1994), BC was found clearly to induce hemorrhagic cystitis with hema-

turia at doses of 5 and 10 mg/kg when administered subcutaneously as a single injection. However, the mechanism responsible for the hemorrhagic cystitis occurring in bracken poisoning has not yet been clearly elucidated, whereas Ushijima *et al.* (1983) considered that toxic factors pass through the kidney, are reactivated by β -glucuronidase in the bladder, and act from the mucosal side similar to the urotoxic factors such as certain carcinogens (Boyland *et al.*, 1957) or ifosfamide (Muraoka *et al.*, 1990).

In order to determine whether the hemorrhagic cystitis induced by BC is due to the urotoxic effects or not, BC was administered to the guinea pigs in which the flow of urine to the urinary bladder had been intercepted.

MATERIALS AND METHODS

1. Animals.

Twenty male Hartley guinea pigs weighing 245–295 g were used in this study. The animals were provided with commercial food (RG-RO, Japan Agricultural Industries) and tap water *ad*

Correspondence : Toshiyuki SAITO at the above address.

libitum.

2. Preparation of the ureteral cannulation model.

Sixteen guinea pigs were used in the experiments. The animals were anesthetized with urethane (1 g/kg), and laparotomy was then performed with additional local anesthesia of the abdomen (1% xylocaine). Eight of the animals were divided into 2 groups of four each, Group 2 (laparotomized control) and Group 3 (laparotomized-BC administration). In the remaining eight animals, the ureters were ligated bilaterally near the urinary bladder and then cannulated into the proximal ends. Urine was collected through the cannula (polyethylene tube, 1.3 mm o.d.) into small tubes, and the urinary output was determined. Four of these animals were assigned to Group 4 (ureteral cannulation control) and four to Group 5 (ureteral cannulation-BC administration). The animals were immobilized on a heating plate adjusted to 38–40°C from the beginning of anesthesia to autopsy, and additional urethane was administered when necessary with monitoring of the level of anesthesia.

3. Preparation and administration of braxin C.

By HPLC using an improved method (Yoshida and Saito, 1994), BC was isolated from bracken fern collected in Hyoen pasture in a rural part of Tottori Prefecture, Japan, and was preserved in methanol containing 0.02% acetic acid at –20°C. Prior to administration, the solvent of the required portion of preserved BC was removed by evaporation. Animals underwent laparotomy, and their ureters were ligated and cannulated as described above. After stabilization of their condition, the animals of Groups 3 and 5 were administered 10 mg/kg of BC dissolved in 0.9% NaCl solution (about 1.0 ml/animal) subcutaneously in the back, while those of Groups 2 and 4 were similarly administered 1.0 ml/animal of 0.9% NaCl solution. Four other males (Group 1) were untreated controls.

In the preliminary evaluation, it was confirmed in a guinea pig that the toxicity of BC is not influenced by the anesthesia (urethane).

Autopsy: Animals were observed for 12 hours following the administration of test chemical described above, and were then killed by exsanguination. The urinary bladder was resected, its wet weight was measured, and its relative organ weight was calculated. The re-

sected urinary bladder was fixed with 10% neutral-buffered formalin. For microscopic examination, paraffin specimens were prepared from these tissues and stained with hematoxylin-eosin. Statistical comparisons of urinary bladder weight and body weight were made between Group 1 and Groups 2–5, between Groups 2 and 3, and between Groups 4 and 5 using Student's t-test.

RESULTS

1. Condition during the 12-hour observation period.

Congestion of the digestive tract was observed in some animals of Groups 2–5 during the 12-hour observation period. There were no differences in degree of digestive tract congestion among 4 groups. Discharge of urine from the cannulas inserted into the ureters was confirmed, but hematuria was not observed in each groups. There were no differences in urine volume among the Groups 2–5.

2. Weight of urinary bladder.

Weights of the urinary bladder are listed in Table 1. Compared with Group 1 (untreated control), weight of the urinary bladder was increased in Group 3 and Group 5, which were administered BC, but the same in Group 2 and Group 4, which were administered 0.9% NaCl solution. These findings showed that neither laparotomy nor ligation and cannulation of the ureters had an effect on the weight of the urinary bladder. Both the absolute and relative weights of the urinary bladder in Group 3 (laparotomized-BC administration) were significantly higher than in Group 2. The relative weight of the urinary bladder in Group 5 (ureteral cannulation-BC administration) was significantly higher than in Group 4.

3. Histopathological findings of the urinary bladder.

Edema was observed in the lamina propria of the urinary bladder in Group 3 and Group 5 (Photo. 1–B). Edema was observed in 3 of the 4 animals in Group 3. In Group 5, also, edema was observed in 3 of 4 animals; and it was moderate in degree and accompanied by hemorrhage in the animal (Photo. 1–C) with the greatest increase in weight of the urinary bladder of the 4 in this group. Mild edema was observed in 1 of the 4 animals in Group 4; it was milder in degree than that observed in the animals of

Group 5 administered BC. No edematous changes were noted in any of the other animals of Group 4 (Photo. 1-A), nor in any of the animals of Group 2 or Group 1. Mild congestion and inflammatory cell infiltration were noted in the

lamina propria and lamina adventitia in various groups including Groups 2 and 4, and were considered to be adaptive responses to experimental manipulations including the surgical procedures.

Table 1. Body and urinary bladder weights.

Group	Individual values						Mean	SD
1	body weight	(g)	260	274	278	253	266.3	11.7
	bladder	AB	178	218	220	248	216.0	28.8
		RE	68.5	79.6	79.1	98.0	81.3	12.3
2	body weight	(g)	268	245	247	254	253.5	10.4
	bladder	AB	193	230	212	215	212.5	15.2
		RE	72.0	93.9	85.8	84.6	84.1	9.0
3	body weight	(g)	275	273	248	250	261.5	14.5
	bladder	AB	378	280	267	245	292.5 [#]	58.8
		RE	137.5	102.6	107.7	98.0	111.5 ^{*#}	17.8
4	body weight	(g)	295	270	258	250	268.3	19.6
	bladder	AB	290	232	204	199	213.3	41.8
		RE	98.3	85.9	79.1	79.6	85.7	8.9
5	body weight	(g)	280	270	269	255	268.5	10.3
	bladder	AB	326	350	276	250	300.5 [*]	45.6
		RE	116.4	129.6	102.6	98.0	111.7 ^{*\$}	14.3

AB : Absolute organ weight (mg).

RE : Relative organ weight (mg/100 g B.W).

* : Significantly different from Group 1, $P < 0.05$.

: Significantly different from Group 2, $P < 0.05$.

\$: Significantly different from Group 4, $P < 0.05$.

A

B

C

Photo. 1. Urinary bladders of male guinea pigs subjected to laparotomy and ureteral ligation and cannulation under anesthesia.

A : Animal subcutaneously administered 0.9% NaCl solution (Group 4). H. E. Staining, $\times 15$.

B : Animal subcutaneously administered 10 mg/kg of braxin C (Group 5).

Edema is observed in the lamina propria. H. E. Staining, $\times 15$.

C : Animal subcutaneously administered 10 mg/kg of braxin C (Group 5).

Hemorrhage and edema are observed in the lamina propria. H. E. Staining, $\times 180$.

DISCUSSION

It has been suggested that BC was a principle responsible for bracken poisoning as well as braxin A1, A2 and B (Saito *et al.*, 1987, 1989b ; Yoshida and Saito, 1994). In a previous paper (Yoshida and Saito, 1994), it was demonstrated that BC induced hemorrhagic cystitis with hematuria in guinea pigs when administered at doses of 5 and 10 mg/kg in a single s.c. injection. However, the mechanism by which BC induces hemorrhagic cystitis has not yet been clearly elucidated. In the present study, we attempted to determine whether the hemorrhagic cystitis induced by BC is a consequence of its urotoxic effects or not.

Hemorrhagic cystitis has been induced with the anticancer drugs ifosfamide (IF), cyclophosphamide, trofosfamide and sufosfamide in rats and dogs (Brock *et al.*, 1979 ; Muraoka *et al.*, 1982). Muraoka *et al.* (1990) demonstrated that acrolein, a metabolite of IF, induced cystitis in rats by acting on the mucosal surface of urinary bladder. Also, Saito *et al.* (1989a) showed that IF induced edema and hemorrhage in the urinary bladder of guinea pigs, and that ligation of both ureters prevented formation of these lesions. These findings suggest that the lesions of the urinary bladder are mediated by urotoxic factors. Concerning the cystitis in the bracken poisoning, Ushijima *et al.* observed a significant increase in β -glucuronidase activity in the urine and the bladder mucosa of cows suffering from hematuria vesicalis bovis (Ushijima *et al.*, 1968) and of guinea pigs fed bracken (Ushijima *et al.*, 1983), and considered that toxic factors passed through the kidney and were activated in the bladder, on the mucosal side of which they exerted their effects. In the present study, the cystitis was induced with 10 mg/kg BC in the guinea pigs even when the influx of urine to the urinary bladder had been intercepted by ureteral cannulation. These findings suggest that the hemorrhagic cystitis induced by BC is independent of urotoxic factors, although β -glucuronidase may also play a role in the pathogenesis of the cystitis occurring in bracken poisoning.

Saito *et al.* (1982) found that edematous and hemorrhagic changes were present not only in the

urinary bladder itself but also in the ligaments of the urinary bladder, intracerebral adipose tissues and serosa of the abdominal wall in guinea pigs orally administered an ethanol extract of bracken fern. Similar findings were also obtained in a previous study of the acute toxicity of BC in guinea pigs (Yoshida and Saito, 1994). These facts suggest that the edematous and hemorrhagic changes are not induced by urotoxic factors, and support the hypothesis that BC-induced cystitis is not associated with such factors.

In bracken poisoning in cows, the levels of hematogenous factors such as histamine (Evans, 1968), heparin (Evans and Howell, 1962 ; Evans, 1968) and heparin-like substance (Yamane *et al.*, 1975) were increased in the blood in association with disturbances of blood coagulation. Saito *et al.* (1990) demonstrated that braxin A1, a bracken toxin known to induce cystitis (Saito *et al.*, 1987), induced a dose-dependent release of histamine from isolated rat peritoneal mast cells. These studies suggest that the development of hemorrhagic cystitis in bracken poisoning may be mediated by the chemical mediators such as histamine. However, further study for hematogenous factors will be necessary.

In the present study, a single subcutaneous administration of BC at a dose of 10 mg/kg was found to induced cystitis in the non-cannulated guinea pigs subjected to laparotomy as well as in the cannulated animals. These changes were clearly milder than those observed following the administration of a 5 mg/kg dose in a previous acute toxicity study (Yoshida and Saito, 1994). We consider that the main reason for this difference between two studies was the difference in length of observation period, 12 hours in the present study, and 3 days in the previous study, though the influence of anesthesia can not be ignored.

In summary, our findings demonstrated that hemorrhagic cystitis induced by BC was mediated by hematogenous and not urotoxic factors.

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REFERENCES

- Boyland, E., Gasson, J. E., and Williams, D. C. (1957) : Enzyme activity in relation to cancer. The urinary β -glucuronidase activity of patients suffering from malignant disease. *Brit. J. Cancer*, **11**, 120–129.
- Brock, N., Stekar, J., Pohl, J. *et al.* (1979) : Antidot gegen urotoxische Wirkungen der Oxazaphosphorin-derivate Cyclophosphamid, Ifosfamid und Trofosfamid. *Naturwissenschaften*, **66**, 60–61.
- Evans, I. A. (1968) : The radiomimetic nature of bracken toxin. *Cancer Res.*, **28**, 2252–2261.
- Evans, I. A. and Howell, R. M. (1962) : Bovine bracken poisoning. *Nature*, **194**, 584–585.
- Hirono, I., Yamada, K., Niwa, H. *et al.* (1984) : Separation of carcinogenic fraction of bracken fern. *Cancer Lett.*, **21**, 239–246.
- Maeda, T. (1975) : Fundamental studies on the etiology of haematuria vesicalis bovis. I. The induction of carcinomas and haemorrhages of the urinary bladder in guinea pigs by feeding bracken fern (*Pteridium aquilinum*). *Bull. Fac. Agric., Tottori Univ.*, **27**, 79–88 (in Japanese).
- Morita, Z., Senda, H., Yamamoto, Y. *et al.* (1976) : Studies on the toxic factor in bracken fern. II. Hemorrhagic changes in the urinary bladder of guinea pigs by feeding an ethanol extract of bracken fern. *Bull. Fac. Agric., Tottori Univ.*, **28**, 66–72 (in Japanese).
- Morita, Z., Senda, H. and Yamashita, M. (1977) : Studies on the toxic factor in bracken fern. III. Hemorrhagic changes in the urinary bladder of guinea pigs by feeding the fractions of bracken separated with lead-acetate. *Bull. Fac. Agric., Tottori Univ.*, **29**, 12–17 (in Japanese).
- Muraoka, Y., Matsumura, S., Yahara, I. *et al.* (1982) : Acute toxicity study of ifosfamide (Z4942). *Clin. Rep.*, **16**, 431–438 (in Japanese).
- Muraoka, Y., Watanabe, H., Matsui, S. *et al.* (1990) : Ifosfamide-induced urinary bladder lesion and its inhibition by mesna. *Folia. Pharmacol. Japon*, **96**, 73–83 (in Japanese).
- Saito, T., Ishii, K. and Goto, H. (1982) : Acute toxicity of bracken fern glucoside in guinea pigs. *Bull. Fac. Agric., Tottori Univ.*, **34**, 43–49 (in Japanese).
- Saito, T., Kurata, Y. and Takeno, K. (1990) : Characteristics of histamine release from rat mast cells induced by a bracken toxin, braxin A1. *Japan. J. Pharmacol.*, **53**, 165–173.
- Saito, T. and Mochizuki, D. (1986) : Isolation of two active glucosides, braxin A1 and A2, from rhizomes of bracken fern. *J. Toxicol. Sci.*, **11**, 15–27.
- Saito, T., Naito, A., Tsuchiya, H. *et al.* (1989a) : Acute toxicity of ifosfamide on the guinea pig urinary bladder. *Bull. Fac. Agric., Tottori Univ.*, **42**, 181–188 (in Japanese).
- Saito, T., Takeno, K., Nakamura, S. *et al.* (1987) : Acute poisoning with braxin A1, a bracken glucoside, in guinea pigs. *Jpn. J. Vet. Sci.*, **49**, 181–183.
- Saito, T., Takeno, K., Kawata, J. *et al.* (1989b) : Isolation of toxic constituents from bracken rhizomes. *In Basic science in toxicology. 5th Int. Congr. Toxicology, Brighton*, p.21.
- Ushijima, J., Matsukawa, K., Yuasa, A. *et al.* (1983) : Toxicities of bracken fern in guinea pigs. *Jpn. J. Vet. Sci.*, **45**, 593–602.
- Ushijima, J., Yuasa, A., Matsukawa, K. *et al.* (1968) : β -glucuronidase activity in urine and bladder tissue of cattle with haematuria vesicalis. *Jpn. J. Vet. Sci.*, **30**, suppl., 255 (in Japanese).
- Yamane, O., Hayashi, T., Sako, S. *et al.* (1975) : Studies on hemorrhagic diathesis of experimental bovine bracken poisoning. II. Heparin-like substance level in blood. *Jpn. J. Vet. Sci.*, **37**, 341–347.
- Yoshida, M. and Saito, T. (1994) : Acute toxicity of braxin C, a bracken toxin, in guinea pigs. *J. Toxicol. Sci.*, **19**, 17–23.