Reproduction of Progressive Retinal Degeneration (Bright Blindness) in Sheep by Administration of Ptaquiloside Contained in Bracken

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ABSTRACT. The process of occurrence of bright blindness, progressive retinal degeneration (PRD), in sheep was observed using two Suffolk ram lambs fed on a diet containing bracken powder. The first sign of the bright blindness was detected 4 months after the start of experiment. Based on these preliminary results, the amount of bracken powder necessary to induce PRD was estimated (experiment I). In the following experiment, ptaquiloside (PT), a norsesquiterpene glucoside of the illudane type isolated from bracken, which is a bracken carcinogen and a causative principle of cattle bracken poisoning was administered to two Suffolk ram lambs. It was clearly demonstrated in this experiment (experiment II) that PT present in bracken is also a causative principle of PRD.——key words: bracken, bright blindness, ptaquiloside, sheep.

Bright blindness, progressive retinal degeneration (PRD), in sheep was first reported by Watson et al. [12] as a progressive degeneration of the neuroepithelium of the retina in sheep in the West Riding of Yorkshire. Subsequently, it was confirmed that there is a correlation between the blindness and grazing of bracken, i.e., all the sheep in which the disease has been diagnosed had access to bracken [2]. Bright blindness was produced experimentally in a Suffolk Down ram lamb and Dorset Horn ewes fed a ration containing dried bracken [3, 13, 14]. Bright blindness due to ingestion of bracken has been encountered in sheep and cattle, and the causative principle contained in bracken has not yet been elucidated.

In order to elucidate the pathogenesis of this disease and the causative principle in bracken, the present authors performed a preliminary study on the occurrence of bright blindness in sheep fed bracken harvested in Japan (experiment I). With reference to these results, the next experiment (experiment II) was executed to test whether ptaquiloside (PT) (Fig. 1) contained in bracken is the causative principle of bright blindness. We have already reported that PT, a novel norsesquiterpene glucoside of the illudane type, contained in bracken is the causative principle of ileal and urinary bladder carcinoma in rats induced by feeding bracken diet [4, 5, 7-10]. Furthermore, it was also reported that PT is the causative principle in bracken poisoning in cattle [6]. The second experiment was carried out on the speculation that PRD in sheep fed bracken may also be induced by PT.

* The maker was Davol Inc. Subsidiary of C. R. Bard Inc., U.S.A.

Fig. 1. Structure of ptaquiloside.

MATERIALS AND METHODS

Experiment I

Two Suffolk ram lambs (Nos. 114 and 115), aged 4 months and weighing about 33 kg were used in this experiment. The rams were purchased from a farm in Hokkaido, northern Japan, in July, 1988. They were given a powdered basal diet for sheep, mixed with bracken powder (50-200 g/day) and hay, and moistened with a small amount of water. The amount of the bracken powder in the diet was adjusted to improve the test conditions as judged by the whole-body condition of the lambs, such as loose bowels or reduced food intake. Basal diet for sheep was powdered ZC (Oriental Yeast Co., Ltd., Tokyo, Japan) comprising protein, fiber, vitamin, mineral and amino acid. Access to salt licks and water was ad libitum. Bracken fern used in this experiment was collected in Hokkaido in August, 1988. The fresh mature
bracken was first dried in shade and subsequently in a dryer equipped with a blower at less than 40°C. Material thus prepared was then milled. The experiment was terminated 250 days after the start of the administration of the bracken diet.

Sheep were autopsied at death, or sacrificed at the termination of the experiment. Tissues except eyes were fixed in 10% neutral formalin, and processed for staining with hematoxylin and eosin (H-E). The eyes were fixed in cold fixative solution consisting of 12% glutaraldehyde (pH 7.2, 1/15 M phosphate buffer) 50 ml, 10% neutral formalin (pH 7.2, 1/15 M phosphate buffer) 50 ml and phosphate buffer (pH 7.2, 1/15 M) 100 ml. Sections of the eye were also stained with H-E.

Experiment II

Three 2-month-old Suffolk ram lambs (Nos. 1, 2, and 3), weighing about 14 kg were used. These lambs were purchased from a farm in Nagano prefecture in central Japan in January, 1991.

Feeding of lambs: Milk substitute (A-3) for sheep (Kyodo Feed Co., Ltd., Yokohama, Japan), 800 ml/head/day, was given with a feeding bottle three times a day. The original powdered milk substitute was dissolved in hot water to make a 5 fold volume ratio. The amount of milk was gradually increased. Ten days later, the lambs received 1600 ml/head/day together with a small amount of hay and ZC pellet (basal diet for sheep, Oriental Yeast Co., Ltd.). Access to salt licks and water was *ad libitum*.

The sheep were weighed weekly. The amount of milk and diet to be given were adjusted according to the body weight. They were weaned 4 months after birth and switched to a new diet; i.e., hay (200-300 g/head/day) and ZC pellet (100-170 g/head/day).

Installation of the intestinal catheter: The Broviac catheter (catalog No. 60010) was used. It had an inner diameter of 1.0 mm and was 90 cm in length, and made of silicone*. Lamb No. 1 was operated to install the silicone catheter about 2 weeks after arrival at our laboratory. The laparatomy and ensuing tube-jejunostomy were carried out under a general anesthesia. About 30 cm of catheter was inserted into the small intestine, and the other end of the catheter was directed towards the dorsum of the animal. The distal end of the catheter was fitted with a screw stopper.

Administration of PT through the catheter: The first administration of PT through the catheter was started 5 days after the operation. As a rule, PT was given every other day. An indicated dose of PT was freshly dissolved in 3 ml of physiological saline kept in an incubator at 37°C and injected through the catheter.

Intravenous injection of PT: Lamb No. 2 received a weekly intravenous injection of PT into the jugular vein. PT was dissolved in 3 ml of physiological saline, sterilized with Millipore filter, and then injected. The first injection was given on the same day as the administration of PT through the catheter in lamb No. 1.

Extraction of PT from bracken: PT was extracted by means of the improved method of Ojika *et al*. [11]. Bracken was collected in Hokkaido in July, 1990, dried at room temperature and milled. Powdered bracken thus prepared was used for the extraction of PT. The PT was determined to be more than 95 percent pure, based on high-performance liquid chromatography analysis and 1H nuclear magnetic resonance spectroscopy. Since PT is unstable at room temperature, it was kept at −20°C in a freezer and dissolved immediately prior to administration. Lamb No. 3 was served as control.

Sheep were autopsied after sacrificed at the termination of the experiment. The eyes and other organs were fixed in the same way as in experiment I, respectively, and sections were stained with H-E.

RESULTS

Experiment I

Ram No. 115 died 132 days after the start of feeding bracken. Total amount of ingested dry bracken powder was 18 kg (average 136 g/day). The ram was autopsied immediately after death. The body weight was 26.2 kg. Several days prior to death, a blue or green unusual brightness appeared in the pupil, probably the coloration of the fundus. At autopsy, hemorrhage was observed in the lung, liver and mucous membrane of the intestine. Severe congestion with dilatation of small blood vessels in the alveolar wall of the lung, and hemorrhagic necrosis as well as degeneration of the liver cells were observed histologically. Hemorrhage was also observed in other organs. The sternal bone marrow was widely replaced by fat marrow and only small foci of erythropoietic cells remained intact (Fig. 2). In the retina near the optic disk, the layer of rods and cones almost completely disappeared. Most cells in the outer nuclear layer also disappeared with only a few cells remaining (Fig. 3). Main pathological changes were confined to the layer of rods and cones and the outer nuclear layer of the retina.

In ram No. 114, unusual brightness of the eyes was

Fig. 2. Sternal bone marrow of sheep No. 115 fed bracken diet. Bone marrow is widely replaced with fat and only small foci of haematopoietic cells are seen. H-E stain. × 170.
observed in less than 4 months after the start of feeding. Despite such clinical findings, the feeding of the bracken
diet was continued, and the ram was sacrificed 250 days
after the start of experiment. The total amount of bracken
powder ingested was 26 kg (average 104 g/day). Body
weight at autopsy was 32 kg. Grossly, slight hemorrhage
was noticed on the parietal and intestinal serosal surface.
Histologically, focal hemorrhage and necrosis were found
in the liver. The sternal bone marrow was widely replaced
by fat marrow and granulopoiesis was poor. Changes in
the eyes were confined to the retina as in ram No. 115. In
this case, however, the layer of rods and cones dis-
appeared completely, and the outer nuclear layer was also
almost completely destroyed (Fig. 4). Thus, the findings
of acute bracken poisoning were not remarkable in ram No.
114 as compared with those in No. 115, and the changes in
the retina in No. 114 were, contrary to acute bracken
poisoning, more severe than those in No. 115.

Experiment II

Administration of PT through catheter: The dose of PT
required to induce PRD was first roughly estimated from
experiment I. During the experimental period, PT was
administered every other day, and each time 80–130 mg of
PT was given during the 6 months after initiation.
However, the administration of PT was sometimes halted
due to diarrhoea and/or anorexia. The total amount of PT
administered was 7.83 g. On the 168th day after the start
of the administration of PT, both eyes of this lamb were
affected, and the dilated pupils became circular, respond-
ing poorly to light; and an alert attitude mentioned by
Watson et al. [13] peculiar to the blind sheep was also
observed. Subsequently, the dose of PT was increased to
140–150 mg and administered 11 times. After the sheep
had slushy feces and anorexia, the dose of PT was reduced
to 70–80 mg on the 230th day. By this time, it was noticed
that the pupils appeared to be blue and transparent. These
changes were the same as those of the two sheep in
experiment I. The total amount of PT after the administra-
tion of 70–80 mg (16 times) was 10.67 g. After that, 100
mg of PT was given weekly (9 times) up to the termination
of experiment. Sheep No. 1 was sacrificed by injection of
Nembutal into the jugular vein 365 days after the start
of PT administration. The total amount of PT administered
was 11.6 g.

Ophthalmoscopic examination: On 243 days after the
initiation of PT administration, the retinal vessels became
simply narrower, which was more prominent 345 days
after the start of experiment (Figs. 5 and 6).

Other findings: Remarkable changes were confined to
the retina (Fig. 7). The layer of the rods and cones almost
completely disappeared and cells of the outer nuclear
layer remained in one layer. Swollen round or pyramidal
cones which stained deeply with eosin were scattered.
Intermingled with these cones were a small number of
round, nucleated cells, presumably formed by migration of
the nuclei from the outer nuclear layer.

Intravenous injection of PT: Weekly intravenous injec-
tion of PT into the jugular vein in sheep No. 2 was started
on the same day as the initiation in sheep No. 1. The
amount of PT injected varied according to physical
condition of animal, and the amount and the number of
administration are summarized as follows: 46–59 mg (5
times), 70–75 mg (12), 105–140 mg (16), 165–200 mg (20),
and 250–400 mg (6). The total amount of PT injected was
8.83 g. Sheep was sacrificed 414 days after the start
of experiment. During the experiment, an alert attitude and
circular dilatation of the pupils were first observed on the
168th day after the start of the experiment. Crystal-clear
brightness of both pupils was noticed about one month
later.

Ophthalmoscopic examination: Both arteries and veins
appeared to be slightly narrower than before the start of
the experiment. However, the change was not so severe as
seen in sheep No. 1.
Autopsy findings: Both eyes were fixed immediately. There were no significant gross lesions. Histologically, sternal bone marrow was replaced with fat, and small foci of haematopoietic cells were seen. In the outer nuclear layer of the retina close to the optic disk in both eyes, cell density was generally low and vacant spaces probably induced by disappearance of degenerated cells were also observed (Fig. 8). Control animal was sacrificed 390 days after the start of experiment. No changes were detected in both eyes.

In the present experiment, the sign of bright blindness first appeared in both sheep (Nos. 115 and 114) about 4 months after the initiation of bracken diet. It was clear that this clinical sign was due to PRD, judging from the histological changes of the retina. Retinal changes in sheep No. 114 which survived longer were more severe than No. 115. It may be due to the fact that No. 114 survived longer, thus receiving a larger amount of bracken compared with No. 115 which died of acute bracken poisoning. Sheep fed on bracken diet in the present experiment developed bright blindness within a shorter period than that reported previously [14]. The reason may be that bracken was dried at room temperature in our experiment, whereas it was dried at a temperature in excess of 200°C and again heat cubed in the previous
report. Furthermore, these facts suggest that the causative principle contained in bracken may be heat labile.

The occurrence of PRD in sheep No. 1 which was administered PT through a catheter was confirmed by the clinical appearance of the eyes, ophthalmoscopical examinations and histological changes of the retina as mentioned above. PT was administered directly into the small intestine using a catheter in sheep No. 1 because a huge amount of PT is necessary in the case of drench, due to destruction of PT in the stomach, as shown in our previous calf experiment [6]. On the other hand, sheep No. 2 which received intravenous injections of PT did not show clear changes as seen in No. 1. Such a difference between the two is considered not due to the difference in route of administration, but rather due to the doses and intervals of administration of PT. Anyhow, it was clearly demonstrated in experiment II that PT contained in bracken is the causative principle of PRD. On the mechanism of occurrence of PRD induced by bracken feeding or administration of PT, a disturbance of the blood circulation due to narrowing of the blood vessels may be regarded as one of the important factors. The narrowing of the retinal blood vessels in sheep affected with bright blindness has been well known [13, 14] and it was also observed in sheep No. 1 which was given PT.

However, the mechanism of the narrowing of the retinal vessels and the causal relationship between the narrowing and retinal changes, including degeneration and atrophy, are still unknown.

We observed previously that microcirculatory changes were induced by the intravenous injection of PT in rabbits [1]. It may be conceivable that such a disturbance of the microcirculation is induced by bracken even in sheep. Furthermore, it is also speculated that PRD may also be induced in rabbits by feeding bracken or PT, if disturbance of the retinal blood circulation is a causative factor of PRD. However, all these questions demand further investigation.

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REFERENCES