CYCAD POISONING IN CATTLE IN JAPAN —STUDIES ON SPONTANEOUS AND EXPERIMENTAL CASES—

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INTRODUCTION

Grazing cattle suffering from ataxia and paralysis in hind quarters and horn damage were found in some detached islands of Okinawa prefecture, Japan. Clinical syndromes resembled the typical ones of the cycad poisoning reported previously in Papua, New Guinea, Puerto Rico, Dominican Republic, and Australia. Among these diseased cattle, two cows with severe clinical signs were examined pathologically. The lesions in the spinal cords of these cattle also strongly suggested the cycad poisoning. To ascertain the etiology of this "cycad ataxia"-like disease, we fed two cows experimentally with leaves of *Cycas revoluta* Thunb., indigenous to the region. Subsequently, for an approach to establish the neurotoxic materials, two calves were orally administered with a toxic principle, methylazoxymethyl β -D-glucoside (cycasin), of the cycad.

In this report the cycad poisoning in cattle in Japan are surveyed pathologically and an approach to confirm the neurotoxicity of cycasin to cattle is attempted.

MATERIALS AND METHODS

Clinical and field observations

Outbreaks of the ataxic disease in grazing cattle were surveyed in seven detached islands in Okinawa prefecture during 1957-1982. The environment of meadows and symptoms of diseased cattle were observed in each island. Of the diseased cattle examined clinicopathologically, two cows of Japanese Black breed (Case A: 1 year old and Case B: 3 years old) reared in Kuroshima island and showing severe clinical signs reared were sacrificed for pathological examination.

Feeding experiments

Fresh cycad leaves

Fresh leaves were collected from the cycad, *Cycas*

revoluta Thunb. in the campus garden. Two young cows of Japanese Black breed (Case C: 1-year and Case D: 1-year) were compelled to feed on minced young cycad leaves admixed with hay, straws and a commercial diet. Average 263 g/day/head of cycad leaves were given on halves twice a day. In total, Case C was fed 9,465 g of leaves in 36 days and Case D 18,740 g, in 82 days.

Cycasin

Specimen of cycasin was isolated from the seeds of *C. revoluta*. Two calves (Case E: Holstein Cattle, male, 5-months and Case F: Japanese Black Cattle, female, 1-year) were orally administered with a solution of cycasin, 3.18-4.17 mg/day.

All experimental animals were observed scrupulously for detection of emaciation and prostration, and submitted to autopsy for the pathological examination.

RESULTS AND DISCUSSION

Field observations

Sickness in grazing cattle was first noticed in 1975 on the Okinawa islands. Sixty nine cows on 5 islands were recognized to be poisoned during 1975-1977. Five more cases were found in 1981 on another island. Further, an outbreak of more than 77 cases among 1700 grazing cattle was found on Kuroshima island in the early summer of 1982 (Table I). The incidence was characteristically found from April to June when there was insufficient grass in the meadow and the cycad plant shot its young sprouts. There were many bite traces recognized on the cycad leaves. Ingestion of cycad leaves by cattle due to scarcity of green grass was proved by field surveys.

Of these diseased cattle, two cows with severe clinical signs were examined clinicopathologically, hematologically and pathologically. Clinical characteristics of the affected cattle were marked emaciation, lumbar paralysis, swinging-leg-lameness and falling-out of horns (Fig. 1). Hematological study revealed marked irreversible decrease in serum choline esterase levels and a tendency to increase in osmotic fragility of erythrocytes. Pathologically, significant microscopic

lesions were concentrated in the spinal cord, among which the most prominent was production of glial tissue, so-called, fibrillary gliosis. In the anterior parts of the spinal cord, fasciculus gracilis and the lateral funiculi were involved and in the posterior parts ventral funiculi were selectively injured (Fig. 2). These sites of fibrillary gliosis corresponded respectively to the ascending and the descending pathways, and the productive lesions were presumed to be the cause of the neural disturbance. Similar poisoning of cattle cases caused by ingestion of the leaves of cycad plants of the family Zamiaceae were known and studied in central America and Australia.

Feeding experiments

Fresh cycad leaves

In corroboration of the "cycad ataxia"-like disease, we experimentally fed two cows with leaves of the cycad plant, *C. revoluta*, indigenous to the area, so as to reproduce some dyskinesia in hind quarters associated with spinal lesions. The first ataxic had been given sign in hind quarters was noticed when Case C had been given 9, 465 g of leaves in 36 days and Case D 18, 740 g in 82 days. A temporary increase in serum γ -GTP level was observed immediately after ingestion of cycad leaves, while serum levels of the other items remained normal throughout the experimental period. On microscopic examination, Case C being sacrificed long after the first sign of dyskinesia, showed vacuolation and fibrillary gliosis in the spinal cord, whereas Case D sacrificed soon after the first dyskinetic sign revealed spheroid bodies and vacuolation. The difference of the lesions between the two was inferred to be dependent on the lapse of time after the appearance of

the first sign. The features and the distribution of these spinal lesions were almost identical with ones in the spontaneous cases. Thus, cycad poisoning of cattle due to ingestion of *C. revoluta*, the sole cycad plant in Japan, was experimentally demonstrated.

Cycasin

Two calves were orally administered with cycasin 2.5-5.0 mg/kg/day; 42 times in 59 days for Case E and 40 times in 52 days for Case F, the total dose being 16.01 g and 21.67 g, respectively. The two calves showed depression, anorexia and decrease in weight gain but no such motor disturbances as seen in cycad

Fig. 1. Diseased cattle in Kuroshima island.

Table 1. Number of the diseased cattle in each island.

Year	Island	Grased	Poisoned (Age)	Dead
1975	Iheyajima	61	48(1-5y)	15
	Ishigakijima	50	4(1-3y)	0
	Izenajima	58	11(1-3y)	0
1977	Yonagunijima	50	3(1-3y)	0
	Kuroshima	40	3(1-5y)	0
1981	Minnajima	112	5(1-3y)	2
1982	Ishigakijima	43	2(1-2y)	0
1982	Shimojishima	32	3(1-3y)	0
1982	Kuroshima	240	62(1-6y)	9
1982	Kuroshima	130	15(1-6y)	3
Total			116	29

Fig. 2. Distribution of lesions in the spinal cord of spontaneous cases.

poisoned cattle were recognized. Hematocrit values and RBC counts were evidently decreased in Case E, while Case F which was anemic from the start presented no obvious changes. In both calves were observed increases in levels of GOT, Al-p, γ -GTP, LDH, total bilirubin and serum γ -globulin as well as decreases in serum albumin and A/G ratio. Case E died from weakening and Case F was killed at the moribund stage. At autopsy, anemia and hemorrhages were noticed in Case E and icterus was manifest in Case F. Either of them had a pale, hard, shrunken liver, which revealed under microscopy marked increase in amount of fibrous connective tissue, stenosis of the central vein and sinusoids, derangement and shrinkage of the hepatic cell cords, irregularity of the size of hepatocytes and their nuclei and bile ductule proliferation. Bile thrombi were noted in Case F. In Case E hemosiderosis was evident in the liver, kidney and spleen. The bone marrow in Case E showed depression in erythroid and platelet

hemopoiesis. Brain and spinal cord of the two calves exhibited extensive spongy vacuolation in white matter, being severe in the reticular formation of the brain stem, around the cerebellar nuclei and adjacent to the gray matter both in the telencephalon and spinal cord. These results indicated that calves were sensitive to cycasin beyond our expectation, showing severe liver cirrhosis that resulted in hepatic encephalopathy. In the spinal cord of these calves administered with cycasin such lesions as seen in cycad poisoned cattle were not observed.

In this study, it was evidenced pathologically that the ingestion of cycad leaves caused damage to the central nerve system in cattle and that the neurotoxicity of cycasin, a toxic principle of the cycad, was not proved in calves. Further studies on the neurotoxic materials in cycads and its mechanisms are to be continued.