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Hepatic and Spinal Lesions in Goats Chronically Intoxicated with Cycasin

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ABSTRACT. Two of the 4 goats given a daily dose of 4–6 mg/kg cycasin for 88 to 124 days presented weakness and paretic signs in the hindquarters. All the 4 goats showed anorexia, weight loss, anemia and decreases in serum albumin and γ -glutamyl transpeptidase (γ -GTP) levels. Two of them had severer hepatic lesions and leukomyelopathy consisting of demyelination and axonal swelling or disappearance in the spinal cord, which were similar to those seen in cycad poisoning of cattle.—**KEY WORDS:** cycasin, goat, spinal lesion.

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Cycasin, methylazoxymethyl- β -D-glucoside [13], is a toxic glucoside contained in the nuts and leaves of cycad plants. Its hydrolysate, methylazoxymethanol (MAM), has been known to have toxic properties as an alkylating agent such as hepatotoxicity and carcinogenicity in various experimental animals [10, 11, 14, 17]. Natural cases of grazing cattle result from ingesting leaves and young shoots of the cycad plant in Australia, Papua, Dominica, Puerto Rico, and Japan [3, 4, 9, 12], and they were characterized by ataxia in the hindquarters and degeneration of myelinated nerve fibers of the spinal cord funiculi [8, 12]. While a neurotoxin other than cycasin was suspected of being responsible for cycad poisoning in cattle, acute intoxication in sheep with marked hepatic and cerebral injury suggested cycasin to be the main toxic principle [7]. In goats, there have been few reports on cycad poisoning [1]. Several months after a single intraruminal administration of cycad leaves to goats, we found the production of spheroids in the spinal white matter, and also a goat orally given 20 mg/kg cycasin 3

times at 7 day-intervals was shown to have remarkable hepatic necrosis and spongy degeneration in the thalamus (data unpublished). In this study we attempted to see effects of a long-term administration of a lower dose of cycasin to goats.

Extraction and purification of cycasin were conducted according to the method previously described [13, 15]. Six Japanese Saanen goats were purchased from a breeding colony in Kumamoto Prefecture. Before use they were checked for serum glutamic-oxaloacetic transaminase (GOT) levels being below 76.0 (average 61.0 Karmen unit) and treated with a single dose of 0.05 g/kg anthelmintic Rintal® (Bayer Japan, Tokyo). Two adult female goats weighing 25.5 and 42.5 kg served as control. Four animals (goats 1 to 4) weighing 28 to 32 kg (average 29.5 kg) were orally given 4 to 6 mg/kg cycasin in water for 88 to 124 days. The total doses ranged for 13.4 to 17.5 g (average 15.6 g) per animal. Goats 1 and 2 which had received a daily dose of 4 mg/kg for 24 days had non-treatment for 7 days due to marked anorexia and emaciation,

after that they were given 6 mg/kg each day. Goats 3 and 4 received a daily dose of 6 mg/kg throughout the experimental period. All the test animals were fed twice a day 100 g Zenraku No. 1 diet (Zenrakuren, Kumamoto) supplemented with some rice straw or hay and given tap water *ad libitum*. The body weight and blood were checked at intervals of 1 week. The goats were sacrificed by exsanguination 4, 25, 1 and 1 day after the last toxin administration and examined for pathological changes.

All the 4 cases showed depression, anorexia, and weight loss; goats 1 and 3 were inactive and hunched down on days 65 and 60 respectively, showing weakness of the hind legs. All the cases had lowered levels of hematocrit, erythrocyte count, and serum albumin and alkaline phosphatase but increased levels of serum γ -GTP. In 3

cases increased levels of GOT, lactic dehydrogenase and total bilirubin were found. There were no remarkable changes in levels of glutamic-pyruvic transaminase, blood urea nitrogen, and choline esterase in the serum, nor in total differential counts of leukocytes.

At autopsy day goat 1 exhibited a lower mean corpuscular volume (MCV) value while goat 3 a lower mean corpuscular hemoglobin concentration (MCHC) value than in other cases. Goats 1 and 3 were shown to have the shrunken and chocolate-colored liver with slightly granular surface. The liver/body weight ratios were 1.1 and 1.0%, respectively. In goats 2 and 4 changes were less severe. Goat 3 had mild fibrinous adhesions of some pulmonary lobes. There was no significant parasitism of nematodes in the digestive tracts.

Main organs were fixed in 10% buffered

Fig. 1. Marked accumulation of hemosiderin around the central hepatic vein and irregularity in size and shape of hepatocytes with unusually large nuclei (upper right). Goat 1, given 15.8 g cycasin for 124 days. HE. $\times 170$.

Fig. 2. Swelling of hepatocytes in the paracentral area of a shrunken lobule and early centrilobular fibrosis. Goat 3, given 13.4 g cycasin for 88 days. HE. $\times 170$.

formalin solution, embedded in paraffin, and sections at 3–5 μm thick were made and stained for microscopy with hematoxylin and eosin (HE), Berlin blue, and luxol fast blue and HE (LFBHE). In the central to midzonal areas of the hepatic lobule were swollen and degenerated hepatocytes irregular in size and shape with unusually large nuclei (Fig. 1), showing acidophilic necrosis of hepatocytes (Fig. 3). Hemosiderosis and erythrophagia were remarkable (Figs. 1 and 4). These changes were prominent in goats 1 and 3, moderate in goat 4 and slight in goat 2 (Table 1). In goats 1 and 3 which were killed at days 128 and 89, respectively, shrinkage of hepatic lobules and proliferation of small bile ductules at the periphery of hepatic lobules were evident (Fig. 4). In goat 3 some central veins showed mild fibrosis (Fig. 2).

Mild reduction in size of pancreatic acinar cells and decreased zymogen granules were

noted in all the 4 goats. Reflecting the latest administration of the toxin, the incidence of mitotic figures was decreased in the intestinal mucous membrane. In all cycasin-treated cases striking hemosiderosis was noticed in the proximal renal tubules and splenic red pulp. In the whole length of spinal cord white matter of all treated cases axons were swollen or disappeared with demyelination in the lateral and ventral funiculi (Figs. 5 and 6). The cord lesions were remarkable in goats 1 and 3, but slight in goats 2 and 4. There was no evidence for cerebrospinal nematodiasis. All treated goats had fatty marrow in the femoral bone, and goat 3 showed small foci of reactive hematopoiesis. No extramedullary hematopoiesis was observed in the spleen of all cases.

No significant changes were seen in the control animals. As an alkylating agent, cycasin had suppressive effects on hemato-

Fig. 3. Remarkable acidophilic necrosis in the paracentral area of the liver. Goat 3. HE. $\times 170$.

Fig. 4. Early proliferation of bile ductules in the portal area (middle to lower left) and centrilobular hemosiderosis (top) in the liver. Goat 1. $\times 170$.

Table 1. Grading of microscopic lesions in the liver of cycasin-administered goats

Lesion	Goat 1 (Day 135) ^{a)}	Goat 2 (Day 156)	Goat 3 (Day 89)	Goat 4 (Day 103)
Swelling and degeneration of hepatocytes	+++ ^{b)}	+	+++	++
Acidophilic necrosis	++	+	+++	++
Irregularity of hepatocytes with unusually large nuclei	++	+	+++	++
Atrophy of lobule	++	—	++	+
Hemosiderosis	+++	+	++	++
Bile ductule proliferation	+	—	+	—
Centrilobular fibrosis	—	—	+	—

a) Killed on indicated day of experiments.

b) Severity of lesions.

Fig. 5. Swelling of axons and vacuolation in the lateral funiculus near the posterior horn of the spinal cord C₁. Goat 3. LFBHE. ×170. Fig. 6. Swollen axons with almost disappearing myelin sheath (center) and an activated macrophage (lower right) at the lateral funiculus of the spinal cord C₁. Goat 1. LFBHE. ×510.

poiesis and hepatic functions in all the present experimental cases. Most features of hepatic and renal lesions in the present cases were common to those in chronic hepatitis of sheep fed nuts of *Macrozamia redlei* containing macrozamin, whose toxic moiety is MAM [2], while there were no

information on pathology of cycad-poisoned goats in Australia [1].

The spinal cord lesions were remarkable in 2 of the 4 goats, and these might result in motor disturbances of the hindquarters. Swelling of axons and myelin degeneration

in the spinal cord were qualitatively similar to those seen in cycad poisoning of cattle [8, 12], and distributed rather diffusely in the lateral and ventral funiculi, showing no obvious predilection to the corticospinal and spinocerebellar tracts as reported in some bovine cases [4, 6, 16]. The spinal cord lesions we observed might be of subacute variation of hepatocerebral disorders. Anemia observed in the goat cases was not described in cycad-poisoned cattle [12] and sheep [2]. The hepatic dysfunction and anemia might have contributed to the leukomyelopathy in the present goat cases. Ischemic necrosis associated with anemia was reported to cause spongy changes in the central nervous system of cattle and sheep [5].

Further studies are required for complete elucidation of cycasin induced spinal lesions in goats.

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要 約

サイカシン長期投与ヤギにみられた肝臓および脊髄の病変について(短報): 清水 孜・安田宣紘・河野猪三郎・八木史郎¹⁾・田寺謙次郎¹⁾・小林 昭¹⁾ (鹿児島大学農学部家畜病理学教室, ¹⁾生物化学・栄養化学教室) —サイカシン 4~6 mg/kg を88~124日連続経口投与した成ヤギ4頭全例に貧血と肝障害を認めた。うち2頭では後肢のふらつき, 起立困難などがみられ, 肝病変がとくに強く, 脊髄白質には脱髄, 軸索の膨化・消失など, 牛のソテツ中毒例類似の病変が認められた。