

Biochemical and Pathological Observations on Sheep Showing Various Clinical Manifestations of Experimental Cerebrocortical Necrosis

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ABSTRACT. For observing chronological changes of biochemical and pathological aspects in experimental cerebrocortical necrosis, 14 sheep were given amprolium (600 mg/kg/day), and killed at various clinical stages. At the onset of abnormal electroencephalograms, total thiamine concentrations in the cerebrum, liver, heart and blood were reduced significantly. After anorexia was noted, total thiamine concentrations in them did not decrease further as symptoms developed severely. Autofluorescence and necrotic lesions in the cerebral cortex were observed in animals killed one day after the onset of abnormal electroencephalograms.—**KEY WORDS:** cerebrocortical necrosis, sheep, thiamine.

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Cerebrocortical necrosis (CCN) is a disease of domestic ruminants induced by thiamine deficiency [6]. Affected animals respond well to thiamine treatment [6, 7]. This disease was experimentally reproduced by administration of amprolium, a thiamine antagonist [4]. In our previous reports, we described changes of electroencephalogram (EEG) [3] and thiamine-related blood biochemical aspects [2] during the course of disease until death in sheep and calves affected with amprolium-induced CCN. Determination of total thiamine concentration in tissues, pathological examination and observation of cerebral autofluorescence under ultraviolet light were also undertaken at death [2]. However, changes in tissue thiamine levels and pathological aspects during the course of disease have not been precisely examined yet. In the

present study, therefore, we similarly observed those various aspects in amprolium-dosed sheep killed at various clinical stages.

Fourteen Suffolk sheep, 14 to 18 months of age, were employed (Table 1). Methods of amprolium administration and EEG recording were the same as in the previous paper [3]. Thiamine tetrahydrofurfuryl disulfide (TTFD, Alinamin-F 50 Injection, Takeda Industries Co., Ltd., Osaka) at a dose of 50 mg was injected subcutaneously to sheep Nos. 22 and 23 every 4 days. The dose of the drugs and the allotment design are summarized in Table 1. The sheep were killed by overdosage of sodium pentobarbital at various clinical stages. Two apparently normal Suffolk and one Merino sheep both of which were intact and not listed in Table 1 were served as a part of the control

Table 1. Experimental animals and starting days of abnormal EEG and behaviors

Sheep ^{a)}				Days after administration				
No.	Sex	Month after birth	Body weight (kg)	Abnormal EEG	Anorexia	Ataxia	Convulsion ^{b)}	Euthanasia
Amprolium 600 mg/kg/day								
10	♀	17	46	28				28
11	♂	17	52	29				30
12	♀	14	37	36	37			37
13	♂	14	47	39	40	41		41
14	♀	14	39	29	30	32		33
15	♀	14	44	31	33	33		34
16	♂	17	48	36	36	37	38	38
17	♂	17	47	44	45	46	46	46
18	♀	14	40	ND	ND	27	27	27
19	♂	18	58	41	43	45	45	46
Amprolium 600 mg/kg/day in ACFS ^{c)}								
20	♀	17	53	30	31	34	34	34
21	♂	15	43	31	35	35	35	36
Amprolium 600 mg/kg/day in ACFS and TTFD ^{d)} 50 mg subcutaneously/4 days								
22	♀	16	44	—	—	—	—	60
23	♀	16	41	—	—	—	—	60

a) Suffolk breed. Each animal was equipped with rumen fistula and EEG electrodes, as previously described [3].

b) If euthanasia was not undertaken, abnormal-EEG stage was followed by anorexia, ataxia and convulsion stages in order. Coma and death followed convulsion stage [3].

c) Amprolium-containing feed supplement.

d) Thiamine tetrahydrofurfuryl disulfide.

ND: Not determined.

—: Normal.

animals.

Organs and tissues were collected immediately after euthanasia. Determination of total thiamine concentration, erythrocyte transketolase activity (ETKA), thiamine pyrophosphate effect on this activity (TPP effect), pathological examination and observation of cerebral autofluorescence were made similarly to those reported in the previous paper [2].

Clinical symptoms at euthanasia: Sheep Nos. 10 to 21 were killed immediately or one day after the onset of various clinical stages including abnormal EEG, anorexia, ataxia and convulsion or violent struggling (Table 1). Sheep No. 19 was killed at an early stage of coma. No abnormal behaviours were found in sheep Nos. 22 and 23 given both amprolium-containing feed supplement (ACFS; Amprol Plus; amprolium 25%, ethopabate 1.6%; Dainippon Pharmaceutical Co., Ltd., Osaka) and TTFD.

EEG: Normal patterns of EEG [1] had been recorded in sheep Nos. 10 to 21 before showing abnormal EEG and in sheep Nos. 22 and 23 throughout the observation periods.

Abnormal EEG seen in sheep Nos. 10 to 21 consisted of continuous slow waves of high amplitude and intermittent spindles bursts of high amplitude (spindles), similarly to those reported in the previous paper [3]. The spindles appeared in 5 of 6 sheep affected with experimental CCN in the previous study [3], but did in 5 (Nos. 11, 16, 18, 19 and 21) of 12 sheep affected in the present experiment. Causes of these different rates were not known.

Blood total thiamine concentration: The thiamine level was 25.2 ± 6.8 (mean \pm standard deviation) ng/ml before the amprolium administration in all the animals examined. The affected sheep Nos. 10 to 21 showed much lower level of blood total thiamine (6.0 ± 3.0 ng/ml) at euthanasia. After the onset of abnormal EEG, the thiamine level was not lowered further until euthanasia. This change was similar to that observed at spontaneous death in the previous study [2]. Thiamine level in sheep Nos. 22 and 23 given both ACFS and TTFD was high (46.1 ± 4.2 ng/ml) at euthanasia. Thiamine level in the 3 intact normal sheep were 25.2 ± 1.0 ng/ml at euthanasia.

ETKA and TPP effect: ETKA and TPP effect were 126.1 ± 38.4 nanomoles of sedoheptulose-7-phosphate formed per ml of erythrocyte per min (nmol-S7P/ml-ery/min) and $95.2 \pm 20.8\%$, respectively, before amprolium administration. The ETKA and TPP effect in affected sheep Nos. 10 to 21 were 40.1 ± 16.5 nmol-S7P/ml-ery/min and $296.0 \pm 70.8\%$, respectively, at euthanasia. These values obtained after the onset of abnormal EEG were similar to those seen at spontaneous death reported previously [2]. No further decrease in ETKA nor an increase in TPP effect was noted after the onset of abnormal EEG. The ETKA and TPP effect at euthanasia in sheep Nos. 22 and 23 given both ACFS and TTFD and the 3 intact normal sheep were similar to those measured before amprolium administration.

Total thiamine levels in tissues: Total thiamine concentrations in the 3 intact sheep ranged from 2.833 to 3.098 in

the liver, 3.687–5.874 in the heart, 1.142–1.277 in the cerebral cortex and $0.921\text{--}0.963$ $\mu\text{g/g}$ wet weight in the cerebral medulla. Although the thiamine level in the cerebral cortex seemed to be higher than that in the medulla, the difference in the level between the cortex and medulla was small as compared with that observed in intact normal calves employed in the previous study [2]. Tissue thiamine levels in the 2 sheep (Nos. 22 and 23) given both ACFS and TTFD were 3.627 in the liver, 3.407 in the heart, 1.164 in the cerebral cortex and 0.856 $\mu\text{g/g}$ wet weight in the cerebral medulla.

Total thiamine concentrations in the liver, heart and cerebrum of affected sheep Nos. 10 to 21 killed after the onset of abnormal EEG were markedly lower than those of sheep Nos. 22 and 23 (Fig. 1). The reduced thiamine values in the liver and heart indicated no further decrease after the onset of abnormal EEG (Fig. 1a). However, the thiamine concentrations both in the cortex and medulla of sheep Nos. 10 and 11 which were killed at the stage of abnormal EEG were higher than those of the remaining affected animals (Fig. 1b). This might suggest that a mechanism by which the decrease of total thiamine level in the cerebrum is delayed against the decrease of blood thiamine level. No such a mechanism might exist in the liver and heart. This observation has to be confirmed by repeated experiments.

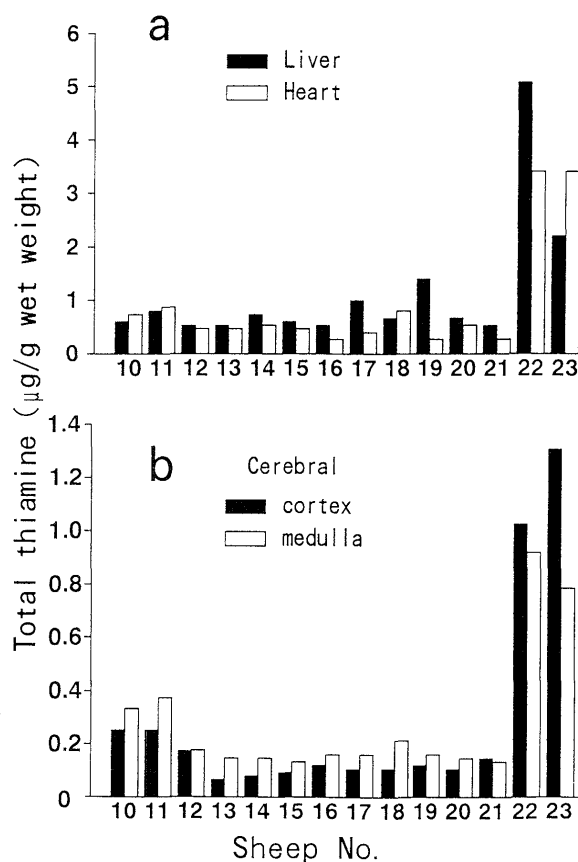


Fig. 1. Total thiamine concentrations in liver and heart (a), and cerebrum (b) at various clinical stages indicated in Table 1.

The total thiamine level in the cortex of the affected animals excluding No. 21 was lower than that in the medulla (Fig. 1b), in contrast with those in sheep Nos. 22 and 23. In this respect, water contents in the cerebral cortex and medulla were measured. No difference in water contents could be found between sheep Nos. 20 and 21 killed at convulsion stage and sheep Nos. 22 and 23 not affected with CCN.

Autofluorescence in the cerebral cortex: Ruminants affected with CCN show the autofluorescent spots in the cerebral cortex under ultraviolet light [5]. In the present study, the discrete pattern of autofluorescence seen on the cerebral surface was defined to 3 levels by visual inspection (Fig. 2). Similar definition was applied to the cut surface of several parts of the cortex, and the maximum levels observed are presented in Table 2.

Sheep Nos. 22 and 23 without CCN and sheep No. 10 killed immediately after the onset of abnormal EEG showed no fluorescent spot. All the other affected sheep showed fluorescence on the surface and/or cut surface of the cortex. Thus the fluorescent spot appeared some times after the onset of abnormal EEG and was increasing in number and space towards death (see the legend b for Table 2).

Histopathological observation: Similarly to the previous experiment [2], rough classification of the brain lesion from - to +++ was applied in the present study (Table 2). Sheep Nos. 22 and 23 without CCN and sheep No. 10 killed immediately after the onset of abnormal EEG had

no lesion in the brain. The pathological changes in the cerebral cortex of sheep No. 11 killed on day after the onset of abnormal EEG was mild compared with those of animals killed after the onset of anorexia. The lesions were found in the midbrain and cerebellum of all sheep dying of amprolium-induced CCN in the previous paper [2], but did in 4 of 12 sheep killed at the various stage in the present study.

Malacic change was not so widely detected as patterns seen at death [2] (see the legend b for Table 2). No severer histopathological change was found in a course from anorexia to convulsion stage. These results suggest that

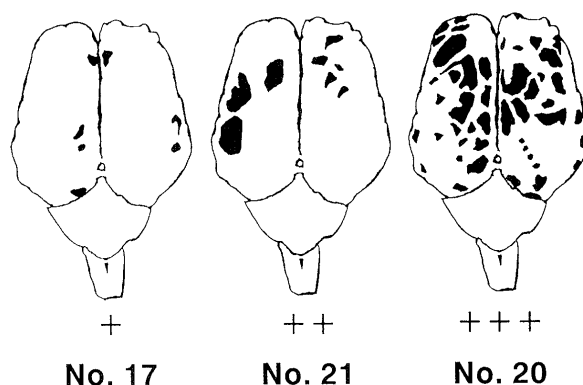


Fig. 2. Illustration for defining levels of autofluorescent spot seen on the brain surface under ultraviolet light.

Table 2. Histopathological changes and autofluorescence detected in brains of amprolium-dosed sheep

Terminal symptom	No.	Cerebral cortex (lobes)				Fluorescence of cerebral cortex		Thalamus	Corpora quadrigemina	Cerebellum	Pons and medulla oblongata
		Frontal	Temporal	Parietal	Occipital	Surface	Section				
at euthanasia	10	-	-	-	-	- ^{a)}	- ^{a)}	-	-	-	-
	11	-	+	+	+	-	++	-	-	-	-
Anorexia	12	+	++	++	++	+	+	-	-	-	-
Ataxia	13	-	++	++	++	+	+	-	-	-	-
	14	++	++	++	++	+++	++	-	++	+	-
	15	++	++	++	++	+++	+++	+	-	-	-
Convulsion	16	-	++	++	+	-	++	-	-	++	-
	17	-	++	++	++	+	+	-	-	-	-
	18	++	+	+	+	++	+++	-	-	-	-
	19	++	++	++	++	+++	+++	+	++	+	-
	20	+++	++	++	++	+++	+++	-	+	++	-
	21	++	+++	++	++	++	++	-	-	-	-
Death ^{b)}		++	+++	+++	+++	+++	+++	-	+++	+++	+
Normal ^{c)}	22,23	-	-	-	-	-	-	-	-	-	-

-: Normal.

+: Necrotic change of nerve cells and general looseness of neuropils.

++: Necrotic change with increasing of vascular endothelial cells in size and number.

+++ : Malacic change with increase of glial cells and migration of macrophages and neutrophils.

a) Fluorescent areas were determined by visual inspection illustrated in Fig. 2.

b) Changes seen in more than 2 of 5 sheep examined in our previous study [2].

c) Sheep Nos. 22 and 23 were given both amprolium and thiamine.

most malacic changes would appear after the convulsion stage.

Any relation was not found between the spindles of EEG observed in 5 of 12 affected sheep and histopathological lesions seen in the thalamus, corpora quadrigemina and cerebellum. More detailed histopathological definition and localization should be needed to find some relations between the spindles or abnormal animal behaviours and pathological changes in the central nervous system.

In this series of study [2, 3], the thiamine level in blood and tissues were already lowered markedly at the onset of abnormal EEG, and the nervous symptoms and necrotic changes in the brain became apparent shortly after the onset of abnormal EEG. Pathological changes in the cerebral cortex, corpora quadrigemina and cerebellum observed at various nervous symptoms seemed to be milder than those of spontaneous death [2]. On the other hand, findings of cortical autofluorescence and histopathological examination in all the affected cases were not similar to each other even when the animals were killed at similar clinical stages (Tables 1 and 2). The difference

might be related to responsiveness of affected animals to thiamine treatment. It was reported in this respect that cows affected with CCN responded to parenteral thiamine therapy but some cases did not [7].

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