Changes in Brain Lipid Composition in Thiamine Deficient Rats

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Abstract—Brain lipid composition was studied in thiamine deficient rats treated with thiamine antimetabolites (oxythiamine: OT, and pyrithiamine: PT) and thiamine deficient diet (TDD). After intraperitoneal injection of OT (40 mg/kg/day) or TDD feeding for 6 days, body weight gain decreased. However, the PT (500 µg/kg/day) treated rats or the pair fed control (PFC: TDD+thiamine of 5 mg/kg, i.p.) showed no decrease in body weight gain compared with the regular diet control (C). Brain lipid levels (total lipid, total cholesterol, triglyceride, phospholipid, sphingomyelin and cerebroside) were examined in four brain regions (cerebral cortex, subcortical structure, brain stem and cerebellum). Total lipid level increased in four regions in OT or TDD treated rats, but total lipid level in the cerebellum in PT treated rats decreased. Total cholesterol level increased in all treated rats, while the triglyceride level in the brain stem decreased dramatically in OT or TDD treated rats. Cerebroside levels of four regions in the PT, OT or TDD group remarkably decreased, and PFC rats showed a significant improvement of the decrease in cerebroside level. It is conceivable that these changes in brain lipid composition provided some clues for the histological and morphological changes of the brain as manifested by the myelin degradation in acute thiamine deficiency.

Thiamine deficiency still occurs frequently in countries where polished rice is the main dietary constituent (1). In addition, chronic alcoholism has been reported to cause thiamine deficiency because ethanol impairs the intestinal absorption of thiamine (2). Severe thiamine deprivation affects the nervous system by causing peripheral neuropathy (3–5), and central nervous system lesions such as those in the Wernicke-Korsakoff syndrome and Leigh’s disease (6, 7). Wernicke’s encephalopathy is a disorder of thiamine deficiency manifested by mental confusion, ataxia and coma. In the lesions of Wernicke’s encephalopathy, edematous necrosis of the astroglia, oligodendroglia, myelin, and neurons combined with petechial hemorrhage appear predominantly (8). Similar clinical manifestations and brain morphological changes have been described in experimental animals treated with a thiamine deficient diet (TDD) and/or thiamine antimetabolites (9, 10). We already reported that morphological changes were observed in the rat brain in acute thiamine deficiency induced by administration of oxythiamine (OT), pyrithiamine (PT) and/or TDD (11). The central nervous system lesions in thiamine deprived rats consisted of abnormal endothelial cells and pericyte swelling or vacuolation of astrocytes, nerve cells containing distorted organelles and myelin degeneration. Especially, PT or TDD treated rats showed the characteristic central nervous tissue alterations of myelin degeneration. However, the pathogenesis of the encephalopathy induced by thiamine deficiency is not fully understood.

The myelin sheath consists of various kinds of lipids, and thiamine diphosphate works as a coenzyme in lipid and glucose metabolism. Therefore, an alteration of lipid metabolism may be related to the pathogene-
sis of thiamine deficiency encephalopathy. The present study was undertaken to investigate the alteration in brain lipid composition in OT-, PT- or TDD-induced thiamine deficient rats and to discuss the pathogenesis of morphological changes in the brain induced by thiamine deficiency.

Materials and Methods

Animal and drug administration: Male Sprague-Dawley rats, 4 weeks of age at the start of the experiments, were fed with a stock diet (MF, Oriental Kobo Co., Ltd.) and tap water ad libitum under standard laboratory conditions (23±2°C, 55±15% humidity). The rats were divided into five groups: regular diet (C), PT treated, OT treated, TDD and a pair-fed control (PFC) groups.

Thiamine-HCl, oxythiamine-HCl and pyritrhiamine-HBr were kindly supplied from Takeda Chemical Ind., Ltd. (Osaka, Japan). TDD was purchased from Nippon Clea Co., Ltd. (Tokyo, Japan). TDD was a purified powder containing a subproportional level of thiamine (thiamine: 0.5 mg/kg of diet). The treatment period was 9 days for all groups. In the OT or PT group, rats were fed with a regular diet (thiamine: 9 mg/kg of diet) for 3 days and then injected intraperitoneally with PT (500 μg/kg/day) or OT (40 mg/kg/day) for 6 days while maintained on a regular diet. In the TDD group, rats were fed with TDD during the same periods (9 days). The PFC group was fed with TDD for the first 3 days and supplemented with adequate amounts of thiamine (5 mg/kg/day, i.p.) for the subsequent 6 days while maintained on a TDD.

Lipid extraction: Animals were sacrificed by decapitation on the day after the 9-day treatment was over. Brains were rapidly removed and dissected into four areas (cerebral cortex, subcortical structures, brain stem and cerebellum). These four areas were obtained as described by Henderson and Schenker (12). Lipids of the individual brain regions were extracted essentially as outlined by Folch et al. (13). Briefly, an individual brain region was homogenized by hand in 20 volumes of chloroform/methanol (2:1, v/v) with butylated hydroxytoluene (20 mg/100 ml of solvent) in a tissue grinder. That is, the brain homogenate from 1 g of wet tissue weight was diluted to a volume of 20 ml. The volume of a tissue sample was calculated on the assumption that the volume of 1 g of tissue was 1 ml. After the residue was reextracted with the same solvent mixture, the washing procedure to remove all the non-lipid contaminants from the crude extracts was carried out by adding 0.9% NaCl solution. After rewashing the lower phase of the lipid from the homogenized mixtures, the solvent was evaporated at low temperature (less than 40°C) under a stream of nitrogen. The residue was dissolved in a volume of chloroform/methanol (2:1, v/v) equivalent to one-tenth the original volume, because each residue weight was about one-tenth of the original wet weight of brain tissue.

Chemical assays: Total lipid content of the brain lipid extracts was determined by the method of Bragdon (14). Total cholesterol content of the brain lipid extracts was determined by a colorimetric method (Cholesterol-B Test). Phospholipid and triglyceride contents of the brain lipid extracts were determined enzymatically (Phospholipid-B Test and Triglyceride-G Test, respectively). These assays were performed using commercial kits (Wako Pure Chemical Ind., Ltd., Tokyo). The results on all lipid contents were expressed in terms of wet tissue weight. The yield of total lipid, total cholesterol, triglyceride and phospholipid expressed as a percentage of the dry weight was about 40–50%, 10%, 10% and 30–40%, respectively.

Sphingomyelin and cerebroside assay: Sphingomyelin and cerebroside were assayed by first isolating them from lipid aliquots by thin-layer chromatography (TLC plate silica gel 60, Merck) using chloroform/methanol/water (65:25:4, v/v) as the developing solvent. The plates were allowed to dry at room temperature for 1–2 hr. Before the experiment, the plates were activated at 120°C for 2 hr. Samples (50 μg or less) were applied to the thin-layer chromatography plate with standardized micropipettes. Reference compounds (sphingomyelin and cerebroside, Sigma Chemical Co., St. Louis), ranging in amounts from 15 to 50 μg, were applied together with tissue lipid extracts. Spots were visualized by exposure to H₂SO₄ vapors (3 ml of 50% solution) and after drying, the silica
gel plate was heated on a hot plate (2 kW) for 30–40 min. The densities of sphingomyelin and cerebroside spots were measured in comparison with the values of the known amounts of reference compounds using a densitometer (F-808, Cosmo Co., Ltd., Japan). The results on sphingomyelin and cerebroside contents are expressed in terms of wet tissue weight.

**Statistical analysis:** Results were expressed as the mean±S.D. from 4 to 7 rats per group. Statistical significance was evaluated using Student's t-test.

**Results**

1. **Physical changes and neurological symptoms:** The body weight gain of the OT or TDD group rats was slightly lower compared with that of the regular diet control rats (Fig. 1). Weights of whole brain were similar in all groups in this experimental period (data not shown). PT, OT or TDD treated rats developed anorexia and early neurological signs such as mild ataxic gait and tendency to walk backwards by the 8–10th day. However, marked physical changes and neurological symptoms were not observed in any treated group during this experimental period.

2. **Total lipid:** Total lipid levels in four brain regions in PT, OT or TDD treated rats and PFC rats are shown in Fig. 2. Total lipid levels in the cerebral cortex and the subcortical structure significantly increased in OT and TDD group rats, but PFC did not ameliorate the increase in total lipid levels observed in the TDD group. OT treatment also increased the total lipid level in the brain stem or the cerebellum. On the other hand, PT treatment decreased the total lipid level in the cerebellum remarkably.

3. **Total cholesterol:** As shown in Fig. 3, total cholesterol levels in four brain regions show the tendency to increase in all groups of treated rats. PFC did not ameliorate the increase in total cholesterol level observed in TDD treatment.

4. **Triglyceride and phospholipid:** Changes in triglyceride levels in four brain regions in PT, OT or TDD treated rats and PFC rats are shown in Fig. 4. In the brain stem, OT, TDD and PFC groups showed a dramatic decrease in triglyceride levels. However, no apparent changes were observed in other brain regions, and PT treatment induced no changes in any of the brain regions. No significant changes in phospholipid levels after treatments with PT, OT and TDD were observed in any of brain regions (data not shown).

5. **Cerebroside and sphingomyelin:**

![Graph](image.png)
Changes in cerbroside levels in four brain regions in all groups are shown in Table 1. In PT, OT and TDD group rats, cerbroside levels decreased drastically, while PFC amel-
iorated the decrease in cerebroside levels observed in the TDD group. Changes in sphingomyelin levels in four brain regions in all groups were studied. In the subcortical structure and the brain stem, sphingomyelin levels of the OT group tended to show a mild decrease, but this was not significant because of the wide variation of the control values (data not shown).

**Discussion**

It is well-recognized that thiamine-deficient rats show marked biochemical and structural alterations in the central nervous system. In experimental animals, a lack of dietary thiamine induces well-defined region-specific lesions in the central nervous system that correlate with neurological symptoms (15, 16). In the present study, no severe neurological symptoms were observed on the day after the 9-day treatment with TDD, OT, PT or PFC was over, except early neurological signs such as mild ataxic gait and tendency to walk backwards. Several factors can be considered to have caused these mild neurological symptoms. One is the short period of treatments and another is the age of used rats. In addition PT or OT treated rats were fed on

![Fig. 4 Triglyceride levels in four brain regions in PT, OT or TDD treated rats and PFC rats. Triglyceride contents were shown in mg/g of wet tissue weight, and each value represents the mean±S.D. of 6–7 specimens. ***, *: Significant difference from the control value with P<0.001, P<0.05 (Student's t-test).](image_url)

Table 1. Changes in cerebroside levels in four brain regions in PT, OT or TDD treated rats and PFC rats

<table>
<thead>
<tr>
<th></th>
<th>Cerebral cortex</th>
<th>Subcortical structure</th>
<th>Brain stem</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>15.74±4.74</td>
<td>25.35±5.51</td>
<td>53.75±13.13</td>
<td>27.71±12.25</td>
</tr>
<tr>
<td>PT</td>
<td>6.09±1.93**</td>
<td>11.63±3.96**</td>
<td>21.48±6.30***</td>
<td>12.66±11.96</td>
</tr>
<tr>
<td>OT</td>
<td>7.82±3.58*</td>
<td>11.13±6.52**</td>
<td>18.59±4.78***</td>
<td>12.18±2.97*</td>
</tr>
<tr>
<td>TDD</td>
<td>6.87±3.26**</td>
<td>9.77±4.61**</td>
<td>20.47±17.50**</td>
<td>12.58±10.35</td>
</tr>
<tr>
<td>PFC</td>
<td>9.68±6.26</td>
<td>18.42±5.74</td>
<td>38.19±14.34</td>
<td>27.66±12.35</td>
</tr>
</tbody>
</table>

Cerebroside contents were shown in mg/g of wet tissue weight, and each value represents the mean±S.D. of 5–6 specimens. ***, *: Significant difference from the control value with P<0.001, P<0.01, P<0.05 (Student's t-test).
regular diet containing thiamine in the present study. Our previous paper indicated that thiamine antimetabolites injection with TDD feeding induced more severe thiamine deficiency and neurophysiological changes (3, 4, 5, 11). Especially, PT causes a significant central muscarinic, cholinergic and serotoninergic defect (17, 18). Many factors could be conceived as causes for the various changes of neurological symptoms.

However, our previous report demonstrated that the pathological change in the brain of TDD or PT treated rats preceded apparent neurological symptoms. Spongy re:ticulation and degeneration of nerve cells were found in the anterior and posterior floor of the fourth ventricle and inferior cerebellar peduncle (11). In addition, electron micro:graphs of the OT, PT and/or TDD treated rat brain revealed irregular myelin sheath, vac:uolation of glial foot process or neuropile, swelling of extracellular space or change of myelinated axon filled with the collection of membranous structures (11). Collins also reported that the microscopic appearance of the central nervous system lesions in thiamine deprived animals revealed selective destruction of myelinated structures (15). Moscatelli et al. reported that the low thiamine and low protein diet produced a decrease in myelin sphingomyelin expressed as relative molar amounts in the rat brain (19). Our data also showed the tendency of decrease in sphingo:myelin in the brain stem and the subcortical structure of the rat brain without severe neurological symptoms. These parts of the brain are more vulnerable in thiamine deficiency induced by PT, OT and/or TDD. Sphingomyelin and cerebroside are generally regarded as characteristic lipids of myelin (9), and their levels can be regarded as a measure showing the degree of myelination. In the present study, cerebroside levels in thiamine deficient rats were shown to decrease remarkably and the PFC group showed the ameliorating effect. These results on cere:broside and sphingomyelin provide evidence for changes of the myelin degradation in the central nervous system induced by thiamine deficiency.

The present study revealed that levels of all brain lipids except phospholipid were changed significantly in thiamine deficient rats. As to the total phospholipid concentr:ation, Geel et al. also reported that no changes were evident in the whole brain of thiamine deficient rats (20). In the present study, we found that the total cholesterol level of all treated rats and total lipid level of OT or TDD treated rats increased. Many reports indicated that thiamine deficiency reduced the activity of brain transketolase and pyruvate decarboxylase in animals (21, 22). Thiamine diphosphate is an essential coenzyme for transketolase which catalyzes reactions necessary for the operation of the hexose monophosphate (HMP) shunt of the major pathway of NADPH synthesis. In addition, thiamine diphosphate works as a coenzyme for py:ruvate decarboxylase which catalyzes the oxidative conversion of pyruvate to acetyl-CoA (22). Therefore, the increases in brain total cholesterol and total lipid level observed in this experiment are intriguing results. These increases in brain lipids might result from an adaptive response to thiamine deficiency, considering that Itoh and coworkers suggested that the increase in lipogenesis occurred despite of the decrease in liver total cholesterol and total lipid in thiamine deficient animals (23).

Thiamine antagonists, OT and PT, have been reported to block the coenzyme functions of thiamine. PT readily crossed the blood-brain barrier and PT diphosphate directly inhibited the activity of thiamine pyrophosphokinase (24). In PT treated rats, our result showed the decreases in cerebroside level in three brain regions and total lipid level in the cerebellum, while the triglyceride level in the brain stem remained constant. The pattern of changes was different from that observed in other treated rats. Moreover, our previous paper (11) revealed that myelin degradation in the brain stem was especially severe on PT or TDD treated rats as compared to that in the OT treated animals. On the other hand, OT competed with thiamine diphos:phate for apo-enzyme by OT diphosphate and induced a decrease in thiamine availability (24-26). OT treated rats and TDD rats showed the similar changes in brain lipids and the decrease in body weight gain due to the apparent anorexia. Bai et al. (27) suggested that the development of anorexia symp-
toms correlated most closely with the decrease in transketolase activity. Since malnutrition due to anorexia has been shown to alter lipid composition in the developing rat brain (28), we cannot rule out the possibility that these changes in brain lipid composition in OT or TDD treated rats are related to malnutrition.

Dreyfus (9) reported that cerebroside concentrations in the whole brain of 25-day-old thiamine deficient rats were reduced, and pair-fed control rats showed a more severe decrease. These studies were performed in immature animals whose thiamine deficiency was induced by feeding pregnant mothers with a diet containing suboptimal amounts of thiamine (0.5 mg/kg of diet). Using 4 week-old rats, we found that the cerebroside level in the cerebral cortex, subcortical structure and brain stem decreased after PT, OT or TDD treatment and demonstrated that thiamine given in adequate amounts to TDD treated rats showed ameliorative effects. From these studies, we can imagine that thiamine deficiency in suckling or weanling rats induces more severe damage in the brain and this cannot be restored by thiamine addition. Our previous paper (11) demonstrated that brain thiamine levels of PT (500 μg/kg, i.p. × 6 days) treated rats fell to 42.9% of those of the regular-diet control in whole brain. TDD treatments induced a mild decrease in the brain, while OT treated rats showed no change in brain thiamine levels without the apparent myelin degradation. By the report of Rindi et al. (25), OT does not penetrate the brain and therefore OT diphosphate was not observed there. As to the relationship between the brain thiamine contents and changes in brain lipids, no simple correlation was observed. In thiamine depleted rats, the total thiamine level is much better maintained in the brain than the total thiamine level in other organs. This control of entry of thiamine into the cerebrospinal fluid and brain is an important part of the homeostatic mechanisms of total thiamine regulation in the brain (29). McCandless et al. reported that thiamine-deficient encephalopathy and the impairment of cerebral transketolase and pyruvate decarboxylase activities occurred primarily in the brain stem and cerebellum, the sites of the morphological changes (21). The determination of activities of thiamine dependent enzymes or contents of thiamine diphosphate in each brain region will reveal the more specific relationship between thiamine deficiency and changes in brain lipids.

In conclusion, this study shows changes in brain lipids in rats with acute thiamine deficiency. Especially, it was revealed that the cerebroside level was remarkably reduced in PT, OT or TDD treated rats and that PFC significantly ameliorated the reduction of cerebroside. These results suggest that the changes in brain lipids are involved in the pathogenesis of some of the neurologic manifestations of acute thiamine deficiency.

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