Effect of Lead Intoxication on Thiamine Content and Transketolase Activity in the Brain of Rats.

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Abstract—In the present study, we tested whether lead intoxication could change the thiamine content and the activity of transketolase, one of thiamine-dependent enzymes, in the brain of rats. It was also tested whether administration of excessive thiamine can reverse the toxic manifestation of lead in the lead intoxicated rats.

Four groups of Wistar rats were prepared: 1) control group, 2) lead treated group, 3) lead plus thiamine treated group and 4) thiamine deficient group. Each group of animals was divided into three subgroups based on ages: 3, 7 and 16 weeks. Lead concentration, thiamine content and the activity of transketolase in three different brain regions, i.e., telencephalon, brain stem and cerebellum, were measured in each group. Lead concentrations in brain regions of the lead treated group were significantly higher than those of the control group, and those of the lead plus thiamine treated group were significantly decreased from those of the lead treated group. Thiamine contents in the brain regions of the lead treated group were significantly lower than those of the control group, and those of the lead plus thiamine treated group were recovered back to those of the control group. Activities of transketolase in the brain regions of the lead treated group and the thiamine deficient group were significantly lower than those of the control group, while those of the lead plus thiamine treated group were higher than the lead treated group. The results from the present study suggest that neurotoxicity following lead intoxication in rats may be mediated, at least in part, through the changes of thiamine status and consequently thiamine-dependent biochemical reactions such as the activity of transketolase.

Keywords □ lead, thiamine, transketolase, rat, brain

Lead intoxication induces hazardous effects on various tissues and organs such as kidney, liver, erythropoietic, reproductive, cardiovascular and nervous system. Among these organs and tissues, the central nervous system is the most sensitive target of lead toxicity (Alperstein et al., 1991; Goyer, 1992). For instance, encephalopathy with seizures and coma is one of the most striking and serious complications of lead poisoning (Needleman, 1990).

Lead toxicity has been suspected to be mediated through interactions between lead and endogenous substances that have high affinity to lead such as molecules that containing -SH group (Needleman and Bellingner, 1991). It was also reported that thiamine, an endogenous -SH containing molecule, can reduce the absorption of lead in gastrointestinal tract and to enhance the elimination of lead from soft tissues, such as brain, kidney and liver (Ghazaly, 1991). Neurological defects such as peripheral neuritis and encephalopathy that occur frequently in lead intoxicated animals, have been observed in thiamine deficient animals, further supporting that the interactions between lead and thiamine exist to some extent (Keyser and De Bruijin, 1991).

Transketolase, one of the thiamine dependent enzymes, is distributed in brain as well as liver, erythrocytes and leukocytes (Gaitonde et al., 1983). Among the thiamine dependent enzymes, transketolase activity is the most sensitive to thiamine deficiency (Friedrich, 1988). Therefore, the measurement of transketolase activity has been widely used as a biochemical parameter to assess thiamine status in tissues (Friedrich, 1988; Hass, 1988).

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In the present investigations, we studied possible relationship between lead intoxication and thiamine. For the purpose, we examined whether lead administration through drinking water influences the thiamine content in the central nervous system and if so, whether lead intoxication can alter the thiamine dependent biochemical reaction such as the activity of transketolase in the brain of rats. Further, it was also tested whether administration of excessive thiamine could reverse the reduced thiamine activity in lead intoxicated animals.

Materials and Methods

Materials

Trichloroacetic acid, α-amylase, sodium hydroxide, thiamine hydrochloride, thiamine pyrophosphate, glycylglycine hydrochloride, ribose-5-phosphate disodium salt, cystein, sedoheptulose-7-phosphate barium salt, folin reagent, bovine serum albumin were purchased from Sigma Chemical Co., Mo., U.S.A. Lead acetate, ethylether, isobutanol, brom, nitric acid and sulfuric acid were purchased from Junsei Chemical Co., Japan. Sodium bicarbonate anhydrous, copper sulfate and sodium potassium tartrate were purchased from Yakuri Pure Chemicals Co., Ltd., Japan. Thiamine tetrahydrofurfuryl disulfide (99.8%) was obtained from Il-Dong Pharmaceutical Company, Korea. Thiamine deficient diet was purchased from Sunkyo Farm Co., Japan.

The water used in this study was either deionized and distilled water or deionized and double-distilled water.

Animals and treatment

Wistar rats of both sexes were supplied from the Laboratory Animal Center of Seoul National University. Four groups of animals were prepared: 1) Control group, 2) Lead treated group, 3) Lead plus Thiamine treated group, and 4) Thiamine deficient group. The control group received a normal diet and tap water, and the lead treated group received a normal diet and deionized and distilled water containing 0.2% lead acetate. The lead plus thiamine treated group was fed on a thiamine sufficient diet containing 2 mg thiamine tetrahydrofurfuryl disulfide per 1 kg ad libitum, and given deionized and distilled water containing 0.2% lead acetate. The thiamine deficient group was fed on a thiamine deficient diet and tap water. Until experimental animals became 3-week old, lead and/or thiamine were administered to them through milk by giving lead and/or thiamine to their dams. After weaning, lead and/or thiamine were administered directly to experimental animals through drinking water or diet as described above. Animals were sacrificed by decapitation when they became 3-, 7- and 16-week old. Brains were rapidly removed from animals and dissected into three regions; telencephalon, brain stem (diencephalon/midbrain and pons/medulla), and cerebellum by the method of Glowinski and Iverson (1966) just before experiments.

Measurement of Lead Concentration

Separated brain tissues were lyophilized and digested overnight in an acid mixture of nitric acid and sulfuric acid (1 : 1, v/v). The resulting yellowish clear solution was added with 10 volumes of perchloric acid and nitric acid (4 : 1, v/v), and heated on a hot plate to remove organic materials and sulfuric acid. Lead concentration was measured by an ICP (inductively coupled plasma)-MASS (VG Plasmaquad) (Taylor and Garbino, 1988). Nominal recoveries for lead was 97 × 1.2% and recovery efficiency for lead were determined at 1 ppm. The lower limit of sensitivity was 0.1 μg/ml and lead concentration was expressed as ng/g wet tissue.

Quantitation of Thiamine Content

Thiamine content in brain tissue was measured by the thiochrome method (Rindi and deGiuseppe, 1961). Briefly, separated brain tissues were homogenized in ice-cold 5% TCA, centrifuged, and the resulting supernatant fraction was divided into two aliquots. Free thiamine content was measured in one aliquot. After the supernatant was washed with water-saturated ethylether, cyanogen bromide and ice-cold 15% sodium hydroxide was added to produce thiochrome and further extracted by isobutanol. Total thiamine content was measured in the other aliquot after the supernatant was digested by α-amylase at 48～50°C for 30 min. Thiamine content was estimated using a spectrofluorometer (FP-777, Jasco international Co., Ltd.) at 425 nm with exciting at 358 nm (Edwin, 1979) and was expressed as μg/g wet tissue.

Determination of Transketolase Activity

Transketolase activity was estimated by measuring the rate of sedoheptulose-7-phosphate elaboration during the incubation of tissue homogenate in the presence of excess ribose-5-phosphate substrate (Dreyfus and Moniz, 1962). Separated brain tissues were homogenized in 10 volumes of ice-cold 0.04 M glycylglycine hydrochloride buffer, pH 7.6. The enzyme reaction was started by addition of 24 mM ribose-5-phosphate substrate to the homogenate and continued at 37.5°C for 30 minutes. After the reaction was terminated by the addition of 20% TCA, the reaction mixture was centri-
fuged, and a clear and colorless supernatant fraction was obtained. An aliquot of the supernatant was vacuum desiccated and the resulting residue was dissolved in redistilled water/concentrated sulfuric acid (2.5/6, v/v). Subsequently, the resulting solution was heated at 100°C for 4 minutes, with thorough agitation, and 3% aqueous cysteine solution was added with shaking. After 5 hours, the spectrophotometric determination was carried out with the sample. Differences in absorbance between 510 nm and 540 nm was converted to total heptose concentration using known concentrations of sedoheptulose as standards.

Protein content of tissue homogenate was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

**Statistical Analysis**

Data were expressed as the mean ± S.E.M. For statistical evaluation of data, ANOVA test and Newman-Keuls test were used. Differences were considered statistically significant when p<0.05 was obtained.

**Results**

**Measurement of Lead Concentration**

Lead concentrations in each brain region were summarized in Table I. Lead concentrations in all brain regions of the lead treated group were higher than those of the control group, and those of the lead plus thiamine treated group were significantly decreased from those of the lead treated group.

**Quantitation of Thiamine Content**

Thiamine contents in each brain region were shown in Fig. 1. Thiamine content in all brain regions of the lead treated group was significantly lower than those of the control group and was about the same as those of the thiamine deficient group. In the lead plus thiamine treated group, thiamine contents were recovered to those of the control group.

**Determination of Transketolase Activity**

Activities of transketolase in each brain region were shown in Fig. 2. The activities of transketolase of the lead treated group as well as the thiamine deficient group were significantly lower than those of the control group. In the lead plus thiamine treated group, transketolase activities were higher than those of the

![Fig. 1. Total thiamine contents in brain regions of 3, 7 and 16 week old rats. Each bar represents the mean ± S.E.M. of data from 5 experiments. Panels A, B and C show the results obtained from 3 week old, 7 week old and 16 week old rats, respectively. * indicates a significant difference from the control group (*, p<0.05 and ***, p<0.01). ◆ indicates a significant difference from the lead-treated group (◆, p<0.05 and ◆◆, p<0.01). Empty bar, control group; filled bar, lead treated group; hatched bar, lead plus thiamine treated group and gray bar, thiamine deficient group.](image-url)
Discussion

The present study showed that lead treatment through drinking water caused accumulation of lead in the brain of rats (Table 1), in consistent with previous observations (Alice and Kennedy, 1989; Kim et al., 1990a). Importantly, however, supplementation of thiamine through diet significantly reduced the accumulation of lead in the brain, suggesting possible interactions between lead and thiamine. Similarly, administration of excessive thiamine eliminates lead from soft tissues in lead intoxicated rodents (Kim et al., 1991; Ghazaly, 1991). It has been also suggested that thiamine may facilitate the removal of lead from body fluids and other tissues by the formation of readily excretable complexes (Flora and Tandon, 1986). When given simultaneously with lead, thiamine may interfere with the absorption of lead in tissues, possibly via the formation of a lead-thiamine or lead-thiamine metabolite complex (Sasser et al., 1984). Thiamine has been shown to form complex in vitro with other heavy metals such as, copper and cadmium (Cramer et al., 1981). Similar complex of lead and thiamine may be formed in the body, which could result in decreased tissue deposition. However, the detail mechanism of the interaction between lead and thiamine remains to be established.

The present study demonstrated that total contents of thiamine are decreased in the brain of lead-intoxicated rats (Fig. 1). The decrement was more clearly shown with longer duration of lead treatment. The reduced thiamine content in the brain may induce pathological consequences since thiamine has important roles in the central nervous system. Thiamine is an essential factor in brain energy metabolism and biosynthetic pathways (Gaitonde et al., 1983; Gibson et al., 1984; Parker et al., 1984). In addition, thiamine is involved in the maintenance of normal membrane function and nerve conduction. Many cases of thiamine deficiency in animal studies further demonstrated the important roles of thiamine in the nervous system. Recently, the early morphological brain lesions in thiamine deficient rats are reported to be linked to energy deficiency. Thiamine depletion affects neurons and their functions in selected areas of the central nervous system (Cooper and Pincus, 1979). Neurological defects, such as peripheral neuritis and encephalopathy, have been recognized in association with thiamine deficiency (Takashashi, 1981).

One of the possible factors that could mediate the neurological abnormalities in the thiamine deficient status could be the change of thiamine dependent biochemical reactions such as the reduced transketolase activity. As expected, the present study demonstrated that the thiamine deficiency caused by lead intoxication reduced the transketolase activity (Fig. 2). Transketolase is one of the enzymes that involved in hexose monophosphate (HMP) shunt that generating NADPH required for lipid synthesis. Transketolase and possibly the entire HMP shunt are important in the development and maintenance of the myelin sheath (Clarke
and Sokoloff, 1993) and the oligodendroglial metabolism in the CNS (Dreyfus and Moniz, 1962). Other thiamine dependent biochemical factors such as pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, although they are less sensitive to thiamine deficiency than transketolase, may also contribute to energy impairment and morphological changes in thiamine deficiency. Taken together, the neurological alterations following lead intoxication may be mediated, at least partly, through the changes of thiamine dependent biochemical reactions due to lead intoxication.

The decreased level of thiamine content in the brain of the lead treated group was recovered toward control level in the brain of the lead plus thiamine treated group (Fig. 1). This phenomenon was also observed in the case of transketolase activities (Fig. 2). Recovery of transketolase activities in the brain regions of lead intoxicated animals toward control level following thiamine treatment may be interpreted as a result of replenishment of thiamine for transketolase activities. The results suggest the role of thiamine in the treatment of lead intoxication. First, administration of excessive thiamine reduces lead concentration in the body by blocking lead absorption and enhancing lead excretion. Second, it prevents deficiency of thiamine caused by lead intoxication. Generally, for the treatment of lead poisoning, chelating therapy has been widely used, but it was unsuccessful, especially, in removing lead from soft tissues such as brain and liver (Ghazaly, 1991).

It was reported that simultaneous administration of thiamine improved the efficiency of CaEDTA treatment (Goyer and Cherian, 1978; Tandon et al., 1987). The results from the present study indicate a possible use of thiamine as preventive or therapeutic agent, or as a complimentary use to other preventive or therapeutic agents against lead intoxication.

References


