Thiamine deficiency as one of the mechanisms for neurotoxicity induced by lead intoxication in rats.

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ABSTRACT

In this study, it was tested whether lead intoxication could change thiamine content and the thiamine related biochemical factor such as activity of transketolase in the brain, and whether the changes of the myelin composition as well as the seizure threshold induced by lead intoxication in rats be related to these changes of thiamine status and thiamine related biochemical factors. In addition, it was also tested whether administration of excessive thiamine can reverse the toxic manifestation of lead in lead intoxicated animals.

Five groups of Wistar rats were prepared: 1)Control group, 2)lead treated group, 3)thiamine treated group, 4)lead plus thiamine treated group and 5)thiamine deficiency group. Each group of animals was divided into three subgroups based on ages: 3, 7 and 16 weeks of age subgroups. Lead concentration, thiamine content, the activity of transketolase and myelin composition in brain areas and threshold of electric shock seizure were tested in each group.

Lead concentrations in all brain regions of lead treated group were higher than those of control group, and those of lead plus thiamine treated group were significantly lower than those of lead treated group. Thiamine contents in the brain regions of lead treated group were significantly lower than those of control group, and those of lead plus thiamine treated group were recovered back to those of control group. Activities of transketolase of lead treated group were significantly lower than those of control group, while those of lead plus thiamine treated group were recovered back to those of control group. The cases of which was observed with the concomitant changes of thiamine content and transketolase activity in myelin content or constituent of all the brain regions tested were total amount of myelin protein in the cerebellum of 3 week old rats, and phospholipid in the cerebellum of 3 week old rats and the telencephalon of 16 week old rats. Thresholds of the electroshock seizure of lead-treated group and thiamine-deficient group in 3, 7 week old rats were significantly lower than those of control group, while those of the lead plus thiamine-treated group were similar to those of control group. Changes of the electroshock seizure threshold induced by lead intoxication were observed in 3 week and 7 week old animals with the concomitant decrement of thiamine content in all the brain regions tested. These observations were reversed by the supplementation with thiamine to those animals.

However, the changes of seizure threshold induced by lead intoxication corelated with the

changes of thiamine contents as well as transketolase due to lead intoxication. The changes of myelin phospholipid as one of myelin composition and those of myelin protein content only in the cerebellum of 3 week old rats correlated with the changes of the seizure threshold as well as thiamine content due to lead intoxication. The results from the present study may indicate that neurotoxicity of lead in rats may be mediated at least in part through the changes of thiamine status. Such changes of thiamine status may induce the changes of myelin composition such as myelin phospholipid and those of myelin protein content especially in the cerebellum of 3 week old rats which may eventually affect the threshold of seizure.

Key word : lead toxicity, thiamine, transketolase, myelin, rat brain, seizure

INTRODUCTION

Central nervous system is the sensitive target of lead intoxication and encephalopathy with demyelination, abnormal conduction velocity, decrement of I.Q. score, seizures and coma is the most serious complication of lead poisoning. Seizures are among the most severe signs of lead poisoning. Alterations of seizure threshold and seizure responsiveness in lead exposed rats have been reported(Silbergeld, E.K. et al., 1979). Significant reductions of the doses required to produce seizures were found in lead-exposed rats for the convulsants, picrotoxin, strychnine, but no change in response to pentylenetetrazol was found in lead-exposed mice or rats. Intensity of maximal electroshock induced seizures was increased in lead-exposed rats(Silbergeld, E.K. and Hruska, R.E., 1980). The maximal electrshock seizure(MES) is an experimental model of grand-mal convulsions and the MES test was used to evaluate changes in seizure development of lead-exposed rats. Factors which influence the development of electroshock seizure pattern include synaptogenesis, myelination, maturation of monoaminergic systems, and electrolyte and amino acid changes in the rat brain(Fox, D.A. et al., 1979). The mechanism of neurotoxicity, such as seizure and demyelination, induced by lead was not clear. It is suggested that lead toxicity may be occurred through interaction betweem lead and -SH containg molecules in the tissue. Thiamine, which is endogenous -SH containing molecule distributed in all tissues, has been reported to reduce the absorption of lead in G-I tract and enhance the elimination of lead from soft tissues, including brain, kidney and liver. Neurological defects due to thiamine deficiency, peripheral neuritis and encephalophacy, has been observed to accompany lead intoxication. This coincidence of neurological alterations may also suggest that interactions between lead and thiamine may occur to some extent. thiamine is related to energy metabolism and the oxidative decarboxylation of keto acids. Among thiamine dependent enzymes, transketolase was the most sensitive to thiamine deficiency. The measurement of the activity of transketolase is one of a number of biochemical methods used to assess thiamine activity in tissues (Hass, 1988).

If lead intoxication may affect the thiamine status in the soft tissues including brain, lead may also affect biochemical reaction and neurobehavioral function which are related to thiamine status in the brain. In the present study we examine whether lead intoxication

affects the thiamine content and the thiamine dependent biochemical reaction such as the activity of transketolasein the central nervous system and if so, whether the changes of the myelin composition as well as the changes of the seizure threshold induced by lead intoxication in rats be related to these changes of thiamine status and thiamine related biochemical factors. In addition, it was also tested whether administration of excessive thiamine can reverse the toxic manifestation of lead in lead intoxicated animals.

METHODS

Five groups of animals were prepared : 1)Control group, 2)Lead treated group, 3)thiamine treated group, 4)Lead plus thiamine treated group and 5)thiamine deficient group. Control group received a normal diet and tap water, and lead treated group a normal diet and deionized and distilled water containing 0.2% lead acetate. Lead plus thiamine treated group was fed on a thiamine sufficient diet (2±0.2mg thiamine tetrahydrofurfuryl disulfide per 1kg body weight per a day), and given deionized and distilled water containing 0.2% lead acetate. thiamine deficient group was fed on a thiamine deficient diet and tap water. Each group was divided into 3 subgroups based on ages; 3, 7 and 16 weeks of age. Animals were sacrificed by decapitation when they became 3, 7 and 16 weeks of age. Brains were rapidly removed from animals and dissected into three regions ; telencephalon,brain stem (diencephalon/midbrain and pons/medulla) and cerebellum.

Lead concentration in separated brain tissues was measured by the inductively coupled plasma-mass spectrometer (VG Plasmaquad) (Franzblau, A., et al., 1988). Thiamine content in brain tissue was measured by thiochrome method using spectrofluorometer (FP-777, Jasco international Co. Ltd.) (Edwin, 1979). 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). Myelin in the brain tissue was isolated by Norton and Poduslo's method(Norton and Poduslo, 1973). The contents of total protein, myelin basic protein I, II, cholesterol and phospholipid were mesured.

Seizure evoked by constant current stimulator and the resulting seizure was determined by overt hindlimb extension. Electroshock threshold was determined by giving individual animals shocks of 0.2-sec stimulus duration with 2mA increments or decrements in current intensity(Browning et al., 1990). CC_{50} was calulated by Litchfield wilcoxons's method.

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

Lead concentrations in all brain regions of lead treated group were higher than those of control group, and those of lead plus thiamine treated group were significantly decreased from those of lead treated group. Thiamine contents in lead treated group were significantly lower than those of control group in all brain regions at 3, 7 and 16 weeks of age except in

telencephalon of 3 weeks of age. In lead plus thiamine treated group, thiamine contents were similar to that of control group. The activities of transketolase of lead treated group as well as thiamine deficient group were significantly lower than those of control group in all the brain regions. In lead plus thiamine treated group, transketolase activities were higher than those of lead treated group. The cases of which was observed with the concomitant changes of thiamine content and transketolase activity in myelin content or constituent of all the brain regions tested were total amount of myelin protein in the cerebellum of 3 week old rats, and phospholipid in the cerebellum of 3 week old rats and the telencephalon of 16 week old rats. Electroshock seizure threshold of lead-treated group and thiamine-deficient group in 3, 7 week old rats were significantly lower than those of control group, while those of the lead plus thiamine-treated group were similar to those of control group. Electroshock seizure threshold of lead-treated group and thiamine-deficient group in 16 week old rats were lower than those of control group but the difference was not significant.

DISCUSSION

Lead concentrations in brain regions in lead treated group increased significantly from those of control group. It has been reported that lead level was significantly increased in soft tissues, including brain, of animals intoxicated through water containing lead (Kim et al., 1990). In animals of lead plus thiamine treated group, lead concentrations in all brain regions were significantly lower than those of lead treated group. This agrees with the observations of others (Ghazaly, 1991). It was demonstrated that administration of excessive thiamine eliminates lead from soft tissues in rodents exposed to lead (Tandon and Flora, 1989). In this study, thiamine tetrahydrofurfuryl disulfide, which can easily be absorbed in gastrointestinal tract, was used as a source of thiamine. Absorbed thiamine tetrahydrofurfuryl disulfide enters erythrocytes, and is decomposed to thiamine backbone and -SR group, and then the -SR group combines with metal to produce stable compound, mercaptide. However, it is also possible that elimination of lead from body after administration of thiamine tetrahydrofurfyryl disulfide may be through the action of thiamine itself (Kim, et al., 1990).

Results from the present study demonstrated that total contents of thiamine in the brain of lead treated group were lower than those of control group. The decrement of thiamine content by lead intoxication was more clearly shown in chronically lead treated group. thiamine content in blood and liver decreased in lead intoxicated rats. In the present study, the decreased level of thiamine content in the brain of lead treated group was recovered toward control level in the brain of lead plus thiamine treated group. This suggests dual roles of thiamine in the treatment of lead intoxication. Administration of excessive thiamine reduces lead concentration in the body and also prevents deficiency of thiamine caused by lead intoxication.

Data from the present study show that activities of transketolase in telencephalon, brain stem and cerebellum of lead treated group of animals at 3, 7 and 16 weeks of age were significantly lower than those of corresponding control group. The decrement of transketolase activities in telencephalon, brain stem and cerebellum was observed in thiamine deficient group.

Transketolase is one of the enzymes of the hexose monophosphate(HMP)shunt. The main contribution of HMP shunt is probably to produce theNADPH required for reductive reactions necessary for lipid synthesis. It was reported that the activity of some of HMP shunt enzymes tends to be greater in CNS white matter than in gray matter (Dreyfus and Moniz, 1962). It is conceivable that transketolase and possibly the entire HMP shunt is important to the development and maintenance of the myelin sheath in the CNS. Also these enzymes relate to oligodendroglial metabolism (Dreyfus and Moniz, 1962). Activities of transketolase were affected with the contents of cofactor, thiamine pyrophosphate, in tissues. Poor myelin maintenance in thiamine deficiency may affect the failure of this pathway to provide sufficient NADPH for lipid synthesis (Clarke and Sokoloff, 1993).

In the present study, the decreased activities of transketolase in the brain regions of lead treated group as well as thiamine deficient group were recovered by simultaneous administration of thiamine. 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 repleshment of thiamine for transketolase activities in such brain regions.

The changes of myelin phospholipid as one of myelin composition and those of myelin protein content only in the cerebellum of 3 week old rats correlated with the changes of the transketolase activities as well as thiamine content due to lead intoxication.

Electroshock seizure threshold was decreased by lead intoxication. The previous report demonstrated that intensity of maximal electroshock induced seizures was increased in lead exposed rats(Silbergeld, E.K. and Hruska, R.E., 1980). Decrements of electroshock seizure thrshold induced by lead intoxication were similar to it induiced by thiamine dificiency. Changes of electroshock seizure threshold induced by lead intoxication were observed in 3 week and 7 week old animals with the concomitant decrement of thiamine content and transketolase activity in all the brain regions tested. These observations were reversed by the supplementation with thiamine to those animals. Electroshock seizure threshold of lead-treated group and thiamine-deficient group in 16 week old rats were lower than those of control group but the difference was not significant. Significant changes electroshock seizure threshold in 3, 7 week old rats and not significant changes in 16 week old rats may be due to general adoptation mechanisms that was caused by the same repetive stimulus. These adoptation in the other neuro-response was reported(Hope et. al., 1994). Factors which influence the development of electroshock seizure pattern include synaptogenesis, myelination, maturation of monoaminergic systems, and electrolyte and amino acid changes in the rat brain(Fox, D.A. et al., 1979). Demyelination and delays in myelin formation have been reported following lead intoxication and thiamine deficiency. The changes of myelin phospholipid as one of myelin composition and those of myelin protein content only in the cerebellum of 3 week old rats correlated with the changes of the seizure threshold as well as thiamine content due to lead intoxication. The changes of electroshock seizure threshold induced by lead intoxication may be concerned with changes of myelin formation caused by thiamine deficiency and lead intoxication.

Although the mechanism of the thiamine-lead interaction in the body remains unclear, it has

been suggested that thiamine may facilitate the removal of lead from body fluids and other tissue by the formation of readily excretable complexes (Flora and Tandon, 1986). Thiamine, when given simultaneously with lead, 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 complex in vitro with other heavy metals, such as copper and cadmium (Cramer et al., 1981). Therefore, similar complex formation may be anticipated with lead and thiamine, resulting in decreased tissue deposition. It was proposed that because thiamine structurally contains a pyrimidine and a thiazole nucleus and a hydroxyl group and a sulfur atom, the thiazole nuclei from two thiamine hydrochloride molecules might combine to form a lead citrate (Flora and Tandon, 1986). The present results sugest that the changes of electroshock seizure threshold by lead intoxication may be causede by changes of thiamine activity.

In summary, thiamine activities in all regions of rat brain were decreased following lead intoxication. Decrements of thiamine activities were reversed by simultaneous administration of thiamine with lead. Changes of electroshock seizure threshold induced by lead intoxication for 3 week and 7 week in neonatal rats was observed with the concomitant changes of thiamine content and transketolase activity in all the brain regions tested. The changes of myelin phospholipid and those of myelin protein content only in the cerebellum of 3 week old rats was observed with the concomitant changes of the seizure threshold as well as thiamine content due to lead intoxication.

The results from the present study suggest that neurotoxicity following lead intoxication may be mediated at least in part through the changes of thiamine dependent biochemical reaction due to lead intoxication. Such changes of thiamine activity may induce the changes of myelin composition such as myelin phospholipid and those of myelin protein content especially in the cerebellum of 3 week old rats which may eventually affect the threshold of seizure.

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Table 2. Transketolase activity in brain areas of 3, 7 and 16 Weeks age of Rats.

Brain region		Enzyme activity(nM/min/mg protein)			
		Control	Lead	Lead+Thiamine	Thiamine Def.
Telencephalon	3 weeks	5.78 ± 0.23	4.49 ± 0.10**	6.62 ± 0.12†	3.72 ± 0.21**
	7 weeks	5.10 ± 0.19	3.72 ± 0.14**	6.62 ± 0.12†	3.72 ± 0.21**
	16 weeks	6.97 ± 0.32	3.62 ± 0.25**	7.20 ± 0.13†	3.67 ± 0.25**
Brain stem	3 weeks	7.60 ± 0.38	5.71 ± 0.17**	7.92 ± 0.50†	4.64 ± 0.10**
	7 weeks	5.97 ± 0.36	4.13 ± 0.09**	6.81 ± 0.19†	4.06 ± 0.26**
	16 weeks	9.60 ± 0.67	5.37 ± 0.17**	9.44 ± 1.57†	5.36 ± 0.21**
Cerebellum	3 weeks	9.56 ± 0.44	7.70 ± 0.18**	8.00 ± 0.38†	4.73 ± 0.27**
	7 weeks	6.97 ± 0.30	5.04 ± 0.27*	8.05 ± 0.53†	4.92 ± 0.32*
	16 weeks	11.21 ± 0.86	7.05 ± 0.33**	11.01 ± 0.38†	6.91 ± 0.70**

Each value represents the mean ± S.E. of data from 5 experiments. * indicates a significant difference from control group (* : $p < 0.05$, ** : $p < 0.01$). † indicates a significant difference from lead treated group († : $p < 0.05$, ‡ : $p < 0.01$).

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	7 weeks	5.97 ± 0.36	4.13 ± 0.09**	6.81 ± 0.19†	4.06 ± 0.26**
	16 weeks	9.60 ± 0.67	5.37 ± 0.17**	9.44 ± 1.57†	5.36 ± 0.21**
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Each value represents the mean ± S.E. of data from 5 experiments. * indicates a significant difference from control group (* : $p < 0.05$, ** : $p < 0.01$). † indicates a significant difference from lead treated group († : $p < 0.05$, ‡ : $p < 0.01$).

Table 3. Effects of Lead intoxication on Electro-shock seizure.

Treatment	Convulsive Current (mA)				
	Control	Lead	Lead+Thiamine	Thiamine Def.	Thiamine Exc.
3 Weeks of age	13.05 (10.59-16.09)[a] N = 37[b]	9.45* (8.10-11.03) N = 30	15.18† (13.44-17.14) N = 16	8.32* (8.12-10.54) N = 13	12.42 (10.00-15.44) N = 37
7 Weeks of age	28.36 (25.38-31.68) N = 31	19.80* (17.41-22.52) N = 16	26.36† (25.31-27.46) N = 18	30.53 (29.55-31.54) N = 17	25.74 (24.37-27.19) N = 16
16 Weeks of age	49.31 (47.29-51.41) N = 20	41.88 (38.13-46.00) N = 17	44.89 (41.63-48.41) N = 17	38.36* (35.87-41.03) N = 18	45.10 (42.48-47.89) N = 18

[a] 95% confidence limits are shown in parenthesis. [b] N equals the number of anmals between 16% and 84% expected effects. * Significantly different from control CC_{50} ($P < 0.05$) † Significantly different from Lead CC_{50}($P < 0.05$)