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Elimination of Lead by TTFD and TPD from Central Nervous System of Postnatally Lead-exposed Rats

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출생후 납중독흰쥐에서 TTFD 및 TPD에 의한 중추신경계 납의 제거 작용에 관한 연구

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ABSTRACT

Amount of lead burden in a tissue reflects poisoning of lead in that tissue, so is the removal of lead directly connected to curement of lead poisoning. The purpose of present study was to investigate the relative effects of penicillamine and thiamine tetrahydrofurfuryl disulfide (TTFD) or thiamine propyl disulfide (TPD) in the removal of lead from rat brain tissue treated with excessive lead. Wistar rat pups of both sexes were used in this experiment. Within 1 day of parturition, experimental mothers nursing their pups as well as rat pups were given drinking water containing 0.2% lead acetate, TTFD 20 mg/1.2 L (2 mg/kg/day), TPD 20 mg/1.2 L (2 mg/kg/day), penicillamine 40 mg/1.2 L (40 mg/kg/day), 0.2% lead acetate+TTFD 20 mg/1.2 L (2 mg/kg/day), 0.2% lead acetate+TPD 20 mg/1.2 L (2 mg/kg/day) or 0.2% lead acetate+penicillamine 40 mg/1.2 L (40 mg/kg/day) ad libitum, throughout the entire period of experiment. Rat pups in the control group received normal tap water. The animals were sacrificed by decapitation on the day when they become 2 or 8 weeks of age. Brains were dissected into five regions: telencephalon, diencephalon, midbrain, pons/medulla and cerebellum. The dissected brain tissues were lyophillized and then solubilized by acid mixture (nitric acid+sulfuric acid). Lead levels in the solubilized brain tissues were measured by the

inductively coupled plasma.

In lead-exposed rats, lead levels were significantly higher than those of control group in all brain regions, lead levels in brain regions of TTFD or TPD group were generally lower than those of control group. The simultaneous administration of lead with TTFD or TPD to animals caused significant decrement of lead from all brain regions. In the elimination of lead from brain regions, effectiveness of TTFD or TPD was equivalant to penicillamine.

INTRODUCTION

Lead (Pb) is one of the most commonly incriminated environmental pollutants, both organic and inorganic form of lead are absorbed in blood by active and passive transport system [1,2]. In the circulation, 95% of lead exists in the red blood cells[1,2]. The lead in erythrocytes has a half life of 35 days and distributes into soft tissue or bone stores[1,2]. About 10% of the total body lead burden equilibrates in soft tissues (kidney, liver, nervous tissues, etc.)[1,2]. The pathogenesis of lead intoxication has not been fully elucidated, but it has been known that the central nervous system is the most sensitive target of lead intoxication by increased lead absorption, and thus clinical and experimental studies of lead poisoning has focused on the neuropsychiatric effect[3~5]. Lead poisoning related with lead concentration and then amount of the tissue lead burden reflect lead poisoning of the tissue[6~8].

Artificial chelating agents are utilized in treatment of lead poisoning, but these compounds are by no means totally effective and not safe[2]. So, vitamines, a naturally occuring body compounds which readily inter the cell, which are not toxic and which effectively interact with lead may play a role in lead poisoning[9~11]. With this background we designed the present experiment to investigate the relative effects of penicillamine and thiamine tetrahydrofurfuryl disulfide (TTFD) or thiamine propyl disulfide (TPD) in the removal of lead from rat brain tissue treated with excessive lead.

MATERIAL AND METHOD

Wistar rat pups of both sexes which male and female rats supplied from the Laboratory Animal Center of Seoul National University breed at 13 weeks of age were used. Within 1 day of parturition, experimental mothers nursing their pups and rat pups were given drinking water containing lead acetate 0.2%, TTFD 20 mg/1.2 L (2 mg/kg/day), TPD 20 mg/1.2 L (2 mg/kg/day), penicillamine 40 mg/1.2 L (40 mg/kg/day), 0.2% lead+TTFD 20 mg/1.2 L (2 mg/kg/day), 0.2% lead+TPD 20 mg/1.2 L (2 mg/kg/day), or 0.2% lead+penicillamine 40 mg/1.2 L (40 mg/kg/day) ad libitum, throughout the experiment. In all cases the pups were normalized to 10 beings and were separated from their dams at 3 weeks after birth. Rat pups in the control group received normal tap water. The animals were sacrificed by decapitation between 10 and 11 A.M. of the day when they become 2 and 8 weeks of age. Brains were rapidly removed from animals and dissected by the method of Glowinski and Iverson (1966) into five regions: telencephalon, diencephalon, midbrain, pons/medulla and cerebellum (At 2 weeks of age, brains were dissected

into four regions; telencephalon, diencephalon/midbrain, pons/medulla and cerebellum because of the small size of whole brain)[17]. Process to digest brain tissue was basically adopted from that or Barry (1975). Whole brain tissues were lyophillized and then digested overnight in acid mixture of nitric acid and sulfuric acid. After standing, the sample which was yellowish solution with foam was sonicated for 2 hours, and then the sample which was yellowish clear solution was diluted by distilled and deionized water (DDW) to make the total volume of solution 80 ~100 ml. Concentration of lead tissue was measured by the ICP (Perkin-Elmer, USA) method. The sample was analyzed with ICP for lead at the emission wavelength: 220.353. Selected wavelength was recommended by the instrument manufacturer, and represents the wavelength giving the stronggest signal to noise ratio. The instrument was calibrated daily with external standard (Junsei chemical Co., Japan). Nominal recoveries for lead was 97+1.2% and recovery efficiency for lead was determined at 1 ppm. The lower limit of sensitivity was 0.1 μg/ml and lead level were expressed ng/g wet tissue weight[9,12].

Results were analysed by the Anova test and Post-Hoc test (Newman-Keuls test). Differences between means were considered statistically significant when the calculated p values were less than 0.01.

RESULTS

The effect of vitamine to remove lead from various brain regions of rats intoxicated to lead was summarized in Table 1 and Table 2. In lead exposed rats, lead levels were significantly higher than those of control group in all brain regions, lead levels of TTFD or TPD group were generally lower than those of control group. Simultaneous treatment of lead-exposed animals with TTFD or TPD caused significant decrease in lead concentrations from all brain regions. In the uptake prevention of lead by brain regions, effectiveness of TTFD or TPD is equivalant to penicillamine, there is no difference between effectivenesses of TTFD and TPD, or between brain regions. The order of

Table 1. The effect of TTFD and TPD on lead levels in brain regions of rat exposed to lead for two weeks.

Brain Region	Brain Lead Level (ng/g tissue)						
	Control	TPD	TTFD	Lead	Lead+ TPD	Lead+ TTFD	Lead+ Penicillamine
Telencephalon	280.6± 40.7	145.5± 4.0	218.2± 20.0	698.2± 21.5	335.9± 7.9*	342.9± 15.2*	330.9± 30.1*
Diencephalon & midbrain	277.9± 21.1	193.4± 12.0	213.3± 9.4	694.5± 13.9	376.2± 17.7*	361.3± 7.1*	319.9± 11.7*
Pons-Medulla	258.1± 13.1	159.5± 4.6	235.7± 29.4	795.8± 34.4	345.1± 11.4*	362.2± 7.1*	320.1± 21.3*
Cerebelium	293.5± 30.2	179.9± 17.5	186.8± 24,1	741.3± 12.3	363.9± 41.2*	350.7± 10.5*	300.5± 12.2*

Lead intoxication to rats was carried out by supplying drinking water containing 0.2% lead acetate.
TTFD or TPD (2 mg/kg body weight/day) was administered to rats through drinking water.
Each value represents the mean±SE of data from 3~5 experiments.
Each experiment was carried out using tissues pooled from 5 animals.
*$p<0.01$ by comparision with lead group.

Table 2. The effect of TTFD and TPD on lead levels in brain regions of rat exposed to lead for eight weeks.

Brain Region	Brain Lead Level (ng/g tissue)						
	Control	TPD	TTFD	Lead	Lead+TPD	Lead+TTFD	Lead+ Penicillamine
Telencephalon	327.8±27.2	205.7±46.2	278.3±21.2	1011.8±156.0	331.4±10.5*	307.4± 9.2*	339.5±52.3*
Diencephalon	284.1± 9.1	201.8±12.6	241.9±13.2	932.9± 99.5	413.4±53.3*	321.2±33.6*	337.2±20.5*
Midbrain	379.7±20.7	241.0± 8.6	215.8±50.9	628.2± 50.5	311.4± 8.9*	320.0±50.6*	322.0±14.9*
Pons-Medulla	364.4±57.0	241.9±19.6	229.2±36.0	682.4± 36.9	391.6±14.9*	343.4±46.6*	337.2±38.7*
Cerebellum	343.9±24.8	195.3±15.8	232.0±30.0	619.9± 34.0	428.6±30.5*	297.1±22.2*	335.4±34.1*

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*$p<0.01$ by comparision with lead group.

lead level in the brain regions between groups of animals was TTFD≒TPD<Control≒TTFD+Lead≒TPD+Lead≒Penicillamine+Lead< Lead group.

DISCUSSION

Thiamine has been found to prevent tissue accumulation of lead, clinical signs of lead poisoning and death in calves[8,9]. In the lead removal effect, combination of thiamine and ascorbic acid is more effective than single treatment of thiamine, and the lead removal action of thiamine is impertinent to prevent and treat lead intoxication[8]. But this study suggests that the lead removal effect of thiamine alkyl disulfide, TTFD or TPD is pertinent to prevent as well as to treat lead intoxication with respect to lead concentration in the brain tissue. Absorbed thiamine alkyl disulfide enters to red blood cell, and is decomposed to thiamine and -SR group, and then the -SR group combine with metal to produce stable compound, mercaptide[13,14]. Other reports showed that -SR group released from TTFD or TPD in the red blood cell bind to methyl mercury and represents effect of mercury removal without action for essential minerals[15,16]. In the present study, lead-removal action of TTFD or TPD in the brain tissue may also be caused by -SR group which might directly bind to organic or inorganic lead.

CONCLUSION

In the rat, lead administered through drinking water evenly accumulate to whole brain regions. Simultaneous treatment of lead-exposed animals with TTFD or TPD caused significant removal of lead from all brain regions following either short term treatment (2 weeks) or long term treatment (8 weeks). In the removal of lead from brain regions, effectiveness of TTFD or TPD is equivalant to that of penicillamine.

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