12562 J. Agric. Food Chem. 2010, 58, 12562–12567
DOI:10.1021/jf1034509

JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY
ARTICLE

Inhibition Effect of Poly(γ-glutamic acid) on Lead-Induced Toxicity in Mice

T. L. WANG,[†,∥] T. H. KAO,[‡,∥] B. STEPHEN INBARAJ,[‡] Y. T. SU,[‡] AND B. H. CHEN*[,‡,§]

[†]Department of Emergency Medicine, Shin Kong Hospital, Taipei, Taiwan, [‡]Department of Food Science, Fu Jen University, Taipei, Taiwan 242, and [§]Graduate Institute of Medicine, Fu Jen University, Taipei, Taiwan 242. [∥] These authors contributed equally to this article.

The objectives of this study were to evaluate the efficiency in treatment of lead-induced intoxication in mice with γ-PGA as chelating agent and compare with the drug (*meso*-2,3-dimercaptosuccinic acid). The results showed the incorporation of γ-PGA at 200 and 400 mg/kg could reduce the accumulation of lead in the liver, heart, and testis; however, the latter was more effective in decreasing the lead content in the kidney and spleen. Nevertheless, both doses failed to inhibit the lead accumulation in the lung and brain. Additionally, both doses of γ-PGA could reduce TBARs in the kidney and brain, as well as elevate δ-aminolevulinic acid dehydrase (δ-ALAD) activity in blood and decrease glutamic pyruvic transaminase (GPT) and lactic dehydrogenase (LDH) activities in the serum. For hematological parameters, both white blood cells (WBCs) and hematocrite (HCT) were raised by 400 mg/kg of γ-PGA, while for both doses of γ-PGA, a slight decline in hemoglobin (HGB), mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) was observed, with the red blood cells (RBCs) being unaffected.

KEYWORDS: Poly(γ-glutamic acid); chelating agent; lead-induced intoxication; *meso*-2,3-dimercaptosuccinic acid

INTRODUCTION

Owing to rapid industrialization and agriculture modernization, environmental pollution, especially for heavy metal contamination in foods, has posed a major threat to human health. It has been well established that the nonessential heavy metals such as mercury, lead, and cadmium can be highly toxic even at low concentration, leading to the dysfunction of the kidney, liver, heart, and immune and nervous systems through the interaction between heavy metals and other metal ions in the human body (*1*).

Patients with heavy metal poisoning can be properly treated through the intake of chelating agents, including British anti-lewisite (BAL), *meso*-2,3-dimercaptosuccinic acid (DMSA), sodium 2,3-dimercaptopropane 1-sulfonate (DMPS), tetrahydrofurfural disulfide (TTFD), and ethylenediamine tetraacetic acid (EDTA). However, these synthetic chelating agents may produce side effects (*2*). To remedy the problem, in the past decade the application of natural polymers such as cellulose in wheat bran and fruit as chelating agent was explored (*3*). But, the chelating efficiency remains questionable.

Many studies have pointed out that during lead poisoning, a high oxidative stress can be produced to cause the dysfunction of human organs and tissues (*4*), mainly through the formation of a large amount of reactive oxygen species (ROS) such as hydroxyl radical (HO$\cdot$), hydrogen peroxide (H_2O_2), superoxide radical ($O_2\cdot$), and lipid peroxide (LPO). The presence of ROS at high concentration can be cytotoxic, for instance, the δ-ALAD activity can be inhibited, resulting in the accumulation of aminolevulinic acid (ALA) and in turn producing more free radicals for lipid oxidation on cell membrane (*5*).

Poly(γ-glutamic acid) (γ-PGA), a natural biopolymer synthesized by microorganism *Bacillus* sp., is composed of many D- and L-forms of glutamic acid units in γ-peptide linkage. As γ-PGA is biodegradable and nontoxic, it finds its application in a wide range of fields. For example, γ-PGA can be a thickening and stabilizing agent in the food industry, an encapsulating agent in both the food and pharmaceutical industries, humectants in the cosmetic industry, and a metal chelating agent in the environmental industry (*6*, *7*). The adsorption efficiency of γ-PGA by binding with toxic compounds such as organic dyes and heterocyclic amines has been well documented (*8*−*11*). In a recent study, Siao et al. (*12*) demonstrated γ-PGA to be effective in chelating lead and cadmium in vitro. However, the efficiency of γ-PGA in chelating heavy metals in vivo remains unknown. The objective of this study was thus undertaken to evaluate the chelating efficiency of lead by γ-PGA with mice as the animal model.

MATERIALS AND METHODS

Chemicals and Reagents. Poly(γ-glutamic acid) (Na-form, MW 1230 KDa) was a gift from Vedan Enterprise Corporation (Taichung, Taiwan). Lead acetate was procured from J.T. Baker Co. (Philipsburg, NJ, USA). D-(+)-Dextrose, *meso*-2,3-dimercaptosuccinic acid (DMSA), dimethyl sulfoxide (DMSO), hydrogen peroxide, ethylenediamine tetraacetic acid (EDTA), sodium dodecyl sulfate (SDS), and sodium dihydrogen phosphate were purchased from Sigma (St. Louis, MO, USA). Dimethyl aminobenzaldehyde, acetic acid, 2-thiobarbituric acid, perchloroic acid, and disodium hydrogen phosphate were from Fluka Chemical Co. (Buchs, Switzerland).

*To whom correspondence should be addressed. Phone: 886-2-29053626. Fax:886-2-29021215. E-mail: 002622@mail.fju.edu.tw.

 Published on Web 11/10/2010 © 2010 American Chemical Society

Seventy percent nitric acid was from Lan-Scan Asia Co. (Bangkok, Thailand). 5-Aminolevulinic acid hydrochloride and trichloroacetic acid were from Merck (Darmstadt, Germany). GOT, GPT, and LDH test kits were from Fujifilm Co (Saitama, Japan). Ninety-five percent ethanol was from Taiwan Tobacco and Wine Co. (Tainan, Taiwan). Deionized water was obtained using a Milli-Q water purification system from Millipore Co. (Bedford, MA, USA).

Instruments. The atomic absorption spectrophotometer (model 932) was from GBC Co. (Victoria, Australia). The ultrasonic cell disruptor/homogenizer (digital sonifier) was from Branson Co. (Danbury, CT, USA). The ELISA reader (VersaMax) was from Molecular Devices Co. (Sunnyvale, CA, USA). The hematology analyzer (Vet abc) was from SCIL Co. (Viernheim, Germany). The clinical analyzer (FUJI DRI-CHEM 3000) was from Fujifilm Co. (Saitama, Japan).

Preparation of Reagents. *Lead Acetate Solution.* Six hundred milligrams of lead acetate powder was mixed with 60 mL of 5% D-(+)-dextrose solution to obtain a concentration of 10 mg/mL. Then mice were injected with 0.1 mL of lead acetate solution subcutaneously with a dose of 50 mg/kg BW.

γ-PGA Solution. Two hundred milligrams and 400 mg of γ-PGA powder were mixed with 5 mL of sodium phosphate buffer solution separately to obtain a concentration of 40 and 80 mg/mL. Then mice were injected with 0.1 mL of γ-PGA solution intraperitoneally with a dose of 200 mg/kg BW and 400 mg/kg BW.

DMSA (meso-2,3-Dimercaptosuccinic Acid) Solution. One hundred milligrams of DMSA powder was mixed with 5 mL of DMSO to obtain a concentration of 20 mg/mL. Likewise, mice were injected with 0.1 mL of DMSA solution intraperitoneally with a dose of 100 mg/kg BW.

Experimental Animals. The animals used were SPF (specific pathogen free) male BALB/c mice (8 week old) and were purchased from BioLASCO (Taipei, Taiwan). A total of 40 mice were housed in ventilation cages in the animal center of the Department of Nutrition, Fu Jen University, with the temperature at 22 °C and the relative humidity at 60 ± 20%. Mice were fed a laboratory rodent diet 5001 (LabDiet Co, St. Louis, MO, USA) ad libitum. Lead-free distilled water was replaced every week. After 1 week of adaptation, 8 mice were randomly collected and used as normal treatment (Group I), which were subjected to subcutaneous injections of 0.1 mL of 5% D-(+)-dextrose solution for 15 days, followed by intraperitoneal injections of 0.1 mL of sodium phosphate buffer solution for 5 days, blood collection, and then sacrificing. The other 32 mice were injected with lead acetate solution subcutaneously at a dose of 50 mg/kg BW everyday for 15 days, after which they were divided into 4 groups randomly with 8 each for the following treatments for 5 days: Group II (control treatment), IP injection of 0.1 mL of sodium phosphate buffer solution; Group III, IP injection of 200 mg/kg BW of γ-PGA solution; Group IV, IP injection of 400 mg/kg BW of γ-PGA solution; Group V, IP injection of 100 mg/kg BW of DMSA solution. Next, blood was collected from each group and the mice sacrificed.

Animal Sample Collection. Blood was collected from the submandibular region of mice by using an animal lancet, followed by pouring into a microcentrifuged tube containing 1% EDTA solution and mixing, storing at 4 °C, and measuring δ-ALAD and hematological parameters within 48 h. In addition, the whole blood was stored at 4 °C overnight and then centrifuged at 2000 rpm for 10 min at 4 °C for serum separation, which was used for the determination of GOT, GPT, and LDH. After sacrificing, the mice organs including the brain, heart, lung, liver, kidney, and testis were collected and stored at −80 °C prior to use.

Analysis of Tissue Lead Concentration. All of the organs were dried in an oven at 80 °C for 24 h, after which each organ was weighed and recorded. Then each organ was poured into a glass test tube, followed by the addition of 1 mL of 70% nitric acid solution and digestion in a 80 °C water bath for 6 h. Next, 1 mL of hydrogen peroxide was added to each tube, and nitric acid was evaporated by heating. Finally, the residue was diluted with 2% nitric acid solution to 2 mL and filtered through a 0.2 μm membrane filter, and 1 mL was collected for lead determination by an atomic absorption spectrophotometer.

Analysis of Tissues TBARs. A method based on Saxena et al. (*13*) was modified. Each organ was mixed with a 5-fold volume of 0.15 M sodium chloride solution, and the mixture was homogenized for 30 s and centrifuged at 6200 rpm for 10 min at 4 °C, after which the supernatant was collected for use. Then 7 concentrations of 100, 50, 25, 12.5, 6.25, 3.125, and 1.563 mM of tetramethoxypropane in sulfuric acid solution were prepared, and 0.1 mL each was collected and mixed with the supernatant in a microcentrifuged tube, followed by the addition of 20 μL of 8.1% SDS solution, 150 μL of 20% acetic acid solution (pH 3.5), 150 μL of 0.8% TBA solution, and 80 μL of deionized water, after which the mixture was heated in a 95 °C water bath for 1 h, cooled in ice for 2 min, and centrifuged again at 4000 rpm for 10 min at 4 °C. The supernatant (200 μL) was collected for absorbance measurement at 532 nm. The TBARs of each organ was obtained on the basis of the standard curve of the tetramethoxypropane solution.

Table 1. Effect of γ-PGA and DMSA on Tissue Lead Concentration in Lead-Exposed Mice[a]

	lead concentration (μg/g)				
	normal	control	DMSA (100 mg/kg)	γ-PGA (200 mg/kg)	γ-PGA (400 mg/kg)
liver	ND	2.33 ± 0.18 A	1.23 ± 0.13 D	1.73 ± 0.16 B	1.44 ± 0.08 C
lung	ND	0.17 ± 0.06 C	0.14 ± 0.05 C	0.63 ± 0.18 A	0.31 ± 0.07 B
kidney	ND	5.04 ± 0.52 A	2.04 ± 0.23 C	5.02 ± 0.36 A	3.96 ± 0.40 B
spleen	ND	1.39 ± 0.13 A	1.35 ± 0.08 AB	1.40 ± 0.16 A	1.27 ± 0.10 B
heart	ND	0.13 ± 0.05 A	0.03 ± 0.02 B	0.02 ± 0.01 B	0.03 ± 0.01 B
brain	ND	0.08 ± 0.01 B	1.19 ± 0.02 A	0.08 ± 0.02 B	0.09 ± 0.03 B
testis	ND	0.03 ± 0.00 B	0.04 ± 0.01 A	0.01 ± 0.00 C	ND

[a] Mean of duplicate analyses ± standard deviation of eight animals per group. ND: not detected. Data with different letters (A−D) in the same row are significantly different ($p < 0.05$).

Analysis of Blood δ-Aminolevulinic Acid Dehydratase (δ-ALAD) Activity. A method based on Saxena et al. (*13*) was modified. Mouse blood (0.05 mL) was mixed with 0.1 mL of deionized water, and the mixture stood at room temperature for 10 min. Then 0.05 mL of 0.1 M ALA solution was added, and the mixture was reacted at 37 °C in a water bath for 30 min, followed by the addition of 0.2 mL of Ehrlich's reagent, homogenization, and standing at room temperature for 15 min, collecting 0.15 mL, and measuring the absorbance at 553 nm. The δ-ALAD activity was obtained on the basis of the ratio of each treatment to normal treatment.

Analysis of Serum GOT, GPT, and LDH Levels. Ten microliters of serum sample was collected for the determination of GOT, GPT, and LDH levels by a clinical analyzer following the instructions by Fujifilm Co.

Analysis of Clinical Hematological Parameters. The whole blood of each mouse was collected for the analysis of WBC, RBC HGB, HCT, MCV, MCH, and MCHC by a hematology analyzer.

Statistical Analysis. All of the analyses were done in duplicate, and the data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test for mean comparison (α = 0.05) by using SAS (*14*).

RESULTS

Effect of γ-PGA and DMSA on Tissue Lead Accumulation. **Table 1** shows the lead accumulation in various organs of lead-poisoning mice after treatment of DMSA and γ-PGA for 5 days. Following the subcutaneous injection of lead acetate, a large amount of lead (5.04 μg/g) was deposited in the kidney, followed by the liver (2.33 μg/g), spleen (1.39 μg/g), lung (0.17 μg/g), heart (0.13 μg/g), brain (0.08 μg/g), and testis (0.03 μg/g). After DMSA treatment, the lead contents in the liver, kidney, and heart were reduced significantly, but the other organs showed no significant change. Similarly, with 200 or 400 mg/kg of γ-PGA, the lead levels declined substantially in the liver, heart, and testis; however, for the kidney and spleen, only a dose of 400 mg/kg γ-PGA was effective. Interestingly, both doses of γ-PGA remained ineffective in reducing the lead level in the lung and brain. Comparatively, γ-PGA was more efficient in lead reduction in the brain and testis than DMSA, whereas the latter was more effective in the liver, lung, and kidney.

Effect of γ-PGA and DMSA on Tissue TBARs. **Table 2** shows the effect of γ-PGA on tissue TBARs in lead-exposed mice. For the normal treatment, a peak of TBARs was found in the kidney,

Table 2. Effect of γ-PGA and DMSA on Tissue TBARs in Lead-Exposed Mice[a]

	TBARs (nmol/g)				
	normal	control	DMSA (100 mg/kg)	γ-PGA (200 mg/kg)	γ-PGA (400 mg/kg)
kidney	1326.7 ± 81.4 B	1641.3 ± 76.7 A	1341.3 ± 90.7 B	1331.4 ± 69.9 B	1316.6 ± 42.5 B
brain	981.3 ± 90.4 B	1325.1 ± 171.0 A	646.7 ± 205.5 C	644.9 ± 53.6 C	438.5 ± 22.8 D
liver	427.9 ± 12.0 B	459.3 ± 52.3 AB	426.7 ± 18.2 B	477.9 ± 20.0 A	474.4 ± 19.7 A

[a] Mean of duplicate analyses ± standard deviation of eight animals per group. Data with different letters (A−D) in the same row are significantly different ($p < 0.05$).

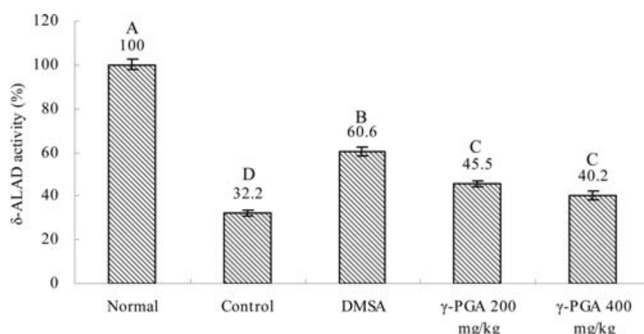

Figure 1. Effect of γ-PGA and DMSA on blood δ-ALAD activity in lead-exposed mice. The data are presented as the mean of duplicate analyses ± standard deviation of eight animals per group.

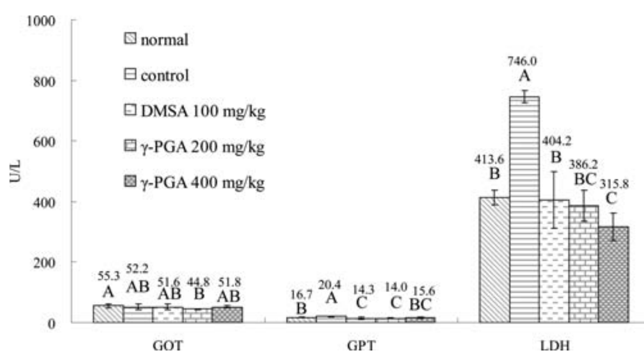

Figure 2. Effect of γ-PGA and DMSA on serum GOT, GPT, and LDH in lead-exposed mice. The data are presented as the mean of duplicate analyses ± standard deviation of eight animals per group.

followed by the brain and liver. But, with DMSA or γ-PGA treatment, the TBARs in the kidney and brain were reduced markedly. Interestingly, there was no significant difference in TBARs in the kidney between DMSA and γ-PGA. Conversely, a high dose of γ-PGA (400 mg/kg) showed a more distinct effect in inhibiting TBARs formation in the brain than a low dose of γ-PGA (200 mg/kg) and DMSA (100 mg/kg). Nevertheless, both DMSA and γ-PGA remained ineffective in inhibiting TBARs in the liver.

Effect of γ-PGA and DMSA on Blood δ-ALAD Activity. Figure 1 shows the effect of γ-PGA and DMSA on blood δ-ALAD activity in lead-exposed mice. One hundred percent activity was shown in mice blood without lead injection (normal treatment) but reduced to 32.2% after IP injection of 50 mg/kg BW of lead acetate for 15 days (control treatment). After treatment of DMSA at 100 mg/kg BW and γ-PGA at 200 and 400 mg/kg BW, the activities were raised to 60.6, 45.5, and 40.2%, respectively, all of which were significantly higher than the control treatment.

Effect of γ-PGA and DMSA on Serum GOT, GPT, and LDH Levels. Both GOT and GPT activities are frequently used as an index of liver function. The effect of γ-PGA and DMSA on serum GOT, GPT, and LDH levels is shown in **Figure 2**. No significant difference in GOT activity was found for all of the treatments, but with lead treatment, both GPT and LDH activities were raised significantly and then declined significantly after DMSA or γ-PGA treatment. Furthermore, with a dose of γ-PGA at 400 mg/kg BW, the GPT activity could be reduced to a normal level; so was the LDH activity when treated with γ-PGA at two doses or DMSA. This outcome indicated that both γ-PGA and DMSA were effective in preventing liver dysfunction in mice induced by lead.

Effect of γ-PGA and DMSA on Clinical Hematological Parameters. Table 3 shows the effect of γ-PGA on hematological parameters in lead-exposed mice, including WBC, RBC, HGB, HCT, MCV, MCH, and MCHC, which were in the range from 4.07 to $6.05 \times 10^3/mm^3$, $9.05-9.66\times10^3/mm^3$, 13.22−15.03 g/dL, 44.70−48.47%, 49.50−51.00 fL, 14.57−16.07 pg and 29.25−31.23 g/dL, respectively. All of these values were in the normal range, implying no significant change in these clinical hematological parameters after treatments of DMSA, γ-PGA, or lead. Nonetheless, a slight decline in HGB, MCV, MCH, and MCHC occurred for lead treatment. In addition, a high dose of γ-PGA (400 mg/kg BW) caused a rise in WBC and a decrease in MCHC when compared to DMSA and a low dose of γ-PGA (200 mg/kg BW).

DISCUSSION

Lead is widely present in the environment and has been reported to be highly toxic to the kidney, liver, skeleton, and central nervous system (*15*). The chelating agents used to treat lead-poisoning patients clinically include $CaNa_2EDTA$, dimercaprol (British antilewisite, BAL), DMSA, monoisoamyl DMSA (MiADMSA), and 2,3-dimercapto-1-propanesulphonic acid (DMPS). However, as mentioned before, these synthetic chelating agents may produce side effects, and thereby the development of natural chelating agents for lead-poisoning therapy has become an urgent problem to solve (*4*, *16*, *17*).

The effect of DMSA on lead chelating was reported in several studies. With a dose of DMSA at 50 mg/kg, the lead contents in the kidney, liver, and brain of rats were reduced substantially (*18*). Comparatively, a high dose of DMSA (100 mg/kg BW) showed a more pronounced effect in lead chelating than a low dose of DMSA (50 mg/kg BW) in the liver, kidney, and brain of mice (*19*). In our experiment, the biopolymer γ-PGA contains many carboxyl functional groups, being responsible for lead chelating. In a previous study, Siao et al. (*12*) demonstrated the chelating capability of γ-PGA toward lead and cadmium in vitro. In this study, we proved a high dose of γ-PGA at 400 mg/kg BW to be efficient in diminishing lead levels in the liver, kidney, spleen, heart, and testis of mice. Nonetheless, γ-PGA did not show a dose-dependent response toward lead binding, which may be explained as follows: Theoretically, according to the Langmuir isotherm, solutes at low concentration are more readily adsorbed onto a known amount of adsorbent with the binding capacity reaching a peak at a saturation solute concentration and attaining a plateau thereafter. Thus, in our experiment, a dose of 200 mg/kg of γ-PGA was effective in adsorbing lead, indicating that a maximum binding capacity was attained, and thereby a high dose of 400 mg/kg of γ-PGA failed to enhance the lead adsorption. Also, on the basis of a study by Siao et al. (*12*), in an in vitro experiment, a low dose of γ-PGA was relatively more efficient in binding lead than a high dose of γ-PGA;

Table 3. Effect of γ-PGA and DMSA on Hematological Parameters in Lead-Exposed Mice[a]

	normal	control	DMSA (100 mg/kg)	γ-PGA 200 mg/kg	γ-PGA 400 mg/kg
WBC ($\times 10^3$/mm^3)	5.20 ± 0.78 B	4.55 ± 0.51 BC	4.75 ± 0.89 BC	4.07 ± 0.45 C	6.05 ± 0.71 A
RBC ($\times 10^6$/mm^3)	9.60 ± 0.55 AB	9.66 ± 0.27 A	9.32 ± 0.23 AB	9.05 ± 0.42 B	9.56 ± 0.63 AB
HGB (g/dL)	15.03 ± 0.56 A	14.20 ± 0.85 B	14.12 ± 0.57 B	13.22 ± 0.22 C	13.90 ± 0.94 BC
HCT (%)	48.27 ± 1.63 A	48.47 ± 1.34 A	46.62 ± 0.90 AB	44.70 ± 2.00 B	47.68 ± 3.29 A
MCV (fL)	51.00 ± 0.00 A	50.33 ± 0.52 B	50.00 ± 0.00 BC	49.50 ± 0.55 C	49.50 ± 0.55 C
MCH (pg)	16.07 ± 0.40 A	15.35 ± 0.29 B	15.18 ± 0.19 B	14.73 ± 0.37 C	14.57 ± 0.27 C
MCHC (g/dL)	31.23 ± 0.91 A	30.48 ± 0.32 B	30.12 ± 0.43 B	30.07 ± 0.46 B	29.25 ± 0.58 C

[a] Mean of duplicate analyses ± standard deviation of eight animals per group. Data with different letters (A−C) in the same row are significantly different ($p < 0.05$).

thus, it may be postulated that a high concentration of γ-PGA may be more susceptible to agglomeration and aggregation through various intermolecular interactions, leading to a decline in lead binding capacity. This tendency may also result in the desorption of adsorbed metals from the γ-PGA surface, diminishing the binding capacity of lead. Additionally, it is also possible that a dose of 400 mg/kg of γ-PGA may possess a higher viscosity than 200 mg/kg of γ-PGA, resulting in a decrease in adsorption of γ-PGA by the abdominal cavity of mice and causing a decline in the binding capacity with lead. All in all, the complexity of metabolism in mice may affect the dose-dependent relationship between γ-PGA and lead. As γ-PGA is a large molecule with biodegradable characteristics, the absorption in the intestine should be retarded after complex formation of γ-PGA with lead.

It has been well documented that lead poisoning may be associated with oxidative stress, as lead may promote lipid oxidation in the brain and liver (*2*, *20*). Thus, it is quite possible that lead poisoning may be minimized through the inhibition of TBARs formation. Theoretically, the incorporation of antioxidants such as α-tocopherol or ascorbic acid should be the most efficient in retarding TBARs formation (*21*). However, as reported by Liao et al. (*19*), ascorbic acid was shown to be ineffective in lead chelating, implying that an indirect association may exist between lead chelating and TBARs formation.

In addition, the effectiveness of a chelating agent in preventing TBARs formation has been controversial. For instance, Saxena and Flora (*5*) reported that the chelating agents including $CaNa_2EDTA$, DMPS, MiADMSA, $CaNa_2EDTA$ + DMPS, and $CaNa_2EDTA$ + MiADMSA failed to reduce the kidney and brain TBARs in rats. In contrast, the liver TBARs could be reduced by DMSA, MmDMSA, and MchDMSA, while no significant effect was observed in the kidney and brain (*13*). This outcome indicated that the variety of chelating agent should be an imperative factor in inhibiting TBARs formation in various organs.

In our study, a maximum TBARs was found in the kidney, which is expected as the kidney is the main organ for lead metabolism. As illustrated in the preceding section, γ-PGA was effective in reducing the lead content in the kidney, which may be caused by the inhibition of TBARs formation. On the contrary, γ-PGA failed to decrease the lead level in the brain, but was shown to be efficient in inhibiting TBARs formation, implying again that the inhibition efficiency may be varied in different organs. Accordingly, most metals can be accumulated in the liver and kidney, as both are mainly responsible for the metabolism of toxic compounds. Nevertheless, the accumulation of metals in the liver and kidney can be varied depending on variety. For instance, in addition to the liver and kidney, cadmium was accumulated more readily in the lung, brain, testes, heart, and the central nervous system, whereas a large amount of mercury deposited in the kidney (*2*). This phenomenon may be associated with metal structure, organ size, and organ composition. Also, factors such as lead content, fat content, and oxidative stress can be responsible for TBARs differences in different organs. The mechanism in lead-induced oxidative stress may involve the following factors: (1) inhibition of δ-ALAD activity to cause ALA accumulation for ROS production, (2) stimulation of iron-induced lipid oxidation in the cell membrane, (3) combination of lead with phospholipid on the cell membrane, (4) formation of a complex between lead and ROS, (5) bonding between lead and the thiol group in glutathione to reduce antioxidant activity, and (6) enhancement of nNOS activity in the brain for NO formation to promote lipid oxidation (*2*, *15*, *22*, *23*).

It has been well established that the δ-ALAD activity in blood can be a vital index of lead poisoning, as ALA can be catalyzed by δ-ALAD for prophobilonogen formation and in turn generate coproporphyrinogen III for heme synthesis. Thus, a low dose (15 μg/dL) of lead in blood was adequate in inhibiting δ-ALAD activity for ALA accumulation and ROS production (*24*). Additionally, the ALA oxidation product 4,5-dioxovaleric acid may cause DNA damage through alkylation, interfere with the formation of γ-amino butyric acid for dysfunction of central nervous system, and affect hematopoiesis by lowering ferrochelatase activity for bonding inhibition between iron and the protoporphyrin ring (*2*, *20*, *25*).

The association between lead poisoning and δ-ALAD activity as affected by chelating agents was investigated by several authors. Of the various chelating agents studied, the δ-ALAD activity could be reversed by 32−56% after treatments of DMPS, $CaNa_2EDTA$ + DMPS, and $CaNa_2EDTA$ + MiADMSA (*5*). In a later study, Saxena et al. (*13*) reported that with DMSA at 100 mg/kg and MmDMSA at 50 and 100 mg/kg, the δ-ALAD activity failed to be reversed. Interestingly, with MchDMSA at 50 and 100 mg/kg, the δ-ALAD activity was reversed by 69 and 98%, respectively. A similar phenomenon was observed by Santos et al. (*26*), as no δ-ALAD activity was shown after treatments of BAL, DMSA, and DMPS. The authors further pointed out that the presence of chelating agent may inhibit δ-ALAD activity in the kidney and liver, and therefore, it would be more appropriate to measure the δ-ALAD activity in blood instead of the organs (*26*). In a study dealing with the effect of DMSA on δ-ALAD activity, Liao et al. (*19*) reported a dose of 100 mg/kg could reverse δ-ALAD activity by 74%, but a lower dose of 50 mg/kg did not show any effect. All of these results suggested that the δ-ALAD activity may be enhanced depending on dose and variety of chelating agents.

In our study, both doses of γ-PGA were effective in enhancing the δ-ALAD activity, though a dose-dependent response was not shown. Obviously only a portion of δ-ALAD activity could be restored even under treatment of chelating agent at high dose. Theoretically, the higher the chelating effect of γ-PGA with lead, the larger the chelating ability of γ-PGA with some other metal ions. As the δ-ALAD activity can also be affected by a zinc ion, the γ-PGA treatment may result in a decline in δ-ALAD activity because of its binding ability with the zinc ion. This phenomenon was observed by Siao et al. (*12*), showing that the bonding between γ-PGA and lead was inhibited in the presence of some other metal ions including Cu, Zn, Fe, Mn, and Ca. More specifically, a dose of 25 and 50 mg/L of zinc could inhibit the binding of γ-PGA with lead

in vitro, whereas a dose of 100 mg/L of zinc could promote the binding of γ-PGA with lead (*12*). However, since this is an in vitro study, the effect of metal ions on the chelating ability of γ-PGA with lead in mice needs further investigation.

Both GOT and GPT are the key enzymes in liver cells, which will swell to increase cell membrane permeability and denature cytoplasm when invaded by toxins. This would lead to cell necrosis or apoptosis and cause a release of GOT, GPT, and LDH from the cell into blood. Thus, the degree of liver dysfunction can be assessed by the activities of GOT, GPT, and LDH. In a similar study, El-Sayed et al. (*27*) reported that both GOT and GPT levels in the serum of mice were reduced after tannic acid treatment. In contrast, the incorporation of α-lipoic acid failed to decrease GOT and GPT levels in the serum of rats, but with vitamin E treatment, the GOT level could be reversed to a normal level (*18*). By comparison, our experiment demonstrated a better protective effect against liver damage in mice when treated with γ-PGA or DMSA.

Similar outcomes were reported in several other studies. Mugahi et al. (*28*) studied the effect of lead intoxication on blood indices in rats and found a significant drop in MCH and MCV, but a slight decrease in RBC and HGB, as well as a pronounced rise in WBC. Conversely, the hematological parameters RBC, MCV, MCH and MCHC remained unaffected in rats after lead treatment, but the WBC increase could be inhibited by $CaNa_2EDTA$, DMPS, MiADMSA and $CaNa_2EDTA$+DMPS (*5*). In another study Saxena et al. (*13*) observed a decrease in HCT, MCHC and HGB in rats after lead treatment, but MCV did not show any significant change. Additionally, both HCT and MCHC could be raised to a normal level after DMSA treatment. All these results suggested that the hematological parameters in blood may be varied depending on animal model, i.e., rat or mice, dose and variety of lead or chelating agent.

From the physiological point of view, lead can be absorbed through the respiratory system, digestive system, and skin, and then transported into blood, in which 99% lead can combine with RBC, with the remaining 1% lead in plasma being carried to the other tissues and organs to affect the hematopoietic and immune systems (*28*). After metabolism, lead may affect the activity of the erythrocyte enzymes such as glyceraldehyde 3-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase. Also, lead may affect the synthesis of RBC and HGB through the inhibition of enzyme activity associated with the cytoplasm, mitochondria, and heme synthesis. A typical example is the δ-ALAD activity inhibition by lead as described in the preceding section. Additionally, lead accumulation may induce microcytic anemia and hypochromia (*2*, *25*, *28*). It was inferred that lead could inhibit pyrimidine 5-nucleotidase activity for the accumulation of pyrimidine nucleotide in RBC and thereby accelerated RBC destruction. Several reports also pointed out the retardation of HGB synthesis in bone marrow by lead and the free radical formation through the reaction between lead and oxyhemoglobin for RBC lysis, both of which resulted in a decrease in RBC and HGB (*28*,*29*). In addition to RBC, leukocytosis also occurred in mice after lead treatment, which should be caused by lead-induced inflammation (*28*). However, a contradictory outcome was reported by Sharma et al. (*25*), as shown by a decrease in the number of WBCs, monocytes, and lymphocytes. This difference may be accounted for by the variety of stimuli and time of measurement during the experiment since cells may show a different response when stimulated to cause an increment in WBCs. Yet, at the same time, WBCs may be decreased because of lead toxicity. This phenomenon of monocytopenia or monocytosis has been reported by several authors (*28*). Sharma et al. (*25*) further postulated the lead-induced decrease in the number of WBCs to be associated with the necrosis of leucopoietic tissue, accumulation of lymphocytes in lymphoid tissue, destruction of lymphocytes by corticosteroid, and reduction of cell proliferation activity.

In conclusion, γ-PGA was effective in mitigating lead-induced toxicity in mice through the reduction of lead accumulation in various organs, decrease of TBARs formation, and prevention of δ-ALAD inactivation and liver dysfunction. The outcome of this experiment may provide a basis for possible clinical use of γ-PGA as a chelating agent instead of DMSA, a clinically used drug, amid the nontoxic and biodegradable nature of γ-PGA.

LITERATURE CITED

(1) Sabolic, I. Common mechanisms in nephropathy induced by toxic metals. *Nephron. Physiol.* **2006**, *104*, 107–114.

(2) Flora, S. J. S.; Mittal, M.; Mehta, A. Heavy metal induced oxidative stress & its possible reversal by chelation therapy. *Indian J. Med. Res.* **2008**, *128*, 501–523.

(3) Nawirska, A. Binding of heavy metals to pomace fibers. *Food Chem.* **2005**, *90*, 395–400.

(4) Wang, C. H.; Liang, N. C.; Zhang, C. L.; Bi, Y. Y.; Shi, X. L.; Shi, Q. Effect of ascorbic acid and thiamine supplementation at different concentrations on lead toxicity in liver. *Anal. Occup. Hyg.* **2007**, *51*, 563–569.

(5) Saxena, G.; Flora, S. J. S. Lead-induced oxidative stress and hematological alterations and their response to combined administration of calcium disodium EDTA with a thiol chelator in rats. *J. Biochem. Mol. Toxicol.* **2004**, *18*, 221–233.

(6) Ho, G. H.; Ho, T. I.; Hsieh, K. H.; Su, Y. C.; Lin, P. Y.; Yang, J.; Yang, K. H.; Yang, S. C. γ-Polyglutamic acid produced through *Bacillus subtilis* (natto): structural characteristics, chemical properties and biological functionalities. *J. Chin. Chem. Soc.* **2006**, *53*, 1363–1384.

(7) Chiu, Y. T.; Chiu, C. P.; Chien, J. T.; Ho, G. H.; Yang, J.; Chen, B. H. Encapsulation of lycopene extract from tomato pulp waste with gelatin and poly(γ-glutamic acid) as carrier. *J. Agric. Food Chem.* **2007**, *55*, 5123–5130.

(8) Inbaraj, B. S.; Chien, J. T.; Ho, G. H.; Yang, J.; Chen, B. H. Equilibrium and kinetic studies on sorption of basic dyes by a natural biopolymer poly(γ-glutamic acid). *Biochem. Eng. J.* **2006**, *31*, 204–215.

(9) Inbaraj, B. S.; Chiu, C. P.; Ho, G. H.; Yang, J.; Chen, B. H. Removal of cationic dyes from aqueous solution using an anionic poly-γ-glutamic acid-based adsorbent. *J. Hazard Mater. B.* **2006**, *137*, 226–234.

(10) Inbaraj, B. S.; Chiu, C. P.; Chiu, Y. T.; Ho, G. H.; Yang, J.; Chen, B. H. Effect of pH on binding of mutagenic heterocyclic amines by the natural biopolymer poly(γ-glutamic acid). *J. Agric. Food Chem.* **2006**, *54*, 6452–6459.

(11) Inbaraj, B. S.; Chiu, C. P.; Ho, G. H.; Yang, J.; Chen, B. H. Effects of temperature and pH on adsorption of basic brown 1 by the bacterial biopolymer poly(γ-glutamic acid). *Bioresour. Technol.* **2008**, *99*, 1026–1035.

(12) Siao, F. Y.; Lu, J. F.; Wang, J. S.; Inbaraj, B. S.; Chen, B. H. In vitro binding of heavy metals by an edible biopolymer poly(γ-glutamic acid). *J. Agric. Food Chem.* **2009**, *57*, 777–784.

(13) Saxena, G.; Pathak, U.; Flora, S. J. S. Beneficial role of monoesters of *meso*-2,3-dimercaptosuccinic acid in the mobilization of lead and recovery of tissue oxidative injury in rats. *Toxicology* **2005**, *214*, 39–56.

(14) *SAS 9.2 Foundation Software for Microsoft Windows*; SAS Institute, Inc.: Cary, NC, 2010.

(15) Sanders, T.; Liu, Y.; Buchner, V.; Tchounwou, P. B. Neurotoxic effects and biomarkers of lead exposure: a review. *Rev. Environ. Health.* **2009**, *24*, 15–45.

(16) Wang, C.; Zhao, M.; Yang, J.; Li, X.; Peng, S. Synthesis and evaluations of pentahydroxylhexyl-L-cysteine and its dimmer as chelating agents for cadmium or lead decorporation. *Toxicol. Appl. Pharmacol.* **2004**, *200*, 229–236.

(17) Aslani, M. R.; Najarnezhad, V.; Mohri, M.; Azad M. The effect of allicin on blood and tissue lead content in mice. *Comp. Clin. Pathol.* **2010** [online early access], DOI: 10.1007/s00580-010-0964-y.

(18) Pande, M.; Flora, S. J. S. Lead induced oxidative damage and its response to combined administration of α-lipoic acid and succimers in rats. *Toxicology* **2002**, *177*, 187−196.
(19) Liao, Y.; Jin, Y.; Lu, C.; Li, G.; Zhi, X.; An, L.; Yang, J. Selection of micronutrients used along with DMSA in the treatment of moderately lead intoxicated mice. *Arch. Toxicol.* **2008**, *82*, 37−43.
(20) Hsu, P. C.; Guo, Y. L. Antioxidant nutrients and lead toxicity. *Toxicology* **2002**, *180*, 33−44.
(21) Patra, R. C.; Swarup, D.; Dwivedi, S. K. Antioxidant effects of α tocopherol, ascorbic acid and L-methionine on lead induced oxidative stress to the liver, kidney and brain in rats. *Toxicology* **2001**, *162*, 81−88.
(22) Verstraeten, S. V.; Aimo, L.; Oteiza, P. I. Aluminium and lead: molecular mechanisms of brain toxicity. *Arch. Toxicol.* **2008**, *82*, 789−802.
(23) Bokara, K. K.; Brown, E.; McCormick, R.; Yallapragada, P. R.; Rajanna, S.; Bettaiva, R. Lead-induced increase in antioxidant enzymes and lipid peroxidation products in developing rat brain. *Biometals* **2008**, *21*, 9−16.
(24) Zhao, Y.; Wang, L.; Shen, H. B.; Wang, Z. X.; Wei, Q. Y.; Chen, F. Association between delta-aminolevulinic acid dehydratase (ALAD) polymorphism and blood lead levels: a meta-regression analysis. *J. Toxicol. Environ. Health A* **2007**, *70*, 1986−1994.
(25) Sharma, V.; Sharma, A.; Kansal, L. The effect of oral administration of *Allium sativum* extracts on lead nitrate induced toxicity in male mice. *Food Chem. Toxicol.* **2010**, *48*, 928−936.
(26) Santos, F. W.; Rocha, J. B. T.; Nogueira, C. W. 2,3-dimercaptopropanol, 2,3-dimercaptopropane-1-sulfonic acid and *meso*-2,3-dimercaptosuccinic acid increase lead-induced inhibition of δ-aminolevulinate dehydratase in vitro and ex vivo. *Toxicol. in Vitro* **2006**, *20*, 317−323.
(27) El-Sayed, I. H.; Lotfy, M.; El-Khawaga, O. A. Y.; Nasif, W. A.; El-Shahat, M. Prominent free radicals scavenging activity of tannic acid in lead-induced oxidative stress in experimental mice. *Toxicol. Ind. Health* **2006**, *22*, 157−163.
(28) Mugahi, M. N.; Heidari, Z.; Sagheb, H. M.; Barbarestani, M. Effects of chronic lead acetate intoxication on blood indices of male adult rat. *DARU* **2003**, *11*, 147−151.
(29) Razia, B.; Ragini, S.; Nazera, Q. Amelioration of lead-induced alterations in body weight and blood cell counts by antioxidant vitamins. *J. Herb Med. Toxicol.* **2007**, *1*, 59−66.

Received for review September 6, 2010. Revised manuscript received October 26, 2010. Accepted October 27, 2010.