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EFFECTS OF MESO-2,3-DIMERCAPTOSUCCINIC ACID, POTASSIUM IODIDE AND CHLOROPHYLL ON LEAD ACCUMULATION IN MALE MICE

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Abstract

Objectives: Lead (Pb) pollution is a serious public health problem all over the world, it especially plays severe damage role in children's health. Apart from reducing lead-induced damages, the decrease of lead accumulation is also critical. This study has been the first attempt to investigate effects of meso-2,3-dimercaptosuccinic acid (DMSA), potassium iodide (KI) and chlorophyll (Chl) on lead accumulation in male mice. **Material and Methods:** Eighty healthy Kunming male mice were selected and divided randomly into 8 groups. They were treated with lead acetate (PbAc) intraperitoneally, individually and in combination with the DMSA, KI or Chl once daily for 5 days. Meanwhile, the control group was treated with normal saline during the whole exposure period. On 30th day, mice were sacrificed and lead concentrations were detected in the whole blood, livers, kidneys, and testicles of mice by means of the graphite furnace atomic absorption spectrometry. **Results:** In comparison with the control group, lead concentrations increased in mice treated with the PbAc and DMSA, KI and Chl diminished lead accumulation in the whole blood, livers, and kidneys. Chl had specifically the same effects on lead concentrations in the testicles of male mice. **Conclusions:** Potassium iodide and Chl, as food additives, had the same effects as the DMSA to reduce lead accumulation in male mice effectively. Our results provided experimental evidence *in vivo* for the preventive measures of lead poisoning. Int J Occup Med Environ Health 2017;30(1):87–93

Key words:
Potassium iodide, Lead accumulation, Meso-2,3-dimercaptosuccinic acid, Chlorophyll, Food additives, Mice

INTRODUCTION

Lead (Pb) is a heavy metal and toxic to the heart, liver, kidney, reproductive and nervous systems. Industrial and mining activities release substantial amounts of lead and lead compounds into the air and soil, which gives rise to the increase of whole blood lead concentration in the human body [1]. Permanent neurological damage may occur at blood lead levels > 10 μg/dl, especially in children because of its continued use in paint and plastics such as polyvinyl chloride (PVC) and toys [2]. Adults may excrete the majority of lead within several weeks after an acute exposure but children tend to retain more lead for a longer period of time [3]. A great number of studies have suggested that lead exposure had been associated with low intelligent quotient, reduced short-term memory, decreased attention, reading and arithmetic ability, and social engagement in the children [4,5]. Therefore, the control

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of lead-induced toxicity has become an urgent public health issue.

In addition to reducing lead-induced toxic effects, diminishing lead contents accumulated in the body could fundamentally decrease lead toxicity. The therapeutic efficacy of thiol-chelating agent being meso-2,3-dimercaptosuccinic acid (DMSA) in reducing lead levels in the whole blood and other soft tissues has been demonstrated in many studies [6]. Meso-2,3-dimercaptosuccinic acid could be given through oral route but the hydrophilic and lipophilic properties of the DMSA do not allow it to cross the cell membrane, therefore, it is one of the least toxic drugs and ideal for the treatment of lead poisoning [7].

Potassium iodide (KI) is an inorganic compound which is less hygroscopic than sodium iodide, making it easier to be added in daily salt. Potassium iodide is the most common additive used for "iodizing" table salt as a nutritional supplement, to prevent iodine deficiency for populations which get little seafood. Chlorophyll (Chl) is the abundant and widely distributed botanic pigment which is in human diet, especially in vegetables and fruits [8]. Several studies have demonstrated that antioxidative effects of Chl may prevent oxidative damage and subsequent DNA damage [9,10]. Both KI and Chl could be used as food additives in daily diet, however, their effects on lead absorption have not been studied.

MATERIAL AND METHODS

Materials

Lead acetate (PbAc), meso-2,3-dimercaptosuccinic acid (DMSA), potassium iodide (KI) and chlorophyll (Chl) were purchased from Sigma-Aldrich (USA). All other chemicals and solvents were of analytical grade and obtained from Sigma-Aldrich, except for those specifically mentioned.

Experimental animals and treatments

Eight-week-old male Kunming mice weighing 22±2 g were purchased from the Chengdu Dossy Experimental Animals Co., Ltd. (License No. SCXK (Sichuan) 2008-24, China), and were housed separately in the laboratory animal house at 20–25°C, 50–60% humidity, and a 12 h light/dark cycle with the lights off at 7 p.m. The animals were fed according to a standard diet and allowed access to distilled water, and had been acclimated to laboratory conditions for 7 days.

Mice were divided into 8 groups, 10 mice per group. They were treated intraperitoneally with:

- normal saline as negative control group,
- 20 mg/kg PbAc,
- 100 mg/kg DMSA,
- 100 mg/kg DMSA + 20 mg/kg PbAc,
- 30 mg/kg KI,
- 30 mg/kg KI + 20 mg/kg PbAc,
- 100 mg/kg Chl,
- 100 mg/kg Chl + 20 mg/kg PbAc for 5 consecutive days.

After the exposure period (5 days) and the follow-up observation period (30 days), the whole blood samples were collected from femoral artery. Then mice were sacrificed through cervical dislocation and their livers, kidneys and testicles were rapidly removed and stored on ice.

Analysis of lead concentrations

Lead concentrations were determined according to assay by Li et al. [11]. A series of standards were prepared from the 1000 mg/l stock solution by dilution for each assay performed. After subtracting the background value from the standards value, lead concentration had a good linear relationship in 0–100 ng/ml range and the lead standard curve ($y = 0.0036 \times x$, $r = 0.99708$) was made as the basis for lead detection in the experimental samples.

The collected whole blood samples were digested with 10% nitric acid (HNO_3) (1 ml of blood : 2.5 ml of HNO_3) for 3 days in a clean room hood. The supernatants were then used for detecting lead content. Meanwhile, the livers, the kidneys and testicles were dried to constant weight

Table 1. Temperature settings in the graphite furnace atomic absorption spectrometer

Steps	Spectrometer parameters		
	final temperature [°C]	hold time [s]	gas path
Dry	120	15	inert
Ash	700	20	inert
Atomise	2 000	4	none
Clean out	2 200	4	inert
Cool down	0	25	inert

(dry weight) at 80°C for 48 h. Then the dried samples were weighed and placed in 10 ml conical flasks with polypropylenelids containing 3 ml of HNO_3 at room temperature until the solution became clear. Then 1 ml of 30% hydrogen peroxide (H_2O_2) was added to the samples. After the effervescence had ceased, the samples were heated at 80°C until HNO_3 evaporated. The samples were then cooled to room temperature. The final volume was increased to 10 ml with the addition of 2% HNO_3. The blood and tissue samples were then analyzed for lead contents using graphite furnace atomic absorption spectrometry (AA-7003, Echen-Tech, Beijing, China). Lead concentrations were expressed as μg/l (whole blood) or μg/g (wet weight). Temperature settings were shown in the Table 1. The suitable wavelength for lead was 283.31 nm.

Statistics

Using sample tissues from the same mouse, lead detections were repeated at least 3 times. All the data is expressed as mean (M) ± standard error of mean (SEM). The statistical analysis was performed by means of one-way ANOVA using SPSS 17.0 software or, where appropriate, two-way ANOVA, followed by least significant difference (LSD) test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

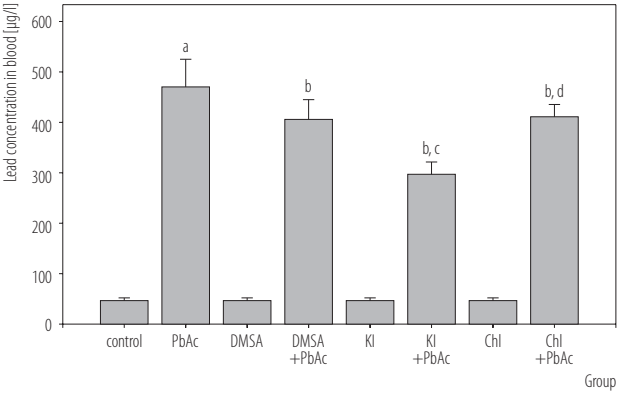
Effects of the DMSA, KI and Chl on lead concentrations in the whole blood of mice

The weight changes of mice in the PbAc-treated groups did not show significant difference as compared with the control group in the Table 2. As shown in the Figure 1,

Table 2. Effects of meso-2,3-dimercaptosuccinic acid (DMSA), potassium iodide (KI) and chlorophyll (Chl) on the body weight of mice treated with lead acetate (PbAc)

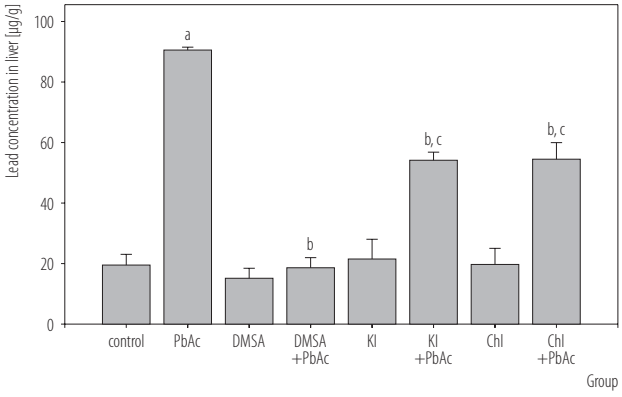
Group	Body weight [g] (M±SEM)		
	before treatment	after treatment	weight changes
Control	23.02±1.85	32.78±0.85	8.89±3.38
20 mg/kg PbAc	21.98±1.81	29.21±2.39	7.53±4.03
100 mg/kg DMSA	22.36±1.32	32.17±2.04	9.81±3.25
100 mg/kg DMSA + 20 mg/kg PbAc	23.93±1.49	33.50±3.49	9.58±4.18
30 mg/kg KI	22.74±3.07	32.04±1.52	9.50±4.22
30 mg/kg KI + 20 mg/kg PbAc	23.56±2.10	32.63±1.02	8.92±3.98
100 mg/kg chlorophyll	22.20±3.12	30.20±3.36	7.82±2.31
Chlorophyll + 20 mg/kg PbAc	23.09±3.47	32.42±3.63	9.73±3.05

M – mean; SEM – standard error of mean.



Results are presented as a mean ± standard error of mean.
PbAc – lead acetate; DMSA – meso-2,3-dimercaptosuccinic acid; KI – potassium iodide; Chl – chlorophyll.
[a] $p < 0.05$ compared to the control group.
[b] $p < 0.05$ compared to the PbAc group.
[c] $p < 0.05$ compared to the DMSA+PbAc group.
[d] $p < 0.05$ compared to the KI+PbAc group.

Fig. 1. Effects of DMSA, KI and chlorophyll on lead concentrations in the whole blood (samples collected from femoral artery) of mice (N = 10/group)



Results are presented as a mean ± standard error of mean.
[a] $p < 0.05$ compared to the control group.
[b] $p < 0.05$ compared to the PbAc group.
[c] $p < 0.05$ compared to the DMSA+PbAc group.
Abbreviations as in Figure 1.

Fig. 2. Effects of DMSA, KI and chlorophyll on lead concentrations in the livers of mice (N = 10/group)

as compared to the control group, lead concentrations increased in the PbAc-treated mice ($p < 0.05$). And lead concentrations in mice treated with the PbAc combined with the DMSA, KI or Chl significantly diminished as compared to the PbAc-treated group ($p < 0.05$). Moreover, lead concentrations in the KI+PbAc group were significantly lower than that in both the DMSA+PbAc group and the Chl+PbAc group ($p < 0.05$).

Effects of DMSA, KI and Chl on lead concentrations in the livers of mice

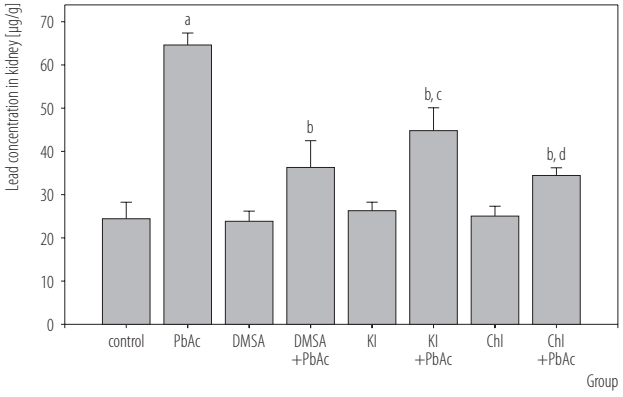
As shown in the Figure 2, lead concentrations in mice exposed to the PbAc were higher than that in the control group ($p < 0.05$). And lead concentrations in mice treated with the PbAc combined with the DMSA, KI or Chl significantly diminished as compared to the PbAc-treated group ($p < 0.05$). Additionally, lead concentrations in the DMSA+PbAc group significantly decreased as compared with both the KI+PbAc group and Chl+PbAc group ($p < 0.05$).

Effects of DMSA, KI and Chl on lead concentrations in kidneys of mice

As shown in the Figure 3, the PbAc-treated mice exerted higher lead concentrations as compared to the control group ($p < 0.05$). And lead concentrations in the kidneys of mice treated with the PbAc plus the DMSA, KI or Chl significantly decreased as compared to the PbAc-treated group ($p < 0.05$). Furthermore, lead concentrations of kidneys in the Chl+PbAc group were much lower than that in the DMSA+PbAc group and the KI+PbAc group and other groups ($p < 0.05$).

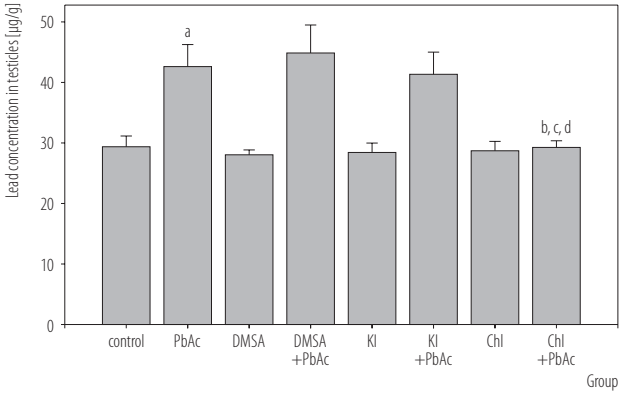
Effects of DMSA, KI and Chl on lead concentrations in the testicles of mice

Lead concentrations in the testicles of mice treated with the PbAc increased as compared with the control group in the Figure 4. Meso-2,3-dimercaptosuccinic acid and KI did not significantly intervene lead increase induced by the PbAc treatment ($p > 0.05$). However, lead concentrations in the testicles of mice in the Chl+PbAc group were significantly less than that of mice in the PbAc group ($p < 0.05$).



Results are presented as a mean ± standard error of mean.
[a] $p < 0.05$ compared to the control group.
[b] $p < 0.05$ compared to the PbAc group.
[c] $p < 0.05$ compared to the DMSA+PbAc group.
[d] $p < 0.05$ compared to the KI+PbAc group.
Abbreviations as in Figure 1.

Fig. 3. Effects of DMSA, KI and chlorophyll on lead concentrations in the kidneys of mice (N = 10/group)



Results are presented as a mean ± standard error of mean.
[a] $p < 0.05$ compared to the control group.
[b] $p < 0.05$ compared to the PbAc group.
[c] $p < 0.05$ compared to the DMSA+PbAc group.
[d] $p < 0.05$ compared to the KI+PbAc group.
Abbreviations as in Figure 1.

Fig. 4. Effects of DMSA, KI and chlorophyll on lead concentrations in the testicles of mice (N = 10/group)

DISCUSSION

Lead poisoning has been known to be associated with structural and functional abnormalities of multiple organ systems. How to limit lead absorption and facilitate lead excretion is a challenging problem. The past research on the treatment of lead toxicity focused on antioxidants (such as ascorbic acid and α-tocopherol) and thiol chelators (DMSA or monoisoamyl DMSA) [12,13].

Many studies have demonstrated that chelation treatment using the DMSA may reduce lead levels in the whole blood, brain and other tissues, which is a classical therapy strategy [14]. Velaga et al. had suggested that the DMSA ameliorated on parameters indication of oxidative stress in the livers, kidneys and blood of lead-exposed rats [7]. Similarly, our results also showed that the DMSA diminished lead accumulation in the whole blood, livers and kidneys in mice. However, thiol chelators (e.g., DMSA) may cause the imbalance of other necessary trace elements in the body, which is especially harmful for children. So how to avoid side-effects and limit lead accumulation needs more work to be done.

This study demonstrated that the DMSA, potassium iodide and chlorophyll all had significant effects on limiting lead accumulation *in vivo*. Interestingly, they did not play equal role in expelling lead in different tissues in mice. Among 3 investigated compounds, the KI displayed the most protective effect in diminishing blood lead level, while the DMSA had the advantage to decrease lead concentration in the livers. And the Chl facilitated the excretion of lead in the kidneys and testicles in mice, while the same effects could not be observed in the testicles of mice treated with the DMSA+PbAc or KI+PbAc.

Although studies on limiting lead accumulation *in vivo* using food-source materials were insufficient, their remarkable effects may be relevant with antioxidative abilities. Lead was found to disrupt the pro-oxidant/antioxidant balance of tissues giving rise to biochemical and physiological dysfunction [15,16]. Reddy et al. suggested that ginger extract diminished lead content in kidneys through increasing the activities of antioxidants [17]. Velaga et al. also found that administration of *Moringa oleifera* seed powder had more advantages in diminishing lead-induced oxidative stress and lead restoration [18].

As for potassium iodide and chlorophyll studied in our research, Qiao et al. found that potassium iodide ameliorated renal ultrastructure and degraded expression of nuclear factor-kappaB and fibronectin induced by lead [19]. Many studies have demonstrated that the KI may prevent oxidative damage of membrane lipids and the DNA, as an ideal antioxidative defense mechanism [20,21]. In addition, the Chl also demonstrated to exhibit antioxidant activity by way of inhibition of lipid peroxidation and protection of membrane damages [22]. The decrease in membrane oxidative damages may lead to the reduction of lead absorption but their specific mechanisms still need further studies.

CONCLUSIONS

Lead is a cumulative toxicant and has adverse health effects on multiple body systems. The prevention of lead poisoning is critical for human health, particularly for young children because lead is dangerous at all levels to children. For the first time, except for the DMSA, we have found that potassium iodide (KI) and chlorophyll (Chl), which could be used as food additives in daily diet, appear to have fewer side-effects and appear to be more effective in alleviating lead accumulation.

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