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ORIGINAL ARTICLE

The therapeutic potential of thiamine for treatment of experimentally induced subacute lead poisoning in sheep

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Abstract Lead poisoning remains a serious problem in veterinary and human medicine. A number of lead chelators have been used for treatment of lead intoxication, but none of them is completely effective to remove lead from all organs. Therefore, alternatives for the treatment of lead poisoning are required. In this study, the efficacy of thiamine on blood and tissue lead contents was evaluated in subclinical lead toxicosis in sheep. Nine female sheep weighing 25–29 kg were orally receiving a daily dose of 80 mg/kg body weight of lead acetate for 5 days. Then, the animals were assigned into two groups. Group 1 did not receive any further treatment and was used as the control group, and group 2 was treated intravenously by 25 mg/kg body weight of thiamine twice daily for 7 days. Within 1 day following treatment, in the treated group, blood lead level (mean 243.5 μg/l) was significantly ($P<0.05$) lower than that in group 1 (mean, 518.16 μg/l). Thiamine treatment significantly reduced ovary lead content. A significant reduction of serum zinc concentration was also observed in thiamine treated animals. These results suggest that thiamine might have some therapeutic effects on lead poisoning, but the zinc status of depletion should be considered during long periods of treatment.

Keywords Lead poisoning · Thiamine · Chelator · Sheep

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Introduction

Lead toxicosis is one of the most commonly reported heavy metal toxicoses in domestic and wild animals (Baker 1987; Miller and Bauk 1992; O'Hara et al. 1995; Burger and Gochfeld 2000; Rumbeiha et al. 2001). It is also the most common metal poisoning encountered in humans today (Kosnett 2001). Among heavy metals, lead also ranks as one of the most serious environmental pollutant all over the world which is incriminated in accidental poisoning of livestock (Radostits et al. 2007). This situation is due to the widespread presence of lead-containing trade products, in industrial and residential settings, and to insidious, nonspecific, multisystemic findings that often characterized lead toxicity (Kosnett 2001). Animals are exposed to lead from numerous sources in the environment. The common sources of lead for ruminants are lead-bearing paints, discarded batteries, used crankcase oil, lead pesticides, environmental pollution, lead shot, and pastures contaminated by engine emissions (Radostits et al. 2007).

Many of the toxicological manifestations of lead poisoning are related, in part, to its pattern of tissue distribution. Following acute oral exposure, lead accumulates in the liver and kidney. Concentrations in other tissues, such as the bone, also increase if the duration of lead exposure is extended (Radostits et al. 2007). There is no threshold for the toxic effects of lead so that even lower levels of lead exposure induce harmful consequences in both laboratory animals and humans (Chiado et al. 2004; Gilbert and Weiss 2006).

Several metal chelators such as D-penicillamine, calcium disodium ethylenediaminetetraacetic acid ($CaNa_2EDTA$), and Meso-2,3-dimercaptosuccinic acid (DMSA) have been used to manage lead toxicity in humans and some domestic animals, but none is fully suitable in reducing lead burden in chronic lead exposure (Osweiler 1999). In addition to

numerous shortcomings, these chelating drugs may have inherent toxic effects. Therefore, they are not suggested to be used at therapeutically adequate doses for prolonged period of time (Denver et al. 2000), and they often fail to remove lead from all body tissues (Bratton et al. 1981; Kostial et al. 1999). On the other hand, none of the chelating agents are approved to be used in food animals (Meldrum and Ko 2003). Therefore, alternatives for the treatment of lead poisoning are required.

Numerous natural products including vitamins, minerals, and plant extracts have had beneficial effects in lead intoxication (Kalia and Flora 2005; Patrick 2006). On the other hand, dietary deficiencies of some minerals, vitamins, and protein have also been reported to increase the toxic effects of lead in animal studies (Houston 2000; Kosnett 2001).

Thiamine, a water-soluble, sulphydryl containing vitamin, has been recommended as a therapeutic agent for lead toxicity. Although, it has been demonstrated that thiamine reduces whole body retention of lead, the exact mechanism and its efficacy in different species are unknown. It is postulated that thiamine reduces absorption and enhances excretion of lead (Kim et al. 1990).

The main aim of this study was to evaluate the therapeutic effects of thiamine on the blood lead levels and the lead content in different tissues following experimental subclinical lead poisoning in sheep.

Materials and methods

Nine female sheep (Baloochi breed), aged between 12 to 14 months, and weighing 25–29 kg were obtained from the Animal Breeding Center of Mashhad. The animals were housed in indoor pens under similar management condition and were fed three times a day with a mixture of alfalfa hay, barley grain, and wheat straw. Fresh water was available freely throughout the study.

Sheep were dewormed with albebndazole (Veterinary Medicine Production Co., Tehran, Iran). Health status of each sheep was evaluated by physical examination and hematology. The sheep were housed and fed under the aforementioned condition for 14 days to ensure proper acclimation before the treatments.

The animals were randomly assigned to two groups. Animals in group A (n=4) received lead acetate (Fluka-Germany) which had dissolved in distilled water, orally by ruminal gavage, at the dose of 80 mg/kg body weight for five consecutive days. Group B (n=5), received equal doses of lead acetate. From day 6, for seven consecutive days, thiamine HCl (Nasr-Fariman Co., Iran) was intravenously administered at the dose of 25 mg/kg twice a day with 12 h interval in these animals.

Blood samples were acquired from jugular vein 6 h following treatment in heparinized vials for measurement of lead. To establish the lead base values of blood samples in each subject, samples of blood were collected 1 day before (day 0) and 6 days after (day 6) commencing the experiment.

On the day after cessation of chelating treatment (day 13), all sheep were killed on ethical grounds. Tissue samples were collected from the liver (caudal portion of the left lobe), left kidney, left ovary, brain (parietal region of the left cerebral cortex), spleen (apex portion), and ribs (10-cm portion from the middle of the left fourth rib). Muscle and periosteum were immediately stripped from the rib to provide a clean sample of bone.

Blood samples were dissolved in nitric acid and ammonium vanadate and then centrifuged at 2,500 rpm for 5 min. Soft-tissue samples were minced with scissors immediately after collection. Small pieces of tissues were weighed and then digested in a 1:1 mixture of 98% sulfuric acid and 70% nitric acid by use of a slight modification of the wet-ashing technique (Ihnat and Miller 1977). The rib samples were cut into small chips and digested using the same method adopted for the soft-tissue samples. Briefly, 10 ml of acid mixture was added per gram of tissue and the mixture was heated at 120°C for 4 h (acid mixture was added as needed in a drop-wise manner to prevent charring until the organic matter was completely destroyed). At the end, the volume of solution reached 50 ml. Lead concentrations were determined by atomic absorption spectrophotometry (Perkin-Elmer 3030) at 283.3 nm wave-length by the use of a graphite furnace. Limit of detection for this analysis was 5 ng/g, and recovery for spiked samples was >%90.

Serum calcium (Ca; methylene thymol blue method), magnesium (Mg; xylidine blue method), zinc (Zn; direct colorimetric method), and copper (Cu; 3, 5-DiBr-PAESA method) were measured by an automated analyzer (Selectra XL, Amsterdam-Netherland) using commercial kits (Man Co. Tehran, Iran for Ca and Cu, Parsazmmon Co. Tehran, Iran for Mg and Colle Prestino Rom, Italy for Zn).

The statistical analyses were carried out using the SPSS software (version 11.5). Student's t test was used to analyze the data of blood and tissue lead contents. Serum minerals were analyzed using the Mann-Whitney procedure. Results were displayed as means±SD, and the difference between groups were considered significant at $P<0.05$.

Results

No clinical signs of toxicosis were observed during the experiment. Rectal temperature, heart, and respiratory rates were normal in all animals. Three days after lead administration, feces of some sheep became transiently loose and fetid.

Blood lead levels of the two groups of sheep are shown in Table 1. Following lead exposure, blood lead values in both groups elevated significantly ($P<0.05$) to greater than 500 μg/l on day 6. After administration of thiamine, blood lead values significantly ($P<0.05$) decreased on day 7.

At day 13, blood lead level of the treated group was lower than that in the control group but the difference was not significant ($P=0.09$).

Following lead administration, the tissue-wise accumulation of lead varied with the highest concentration in the ovary followed by the kidney, bone, spleen, liver, and brain. The tissues' lead concentrations in control and treated sheep are presented in Table 2.

Thiamine administration caused significant reduction in lead content of ovaries when compared with untreated sheep. Treatment with thiamine reduced lead concentrations in the ovary, kidney, bone, spleen, and brain by 45.5%, 43.9%, 41.1%, 9.5%, and 1.4%, respectively. The reduction of lead by thiamine treatment was greater in the ovary and the bone.

Serum zinc, copper, calcium, and magnesium concentrations of animals in both groups are shown in Table 3. Thiamine therapy led to a significant reduction of serum zinc concentration and significant elevation of magnesium concentration ($P<0.05$).

Discussion

Thiamine altered the level of tissue retention of lead in the ovaries. Administration of this vitamin reduced the blood and ovaries' lead contents, but its effect on the spleen and brain's lead content was not noticeable. The effect of thiamine on the tissue's lead content in sheep has not been reported. Lead kinetics is influenced by a number of factors including species, diet, age, dose, and route of entry. Generally, lead is distributed in various body organs with high concentration in the liver, kidneys, bone, and red blood cells. The organ distribution of lead is also influenced by route of administration and dose of chelators. The oral mode of lead exposure utilized in this study is thought to produce a subclinical lead intoxication with toxic levels of blood and soft tissue's lead burden (Radostits et al. 2007). For the control group, the lead distribution was in the following descending order: ovary>kidney>bone>spleen>liver>brain. This pattern of lead distribution in thiamine-treated animals was changed to the following order: ovary>kidney>spleen>liver>bone>brain. Results of the present study for the use of thiamine in sheep are in accord with previous studies reported in goats and cattle (Maiti et al. 1990; Coppock et al. 1991).

Very low dose of thiamine (2 mg/kg once daily IM) has not been effective in reducing the blood's lead concentration in cattle, although treatment with thiamine has been more effective than Ca Na_2 EDTA and thiamine plus Ca Na_2 EDTA in inducing remission of clinical signs of plumbism (Coppock et al. 1991). In the present study, thiamine reduced the blood's lead levels below confirmatory levels associated with lead poisoning on the day after administration, but the reduction of blood's lead level did not continue linearly during treatment period, although it remained lower than the toxic levels (Radostits et al. 2007).

Thiamine is a water-soluble vitamin containing the sulfhydryl group. Although the exact mechanism of thiamine in antagonizing lead toxicity has not been clearly elucidated, it has been supposed that the exposure of sulfhydryl groups in the thiamine molecules permits several possible configurations of thiamine–lead complexes (Olkowski et al. 1991). On the other hand, it was demonstrated that thiamine enhances elimination of lead from the body (Kim et al. 1990, 1992; Olkowski et al. 1991; Radostits et al. 2007). It also reduces lead absorption from gastrointestinal tract and prevents its deposition in some tissues (Bratton et al. 1981; Kim et al. 1992). However, although thiamine when used alone did increase excretion of lead via bile and urine, in some studies, it has not been considered therapeutically effective (Olkowski et al. 1991; Maiti et al. 1990). The low efficacy of thiamine treatment alone on the retention of lead by the tissues has been shown in experimentally induced lead poisoning in rodents (Kim et al. 1992). It has also been shown that a combination of thiamine with Ca EDTA is more effective than the individual treatments (Radostits et al. 2007).

Table 1 Changes in blood lead concentration (μg/l) following lead exposure and chelating therapy

Group	Day 0	Day 6	Day 7	Day 9	Day 11	Day 13
A	80.3±28.1[a]	653.7±159.4[a]	518.16±126.33[b]	438.7±114.49	326.05±106.09	320.28±96.77
B	74.55±18.1[a]	530±95.52[a]	243.5±133.21[a,b]	339.54±105.31	230.48±71.85	203.82±89.50[a]

A control, *B* thiamin treated

[a] Significantly different ($P<0.05$) in each row compared with day 6 value of the same group

[b] Significantly different ($P<0.05$) in each column compared with value of the same day

Table 2 Tissues lead concentration (μg/g) in different groups of sheep

Group	Organ					
	Liver	Kidney	Brain	Bone	Ovary	Spleen
A	10.20±1.49	27.57±14.45	4.22±1.12	19.89±16.03	113.71±34.92[a]	15.85±2.34
B	12.20±5.29	15.48±3.44	4.21±0.94	11.71±2.56	62.02±24.22[a]	14.36±5.49

[a] Significantly different ($P<0.05$) in each column

Interestingly, it was shown that ovaries accumulate the highest concentration of lead. Lead content of ovaries was ten times more than the liver and about four times more than the kidneys. To our knowledge, this is the first report of lead concentration in the ovaries and needs more attention. The ovary may be a better indicator for diagnosis of lead exposure than the liver and kidney. It should be noted that because reference ranges for different assays vary considerably, results must be compared with expected values for a specific assay. This high concentration of lead in the ovary may be responsible for infertility in several lead-poisoned species (Cebra and Cebra 2004). There are no more data for the ovary's lead concentration in sheep to compare with, and further studies are required to examine the exact values of the ovary's lead content in different species.

Lead concentration in the brain is generally quite low (Senapati et al. 2001; Meldrum and Ko 2003) and data from the study reported here also support this findings. Given this low tissue burden, it is difficult to document that chelating agents have much effect on lead concentrations in the brain tissue.

The bone is recognized as a tissue to store a considerable portion of the body burden of lead (Zmudzki et al. 1983). Furthermore, several studies (Cory-Slechta et al. 1987; Tendon et al. 1994; Jones et al. 1997) have revealed that at least some portion of lead in the bones is accessible to chelators, as evidenced by the fact that EDTA and DMSA can reduces lead concentrations in the bone. Although bone analysis in the study reported here showed 58.9% reduction of lead content in thiamine-treated sheep, statistical analysis did not indicate significant difference between the two groups of sheep.

The effect of thiamine on the liver, kidney, and spleen's lead content in the present study is similar to responses that were seen with lead-poisoned mice injected with thiamine (25 or 50 mg/kg) alone, as a lead chelator (Kim et al. 1992). It is reported that a very high dose of thiamine can prevent the tissue's lead accumulation in calves (Bratton et al. 1981), but postexposure treatment has not demonstrated any significant reduction of the blood's lead level in goats and cattle (Maiti et al. 1990; Coppock et al. 1991).

Some degrees of mineral depletion, zinc in particular, is an adverse effect associated with administration of most of current heavy metal chelators (Anderson 1999; Kalia and Flora 2005). Our results show that thiamine reduces the blood's zinc concentration in sheep. To our knowledge, this is the first report of such side effect in thiamine therapy. Although the clinical importance of such effect is not fully understood, it is suggested that teratogenic potential of most chelators is, at least in part, due to induced trace element deficiencies (Taubeneck et al. 1992; Domingo 1998). Our findings also show a significant difference of serum magnesium concentration between control and thiamine-treated sheep. It has been supposed that lead toxicity causes magnesium deficiency. On the other hand, magnesium is an essential cofactor for thiamine pyrophosphate (TPP) in TPP-dependent enzymes (Anetor et al. 2007).

Although these results show some beneficial effects of thiamine therapy for lead intoxication, further investigation to determine the exact dose and duration of thiamine administration is needed. Zinc depletion should also be considered during thiamine therapy particularly in pregnant animals.

Table 3 Serum concentration of Zn, Cu, Ca, and Mg following lead exposure and thiamin therapy

Group	Zn[a]	Cu[a]	Ca[b]	Mg[b]
A	104.75±18.30[c]	77.5±13.89	9.975±0.49	2.05±0.21[c]
B	43.2±8.76[c]	77.6±10.11	9.5±0.35	2.66±0.62[c]

A control, *B* thiamin treated

[a] Concentration as μg/dl

[b] Concentration as mg/dl

[a] Significantly different ($P<0.05$)

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