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Thiamine reduces tissue lead levels in rats: mechanism of interaction

Srinivasa Y. Reddy · Raghu Pullakhandam · B. Dinesh Kumar

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Abstract Lead (Pb) toxicity has been a serious concern in industrialized societies because of its association with functional deficits in nervous, haematopoietic and renal systems. Several studies have shown beneficial effects of thiamine on Pb toxicity. It is speculated that Pb chelation by thiamine may be a possible mechanism. However, the exact nature of these interactions remained elusive. In the present study we have characterized the interaction of Pb with thiamine using UV–Vis as well as fluorescence spectroscopic methods and studied the effect of thiamine treatment on blood and tissue Pb levels during simultaneous or post-exposure to Pb in rat model. The spectroscopic studies revealed that Pb interacts with the pyrimidine ring of thiamine, leading to its solubilization at physiological pH. Further, thiamine reduced the Pb levels in blood, kidney and bone during both simultaneous and post-exposure Pb treatment. Interestingly, thiamine appears to prevent the accumulation of Pb in bone during simultaneous treatment. Together these results suggest that pyrimidine ring of thiamine mediates its interaction with Pb, leading to the prevention of its accumulation and/or increased clearance from tissues.

Keywords Lead toxicity · Thiamine · Metal chelation · Pyrimidine ring · Absorption · Solubilization

S. Y. Reddy · B. Dinesh Kumar (✉)
Food and Drug Toxicology Research Centre, National Institute of Nutrition (ICMR), Jamai Osmania, Hyderabad 500 604, India
e-mail: nindineshlead@rediffmail.com

R. Pullakhandam
Biophysics Division, National Institute of Nutrition (ICMR), Jamai Osmania, Hyderabad 500 604, India

Introduction

Lead (Pb) is a toxic heavy metal. Due to urbanization coupled with industrialization, the environmental Pb levels are rapidly increasing and thus posing a potential health risk (Flora et al. 2006; Toscano and Guilarte 2005; Papanikolaou et al. 2005; Preuss 1993; Goyer 1971). Emerging evidence suggests that chronic Pb exposure often leads to functional deficits in nervous, hematopoietic, renal systems which are most often irreversible (Flora et al. 2006; Toscano and Guilarte 2005; Preuss 1993). Continuous efforts to develop safe and effective therapy to treat Pb toxicity have been made (Flora et al. 2008, 1986). Chelation therapy with calcium-disodium ethylenediamine tetra acetic acid (CaNa(2)EDTA), British Anti Lewisite (BAL), sodium 2,3-dimercaptopropane 1-sulfonate (DMPS), meso 2,3-dimercaptosuccinic acid (DMSA) etc., are considered to be the best known treatment against metal poisoning (Flora et al. 2006, 2004; Saxena et al. 2005). However, the treatment with these chelating agents is reported to be compromised with number of serious side-effects (Flora et al. 2008).

The beneficial role of B complex vitamins particularly thiamine in reducing the toxic effects of Pb is well documented. Bratton et al. (1981) demonstrated that simultaneous administration of thiamine reduces Pb levels. It is possible that thiamine might hamper the gastrointestinal absorption of Pb or it might increases the clearance from the tissues. Interestingly, combined supplementation of thiamine and Pb appears to increase the absorption of Pb (Sasser et al. 1984; Flora 2002). However, subcutaneous administration of thiamine has been shown to decrease the blood and tissue Pb levels in mice, which could have been mediated by the interaction of thiamine and Pb (Louis-Ferdinand et al. 1982). Further, reduction in thiamine levels during Pb intoxicication explains the possible neuropathological effects observed during Pb toxicity (Anetor et al. 2007). Together these studies unequivocally suggest the beneficial effect of thiamine in preventing Pb toxicity, possibly due to interaction of Pb with thiamine. However, the mechanism of Pb-thiamine interaction remains to be characterized. Moreover, treatment with thiamine in occupational groups (with routine exposure) is of concern, particularly due to the enhancing effect of thiamine on Pb absorption (Sasser et al. 1984).

In the present study we investigated the interaction of Pb with thiamine using spectroscopic methods and studied the modulation of blood and tissue Pb levels by thiamine treatment during simultaneous or post Pb exposure in rat model.

Materials and methods

Materials

Lead acetate, thiamine hydrochlroide and all other chemicals were procured from Sigma–Aldrich, Bangalore, India.

UV–visible spectrometry

Thiamine (60 μmole/l in 20 mmole/l sodium acetate, pH 6.0 and 3.5) either in the absence or presence of lead acetate (120 μmole/l) was incubated at 37°C for 30 min and the absorption spectra were recorded (190–700 nm) using Unicam, UV300 spectrophotometer. All the spectra were corrected for respective buffer blanks.

Fluorescence spectroscopy

The oxidation of thiamine (100 μmole/l) with mercuric oxide (2 μmole/l) was studied in the absence and presence of increasing concentrations of lead acetate (0–900 μmole/l). The mercury induced oxidation of thiamine was monitored by fluorescence emission at 444 nm by exciting at 365 nm using Cary Eclipse Fluorescence spectrophotometer. The emission and excitation slits were set at 2.5 nm and all the recording were performed at room temperature.

Pb solubility assay

Lead nitrate (in 0.2% nitric acid) was diluted with 100 mmole/l 2-(*N*-morpholino)-ethane sulphonic acid (MES) buffer pH 6.5 to a final concentration of 100 nmole/l in the absence and presence of thiamine (0–360 nmole/l) and incubated at 37°C for 1 h. At the end of incubation the samples were centrifuged at 12,000 rpm for 25 min. The Pb concentration in the supernatant was determined after acidification with nitric acid (2%) on GF-AAS (Thermo electron) as described below.

Animal experiment

The study design and protocol were approved by the institutional animal ethics committee of National Institute of Nutrition, Hyderabad. Thirty-Six Sprague–Dawley (out bred) male rats, weighing 100–150 g, were housed individually in stainless steel cages in an environmentally controlled room (20–26°C temperature and 45–70% relative humidity). Deionized water and standard cereal pulse based pellet diet was provided ad libitum. To study the effect of thiamine on Pb accumulation, the animals were divided randomly into two groups and treated with Pb and/or thiamine as described below.

Simultaneous supplementation of thiamine and Pb

The first group of animals ($n = 18$) were further subdivided into three groups ($n = 6$) and supplemented with saline or Lead acetate in distilled saline (10 mg/4 ml/kg body weight/day dosage to induce subclinical toxicity) through oral gavage in the absence and presence of thiamine (25 mg/4 ml/kg body weight/day) for 7 weeks, to achieve approximately

twofold molar excess of thiamine compared to lead (which in turn is based on the high solubility of lead at this concentration) and is 2.8 times higher than the recommended clinical therapeutic dose of humans (Laurence and Bacharah 1964).

Post exposure treatment with thiamine

The second group of animals were also subdivided in two groups and supplemented with saline ($n = 6$) or lead acetate (10 mg/4 ml/kg body weight/day) for 7 weeks ($n = 12$). The Pb supplemented animals were further subdivided into two groups ($n = 6$) and supplemented with saline in the absence and presence of thiamine (25 mg/4 ml/kg body weight/day) from 8th to 10th week. The food intake was monitored daily and body weights recorded every fifth day. At the end of the experiments urine samples were collected and the animals were sacrificed to collect blood and tissues.

Pb estimation

Blood Pb levels were estimated in a graphite furnace using atomic absorption spectroscopy as described previously (Subramanian and Meranger 1981) and tissue Pb levels were determined by flame atomic absorption spectroscopy as described previously (Yeazer et al. 1971).

Enzyme assays

The urinary *N*-Acetyl β-D glucosaminidase (NAG) activity was measured by the spectrophotometric method (Horak et al. 1981) and the δ-Amino Levulinic Acid Dehydratase (ALAD) activity in the blood was determined by colorimetric method (Granick et al. 1972).

Statistics

The descriptive statistics such as mean and SD were calculated using Microsoft excel and were subjected to one way ANOVA using SPSS version seven package. The results were considered significant if $P < 0.05$.

Results and discussion

Previous studies demonstrated that simultaneous administration of thiamine reduces Pb levels in tissues and prevents the clinical signs of Pb poisoning in calves (Bratton et al. 1981). In line with these observations, chelation of metal ions such as Cd, Zn and As by thiamine has been reported (Malandrinos et al. 2000). Therefore, it is possible that Pb chelation by thiamine may prevent its transporter mediated intestinal absorption. In contrast, thiamine appears to increase the intestinal absorption of Pb (Sasser et al. 1984; Louis-Ferdinand et al. 1982), which could be attributed to the interaction of thiamine with Pb, leading to its solubilization at intestinal pH and thus increased absorption.

The interaction of Pb with thiamine was studied by UV–Vis spectroscopy. As shown in Fig. 1, thiamine UV absorption spectra showed two distinct absorption maxima as a function of pH, 231 and 270 nm at pH 6 (Fig. 1a), 244 and 262 nm at pH 3.5 (Fig. 1b), representing the pyrimidine and thiazole groups, respectively (Ball 2006). Interestingly, addition of Pb resulted in decrease in the absorption only at 231 nm at pH 6 (Fig. 1a) while the λ max of pyrimidine shifted to 238 from 244 nm (hypsochromic shift) at pH 3.5 (Fig. 1b), suggesting interaction of lead with thiamine through the pyrimidine ring. It is well known that Mg (+2) is a cofactor for thiamine mediated enzyme

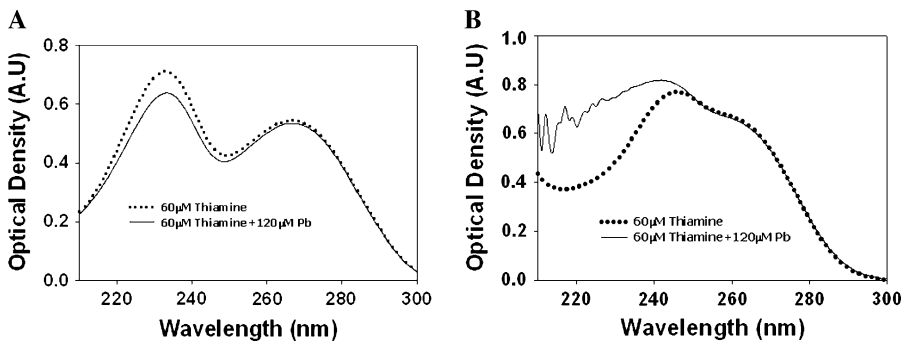

Fig. 1 UV–Vis absorption spectra of thiamine in the presence and absence of lead: UV–Vis absorption spectra of thiamine (60 μmole/l) in 20 mmole/l sodium acetate buffer pH 6.0 (**a**) or 3.5 (**b**) in the absence (*dotted line*) and presence (*solid line*) of Pb (120 μmole/l)

catalysis. Similarly, various other metal ions such as Mn (+2), Co (+2), Zn (+2), Pt (+2) and Cd (+2) also showed activity but with less efficiency (Stamatis et al. 2007; Malandrinos et al. 1998; Dodi et al. 1996; Hu et al. 1999, 2001). These metal ion thiamine complexes reported to exist either as ionic salts with metal-anion bridges or metal complexes where direct metal bonding to thiamine is demonstrated. Further, the composition of metal anion complexes is varied depending on the pH of solution, at pH 3.5 the $M(ThH)Cl_3$ of a zwitterionic formula, with the metals bonded through the phosphate moiety, and at pH 6 complexes of formula $MThCl_2$, with the metals simultaneously bonded through N1 and pyrophosphate oxygens (Stamatis et al. 2007). The fact that thiamine hydrochloride was used in the present study rules out the possibility that Pb interacts with phosphate group at pH 6 and subtle changes in the absorption properties, particularly the hypochromic shift in pyrimidine absorption strongly suggests its involvement in interaction with lead, which could be mediated through its N1 atom (Stamatis et al. 2007; Malandrinos et al. 2006).

Oxidation of thiamine in the presence of mercury (Hg) leading to the formation of fluorescence thiamine adduct has been used to measure the Hg or thiamine levels (Malandrinos et al. 2006; Segura-Carretero et al. 1999; Ryan and Ingle 1980). Furthermore, reduction of Hg (+2) is proposed to be catalyzed by the pyrimidine ring of thiamine (Ryan and Ingle 1980). Similarly, addition of Hg resulted in the oxidation of thiamine, as evidenced by increased fluorescence emission at 444 nm. Interestingly, presence of Pb inhibited the Hg induced increase in thiamine fluorescence in a dose dependent manner, implying that Pb inhibits Hg induced thiamine oxidation (Fig. 2). It's known that mercury (Hg^{+2}) interact with thiamine only at the N1 site of the pyrimidine moiety at pH 6 (Casas et al. 2006). Therefore, it is possible that Pb and Hg perhaps share common binding site on the pyrimidine ring of thiamine. However, owing to the redox-inert nature of Pb, it cannot oxidize the thiamine.

Apart from the quantity, the solubility of metal ions such as iron and Pb determines their uptake at the enterocyte (Pullakhandam et al. 2008; Bosso and Enzweiler 2008). Poor solubility of iron at the intestinal pH attributed to its low absorption (Pullakhandam et al. 2008; Bosso and Enzweiler 2008).

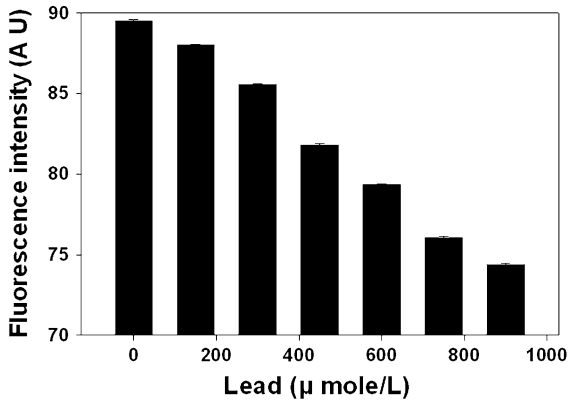

Fig. 2 Effect of Pb on Hg^{+2} induced oxidation of thiamine: Thiamine (100 µmole/l) was incubated with mercuric oxide (2 µmole/l) in the absence and presence of increasing concentration of Pb (0–900 µmole/l). The fluorescence due to mercury induced oxidation of thiamine was monitored at 444 nm emission by exciting at 365 nm using Cary eclipse fluorescence. The *bars* represent the mean + SD of three independent observations. Thiamine alone did not possess any fluorescence under the experimental conditions

Similar to that of iron, Pb is also poorly soluble at near intestinal pH and hence poorly absorbed (Bosso and Enzweiler 2008). In the current study it was observed that thiamine is interacting with Pb, which is likely to modulate its solubility and thus intestinal absorption. As shown in Fig. 3, at physiological pH the Pb is poorly soluble compared to acidic pH. Interestingly, the presence of thiamine dose

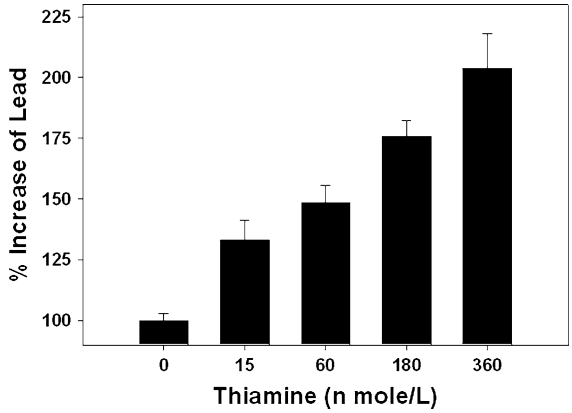

Fig. 3 Effect of thiamine on Pb solubility. Lead nitrate (100 nmole/l) in MES buffer was incubated at 37°C for 1 h in the absence and presence of increasing concentrations of thiamine (0–360 nmole/l). At the end of incubation the samples were centrifuged at 12,000 rpm for 25 min. The Pb concentration in the supernatant was determined. The *bars* are mean + SD of four samples

dependently increased the solubility of Pb, implying that interaction of Pb with thiamine aids in its solubilization (Fig. 3).

Together above results suggest that the interaction of thiamine with Pb leads to its solubilization at intestinal pH, which could be possible mechanism of thiamine induced Pb absorption observed in previous studies (Sasser et al. 1984). Therefore, the thiamine based treatment regime during Pb toxicity remains a concern particularly in those who are exposed to Pb routinely (i.e. occupational exposure). In order to understand the effect of thiamine on Pb accumulation during combined supplementation (simulating the occupational exposure group) or post Pb exposure conditions (accidental Pb poisoning), rats were supplemented with thiamine either together or after Pb exposure. Food intake was found to decline significantly in all the experimental groups (23.7 ± 2.06 g/day) compared to the control groups (29.5 ± 1.37 g/day) from day 20 onwards. Similarly, a proportional decrease in the body weights of Pb exposed group (173.7 ± 7.23 g) was observed compared to the control group (186.8 ± 9.12 d), consistent with the well known anorexic effect of Pb (Bolanos et al. 1996). As shown in Fig. 4, Pb supplemented animals showed elevated Pb levels in their blood, bone and kidney. However, thiamine significantly reduced the tissue Pb levels regardless of treatment regime, consistent with several studies in animal models (Flora et al. 2008). Interestingly, the distribution of Pb in the blood is higher compared to bone during simultaneous treatment with thiamine (Fig. 4a), which is reversed during post-exposure thiamine treatment (Fig. 4b). Therefore, it is likely that thiamine treatment preferentially prevents accumulation of Pb in the bone during simultaneous treatment while it increases the mobilization during post exposure treatment, possibly by increasing the renal clearance (Flora et al. 1986). However, similar to that of lead, thiamine might also increase the clearance of other biologically important metals such as zinc and Cu. Therefore, the status of these nutrients needs to be monitored and addressed during long term thiamine supplementation.

Apart from the accumulation in tissues, the inhibition of δ-amino levulinic acid dehydratase (ALAD) activity in the blood and increased *N*-Acetyl β-D glucosaminidase (NAG) activity in the urine, are considered the markers of sub-clinical Pb toxicity on heamopoietic system and renal damage, respectively (Bolanos et al. 1996). Similarly, Pb exposure resulted in the reduction of blood ALAD activity (Fig. 5a) and increased urinary NAG activity (Fig. 5b), suggesting the Pb toxicity in these animals. Thiamine treatment normalized the Pb induced alterations in ALAD and NAG activity, during both the treatment regimes. However, despite higher blood Pb levels, ALAD activity is significantly higher in simultaneous thiamine supplemented group compared to that of post exposure treatment (Fig. 5a). Therefore, it is likely that the interaction of thiamine renders the Pb biochemically inactive and prevents its accumulations in bone.

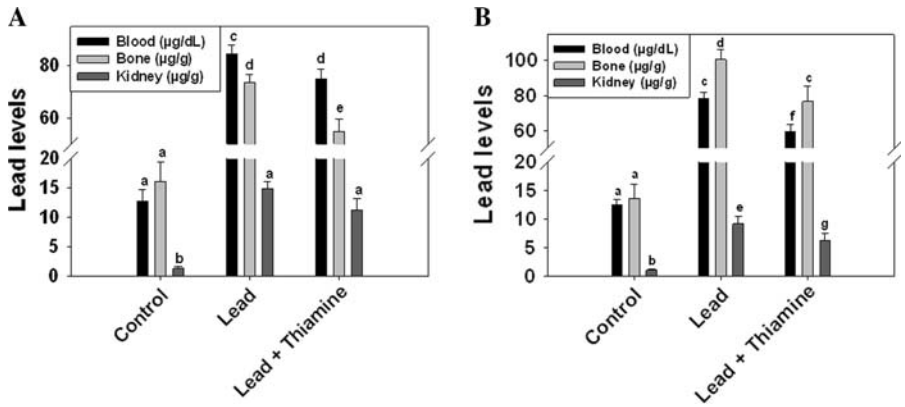

Fig. 4 Effect of thiamine supplementation on blood and tissue Pb levels in Pb exposed rats: Rats exposed to Pb through oral gavage (10 mg/4 ml/kg body weight/day) for 7 weeks and treated with thiamine (25 mg/4 ml/kg/day) either simultaneously (**a**) or post Pb exposure (**b**) from 8th to 10th week. The Pb levels of blood, bone and kidney were measured at the end of treatment. *Bars* indicate mean + SD and *bars* that do not share common *superscripts* differ significantly ($P < 0.05$)

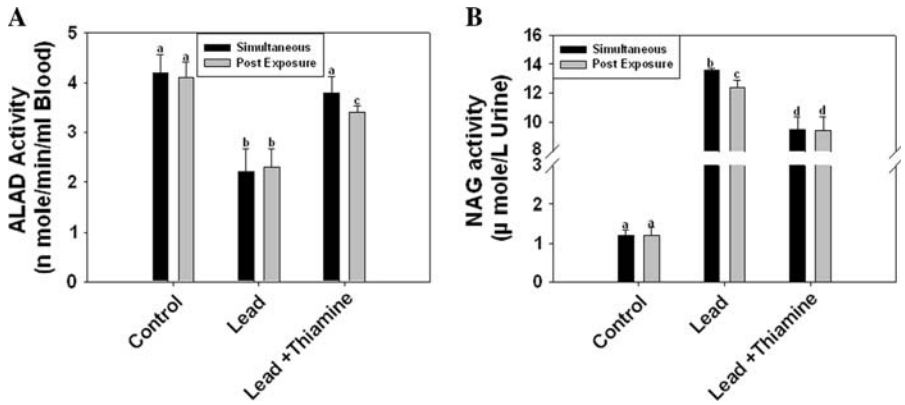

Fig. 5 Effect of thiamine supplementation on ALAD (**a**) and NAG (**b**) activities: Rats exposed to Pb through oral gavage (10 mg/4 ml/kg body weight/day) for 7 weeks and treated with thiamine (25 mg/4 ml/kg/day) either simultaneously or post Pb exposure from 8th–10th week. The ALAD activity in the blood and NAG activity in the urine were measured at the end of the treatment period. *Bars* indicate mean + SD and *bars* that do not share common *superscripts* differ significantly ($P < 0.05$)

In conclusion we provided the evidences for the interaction of Pb with thiamine, which appears to be mediated through pyrimidine ring. Further, enhancement of Pb solubility in the presence of thiamine provides a mechanistic basis for increased intestinal Pb absorption observed during previous studies. However, regardless of treatment regime thiamine prevented the accumulation of Pb in the blood and tissues and normalized the biochemical markers of sub-clinical Pb toxicity.

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