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Original Research Article

Protective role of Vitamin B1 in lead induced poisoning and its effect on the plasma TBARS and hematological parameters in rat

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Abstract

The aim of study was to investigate the effect of thiamine (Vitamin B1) on antioxidative activities of neutrophils and the hematological parameters following the lead toxicity in rats. Forty Wistar rats were randomly assigned into 4 groups (n=10). Group A served as control, Group B received 30 mg/l thiamine, Group C received 8.5 mg/l lead acetate, group D treated by thiamine along with lead acetate by oral gavages for 20 consecutive days. To evaluate the impact of oxidative stress and lipid peroxidation, the levels of thiobarbituric acid reactive substances (TBARS) were measured in serum samples that were collected at time intervals after 0, 10 and 20 days. Compared with basal level on day 0, lead administration (Group C) resulted in a significant increase of TBARS in blood (P<0.05). On 10th and 20th days in group C and 20th day in group D, a significant increase were also observed in TBARS value than control group (P<0.05). PCV and RBC values did not show significant changes. Also, there were significant increases of neutrophil counts and significant decreases of the lymphocyte counts on days 10 and 20 in group C, in comparison with day 0 and controls (P<0.05). The results of the present study in group D indicate thiamine's ability to appease lead-induced alterations in blood tissue. This study demonstrates that exposure with lead results in changes in blood cells. Thiamine supplementation can induces ameliorative effects during this susceptible period.

Keywords: Blood cells, Lead poisoning, Rat, TBARS Thiamine

INTRODUCTION

The most useful and complete model for toxicological studies is the toxicity of lead (Pb) (Silbergeld, 2005). As was detected a decade ago, even exposures at lower concentrations of lead adversely affect development of cognitive, behavioral, and neuro-physiologic (Mahaffey, 1990; Riaz et al, 2011). Although several mechanisms have been suggested for abnormalities induced by Pb and none has yet to be clearly defined, however this element causes damage to multiple body systems and is considered as the oldest and most common environmental contaminants (Meyer et al, 2008; Tian and Lawrence, 1995). One of the effects of Pb exposure is creation of an imbalance between the production and consumption of free radicals that potentially conducts toward oxidative stress (Patrick, 2006). Previous studies have shown that generation of the reactive oxygen species (ROS) relationship with lead toxicity in the rat. Induced ROS can oxidize fatty acids within membrane that finally conducts to proteins changes, damage DNA and lipid peroxidation (Ahamed and Siddiqui, 2007; Bechara, 1993; Hsu et al, 1998). On the other hand, with measurement of some biochemistry values, antioxidant

compounds levels and activity of the antioxidant enzymes, can detect the risk of pb exposure (Kamiński et al, 2009). Some studies have been shown that production of highly ROS, like hydrogen peroxide, lipid peroxide, superoxide radical and hydroxyl radical, which are from the aftermath of lead exposure, is resulted in reduction of the cells antioxidant defense and systematic mobilization (Flora et al, 2004).

Researchers have shown that the simultaneous infusion of thiamin [vitamin B1] and/or vitamin C is more effective in protecting or treating the experimental lead intoxication than either of them individually (Dhawan et al, 1988; Flora et al, 1986). Although studies role of VB1 on lead exposure are well documented, but reports on the impact of administration of VB1 at different times on lead exposure is limited. Therefore, the present study was designed to explore the protective role of VB1 against lead acetate side effects on lipid peroxidation and hematological parameters of rat for 20 days.

MATERIALS AND METHODS

Animals

Forty adult Wistar male rats (Rattus norvegicus albinus) that were 1.5 months old, weighing 210±35 g, were used in this study. The animals were maintained in individual cages at the room with a 12-h light and 12 dark cycles and limited temperature of 20±1°C, and were permitted access to water and standard laboratory pellets ad libitum. The assay protocol for this research was approved by the University Research Committee.

Experiment protocol

After one-week, rats were randomly divided into four groups, A, B, C and D; with 10 rats in each group. The animals in group A served as the control and received the distinct dosage of distilled water. The animals in group B were treated with 30 mg/l thiamine (Fariman Nasr-Iran). The group C received 8.5 mg/l by oral gavage of the lead acetate (fulda-Germany). Group D, animals received simultaneously lead acetate (8.5 mg/l) and thiamine (30 mg/l). Experiment design was followed for 20 consecutive days in all groups.

Blood sampling

Two blood samples were prepared from the hearts of the rats directly and transferred into polyethylene tubes at the outset of the experiment (day 0) and was followed on the days 10 and 20. For hematological parameter, one tube included heparin as the anticoagulation agent analyses, and subsequent tube was coagulation agent, to measure TBARS level.

TBARS evaluation

Serum malondialdehyde concentrations, also known as thiobarbituric acid reactive substances (TBARS), were determined colorimetrically using the method of Buege and Aust, (1978). In brief, 0.1 mL of serum was treated with 2 mL of TBA– TCA–HCl reagent (thiobarbituric acid 0.37%, 0.25 N HCl and 15% TCA) and placed in water bath for 15 min. After cooling, the flocculent precipitate was removed by centrifugation at 112 g for 10 min. The absorbance of supernatant was measured against reference blank at 535 nm. Concentration was calculated using an extinction coefficient of 1.56 9 105 M -1 cm -1 and expressed as nmol L-1.

Hematological factors

Common procedures (Jain, 1993) were used for measuring red blood cell (RBC), packed cell volume (PCV) value, and neutrophils and lymphocytes. PCV levels were determined by a microhematocrit centrifuge (12,000×g for 5 min). Neutrophils and lymphocytes were measured by the manual standard procedures, as leukoctye counts were done on Geimsa-stained blood slides with cross-sectional technique (Jain, 1993).

Statistical analysis

Total obtained results from control, lead-poisoned and thiamine treated animals were compared using one-way ANOVA. A p value <0.05 was considered significant.

RESULTS

TBARS

Data obtained from TBARS values of rats' serum in each group on different days are shown in Table 1. It should be noted that significant differences were not observed in the TBARS values of the four groups at the beginning of the assay (day 0), but there was a significant increase in group C on the 10th and 20th day ($P<0.05$) and in group D on the 20th day in comparison with the basal level (day 0) ($P<0.05$). In groups A and B (control and thiamine respectively), significant differences were not observed in the TBARS value in all of sampling days in comparison to basal level (day 0) and with each other. By comparing the means of the four groups, it was detected that there was no significant difference in the TBARS value on day 0 ($P>0.05$), while in group C, on the 10th and 20th days,

Table 1. The TBARS value (means±SD) in all the groups during the various days of sampling

Time (days)	Groups			
	Group A (Control)	**Group B (Thiamin)**	**Group C (Lead acetate)**	**Group D (Lead, Thiamin)**
0	0.518±0.092	0.516±0.044	0.532±0.055	0.527±0.073
10	0.514±0.071	0.518±0.082	1.02±0.14*,***,****	0.531±0.045
20	0.511±0.084	0.513±0.093	1.14±0.21*,**,***,****	0.742±0.058*,**,***
P value	>0.05	>0.05	<0.05	<0.05

*P<0.05, significant increase to day 0; **P<0.05, significant increase to day 10; ***P<0.05, significant increase to the control group; ****P<0.05, significant increase to the group D

Table 2. PCV value (means±SD) in all the groups during the different days of sampling

Time (day)	Groups			
	Group A (Control)	**Group B (Thiamin)**	**Group C (Lead acetate)**	**Group D (Lead, Thiamin)**
0	30.60±1.23	30.20±1.32	32.21±2.43	32.30±2.12
10	32.00±2.73	32.00±2.64	29.00±1.24	29.10±2.45
20	30.40±1.50	31.00±1.23	28.20±1.33	31.90±1.26
P value	>0.05	>0.05	>0.05	>0.05

Table 3. RBC counts (means±SD) in all the groups during the different days of sampling

Time (day)	Groups			
	Group A (Control)	**Group B (Thiamin)**	**Group C (Lead acetate)**	**Group D (Lead, Thiamin)**
0	9.53±0.60	9.56±0.56	8.60±1.03	9.36±0.60
10	9.16±0.75	9.02±0.61	7.00±0.64*	7.12±0.54*,**
20	8.91±0.85	8.89±1.2	5.80±1.2*,**,***	8.02±0.63
P value	>0.05	>0.05	<0.05	<0.05

*P<0.05, significant decrease to the control group; **P<0.05, significant decrease to day 0; ***P<0.05, significant decrease to day 10

Table 4. Neutrophil counts (means±SD) in all the groups during the different days of sampling

Time (day)	Groups			
	Group A (Control)	**Group B (Thiamin)**	**Group C (Lead acetate)**	**Group D (Lead, Thiamin)**
0	23.00±1.34	24.00±2.65	23.00±1.25	23.00±1.05
10	22.00±2.51	23.00±1.04	29.00±1.70*,****	28.00±1.24*,****
20	23.00±1.74	24.00±1.76	34.00±1.16*,**,***,*****	24.00±1.12*****
P value	>0.05	>0.05	<0.05	<0.05

*P<0.05, significant increase to day 0; **P<0.05, significant increase to day 10; ***P<0.05, significant increase to group D; ****P<0.05, significant increase to the control group; *****P<0.05, significant decrease to day 10

Table 5. Lymphocyte counts (means±SD) in all the groups during the different days of sampling

Time (day)	Groups			
	Group A (Control)	**Group B (Thiamin)**	**Group C (Lead acetate)**	**Group D (Lead, Thiamin)**
0	52.00±1.93	53.00±1.89	51.00±2.58	52.00±2.68
10	53.00±2.15	52.00±2.42	34.00±1.62*,****	35.00±1.22*,****
20	50.00±1.47	52.00±1.05	22.00±2.26*,**,***,*****	45.00±1.71*,****,*****
P value	>0.05	>0.05	<0.05	<0.05

*P<0.05, significant decrease to day 0; **P<0.05, significant decrease to day 10; ***P<0.05, significant decrease to group D; ****P<0.05, significant decrease to the control group; *****P<0.05, significant increase to day 10

and group D on 20th day a significant increase in the TBARS values was seen in comparison with the control group (P<0.05). In group C (lead acetate), the statistical study showed that the values of TBARS were increased to 1.02±0.14 and 1.14±0.21 on the 10th and 20th days respectively, in comparison with the basal level (0.532±0.055). However, there were significant increase in the values of TBARS on the 10th and 20th days in comparison with day 0 (P<0.05). In group D (lead together with thiamine), the TBARS values showed a similar increasing pattern compared to group C, with this difference that obtained values were lesser in group D in comparison with group C. On the 10th and 20th day of group D, the values of TBARS were increased to 0.531±0.045 and 0.742±0.058, respectively, in comparison with the basal level (0.527±0.073). Besides, a significant increase was seen on the 20th day in comparison with day 0 and the 10th day (P<0.05).

Hematological factors

The observed PCV level and counts of RBC, neutrophil, and lymphocyte on different days of sampling are shown in Tables 2 to 5.

The PCV Counts

In all the groups, there were no significant differences in the PCV level at all of the sampling days, neither with the basal level (day 0) nor with each other (P>0.05). Although in group C, PCV counts decreased in the period between days 0 and 29, but this decrease was not significant.

RBC Counts

There was a significant decrease (P<0.05) in the RBC counts in the group C on 10th and 20th day and in group D on 10th day compared to control group. Also RBC counts showed that there is significant decrease (P<0.05) in RBC counts of group C on 20th day and in group D on 10th day in comparison whit basal level (day 0).

Neutrophil counts

As Table 4 shows, the neutrophils number in the group C increased at time interval between days 0 and 20, and in group D increased from day 0 to day 10 and afterward decreased (the P value was <0.05 in each of the four groups). The statistical study in group C (lead) showed that there is a significant increase in the neutrophil counts on the 10th and 20th day in comparison with day 0 and on the 20th day in comparison with the 10th day (P<0.05). In group D (Lead together with Thiamine), there was significant increase in the neutrophil counts on the 10th day in comparison with day 0 (P<0.05), but unlike group C, neutrophils number was significantly decreased on the 20th day in comparison with the 10th day (P>0.05). In the groups A and B, there were no significant differences in number of neutrophils at all of the sampling days, than basal level (day 0) and than each other. By comparing the means of the all of the groups, it was detected that significant difference was no observed in the neutrophil numbers on day 0 (P>0.05). The number of neutrophil in group C showed a significant increase on the 10th and 20th days in comparison with control group (A) (P<0.05). In the group D was observed that only on the 10th day this increase was significant in comparison with control group (P<0.05).

Lymphocyte number

As Table 5 shows, the lymphocytes number in group C decreased from day 0 to 20th day and in group D from day 0 to 10th day. In group C, a significant decrease was observed in lymphocyte counts on the 10th and 20th day in comparison with day 0 (the P value was <0.05 in each of the four groups), on the 20th day in comparison with the 10th day (P<0.05). In group D, there was significant decrease in the lymphocytes number on the 10th day in comparison with day 0 (P<0.05), but unlike group C, there was a significant increase on the 20th day in comparison to the 10th day (P<0.05). In the control group, there were no significant differences in the lymphocyte counts on all of the sampling days, neither with the basal level (day 0) nor with each other. By comparing the means total of the four groups, significant differences not observed in the lymphocyte counts on day 0 (P>0.05).

There was a significant decrease in the lymphocyte counts of group C on the 20th day in comparison with those in group D (P<0.05), and on the 10th and 20th day, in comparison with control group (P<0.05). Also in group D, was observed a significant decrease in the lymphocyte counts on the 10th day, in comparison with the control group (P<0.05).

DISCUSSION

Lead is a toxic heavy metal of the environmental pollutant, which has direct and indirect effects on the biological and biochemical systems and somatic cells. In addition to, being a ubiquitous toxic agent, because of the large amount of resources in the home environment, its toxicity remained as a consequential public health problem (El-Mehi and Amin, 2012). Although there was no well defined mechanism for its toxicity, however studies have been shown that some the effects occur as a consequence of lead propensity for disrupting the

delicate prooxidant/antioxidant balance, which is seen within cells (Donaldson and Knowles, 1993; Monteiro et al, 1986). In the biological samples serum TBARS, containing aldehydes and lipid hydroperoxides that increase in oxidative stress conditions. On the other hand, TBARS plasma concentrations can be an index of lipid peroxidation and oxidative stress, and reverting TBARS to basal levels over time is dependent on the antioxidants (Armstrong and Browne, 1998; Yagi et al, 1998). In this study, the TBARS value in group C (lead) was in a high level till the 20th day, but this value in group D (lead together with thiamine) was increase in a slightly higher value after 10th day. Hence, in group D the increase of TBARS value has been done with more delay. The prominent finding in this study is that the presence of thiamine with lead acetate diminished its injurious effects on the levels of TBARS. In agreement with our findings, Newairy and abdou (2009) indicated that TBARS serum concentration increase when they study defensive effect of flax against lead acetate induced oxidative damage (Newairy and Abdou, 2009). Flora et al. (2004) showed that tissue TBARS contents in kidney significantly increase on lead induced oxidative stress (Flora et al, 2004).

Erythrocytes have a high dependence to the presence of lead acetate, as they can be bind as much as 99 percent to lead in the bloodstream. The cellular membranes are destabilized by lead and this effect in red blood cells (RBC) decreases fluidity of the cell membrane and increase the amount of erythrocyte hemolysis. Hemolysis seems to be the final result of membrane lipid peroxidation and ROS production in RBC (Lawton and Donaldson, 1991). Since that the role of lead in destruction of RBCs has been proved and one of the properties of its destructive has been mentioned as a factor in anemia production (Patrick, 2006), hence this effect has been significant when studying the hematological profiles in the present study. The number of RBC in the group getting lead acetate have been less than group D (lead together with thiamine) and the control group, but decreasing of PCV counts not statistically significant. On the other hand, lead have been reported that can induce immune reactions (Flora et al, 2012; Flora et al, 2004; Gonick, 2011), hence activity of polynuclear cells specifically neutrophils that are the body's first barrier of defensive, can be affected by this chemical element. The lead acetate by influence on the development process of various types of WBCs, can disrupt postnatal development and then lead to a sudden neutrophilic deterioration and abnormal neutrophils (Sharma et al, 2012). When neutrophils are faced with foreign agents, may be released very high oxygen than the normal. Therefore, activity of the NADPH oxidase enzyme on the cell surface membrane increases. The result of interactions in neutrophils is severely destructive, and cause neutrophil membrane desolation (Hodgson et al, 2006). It should be considered that the changes in the number of neutrophils and lymphocytes, that are type of body immunity system response, depending on the individual differences that observe within some the species (Chandra, 1997).

Other result that has been earned about the effect of the lead on blood cells was increasing the number of the neutrophil cells and its keep up a high level for a longer time (20 day) in the group C, which is characterized as a neutrophilia. Increasing neutrophil counts was reported in humans when exposed to lead (Di Lorenzo et al, 2006). What should be noted here is that inducing of rats with lead acetate caused a significant decrease in small lymphocyte count. Treatment of rats with thiamine at a dose of 30 mg/l, to some extent improved the lead-induced changes in neutrophil and lymphocyte counts in the rats of group D. Thus it appears that thiamine has the ability to protect against lead acetate induced immune reactions. Some the researchers mentioned that possibly thiamine by the formation of a lead-thiamine metabolic complex inhibit the absorption of lead in tissues, when both together are used (Ref.).

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CONCLUSION

According to above information, it is concluded that lead can be important agents in demolition of RBCs and decreases lymphocytes, Lead is effective on the counts and the activity of neutrophils and increases them (neutrophilia). The increase in TBARS value was longer and more sever when caused by lead (group C) than lead with thiamine (group D). Lead in combination with thiamine, when given to rats in a dose of 30 mg/l for 20 days, minimized these damaging effects. It can is expressed that append thiamin, counteract the deleterious effects of the environmental exposure to lead.

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