Experimental Research

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The Effect of Vitamin C on the Erythrocyte Antioxidant Enzymes in Intoxicated-Lead Rat Offsprings

Animal Research Section

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ABSTRACT

Objective: Lead exposure or lead poisoning is known to cause a large spectrum of physiological, biochemical, and behavioural disorders in animals. This study was aimed at assessing the effect of vitamin C on the erythrocyte superoxide dismutase, glutathione peroxidase and the glutathione reductase activities in intoxicated- lead rat offsprings.

Methods: This study was performed on the pups from female Wistar albino rats. The rats were divided into 4 groups and the treatments were administered through drinking water. Group1 (control group) consumed distilled water. Group 2 (lead group) consumed a solution of lead acetate (300mg/L). Group3 (lead + vitamin C) consumed a solution of lead (300mg/L) which was supplemented with vitamin C (2g/L). Group4 (vitamin group) consumed a solution of vitamin C (2g/L). The enzyme activities were determined in all the 4 groups.

Results: The administration of lead showed a decrease in the enzyme activities. The superoxide dismutase activity was increased after the administration of lead in combination with vitamin C. The lead treated rats showed significantly lower body weights at birth and at weaning. The vitamin C treatment showed a significant increase in the body weight. The haemoglobin levels were significantly decreased in the lead-treated rats. The addition of vitamin C to the lead treatment and vitamin C alone could elevate the haemoglobin levels significantly.

Conclusion: The results of this study showed that lead alterates the erythrocyte antioxidant enzyme activities. There was an increase in the superoxide dismutase activity following the treatment with vitamin C. This study suggests that the treatment with vitamin C during lactation has a therapeutic effect in the treatment of lead intoxication. The administration of vitamin C prevents haemoglobin reduction in the erythrocytes.

Key Words: Vitamin C, Antioxidant enzymes and lead

INTRODUCTION

Lead exposure or lead poisoning is known to cause a large spectrum of physiological, biochemical, and behavioural disorders in the animals which are investigated in experiments and laboratories and in humans [1-2], (their central and peripheral nervous [3], haemopoietic [4] and cardiovascular systems [5], kidneys [6], liver [7] and male [8] and female reproductive systems are affected) [9]. It has been reported that oxidative stress is connected with the lead toxicity [10]. The modification of the antioxidant systems in the cells are another possible reason for the lead caused oxidative stress. The enzymatic antioxidant systems are superoxide dismutase (SOD), catalase, glutathione peroxidase and glutathione peroxidase [11].There are some non-enzymic antioxidants such as vitamins. They have a role in the treatment of lead poisoning. Vitamin C is one of these vitamins that eliminates the oxidative stress products [12-15] by a very rapid electron transfer [14-15]. The studies on adult animals [16-17] and in newborns following lead exposure [18] showed increased oxidative stress in the brain. Other studies showed that a 500 ppm lead exposure throughout pregnancy and lactation, could cause oxidative stress in the weaned and adult rats in some areas of the brain [19]. It has been shown that the lead exposure in the uterus may involve the foetal growth and development of the post natal behavioural function [20-22]. The foetus and child developing are more sensitive to lead exposure than adults. This can depend on the immaturity of the blood-brain barrier, an elevated gastrointestinal absorption, and hand-to-mouth behaviours, which can elevate the lead exposure [23]. The lead concentration in the maternal blood circulates across the placenta. The lead concentration in the foetal blood is probably the same as that in the maternal blood [24].There is only little information on the effects of lead exposure on the antioxidant enzyme activity. The present study was aimed at assessing the effect of vitamin C on the erythrocyte superoxide dismutase (SOD) glutathione peroxidase (GPX) and the glutathione reductase (GR) antioxidant enzyme activities in intoxicated- lead rat offsprings.

MATERIALS AND METHODS

This study was performed on the pups from healthy female Wistar albino rats (they were obtained from the Pasteur Institute of Iran, which had a body weight of 220–240 g) in 2012. The pregnant animals were maintained in standard laboratory cages at 23 ± 2°C in 12 hr light/dark cycles. They had free access to food (the commercial diet for rodents) and water. The rats were housed individually in cages and they were maintained under supervision, in accordance with the principles of the Health Guide for the care and use of laboratory animals. The rats were divided into 4 groups (2 female rodents in each group) and the treatments were administered through drinking water. The female rodents in Group 1 (control group) consumed distilled water. The female rodents in Group 2 (lead group) consumed a solution of lead acetate (300mg/L) (Chem-Lab [Belgium]). The female rodents in Group 3 (lead + vitamin C) consumed a solution of lead (300mg/L) which was supplemented with vitamin C (2g/L) (Germany). The female rodents in Group 4 (vitamin group) consumed a solution of vitamin

C (2g/L). The solutions were prepared and replaced daily, to minimize the metal precipitation. The treatments lasted throughout the pregnancies and lactations (41-43 day). Each female rodent delivered 4-6 live litters of pups. At weaning (21th day, postnatal), 8 pups in each group were weighed and anaesthetized with a 100mg/kg injectable solution of ketamine 10% and xylazine 2% (alfasan-Holland). A 1.5-ml blood sample was collected by cardiac puncture. The sample of blood was separated into plasma and erythrocytes by centrifugation at 3,000 × g for 10 min. The erythrocyte samples were washed three times with cold physiological saline (PS) and they were then haemolyzed by adding a fourfold volume of ice cold. The haemolyzed erythrocyte samples were centrifuged at 10,000 × g for 10 min, and the supernatants were collected and stored at –70 °C for the measurement of the enzymatic activities. Haemoglobin (HGB) was analyzed by using an automated cell counter, Sysmex.

Superoxide Dismutase (SOD)

The SOD activity was estimated in the erythrocyte samples (cell lysates) by using the commercially available RANSOD Kit of Randox Laboratories Ltd. (Randox, UK), by employing xanthine and xanthine oxidase to generate superoxide radicals. The reaction was measured at 500 nm by using 5 µl of the sample in a total reaction volume of 230 µl. We used an appropriate SOD control (calibrator). The enzyme activity was reported as units per gram Hb (U/g Hb).

Glutathione Reductase (GR)

The glutathione reductase activity of the erythrocytes was measured by using a kit from the Randox Company and an appropriate GR control. Glutathione reductase catalyzes the reduction of glutathione in the presence of NADPH, which is oxidized to NADP+. The decrease in the absorbance at 340 nm is measured. The GR activity was reported as units per gram Hb (U/g Hb).

Glutathione Peroxidase (GPx)

Glutathione peroxidase (GPx) was measured by using a kit which was supplied by Randox Laboratories Ltd., by using the appropriate control (calibrator). The GPx activity was measured at 340 nmby using a sample volume of 5 µl in a total reaction volume of 285 µl.

STATISTICAL ANALYSIS

The results were expressed as mean ± standard deviation. The Kolmogorov test ($p < 0,01$) was used to determinate whether the data were adequate for parametrical analyses. The analysis of variance (ANOVA) was used to determinate the significant differences between the groups. If there were significant differences, we used the Post Hoc (Tukey) test. The differences were considered to be statistically significant if the p value was <0.05.

RESULTS

The results of this study have been shown in [Table/Fig-1] and as mean ± SD. [Table/Fig-1] shows the alterations in the antioxidant enzyme activities following the treatment with lead alone or in combination with the vitamin C treatment. The mean value of erythrocyte superoxide dismutase decreased and it increased significantly in the groups 2 and 3 in comparison with that in group 1, respectively. There was an also a significant increase in the erythrocyte superoxide dismutase activity and a decrease in the erythrocyte glutathione peroxidase (GPx) and glutathione reductase activities in group 4 as compared to those in group 2, respectively. The mean values of erythrocyte glutathione peroxidase (GPx) and glutathione reductase decreased significantly in the groups 2 and 3 in comparison with those in group 1, respectively.

The animal body weights were changed by the treatments with lead, lead in combination with vitamin C and vitamin C alone as shown in [Table/Fig-2]. The lead treated rat offsprings (group 2) showed significantly lower body weights at birth and at weaning as compared to the weights of those in group 1 ($p < 0.001$). The treatment with vitamin C at birth and at weaning showed a significant increase in the body weight in the groups 3 and 4 as compared to that in group 1 ($p < 0.001$). The results of the haemoglobin alterations also have been shown in [Table/Fig-2]. The haemoglobin levels were significantly decreased in the lead-treated rat offsprings (group 2) as compared to those in the offsprings in group 1 p<0.006).The addition of vitamin C to the lead treatment group (group 3) and the vitamin C alone group (group 4) caused significant elevations in the haemoglobin levels ($p < 0.006$).

DISCUSSION

It has been reported that lead poisoning can alterate the vitamin C metabolism [25]. The results of the present study suggest about the

Groups	n	SOD (IU/gHb/min)	GPX(IU/gHb/min)	GRX (IU/gHb/min)
Control (Group 1)	8	0.29^{1} ± 1.15	182.14^{2} ±291.12	$3.83±1.00^{3}$
Lead (Group 2)	8	0.15^{1a} ± 0.60	17.70^{2a} ± 250.17	$2.97±0.13^{3a}$
Lead + vitamin C (Group 3)	8	0.86^{1b}± 1.50	66.16^{2b}± 202.65	$1.70±0.66^{3b}$
Vitamin C (Group 4)	8	0.35^{1c} ± 0.77	38.98^{2c} ± 136.14	$2.14±1.01^{3c}$

[Table/Fig-1]: Erythrocyte antioxidant enzyme activities at weaning in rat offspring
1a and 1b compared with 1; 1c compared with 1a group (P<0.05); 2a and 2b compared with 2; 2c compared with 1a group (P<0.05); 3a and 3b compared with 3; 3c compared with 1a group (P<0.05); 3c compared with 3b group (P<0.05).
Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and Glutathione Reductase (GR)

Groups	n	Body weight at birth (gr)	Body weight at weaning (gr)	Hemoglobin at weaning (mg/dl)
Control (Group 1)	8	0.7^{1}±5.33	1.83^{2} ±21.11	$11.78±4.45^{3}$
Lead (Group 2)	8	0.35^{1a}±2.12	8.0^{2a} ±17.25	$10.75±0.95^{3a}$
Lead + vitamin C (Group 3)	8	0.77^{1b} ±3.0	5.88^{2b} ±32.0	$12.0±3.31^{3b}$
Vitamin C (Group 4)	8	0.88^{1c} ±5.75	1.90^{2c}±27.7	$13.50±4.72^{3c}$

[Table/Fig-2]: Effect of lead, lead and vitamin C and vitamin C alone treatment on body weight and Hemoglobin level of rat offspring at weaning
1a and 1b compared with 1; 1c compared with 1a group (P<0.05); 2a and 2b compared with 2; 2c compared with 1a group (P<0.05); 3a and 3b compared with 3; 3c compared with 1a group (P<0.05); 3c compared with 3b group (P<0.05).

beneficial role of vitamin C on some antioxidant enzyme activities when it exposes to lead. Several studies have shown that different vitamin supplementations can decrease the toxic indications of lead [26-27]. Vitamin C increases the superoxide dismutase activity when animals treated with lead and vitamin C, while the treatment with these supplements decreases the glutathione peroxidase and the glutathione reductase activities. It has been reported that vitamin C shows protective effects against the intoxication with lead [28-29]. Studies have shown that vitamin C has the ability to form a complex with lead [26]. The alterations in the antioxidant enzyme activities are caused by lead. Our study showed that vitamin C has a protective effect on the superoxide dismutase activity. Some researchers reported that the alterations in the superoxide dismutase activities in lead-exposed rats did not show any statistical significance [14]. In the study of Moreira et al., [19] following an exposure of rats to 500 ppm of lead during pregnancy and lactation, the superoxide dismutase, glutathione peroxidase and the glutathione reductase activities were determined in the hypothalamus, hippocampus and the striatum of the male rats at weaning. They showed that the superoxide dismutase activity was slightly decreased in the hypothalamus. This decrease was not statistically significant, while it existed in the lead treated and in the lead and antioxidant treated rats. In studies which were done on animals who had high exposures to lead, a reduced erythrocyte superoxide dismutase activity was reported in many cases [30-32], whereas the superoxide dismutase activity did not show any changes in the brain. Sandhir et al., [33] showed a reduction in the activity of the superoxide dismutase enzyme. Different investigators have reported decreased superoxide dismutase activities [34-35, 19] and these may be caused by an interaction between lead and copper, a metal which is necessary for the complete functioning of the superoxide dismutase enzyme. It has been reported that glutathione reductase is inhibited by lead. Glutathione reductase is the enzyme which is responsible for the recycling of the oxidized enzyme to the reduced form [16].

Some studies have shown that lead increased and decreased the erythrocyte antioxidant enzymes, superoxide dismutase, catalase, and glutathione peroxidase [35-37]. A study showed that the activities of the antioxidant enzymes depended on the lead concentrations. Lower and higher levels of lead exposure can increase and decrease the enzymes activities, respectively. A study which was done on 137 lead-exposed workers with high (over 40 µg/dL) and low blood lead levels (25-40 µg/dL) showed significant decreases and increases in the levels of blood glutathione peroxidase, respectively [38]. The effect of vitamin C on the lowering of the lead exposure has not exactly shown. The workers who were exposed to lead showed higher blood lead levels. The treatment of workers with1 g of vitamin C orally, once daily, five days a week for 20 weeks, showed no effect on the lead levels or the lead metabolism [39]. A study which was done on the effect of vitamin C on the lead levels has shown that vitamin C decreases the intestinal absorption of lead by changing the ferric iron to ferrous iron in the duodenum. Vitamin C elevates the accessibility of iron. It competes with lead for the intestinal absorption [40].

In our study, the weights of the rats which were supplemented with lead, significantly reduced at birth and at weaning. These results showed that lead poisoning delayed the growth of the rat offsprings. Studies have shown that lead reduced the weights of the pups at birth and at weaning when lead was administrated alone or in combination with zinc and vitamins [41-42]. Our study showed that the rats which were treated with vitamin C had body weights which were higher than those of the rats in the control groups. This means that vitamin C is important for the developmental growth of rats. In our study, the weights of the rats which were treated with lead had significantly reduced at birth and at weaning. These results showed that lead poisoning delayed the growth of the rat offsprings. Our results are in agreement with the findings of other studies [41,43].

The haematological system is susceptible to the lead intoxication. [44-45]. The affinity of the erythrocytes to lead is high. The blood stream has more than 90% lead. Our study showed reductions in the haemoglobin levels when the rats were supplemented with lead. There was an increase in the haemoglobin levels when the rats were supplemented with lead and vitamin C and with vitamin C alone. This suggests that vitamin C has a protective effect on the haemoglobin levels and that it can reduce the prevalence of anaemia.

In conclusion, the results of this study show that lead alterates the erythrocyte antioxidant enzyme activities. It was seen that there was an increase in the superoxide dismutase activity following the treatment with vitamin C when the subjects who were treated with lead and vitamin C were compared. This study suggests that the treatment with vitamin C during lactation has a therapeutic effect on the treatment of lead intoxication. The administration of vitamin C prevents the haemoglobin reduction in the erythrocytes.

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