

The Evaluation of Deferasirox on Hematological Parameters after Lead Administration

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Abstract

Background: Metals such as iron, zinc, and copper are critical and necessary for the survival of all living organisms, whereas xenobiotic metals such as lead, cadmium, mercury, and arsenic have no known biologic role. Any metals in high doses can have toxic effects. The aim of this work was to evaluate the hematological changes induced by lead as a toxic metal and characterize the potential efficacy of Deferasirox in removing lead from bodies of male rats.

Methods: Lead was given to rats at two doses of 40 (low dose of drinking lead) and 80 mg/kg (high dose of drinking lead). After 60 days of lead administration, chelation therapy was carried out for two weeks and then clinical, biochemical and haematological parameters were compared with the lead-free control group.

Results: The results showed a decrease in iron level, hematocrit, red blood cells count, hemoglobin concentration, mean cellular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration, after lead administration. Chelation therapy with Deferasirox (DFX) significantly reduced blood lead level, and iron concentrations returned to normal levels simultaneously.

Conclusion: Deferasirox significantly reduced blood lead level along with normalizing iron. The symptoms of toxicity were also reduced and iron deficiency anemia following lead administration was obviated.

Keywords: Deferasirox; Hematological Tests; Iron-Deficiency; Lead Poisoning

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INTRODUCTION

Lead poisoning is a health problem around the world (1). Lead poisoning correlates with blood lead concentration. *Biochemical* and sub-clinical abnormalities often disappear at levels around 10µg/dL and can lead to coma and death at levels over 100µg/dL. Exposure to lead can damage the hematopoietic system, kidneys, cardiovascular and central nervous system (1). Lead reduces the absorption of iron in the gastrointestinal tract (2). The hemoglobin content of blood becomes lower with increasing the concentration of lead in the blood (3, 4). The hematological effects of lead can be attributed to a combination of effects including inhibition of hemoglobin synthesis and shortened life spans of circulating erythrocytes (5). These effects may result in anemia (5, 6). Lead inhibits activity of SH-dependent enzymes involving in heme synthesis (7). High blood lead levels can inhibit protoporphyrin synthesis and increasing the risk of anemia (8). One of the most effective ways to remove toxic elements such as lead from the biological system is chelation therapy. Chelating agents bind to toxic metal ions and promote the excretion of this metal from biological organs (9). Deferasirox (Figure 1) was first reported in 1999 (10). It is a tridentate chelator with high selectivity for iron (III) (11). Deferasirox is absorbed rapidly,

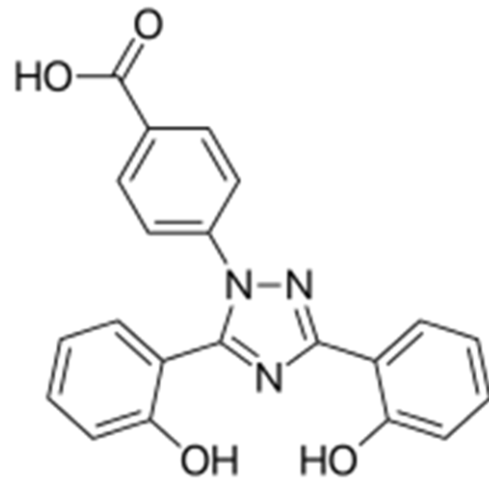


Figure 1. Chemical structures of Deferasirox

achieving peak plasma concentration within 1–3 hours after administration. The terminal elimination half-life ranges from

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8 to 16 hours with repeated doses, which allows a once-a-day regimen (12). In blood circulation, two molecules of deferasirox can form a complex with ferric iron ($\text{Fe}(\text{deferasirox})_2$) (Figure 2); also there is as an unchanged form (13). The results show that deferasirox and its iron complex were 99.2% bound to plasma proteins (14). Haematological analysis, which comprised serum iron, serum ferritin, total iron binding capacity (TIBC), transferrin saturation (TS), red blood corpuscles (RBC), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (PLT) counts, hemoglobin (Hb) and hematocrite (HCT) levels are Diagnostic test that provide information about the haematopoietic system response. These blood tests can serve as diagnostic of iron deficiency (15). Evaluations of some chelators for removal of toxic metal ions in animals have been previously reported (16-23). In this study, we aimed to evaluate the effects of lead on several haematological parameters and protective effect of chelation therapy with Deferasirox in rats.

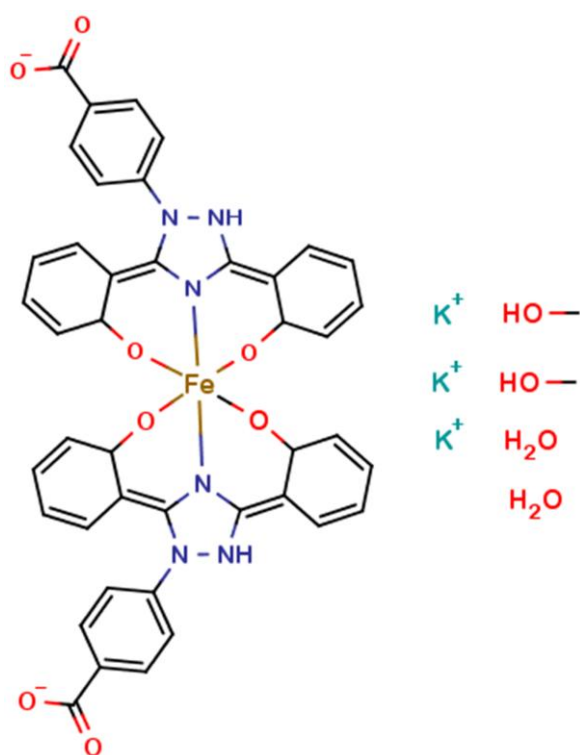


Figure 2. Chemical structures of complex $\text{Fe}(\text{Deferasirox})_2$

METHODS

Maintenance of the animals

Seventy male Wistar rats were bred in the animal house at Mashhad University of medical science, Mashhad, Iran. At the onset of the study, the rats were 6 weeks old, weighing 200 ± 10 g (mean \pm SD). They were housed in a temperature-controlled ($23 \pm 1^\circ \text{C}$), 12/12 light/dark room, and acclimated for 3 days prior to experimentation. The rats were allowed standard animals chow diet as well as pre-prepared drinking water throughout the experiment. This study was approved by the ethics committee of the Ferdowsi University of Mashhad, Mashhad, Iran and Mashhad University of medical science, Mashhad, Iran.

Apparatus

A Varian flame atomic absorption spectrometer (FAAS) was used for measurement of lead and iron concentrations in rats' blood. Hematological indices including red blood cells, mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet counts, hemoglobin and hematocrite levels were measured by a Sysmex Hematology Analyzer.

Drugs and reagents

Lead (II) nitrate and Deferasirox were purchased from Merck Chemicals Co. and Novartis Co. (Basel, Switzerland), respectively.

Experimental design

On the third day after arrival, the animals started to receive lead in drinking water. Seventy animals were randomized into one group of 10 (Group I) and two groups of 30 (Groups II and III) rats and were treated as below for 60 days (Table 1):

Group I: No treatment (control group).

Group II: Drinking group (with low level Lead nitrate).

Group III: Drinking group (with high level Lead nitrate).

Lead nitrate was dissolved in distilled water and administered to group II and III as a drinking solution. Lead was given to the drinking group at two doses of 40 mg/kg body weight (as low level drinking group) and 80 mg/kg body weight (as high level drinking group). Over time, following the lead administration, lead toxicity symptoms gradually appeared. With 10 animals, the control group was given normal food and distilled water to drink. After 60 days, animals of groups II and III were divided into three sub-groups (A to C) of 10 rats in each dose (Table 1):

Sub-group A: Before chelation therapy group

Sub-group B: Without chelation therapy group

Sub-group C: Chelation therapy with Deferasirox

The control group and sub-groups IIA and IIIA were killed at

Table 1. Classification of animals

Period of lead administration	All rats						
	Group I (Control group)	Group II (Low dose drinking of lead)			Group III (High dose drinking of lead)		
Period of chelation therapy		Sub-group IIA	Sub-group IIB	Sub-group IIC	Sub-group IIIA	Sub-group IIIB	Sub-group IIIC

Table 2. Body weights over 60 days for rats in different groups (P < 0.01)

Group	Group I	Group II	Group III
Initial body weight ^a (g) (day 1)	205±6 (10)	197±5 (30)	203±4 (30)
Final body weight ^a (g) (day 70) ^b	276±8 (10)	241±6 (29)	214±8 (28)

Values in parentheses are the number of animals in each group
^a Mean of five determinations ± standard deviation
^b Group I (day 60)

Table 3. Lead concentration (µg/l) in blood serum of various groups of rats sub-group A, sub-group B and sub-group C

Group	Sub-group A (µg/l)	Sub-group B (µg/l)	Sub-group C (µg/l)
Group II (low level)	0.1220 ± 0.0013 (10)	0.1161 ± 0.0018 (10)	0.0721 ± 0.0029 (10)
Group III (high level)	0.1430 ± 0.0041 (9)	0.1283 ± 0.0022 (9)	0.0681 ± 0.0027 (9)

Results are presented as arithmetic means ± SEM, number of animals in parenthesis. Significant at P < 0.05 when compared with control

the end of the lead administration stage (day 60). After 60 days of lead administration, sub-groups IIB and IIIB were given normal food and drink for 10 days. This group was killed at the end of the study to show the effect of time on concentrations of lead and iron in rat blood. In order to evaluate the effect of Deferasirox in removal of lead, this chelator was given immediately after lead administration in low-dose and high-dose categories to sub-groups IIC and IIIC (day 70). Lead exposure was stopped during chelation therapy. Sub-groups IIC and IIIC were killed at the end of the study (day 70).

Dosage of the chelator was calculated based on the rats' body weight (70 mg/kg body weight), and it was dissolved in their drinking water. At the end of the treatment, all animals were euthanized under light anesthesia with Ether and then blood samples were collected for determination of lead and iron contents. Moreover, at the end of this step, some hematological indices such as hemoglobin concentration in red blood cells, serum iron concentration and total iron binding capacity were determined. For biochemical analysis, blood samples were centrifuged and plasma or serum was aspirated until used for analyses. Different analyzed hematological parameters were as follows: red blood corpuscles, mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet counts, hemoglobin, and hematocrit levels.

Statistical analysis

All data are presented as mean±SD and analyzed by SPSS 15.0 statistical software (SPSS Inc., Chicago, IL). Prior to analysis covariance (ANOVA), a homogeneity of variance test was conducted to determine the homogeneity of the tested values. Comparison of two means was then performed using one-way ANOVA test, followed by Dunnett's multiple comparison tests. In all cases, the differences between two means were considered significant if p-values were equal or less than 0.05.

RESULTS

In the present study, the effects of doses of lead were compared to control group in concentrations of lead and iron in rat blood and hematological parameters. There were slight differences between the groups in the initial body weight of the rats (mean 200 g), but following exposure to 40 and 80 mg dosages of lead, the body weight of the rats was found to have been slightly reduced (Table 2). Some of the symptoms of lead toxicity, appeared during lead uptake, were red staining around the eyes, black dots on the liver, weakness, and loss of hair. The results of this study demonstrated significant changes in both the haematology parameters and concentrations of lead and iron in the rat blood.

Changes in concentration of lead and iron

The lead concentration of the diet had a significant effect on iron deposition in blood serum. During lead administration, its concentration increased in blood serum, while iron level decreased. After chelation therapy, blood lead levels in different dose groups were significantly reduced (Table 3), and simultaneously, iron concentrations returned to the normal level and the symptoms of toxicity were also reduced. The results of the serum iron concentrations before and after chelation therapies are summarized in Tables 4 and 5. The difference between iron values before and after chelation therapy was notable.

Changes in haematological parameters

Hematological data showed that toxic metals like Lead affect some of blood indices. Data showed that increasing doses of lead significantly decreased Hb, MCV, MCH, MCHC, RBCs and HCT. In the end of the 60-day period, hematological indices in lead administration groups were compared with the control group (Table 4). Our results showed that other hematological indices such as transferrin saturation, serum iron and serum ferritin decreased due to lead administration, whereas total iron binding capacity and platelet count increased (Table 4). After chelation therapy with Deferasirox,

Table 4. Hematological indices in various groups of rats after lead administration

Hematological indices	Group I	Group II	Group III
Serum iron ($\mu\text{g/dL}$)	140.65 \pm 11.623	93.345 \pm 6.271	67.325 \pm 6.433
TIBC ($\mu\text{g/dL}$)	283.74 \pm 21.88	1670.1 \pm 21.42	1791.2 \pm 33.04
TS (%)	46.821 \pm 7.192	6.07 \pm 0.78	3.87 \pm 0.31
Serum ferritin ($\mu\text{g/L}$)	81.923 \pm 2.810	52.986 \pm 1.294	48.105 \pm 1.245
Hemoglobin (g/dL)	14.987 \pm 1.298	9.050 \pm 1.342	7.879 \pm 1.763
Platelet ($10^9/\text{L}$)	748.18 \pm 51.24	1371.21 \pm 84.43	1365.77 \pm 73.64
RBCs ($10^{12}/\text{L}$)	7.421 \pm 0.933	6.843 \pm 0.965	6.872 \pm 1.402
HCT (%)	41.110 \pm 5.193	28.517 \pm 1.276	26.824 \pm 1.521
MCV (fL)	59.20 \pm 1.41	43.315 \pm 2.922	38.841 \pm 1.248
MCH (pg)	21.580 \pm 0.749	12.923 \pm 1.476	10.848 \pm 0.394
MCHC (g/dL)	35.286 \pm 1.519	34.223 \pm 2.245	31.172 \pm 1.323

Results are presented as arithmetic means \pm SEM, Significant at $P < 0.05$ when compared with control

Table 5. Hematological indices in various groups of rats after DFX administration

Hematological indices	Group I	Group II	Group III
Serum iron ($\mu\text{g/dL}$)	140.65 \pm 11.623	136.34 \pm 10.746	137.85 \pm 10.895
TIBC ($\mu\text{g/dL}$)	283.74 \pm 21.88	278.74 \pm 27.49	284.97 \pm 27.48
TS (%)	46.821 \pm 7.192	45.288 \pm 6.872	47.934 \pm 7.717
Serum ferritin ($\mu\text{g/L}$)	81.923 \pm 2.810	80.856 \pm 3.572	82.513 \pm 3.306
Hemoglobin (g/dL)	14.987 \pm 1.298	13.471 \pm 1.464	14.983 \pm 2.415
Platelet ($10^9/\text{L}$)	748.18 \pm 51.24	742.27 \pm 43.27	738.15 \pm 49.34
RBCs ($10^{12}/\text{L}$)	7.421 \pm 0.933	6.594 \pm 0.822	7.148 \pm 1.205
HCT (%)	41.110 \pm 5.193	38.955 \pm 5.690	40.055 \pm 5.384
MCV (fL)	59.20 \pm 1.41	59.24 \pm 1.38	59.37 \pm 1.28
MCH (pg)	21.580 \pm 0.749	20.478 \pm 0.214	20.824 \pm 0.245
MCHC (g/dL)	35.286 \pm 1.519	37.747 \pm 1.328	36.428 \pm 1.245

Results are presented as arithmetic means \pm SEM, Significant at $P < 0.05$ when compared with control

started immediately after lead administration, symptoms of lead toxicity in rats were removed in short term after drug administration. Therapy with Deferasirox returned iron levels to normal state (Table 5). At the end of this study, hematological indices also returned to normal state (control group values). Transferrin saturation, serum iron, serum ferritin, hemoglobin, hematocrit, RBC, MCH, MCV and MCHC increased significantly to normal state after drug administration. Also, our results in Table 5 showed a decreased platelet count and a marked decrease in total iron-binding capacity.

Effect of time on spontaneous removal of lead from the body

In order to investigate the effect of time on spontaneous removal of lead from the body, one sub-group was treated as sub-groups B. The results of chelation therapy are shown in Table 3. Comparison of the results obtained from both sub-

groups A and sub-groups B indicates that elimination of lead through biological system is not noticeable. Therefore, time has no significant effect on the removal of the lead.

DISCUSSION

Lead can get into the body through inhalation (24), ingestion (25) and carried throughout the body by the blood. Thus, measurement of blood lead levels is the most common method for organizing the degree of exposure in humans (26). Our results are consistent with those observed in other studies, which have found significant association between iron deficiency and high blood lead levels (27-29). Hematological characteristics are an important tool that can be used as an effective and sensitive index to monitor iron association between anemia and blood lead levels. Lead toxicity can cause anemia via impairment of heme synthesis deficiency anemia (30). Our study demonstrates a significant

and increase the rate of the red blood cells destruction. One reason is form heme in the mitochondrial matrix. Insertion of ferrous into the tetrapyrrole macrocycle of protoporphyrin catalyzed by enzyme ferrochelatase is more sensitive to the effects of lead (31). Iron-deficiency anemia is characterized by reduction or absence of iron stores (serum ferritin), low serum concentration of iron, decreased transferrin saturation, an increased platelet counts and a marked increase in TIBC (32). Furthermore, red blood cells may also become smaller in *size* than normal. This leads to hypochromic microcytic anemia (33). Hematological parameters such as RBC, Hb, Hct, MCV, MCH, and MCHC are reduced due to iron deficiency. Blood parameters are useful and sensitive for the diagnosis of Iron-deficiency anemia (33). The inverse relationship between hematological system and lead-toxicity has been intensely investigated (26). In the present study, we reported the hematological changes induced by lead at doses of 40 and 80 mg/kg in order to evaluate the effects of the lead on iron levels, which results in Iron-deficiency anemia. Our results in Table 4 showed that after lead administration, serum iron and serum ferritin decreased while TIBC increased when compared with the control group. Decrease in MCV, MCH, HCT, and MCHC were another sign that confirmed iron deficiency anemia in rats. In addition, our results showed that lead accumulation in blood at higher dose levels was greater than that of the lower dose levels, which is probably due to a significant interference that could take place by the lead through the iron uptake mechanism. In the present study, we suggested that lead affects the hematopoietic system by inhibiting the heme and hemoglobin synthesis.

Chelation therapy has a great importance in the removal of the toxic elements and preventing *metal overload* in the critical organs (14, 16). Clinical evaluations of some chelators for removal of toxic metal ions in rats have been reported previously (16-23). In during the chelation therapy toxic metal may be bound to chelate ligand. The efficacy of a ligand agent is the ability to remove the toxic metal ion from biomolecules such as proteins (19). Complex toxic metal-chelates should be extracted from the body. Deferasirox has a low molecular weight and high lipophilicity, thus it easily distributes in the body with an elimination half-life ranging from 8 to 16 hours. The treatment consists of removal of lead from body using Deferasirox as a chelator (22). Our results showed that use of Deferasirox as a chelator is a potential treatment for complications of lead toxicity. As a chelating agent, Deferasirox reduced serum lead levels and led to normal iron level. The present study suggests significant beneficial effects of chelation therapy with Deferasirox during lead exposure, particularly on decreasing the concentrations of lead and returning the iron levels to normal state in the rat blood. Therefore, all hematological indices that were investigated in this study returned to normal state (control group values).

LIMITATIONS

Lead nitrate was partly insoluble in water; therefore, the suspension of lead was administered to rats.

CONCLUSION

Lead poisoning can result in iron deficiency anemia. Deferasirox has the ability to remove the lead from the body of the rats and therefore has the potential to be researched as a chelator in the elimination of the lead from the body.

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