Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(4):310-314

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

The Effect of Cadmium on Iron Metabolism in Rats: A Combined Therapy by Deferasirox and Deferiprone

Seyed Mojtaba Mashmool Moqadam, Samie Salehi, Soheila Mohammadkhani and Amir Sh Saljooghi*

Department of Chemistry, Ferdowsi University of Mashhad, Faculty of Science, Mashhad, Iran

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ABSTRACT

This study is aimed to evaluate the potential efficiency of deferasirox (DFX) and deferiprone (L1) as an oral drug chelators on the excretion of cadmium (Cd) in cadmium-poisoned wistar rats following drink administration. After a period of 60 days, all the rats administered cadmium were severely anemic and showed toxicity symptoms through loss of hair and increasing in cadmium and reduction in iron levels in blood. Our results showed that the effect of cadmium on hematological indices was statistically important and confirmed the iron deficiency anemia in rats. Chelation therapy period started immediately after cadmium administration during two weeks. The chelation therapy results showed that deferasirox and deferiprone were able to remove cadmium ions from the body while iron concentration returned to normal level and also iron deficiency anemia that caused by cadmium administration obviated.

Keywords: Deferasirox; Deferiprone; Combined therapy; Iron deficiency anemia; Cadmium toxicity

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INTRODUCTION

Heavy metal toxicity has established to be a major problem and there are several health risks related with it [1]. The metals toxicity, even if they do not have any biological role, remains present in some or the other form harmful for the human body and its suitable operative [2]. Cadmium (Cd), as a well-known environmental hazard, exerts a number of toxic effects in human and animal organisms. Dietary intake of cadmium from plants and animals grown on polluted soil results in numerous human diseases. The most important origin of this toxic chemical presented in the soil are fertilizer, industrial waste, mine drainage, and soil amendments [3]. Cd is used in television screens, lasers, batteries, paint pigments, cosmetics, and in galvanizing steel, as a barrier in nuclear fission, and was used with zinc to weld seals in lead water pipes prior to the 1960s [4-6]. The main source of human Cd exposure is cigarette smoking. Surprisingly, study revealed that Blood and kidney Cd levels are higher in smokers than nonsmokers [7]. Effluence with cadmium is mostly irritating because of its stability in the environment and long half-life in human biological system, between 10 and 40 years [8]. There is no clear explanation about the mechanism of cadmium toxicity but its effects on cells are well understood. It is assumed that cadmium concentration increases 3,000 fold when it binds to cystein-rich protein such as metallothionein [9-11]. This toxic metal could cause to the deficiency of iron by its capability to bind with biological active molecules in the human cells like cystein, glutamate, histidine and aspartate. Many studies have examined the bioaccumulation and toxic responses of Cd in animals, plants, phytoplankton and freshwater bacteria.[12] It is shown that Cd can induce apoptosis in mouse liver. Cadmium in liver moves to kidneys where it is excreted and then re-absorbed almost completely. This poor ability of humans to excrete cadmium through kidneys underlies the health implications of cadmium as a nephrotoxin [13,14]. Using chelation therapy methods to eliminate toxic elements such as metal ions from the biological system is an effective way in treatment of patient [15]. Commonly, in cadmium therapy all antibodies specific to cadmium recognize a chelated form of Cd.EDTA. [16,17]. A one-step competitive immunoassay employing an anti-Cd.EDTA antibody has been reportedly applied for the recognition of cadmium in environmental water and human serum [18]. This method is used to recognize other toxic metal such as Hg^{2+}, Pb^{2+} and In^{3+} currently [19]. The most important class of chelators

which are using nowadays includes the commercially available ligands: deferiprone (Ferriproxs), deferasirox (Exjades) and desferrioxamine (Desferal). These organic ligands easily coordinate with metal ions to form a bidentate, tridentate or hexadentate complex, respectively [20]. This study was designed to test the chelation potency of DFX and L1 while given to animals solely or in combination after cadmium loading. The cadmium influence on iron metabolism in rats was investigate by measuring the different parameters involved in iron metabolism such as Serum iron (sFe), total iron binding capacity (TIBC). The result confirmed that the presence of Cd ions in human biological systems have a great role in appearing of iron deficiency while using the chelators decreased the iron deficiency symptoms. Testing was performed by using an acute experimental model on rats with mono or combined chelators given shortly after cadmium application (Figure 1).

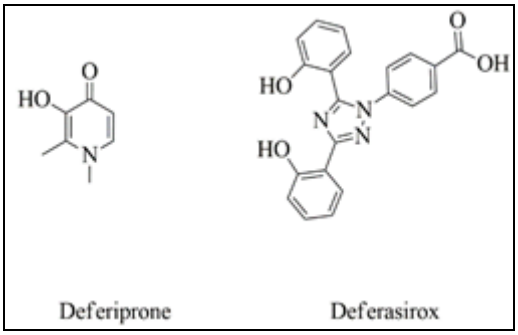

Figure 1: Chemical structures of two iron chelators used in this study

EXPERIMENTAL SECTION

Materials and Methods

Maintenance of the animals:

Male growing Wistar rats with initial mean body weight of 200 g were housed separately in plastic cages with glassgridded bottoms and maintained under controlled temperature (23 ± 1°C), humidity (55%) and light/dark cycle (12/12 hours). Experiments were performed on seven-week-old male Wistar rats. They bred in animal house at Mashhad university of medical science, Mashhad, Iran. This study was approved by the ethics committee of Ferdowsi University of Mashhad, Mashhad, Iran and Mashhad university of medical science, Mashhad, Iran.

Apparatus:

Atomic absorption spectrometer (F AAS and GF AAS) Model Varian was used for measurement of mercury and iron concentrations in rats blood, respectively.

Materials:

Deferiprone and defrosirox were purchased from Novartis Co. (Basel, Switzerland). Cadmium (II) chloride was purchased from Merck.

Experimental design:

During 3 days of adaptation to new surroundings rats were given a cereal-based stock diet and then they were divided into 3 groups. Consequently after acclimatization of animals, we assigned them randomly to control and treated groups. The first group containing 5 rats (control group) was given normal food and distilled water to drink. The second and third groups were given water containing cadmium to the extent of 20 mg Cd^{2+}/kg body weight (low dose drinking of cadmium) and 40 mg Cd^{2+}/kg body weight (high dose drinking of cadmium), respectively. The second and third groups consisted of 15 animals in each group. Oral administration of toxic metal ion was performed once a day. The consumed dosing volume for animals was calculated based upon their weight. During this time since cadmium administration, its toxicity symptoms gradually were appeared. After 60 days, the animals in each group (except control group) were divided into three sub-groups containing 5 rats; before chelation therapy group, without chelation therapy group and chelation therapy with deferiprone. Chelation therapy period started immediately after cadmium administration during 14 days. Cadmium toxicity symptoms observed in rats have been removed in short term after drug administration. Classifications of animals are shown in Table 1.

After 60 days, the first group of rats were anesthetized with ether vapors and immobilized by cervical dislocation. Animals were sacrificed by exsanguinations from abdominal aorta; and blood samples were collected for determination of iron and cadmium concentrations by graphite furnace atomic absorption spectroscopy (GF AAS). After 14 days from chelation therapy period, the second and third groups of rats were killed by exasanguination from abdominal aorta and blood collected for determination of mentioned parameters.

Also at the end of this step, some hematological indices such as hemoglobin (Hb) concentration in red blood cells, serum iron concentration, total iron binding capacity (TIBC), serum cadmium concentration and etc. were determined.

Table 1: Classification of animals

Control group		
All Rats	Low and/or high cadmium doses drinking group	Before chelation therapy Without chelation therapy (vehicle) Single therapy with DFX (140 mg/kg body weight) Single therapy with L1 (300 mg/kg body weight) Combined therapy with DFX (70 mg/kg body weight) + L1 (150 mg/kg body weight)

Statistical analysis

Determination of cadmium and iron in samples were carried out by atomic absorption spectrometry by standard addition method. The values are expressed as mean values (at least three separate determinations) ± standard error of the mean (SEM). The data were subjected to statistical analysis by Student's t-test; $p< 0.05$ was considered significant.

RESULTS AND DISCUSSION

In the present study, the effect doses of toxic metal were compared to control group in concentration of cadmium 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 the exposure to 20 and 40 mg dosages of toxic metal, the body weight of rats were found to have been slightly reduced (Table 2). Some of cadmium toxicity symptoms which appeared during period of cadmium uptake were such red staining around the eyes, black dots on liver and weakness, and loss of hair. The results of this study demonstrated some statistical significant changes in both the haematology parameters and concentration of cadmium and iron in rat blood.

Table 2: Body weights over 60 days for rats in different groups (values in parentheses are the number of animals in each group)

Group	Control	Low dose drinking of cadmium	High dose drinking of cadmium
Initial body weight (g)	205 ± 7(8)	200 ± 4(8)	195 ± 3(8)
Final body weight (g)	270 ± 7(8)	245 ± 8(8)	215 ± 7(8)

Changes in Concentration of Cadmium and Iron

The cadmium concentration of the diet had a significant effect on iron deposition in blood serum. During cadmium administration, its concentration increased in blood serum, while iron level decreased. Furthermore our results showed that cadmium accumulations in blood at higher dose levels were greater than the lower dose levels which are probably due to a significant interference that could take place by cadmium through iron uptake mechanism. After chelation therapy, blood cadmium levels in the different dose groups were significantly reduced (Table 3), and simultaneously, iron concentrations returned to the normal level and the symptoms of toxicity also were reduced. The results of serum iron concentrations after chelation therapy are summarized in Table 4. The difference between iron values before and after chelation therapy is notable.

Table 3: Hematological indices in various groups of rats after cadmium administration in (Results are present as arithmetic means ±SEM, Significant at p<0.05 when compared with control)

Hematological indices	Control	Low dose drinking of cadmium	high dose drinking of cadmium
Serum iron (µg/dL)	138.43 ± 12.729	85.328 ± 5.328	65.225 ± 5.102
TIBC (µg/dL)	285.68 ± 24.15	1673.2 ± 23.68	1788.6 ± 289.5
TS (%)	47.624 ± 7.255	5.45 ± 0.67	3.68 ± 0.34
Serum ferritin (µg/dL)	83.624 ± 2.684	53.219 ± 1.171	49.225 ± 1.220
Hemoglobin (g/dL)	14.680 ± 1.235	9.950 ± 1.445	8.100 ± 1.895
Platelet (109/L)	739.12 ± 52.21	1375.15 ± 83.97	1360.13 ± 73.28
RBCs (1012/L)	7.125 ± 0.775	6.752 ± 0.923	6.554 ± 1.398
HCT (%)	40.812 ± 5.098	29.005 ± 1.785	26.913 ± 1.742
MCV (FL)	58.25 ± 1.22	41.112 ± 3.245	38.325 ± 1.268
MCH (pg)	20.775 ± 0.905	13.982 ± 1.541	10.999 ± 0.375
MCHC (g/dL)	36.500 ± 1.160	35.090 ± 2.358	35.700 ± 1.734

Changes in Hematological Parameters

Hematological data showed that toxic metals like cadmium exerted a certain influence on some of the blood indices studied. Presented data showed that the increasing doses of cadmium caused a significant decrease in

hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), total number of Red Blood Cells (RBCs) and hematocrit (Ht or HCT) at the end of 60 days cadmium administration compared with control group ($P<0.01$). Also our results showed that other of hematological indices such as transferrin saturation (TS), serum iron, serum ferritin decreased in due to cadmium administration, whereas total iron binding capacity (TIBC), platelet count increases in blood serum (Table 4). After chelation therapy with deferasirox and deferiprone that start immediately after cadmium administration, cadmium toxicity symptoms observed in rats have been removed in short term after drug administration. The results of chelation therapy with deferasirox and deferiprone return iron level to normal state (Table 5). Therefore all hematological indices that investigated in this study returned to normal state (control group values). Transferrin saturation (TS), 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 (TIBC) when the other of hematological indices increases.

Table 4: Hematological indices in various groups of rats after DFX+DFP administration (Results are present as arithmetic means ±SEM, Significant at p<0.05 when compared with control)

Hematological indices	Control	Low dose drinking of cadmium	high dose drinking of cadmium
Serum iron (µg/dL)	138.43 ± 12.729	133.25 ± 10.613	137.66 ± 11.924
TIBC (µg/dL)	285.68 ± 24.15	280.68 ± 24.15	286.24 ± 26.70
TS (%)	47.624 ± 7.255	45.320 ± 8.231	46.991 ± 7.355
Serum ferritin (µg/dL)	83.624 ± 2.684	81.314 ± 3.218	82.450 ± 3.998
Hemoglobin (g/dL)	14.680 ± 1.235	13.998 ± 1.1124	14.652 ± 2.472
Platelet (109/L)	739.12 ± 52.21	732.73 ± 51.02	739.11 ± 50.09
RBCs (1012/L)	7.125 ± 0.775	6.968 ± 6.668	7.100 ± 1.190
HCT (%)	40.812 ± 5.098	38.972 ± 5.169	39.920 ± 5.128
MCV (FL)	58.25 ± 1.22	57.55 ± 1.23	58.09 ± 1.87
MCH (pg)	20.775 ± 0.905	19.789 ± 0.235	20.895 ± 0.478
MCHC (g/dL)	36.500 ± 1.160	36.350 ± 1.624	36.005 ± 1.852

Table 5: Cadmium concentration (µg/l) in blood serum of various groups of rats after cadmium and with and without DFX+DFP administration (Results are present as arithmetic means ±SEM, number of animals in parenthesis. Significant at p<0.05 when compared with control)

Group	After cadmium administration (µg/l)	without DFX+DFP administration (µg/l)	With DFX+DFP administration (µg/l)
Control	1.245+0.021(5)	1.198+0.019(5)	0.356+0.023(5)
Drinking (low level)	9.856+0.234(5)	8.992+0.231(5)	3.027+0.127(5)
Drinking(high level)	25.17+4.29(5)	20.37+3.35(5)	2.975+0.239(4)

Effect of Passing Time in Removing Cadmium from the Body Spontaneously

In order to investigate the effect of passing time in removing cadmium from the body spontaneously, one group was treated as without chelation therapy. The results of chelation therapy group are shown in Table 3. Comparison of the results obtained from both (with and without chelation therapy) groups are indicating that elimination of cadmium by the biological system is not noticeable. Therefore, the passing time has no significant effect on removal of cadmium.

CONCLUSION

Our results demonstrate the harmful effect of cadmium on iron metabolism in rats. Dietary cadmium, consumed in amounts of 20 mg Cd^{2+}/kg body weight (Low dose drinking of cadmium) and 40 mg Cd^{2+}/kg body weight (High dose drinking of cadmium) caused a decrease in apparent absorption of iron and it was accompanied by a decrease in iron concentration in blood serum and a development of anemia. Our results in Table 3 verified the iron deficiency anemia in rats.

The aim of this study was removal of cadmium and treatment iron deficiency anemia due to cadmium poisoning by chelation therapy. We tested the chelation potency of DFX and DFP given to animals after cadmium administration. The results of chelation therapy with DFX and DFP return iron level to normal state. On the other hand, at the end of the experimental period, all hematologic parameters studied in the control rats, namely Hb concentration, mean corpuscular volume, serum Fe, red blood cells, hematocrit, platelets, serum ferritin, transferrin saturation, TIBC, etc. were within normal limits in rats. Also iron deficiency anemia that caused by mercury administration obviated. Also iron deficiency anemia that caused by cadmium administration obviated. Therefore the chelation therapy results showed that deferasirox and deferiprone were able to eliminate cadmium ions from body. This process might be useful for preliminary testing of the efficiency of deferasirox and deferiprone in removing cadmium. Therefore after basic preclinical research, this could be recommended for human administration.

ACKNOWLEDGEMENT

The authors are thankful to the head and director of Mashhad University of medical science and Ferdowsi University of Mashhad Faculty Research Funds for their support of these investigations.

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