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Clinical evaluation of Deferasirox for removal of cadmium ions in rat

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Abstract An investigation was conducted to evaluate the ability of Deferasirox (ICL670 or Exjade) following the distribution of cadmium salt in male Wistar rats. Cadmium was introduced to several groups of weanling male Wistar rats through different means, by act of drinking, feeding. A control group was fed on a diet containing normal level of iron. After a period of 30 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. Chelation therapy was carried out to remove the toxic element from the body. The ability of Deferasirox chelator in removing cadmium was investigated this chelator for 1 week to the remaining rats of similar groups. The results showed that the cadmium level present in blood was significantly reduced and at the same time, iron concentration returned to the normal level. It was concluded that Deferasirox chelator is able to remove cadmium from the body and could be used for the treatment of complications and eradication of symptoms of cadmium intoxication.

Keywords Deferasirox · Cadmium toxicity · Chelation therapy · Rats

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Introduction

Cadmium (Cd), as a well-known environmental hazard, exerts a number of toxic effects in human and animal organisms. Other cellular activities would be differentiated by cadmium cell proliferation effects (Waisberg et al. 2003). Cadmium can also enter the aquatic environment naturally from rocks and soils directly exposed to surface water. They can be concentrated from the water and sediments into aquatic mammals (Henkel and Krebs 2004; Huang et al. 2004). Many studies have examined the bioaccumulation and toxic responses of Cd in animals, plants, phytoplankton and freshwater bacteria (Waisberg et al. 2003; Fatemi et al. 2009; Mirimanoff and Wilkinson 2000; Hassler and Wilkinson 2003; Miao et al. 2005; Wilde et al. 2006). A number of Cd-induced effects including deterioration of cell–cell adhesion, DNA-related processes, cell signaling and energy metabolism can imply that this metal acts on the different molecular targets in human organism. It is shown that Cd can induce apoptosis in mouse liver (Fatemi et al. 2009; Shimoda et al. 2001; Ivanoviene et al. 2004). Cadmium in liver moves to kidneys where it is excreted and then re-absorbed almost entirely. This poor ability of humans to excrete cadmium through kidneys underlies the health implications of cadmium as a nephrotoxin (Satarug et al. 2006; Akesson et al. 2005). In long-term high-exposure cases, the hypertension has been understood to arise secondary to the loss of kidney function.

A number of active genes in the kidney are related to the control of blood pressure in physiologic state, however, including those affecting salt excretion and re-absorption, vascular tone, and volume homeostasis (Lifton et al. 2001), and it is plausible that even low level exposure to cadmium may affect the blood pressure control of human body. However, few studies supported the role of cadmium-induced nephropathy including tubular damage, which might induce increase of blood pressure in part. If the kidney's role in the control of blood pressure is affected by cadmium, then the kidney function may act as an effect modifier on the association between cadmium exposure and hypertension (Satarug et al. 2005). One of the most effective ways to remove toxic elements such as cadmium from the biological system is chelation therapy. It involves the use of ligating drugs that binds metal for the treatment of potentially fatal conditions. These ligands promote the excretion and subsequent depletion of this transition metal in biological systems. Deferasirox (4-[3,5-bis(2-hydroxyphenyl)-1,2,4-triazol-1-yl]-benzoic acid, or ICL670, Fig. 1) that was first reported in 1999 is a new drug to remove toxic metal ions from biological systems (Heinz et al. 1999). It is a tridentate chelator with high selectivity for Fe^{3+} (Steinhauser et al. 2004). In vivo, this selectivity is demonstrated by conserved plasma Zn and Cu levels in patients taking Deferasirox, and while its efficacy is rather low for inducing negative iron balance, it is effective and well-tolerated (Nisbet-Brown et al. 2003). In 2005, Deferasirox became the first FDA approved oral alternative for treatment of iron overload and was subsequently approved in the EU in 2006 (Yang et al. 2007). Its relatively long half-life before excretion allows once-daily dosage and good overall patient compliance, as well as cost-effectiveness, and Deferasirox is considered to be superior to deferoxamine (DFO). Iron chelation therapy (ICT) with (DFO), the current standard for the treatment of iron overload in patients with transfusion dependent disorders such as β-thalassemia, requires regular subcutaneous or intravenous infusions. This can lead to reduced quality of life and poor adherence, resulting in increased morbidity and mortality in iron overloaded patients with β-thalassemia (Scott and Orvig 2009).

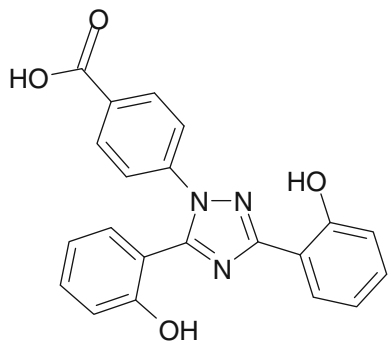

Fig. 1 Deferasirox: multidentate pro-ligand used for treatment of iron overload

The aim of this study was to investigate the chelation potency of Deferasirox given to animals after cadmium loading. Testing was performed using an acute experimental model on rats with the chelator given shortly after cadmium administration. This study indicates that this procedure might be useful for preliminary testing of the efficiency of the chelating agent to ascertain whether this chelator can mobilize and promote the removal of cadmium from rat organs.

Experimental section

Apparatus

A Varian atomic adsorption spectrometer (FAAS, GFAAS) was used for measurement of cadmium and iron concentrations in organs, respectively. Also a Mettler analytical balance Model AE 160 was used in this study.

Maintenance of the animals

Male Wistar rats were obtained from Razi Institute (Karaj, Iran). They bred in animal house at Kerman Neuroscience Research Center, Kerman, Iran. The rats were maintained under a controlled light: dark (12:12 h) schedule at 23 ± 1°C and humidity of 55%. The animals were assigned to control and treated groups and were kept in well cleaned sterilized cages. The rat food was purchased from Razi Institute. This study was approved by the ethics committee of Shahid Bahonar University of Kerman, Kerman, Iran and Kerman Neuroscience Research Center, Kerman, Iran.

Experimental design

In our model, we used two different doses of cadmium (20, 40 mg/kg body weight), followed by an early administration of chelating agent. Experiments were performed on 7 week-old male Wistar rats.

Animals were classified as follows: the first group (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 fourth group (food group) had cadmium (II) chloride incorporated into their food. Food was prepared by adding 50 mg of cadmium salts to 2 kg of normal food.

After 45 days, the animals in each group were divided into three sub-groups; the first group was killed and their blood was taken by cardiac puncture. The serum iron concentration, total iron binding capacity (TIBC) and serum cadmium concentration were determined by graphite furnace atomic absorption spectroscopy (GFAAS). The second sub-group was used for the Deferasirox chelation therapy and the third group was used as a Deferasirox control group (without Deferasirox administration) and after 1 week, the rats were killed and mentioned parameters were measured (Franchini et al. 2000). The samples for cadmium analysis were prepared by adding 0.5 ml blood to the acid washed, EDTA-washed (100 g/l) polyethylene centrifuge tube. To the solution, 0.5 ml water and 50 µl concentrated nitric acid were added and then, the solution was placed on a vortex mixer at medium speed for 1 min. The solution was heated for 10 min at 70°C in a water-bath, placed on the vortex mixer and then centrifuged for 10 min. The supernatant fraction was pipetted into sampling cup and the auto sampler used for injection. In order to remove cadmium from animals, chelation therapy was performed on them. Deferasirox (140 mg/kg body weight) was given orally during 1 week. Cadmium and iron standards were prepared by appropriate dilution with redistilled water from a stock solution (1 mg/ml). Analyses were done by GFAAS and FAAS spectrometry. A deuterium lamp was used to correct the background noise.

Statistical analysis

Determination of cadmium and iron in samples were carried out by GFAAS and FAAS, respectively. 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.

Result

There were no significant differences between the groups in the initial body weight of the rats (mean 200 g), but at the end of cadmium administration experiment, those given cadmium in their diet had significant weight loss [275 ± 7, 255 ± 8, 225 ± 7, 250 ± 6 g for control, 20 mg Cd^{2+}/kg body weight (low dose drinking of cadmium), 40 mg Cd^{2+}/kg body weight (high dose drinking of cadmium) and food group, respectively].

Comparison of the weights in this experiment shows dietary treatment affected the food intake, whereby animals given normal diet consumed more food than those given cadmium. Some of the cadmium toxicity symptoms that appeared during the period of cadmium uptake included darkening of the eyes, yellowish discoloration of hair, flaccid and hypotonic muscles, irritability, aggressiveness and indifference, weakness and loss of hair and weight. The cadmium concentration of the diet had a significant effect on iron deposition in blood serum. The results shown as cadmium concentration increases in blood serum, iron level decreased. The effect of administration of cadmium on iron status [serum iron concentration, total iron binding capacity (TIBC)] summarized in Table 1. Also our results show that iron level is lowest in the group having the highest cadmium concentration which is probably due to a significant interference that could take place by cadmium through iron uptake mechanism. Cadmium transport in blood serum and interaction between cadmium and iron has previously been reported (Shokooh Saljooghi and Fatemi 2010). The effects of Deferasirox administration on cadmium and iron concentrations in blood serum are summarized in Tables 2 and 3. From the obtained data, it is clear that there is a significant increase in cadmium

Table 1 Serum iron level and TIBC in various groups of rats after cadmium administration

Group	Serum iron (μg/dl)	TIBC (μg/dl)
Control	97.65 ± 12.62 (5)	379.12 ± 35.92
Drinking (low level)	92.43 ± 9.89 (5)	312.25 ± 38.17
Drinking (high level)	84.29 ± 11.34 (5)	301.11 ± 45.15
Food	67.73 ± 7.87 (5)	245.41 ± 26.23

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

Table 2 Serum iron level and TIBC in various groups of rats after Deferasirox administration

Group	Serum iron (μg/dl)	TIBC (μg/dl)
Control	97.63 ± 11.43 (5)	409.68 ± 43.38
Drinking (low level)	95.48 ± 10.17 (5)	403.13 ± 37.24
Drinking (high level)	96.35 ± 17.23 (5)	405.27 ± 55.23
Food	87.25 ± 6.95 (4)	323.42 ± 29.15

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

concentration ($P < 0.05$) in various groups compared with the control group. The cadmium accumulation in blood serum at 40 mg/kg dose was more than the 20 mg/kg dose. It is obvious that the cadmium concentration after chelation therapy was significantly decreased ($P < 0.05$). To investigate the effect of passing time in removing cadmium from the body spontaneously, one group was treated without chelation therapy (Fatemi et al. 2007, 2009; Tubafard and Fatemi 2008; Tubafard et al. 2010). Elimination of cadmium by the biological system in the groups without the chelation therapy is not noticeable (Table 3).

After administration of cadmium, iron concentration was significantly decreased. Chelation therapy with Deferasirox cause that the cadmium level present in blood serum was significantly reduced and simultaneously, iron concentrations returned to the normal level and the symptoms of toxicity also were reduced. The difference between iron values before and after chelation therapy is notable. The results passed the *t*-test at 95% confidence level and were significant.

Discussion

Clinical evaluations of some chelators for removal of toxic metal ions in rats have been previously reported by (Fatemi et al. 2007, 2009; Tubafard and Fatemi 2008; Tubafard et al. 2010). Chelation therapy is one of the most effective ways to remove toxic elements from the biological system. In view of these considerations, there is a great need for the development of an alternative orally effective chelating drug. Recently, many candidate compounds have been screened in animal models (Scott and Orvig 2009; Fatemi et al. 2007, 2009; Tubafard and Fatemi 2008; Tubafard et al. 2010). Many of them have poor solubility, numerous side effects and a high production cost. Some of them such as DFO require regular subcutaneous or intravenous infusions. This can lead to reduced quality of life. These properties also may result in diminished medicinal activities in biological model. Deferasirox is a new drug that shows benefit properties. Its relatively long half-life before excretion allows once-daily dosage and good overall patient compliance, as well as cost-effectiveness, and Deferasirox is considered to be superior to DFO (Franchini et al. 2000; Cappellini

Table 3 Cadmium concentration (μg/l) in blood serum of various groups of rats after cadmium and with and without Deferasirox administration

Group	After cadmium administration (μg/l)	Without deferasirox administration (μg/l)	With deferasirox administration (μg/l)
Control	0.131 ± 0.011 (5)	0.119 ± 0.015 (5)	0.100 ± 0.018 (5)
Drinking (low level)	0.756 ± 0.016 (5)	0.699 ± 0.016 (5)	0.119 ± 0.011 (5)
Drinking (high level)	1.285 ± 0.019 (4)	1.161 ± 0.022 (4)	0.158 ± 0.014 (5)
Food	1.874 ± 0.023 (4)	1.597 ± 0.011 (5)	0.201 ± 0.009 (4)

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

2008; Lindsey and Olin 2007). Interactions between cadmium and iron have previously been reported (Shokooh Saljooghi and Fatemi 2010). However our results show that the cadmium concentration of the diet had a significant effect on iron status as assessed by serum iron. These results shown that cadmium concentration increases in blood serum while iron level decreases. Our results show that use of Deferasirox as a chelator is a potential treatment for complication of cadmium intoxication. As a chelating agent, Deferasirox reduced serum cadmium levels and led to arise in normal iron level.

Cadmium affected rats do no appear to lose excessive iron during Deferasirox chelation, which is interesting because Deferasirox is widely used as a chelating agent for the treatment of both chronic and acute iron overload. Iron concentration after administration of cadmium was significantly decreased. The difference between iron values before and after chelation therapy is notable. Our results support the usefulness of this animal model for preliminary in vivo testing of cadmium chelators. The obtained results of chelator treatment confirmed the use of this method.

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