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Original Article

Therapeutic Effect of Deferasirox and Glycine on Chronic Cadmium Toxicosis in Rats

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ABSTRACT

Objective: It has been shown that deferasirox can reduce blood and tissues lead content in animal models. In this study the effect of deferasirox alone or combined with glycine as an antioxidant was evaluated in chronic cadmium toxicosis in rat. **Methods:** Male wistar albino rats were exposed to 200 ppm cadmium in the drinking water for 3 weeks and treated thereafter with deferasirox (140 mg/kg), glycine (1000 mg/kg) and deferasirox (140 mg/kg) + glycine (1000 mg/kg) by oral gavages, twice daily for 7 days. The effect of these treatments on blood, liver, kidney, bone and testis cadmium values and parameters indicative of oxidative stress (superoxide dismutase, catalase, glutathione peroxidase and total antioxidant capacity in blood) were investigated. **Results:** Deferasirox was found to be effective in reducing cadmium level in blood, liver and kidney and glycine reduced cadmium levels in bone. Individual administration of deferasirox or glycine mitigated the effects of cadmium on some indicative parameters of oxidative stress in blood. But the best results were obtained following co-administration of both drugs.

1.INTRODUCTION

Cadmium is a well recognized environmental pollutant with diverse toxicity in various organs of human and animals (IARC, 1993). Prolong cadmium exposures can cause lung fibrosis, kidney tubular dysfunction, hypertension, osteoporosis, cancer, etc. (Peters et al., 1986; Patra et al., 2011). The common sources of cadmium are combustion of coal, mining, smelters and contaminated phosphate fertilizers (Patra et al., 2005; Patra et al., 2007). Major occupational exposure occurs from nonferrous smelters during production and processing of cadmium (Patra et al., 2011). Cadmium was found to result in oxidative stress (Hendy et al., 1992; Somashekaraiah et al., 1992) by inducing oxygen free radical production (Balaknina et al., 2005; Demirevska et al., 2006; Dailiah Roopha et al., 2011). Cadmium causes oxidative damage to erythrocytes and various tissues by stimulating the formation of metallothioneins and reactive oxygen species (ROS) (Sarkar et al., 1998). Long term exposure to cadmium increases lipid peroxidation (LPO) and inhibits superoxide dismutase (SOD) activity, causing oxidative damage in liver, kidney and testis (Patra et al., 1999). The increase in lipid peroxidation due to cadmium toxicity have been attributed to alterations in the enzymatic antioxidant defense system such as glutathione peroxidase (GPH-Px), superoxide dismutase (SOD), and catalase (CAT) (Patra et al., 2011). Thus, cadmium toxicosis treatment should consist of cadmium chelation and free radical scavenging.

Deferasirox (4-[3,5-bis(2-hydroxyphenyl)-1,2,4-triazol-1-yl]-benzoic acid) was first reported in 1999 as a new drug to remove toxic metals from biological systems (Heinz et al., 1999). In 2005, deferasirox became the first FDA approved oral alternative for treatment of iron overload and was subsequently in 2006 approved in the EU (Yang et al., 2007). Chelation therapy with the current expensive chelators such as meso-2,3-

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dimercaptosuccinic acid (DMSA), diethyl dimercapto succinate (DEDMS), a-mercapto-13-(2-furyl) acrylic acid (MFA), a-mercapto-3-(2-thienyl) acrylic acid (MTA), etc., for the treatment of cadmium poisoning, requires regular subcutaneous or intravenous infusions, while deferasirox is a cost effective orally administered chelator (Tandon and Prasad, 2000; Scott and Orvig, 2009). Several studies have been conducted to determine the effect of antioxidant supplementation in cadmium intoxication (Karabulut-Bulan et al., 2008; Gaurav et al., 2010; Kini et al., 2011). Recently, glycine has been given a lot of importance for its antioxidant effects (Mauriz et al., 2001). Alcaraz-Contreras, (2011) investigated the effect of glycine on chronic lead toxicity in rats and reported that glycine protects against lead induced hepatotoxicity by blocking the lipid peroxidation (Alcaraz-Contreras et al., 2011). This protection was evaluated by the reduction of lipid peroxidation and the increase of glutathione (GSH) levels in liver. Okoko, (2010) announced that glycine can reduce cadmium induced alterations in culture cells. This finding supports the important role of this antioxidant (Okoko and Awhin, 2010). The present study was conducted to compare the efficacy of the deferasirox as a chelator and the glycine as an antioxidant administered alone and in combination for the treatment of cadmium intoxication in rat.

2. MATERIALS AND METHODS

2.1. Animals and experimental design

Thirty male wistar albino rats, weighting 220±16 g and 7-8 weeks old, were randomly divided into five groups (n = 6). They were housed in polypropylene cages under conventional conditions (temperature 22±1 °C, humidity 60% and controlled 12-h light-dark cycle). All animals had free access to drinking fluid and a standard rodent pellet diet ad libitum throughout the experiment. After 7 days of acclimatization period, the experiment was conducted as follows: Group I served as negative control and received tap water alone; Animals in groups II–XI received 200 ppm Cd (as $CdCl_2$) in drinking water for 21 days (SaÃ¯d et al., 2010). On day 22 animals of group III, IV and V were orally (by gastric gavage) treated with 140 mg/kg deferasirox (Saljooghi and Fatemi, 2010), 1000 mg/kg glycine (Alcaraz-Contreras et al., 2011) and a combination of both, twice daily for 7 days, respectively. The oral solutions containing deferasirox and/or glycine dissolved in distilled water were prepared just before the administration.

2.2. Sampling

One day after the last treatment, rats were anesthetized with ether and blood samples were collected from heart. Liver, both kidneys and testes and left femur were collected and used for cadmium measurement and biochemical analysis.

2.3. Determination of cadmium in blood and tissues

Blood samples were dissolved in 70% nitric acid and ammonium vanadate and then centrifuged (2,500 rpm for 5 min). Tissue samples were digested in a 1:1 mixture of 98% sulfuric acid and 70% nitric acid, with a slight modification of the wet-ashing technique (Najar-Nezhad et al., 2008). Cadmium concentrations were determined by atomic absorption spectrophotometer (Perkin-Elmer AS800, Massachusetts, U.S.A.) at 228.8 nm wave-lengths by use of graphite furnace. Limit of detection for this analysis was 5 ng/g, and recovery for spiked samples was 90%.

2.4. Evaluation of GSH-Px, SOD, CAT and TAC activities in blood

The activities of erythrocyte glutathione peroxidase (GSH-Px, EC 1.11.1.9.) and superoxide dismutase (SOD, Ec 1.15.1.1) were determined in washed red blood cells obtained immediately after sampling from heparinized blood. Hemolyzed cells were stored frozen at −70 °C awaiting analysis. Glutathione peroxidase activity was measured according to Paglia and Valentine by commercially available kits (Ransel test kit, Randox Laboratories Ltd. G.B.) (Paglia and Valentine, 1967). For evaluation of activity of superoxide dismutase, superoxide radicals generated by the xanthine oxidase reaction convert 1-(4-iodophenyl) 3-(4-nitrophenol)-5-phenyltetrazolium chloride quantitatively to a formazan dye (Ransod test kit, Randox Laboratories Ltd. G.B.). Conversion of superoxide radicals to hydrogen peroxide by superoxide dismutase inhibits dye formation and serves as a measure of superoxide dismutase activity.

The activity of catalase (CAT, EC 1.11.1.6) was determined by colorimetric method, described by Slaughter & O'brien that involves two steps. Since the rate of dismutation of hydrogen peroxide to water and oxygen is proportional to the concentration of catalase, samples were first incubated with a known amount of hydrogen peroxide. The remaining hydrogen peroxide, following a fixed incubation period, was then determined by the oxidative coupling reaction of 4-aminophenazone (4-aminoantipyrene, AAP) and 3,5-dichloro-2-hydroxy-benzenesulfonic acid (DHBS) in the presence of H_2O_2 and catalyzed by horseradish peroxidase (Slaughter and O'Brien, 2000). The resulting quinoneimine dye was measured at 520 nm (Catalase Assay Kit, Oxford Biomedical Research, Inc., USA). Activities of the enzymes were expressed in units per mg of hemoglobin. Total Antioxidant Capacity (TAC) of Blood Plasma levels was determined using a novel automated colorimetric measurement method. 2, 2-Azino-disses-[3-ethylbenzthiazoline sulphonate] incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation. This has a relatively stable blue-green colour, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this colour

production to a degree which is proportional to their concentration. (Total Antioxidant Status Kit, Randox Laboratories Ltd. UK). The results are expressed as nanomol Trolox equivalent per liter.

2.5. Statistical analysis

The analyses were performed with the SPSS 11.5 software (SPSS Inc., Chicago, IL, U.S.A.). The analysis of variance was applied for the comparison of the means of the different treatment groups. Post hoc test was performed for inter-group comparison applying the least significant difference. All values are expressed as mean ± standard error and values of $P < 0.05$ were considered to be significant.

3. RESULT

3.1. Cadmium concentration in blood and tissues

Cadmium concentration in various tissues of rats exposed to cadmium is given in Table 1. For cadmium exposed untreated group (group II), the distribution of cadmium was in the following order: bone > liver > kidney > testis > blood. Cadmium treated animals showed significantly ($P < 0.05$) higher levels of cadmium in blood, liver, kidney, bone and testis compared to control animals. Groups treated with deferasirox (group III) and combination of deferasirox + glycine (group V) had blood, liver and kidney cadmium burden significantly ($P < 0.05$) lower than cadmium exposed untreated group. Moreover bone cadmium concentration of groups treated with glysin (group IV) and combination of deferasirox + glycine (group V) was significantly ($P < 0.05$) lower than cadmium exposed untreated group.

3.2. GSH-Px, SOD, CAT activities and TAC status in blood

Cadmium exposed untreated rats have shown significant ($P < 0.05$) depletion of GSH-Px, SOD and CAT in blood, indicative of oxidative stress consistent with the accumulation of cadmium in blood. Administration of each deferasirox or glycine alone to cadmium exposed animal significantly reversed blood CAT level. The subsequent oral treatment of cadmium exposed animals with deferasirox + glycine, reversed blood GSH-Px, SOD, CAT and TAC levels, significantly (Table 2).

Table 1.

Cadmium concentration in blood (µg/l) and tissues (µg/gr) of various groups

Group	Blood	Liver	Kidney	Bone	Testis
Control	0.02 ± 0.01^{a}	0.05 ± 0.01^{a}	0.02 ± 0.01^{a}	0.04 ± 0.02^{a}	0.02 ± 0.01^{a}
Cd-treated	18.53 ± 0.36^{b}	32.53 ± 5.72^{b}	25.40 ± 1.97^{b}	40.53 ± 5.67^{b}	12.05 ± 0.01^{b}
Cd+Deferasirox	7.94 ± 1.88^{c}	24.51 ± 2.39^{c}	18.62 ± 6.37^{c}	29.71 ± 1.82^{b}	10.62 ± 1.27^{b}
Cd+Glycine	20.73 ± 3.40^{b}	29.67 ± 4.88^{b}	28.55 ± 10.31^{b}	$15.80 \pm 3.17^{b,c}$	11.09 ± 1.52^{b}
Cd+Deferasirox+glysine	$11.47 \pm 1.41^{b,c}$	21.64 ± 2.98^{c}	16.11 ± 9.50^{b}	$21.53 \pm 3.44^{b,c}$	9.31 ± 1.64^{b}

Mean ± S.D. (six values). In each column, a, b and c was significantly differed ($P<0.05$)

Table 2.

SOD, CAT, GSH-Px (U/mg Hb) and TAC (nmol/l) concentrations in blood of various groups

Group	SOD	CAT	GSH-Px	TAC
Control	4564.34 ± 73.35^{a}	2839.01 ± 89.47^{a}	2402.51 ± 207.89^{a}	$1.19 \pm 0.04^{a,b}$
Cd-treated	2796.33 ± 50.40^{b}	1222.67 ± 14.19^{b}	1366.67 ± 160.73^{b}	0.73 ± 0.06^{a}
Cd+Deferasirox	3087.51 ± 401.18^{b}	$1893.68 \pm 280.70^{b,c}$	1474.01 ± 475.49^{b}	1.04 ± 0.43^{a}
Cd+Glycine	3250.22 ± 457.00^{b}	$1822.90 \pm 349.68^{b,c}$	1525.90 ± 285.76^{b}	0.99 ± 0.33^{a}
Cd+Deferasirox+glysine	3927.33 ± 135.43^{a}	2160.67 ± 54.37^{c}	2230.00 ± 125.30^{a}	1.73 ± 0.24^{b}

Mean ± S.D. (six values). In each column, a, b and c was significantly differed ($P<0.05$)

4. DISCUSSION

Diverse doses of cadmium have been used to induce cadmium toxicosis in rats and to study therapeutic efficacy of different chelators and antioxidants (Karabulut-Bulan et al., 2008; Saljooghi and Fatemi, 2010; Kini et al., 2011). A dose of 200 ppm cadmium through drinking water for 3 weeks has significantly increased blood and tissues cadmium values. The highest accumulation of cadmium per milligram of tissue or per milliliter of blood was detected in bone, followed by liver, kidney, testis and blood. This could be due to different bio-kinetic pattern of its distribution in various tissues. Accumulation of cadmium with highest concentration in bone can be indicative of chronic cadmium toxicosis.

Cadmium induced oxidative stress has been demonstrated by the increased lipid peroxidation and inhibition of enzymes required to prevent such oxidative damage (Kelley et al., 1999). Accordingly, depletion of GSH-Px, SOD and CAT in blood, consistent with the accumulation of cadmium in blood and tissues, seem to be due to the generation of reactive oxygen species (ROS) (Bray and Taylor, 1993). It indicates enhanced lipid per oxidation in these cadmium exposed animals (Muller, 1986). The increased activity of GSH-Px, SOD and CAT level in response to cadmium toxicity may be a defensive mechanism towards free radical damage to tissues. Thus, an effective therapy of cadmium intoxication should encompass both metal chelating and antioxidant actions.Evaluations of some chelators for removal of toxic metal ions in animals has been previously reported (Najar-Nezhad et al., 2008; Aslani et al., 2009; Najarnezhad et al., 2010). However, there is a great need for the developing an alternative orally effective chelating drug. Recently, many compounds have been experimented in animal models (Aslani et al., 2009; Aslani et al., 2011). Deferasirox is a new drug that shows benefit properties (Saljooghi and Fatemi, 2010). In this study the administration of deferasirox to cadmium exposed rats lowered cadmium levels in blood, liver and kidneys and increased blood catalase. The observed reversals in blood catalase levels may be due to the reduced tissue cadmium contents than because of any antioxidant property that could be associated with the chelating agent.

The present study is the first report that shows glycine can mobilize cadmium from bone. Alcaraz-Contreras, et al. have previously reported that, glycine is an amino acid effective in mobilizing lead from bone (Alcaraz-Contreras et al., 2011). Blood and tissue concentrations of cadmium were increased to some extent, following administration of glycine. This can be due to redistribution of cadmium from bone following cadmium mobilization. Nishida, (1997) believes glycine can participate in GSH synthesis, and it facilitates the γ-glutamyl cysteinyl ethyl ester mediated increase in intracellular GSH levels. Thus, GSH combines with certain non nutrients, such as drugs and poisons, and promotes their biotransformation (Nishida et al., 1997). However, the mechanism by which glycine decreased the level of cadmium in bone could not be explained explicitly and requires further research, such as the measurement of urinary and fecal cadmium excretion. The results of combined treatments with deferasirox and glycine were more effective both in terms of reduction in tissues cadmium burden and recovery considering parameters of oxidative stress including GSH-Px, SOD, CAT and TAC.

5. CONCLUSION

In conclusion, treatment with deferasirox significantly decreased cadmium levels in blood, liver and kidney and treatment with glycine significantly decreased cadmium levels in bone. Individual administration of deferasirox or glycine mitigated some effects of cadmium on parameters indicative of oxidative stress in blood. But the best results were obtained following co-administration of both drugs.

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