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Oral Cadmium Exposure and Levels of Superoxide Dismutase, Catalase, Lipid Peroxidation and ATPases in the Eye

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Abstract: The purpose of the present study was to evaluate the effect of oral cadmium exposure on the activities of ocular superoxide dismutase, catalase, ATPases and level of lipid peroxidation using the rat as animal model. Forty male albino rats (Wistar strain) were divided into four groups of ten animals each; one group received de-ionized water and acted as control. The other groups, which were the tests, received 50, 100 and 200 mg Cd L⁻¹, respectively in drinking water for one month. The results obtained indicate a significantly ($p < 0.05$) decreased body weight gain of rats exposed to cadmium. There was a dose dependent decrease of ocular superoxide dismutase activity and a corresponding increase in level of lipid peroxidation of cadmium exposed rats as compared to control. No significant ($p > 0.05$) change was observed in the activities of catalase and Mg²⁺ ATPase, but a dose dependent increase in the activities of total and Na⁺/K⁺ ATPase were observed in the eyes of rats orally exposed to cadmium as compared to control. The study suggests that exposure to cadmium enhanced oxidative stress and Na⁺/K⁺ ATPase activity in the eyes of rats.

Key words: Cadmium, super oxide dismutase, catalase, Na⁺/K⁺ ATPase, eye

INTRODUCTION

Cadmium has long been known as an industrial and environmental toxicant (Friberg *et al.*, 1985; Horiguchi *et al.*, 1996). Its diverse use in industries has led to its wide distribution in the environment (Friberg *et al.*, 1986). The oral route is the principal route of cadmium exposure in humans. The element is readily distributed in tissues after exposure, where it inhibits antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and catalase (Gupta *et al.*, 1991; Bagchi *et al.*, 1996). This inhibition can lead to increased oxidative stress in tissues, which could result in peroxidation of membrane lipid and loss of membrane bound enzymes such as the ATPases (Timbrell, 1991; Figueiredo-Pereira *et al.*, 1998). Tissues in which these effects have been reported include testis, heart, kidney, liver, intestine and brain (WHO, 1992; Pal *et al.*, 1993; Sarker *et al.*, 1995; Lall *et al.*, 1997; Elsenhans *et al.*, 1999).

Few reports are available on the effect of cadmium on ocular antioxidant enzymes. In an earlier study, it was shown that exposure of rats to 100 mg Cd L⁻¹ in drinking water increased lipid peroxidation in the eyes, which may compromise vision (Asagba *et al.*, 2002). As a follow up the present communication reports the dose related effect of oral cadmium on ocular levels of superoxide dismutase, catalase, lipid peroxidation and ATPases in rats.

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MATERIALS AND METHODS

Forty male albino rats (Wistar strain) weighing between 180-185 g which were housed individually in wire meshed cages to prevent coprophagy were used for the study. The rats were allowed to acclimatize to laboratory conditions for two weeks. During this period they were allowed water and food freely. After the period of acclimatization, they were distributed into four groups with ten rats in each group. Rats in one group were given deionised water and served as controls while rats in three other groups were given 50, 100 and 200 mg Cd L⁻¹ in drinking water, respectively. All groups of rats were fed *ad libitum* with growers mash (product of Bendel feeds and flour mills, Ewu, Nigeria). They were exposed to this treatment for one month, during which the food and water intakes, as well as fecal output were measured daily, while the weight was recorded weekly. At the end of the treatment period, the rats were weighed and sacrificed under chloroform anesthesia. The pair of eyes of each rat were quickly excised, weighed and placed on ice until required for biochemical analysis. All these animal treatments were carried out in accordance with the principles of laboratory animal care (NIH Publication No. 85-93, revised 1985).

The whole eyes from each rat were homogenized with chilled 0.9% NaCl solution. The homogenate was centrifuged at 3,000 g for 10 min. The supernatant obtained was used for the estimation of the activities of superoxide dismutase, catalase and lipid peroxidation. Superoxide dismutase was assayed by the method of Misra and Fridovich (1972), while catalase was by the method of Beers and Sizer (1952). The activities of both enzymes were expressed in units/g tissue. One unit of superoxide dismutase activity is the amount of the enzyme required for 50% inhibition of the autooxidation of epinephrine to adrenochrome per minute. One unit of catalase activity is the amount of enzyme required for the breakdown of 1 μmol of hydrogen peroxide per minute. A molar extinction coefficient of 40 M⁻¹ cm⁻¹ was used to calculate catalase activity. The level of lipid peroxidation was assayed by the method of Gutteridge and Wilkins (1982). The procedure involved the determination of Thiobarbituric Acid Reactive Substances (TBARS), which are indicators of lipid peroxidation. Values for TBARS are reported as Malondialdehyde (MDA) and quantitated using a molar extinction coefficient of 1.56 × 10⁵ M/cm and expressed as μmole MDA g⁻¹ tissue.

The pellet obtained after centrifugation of the homogenate at 3000 g was resuspended in 5 mL of 0.9% NaCl. The suspension was subsequently used for the assay of Na⁺/K⁺ ATPase by the method of Adam Vizi and Seregi (1982). The Na⁺/K⁺ ATPase activity was taken as the difference between total ATPase and Mg²⁺-ATPase activities. The specific activities of the ATPases were expressed in standard units of μmoles of inorganic phosphate released per min per mg protein. The inorganic phosphate released was measured by the method of Fiske and Subarrow (1925), while the protein concentration of the samples was determined according to the method of Lowry *et al.* (1951).

The results are presented as Means ± SEM. Statistical differences in the means were determined using analysis of variance (ANOVA) and Duncan's multiple range test.

RESULTS

The eye weight of rats exposed to cadmium in drinking water was significantly (p<0.05) increased in a dose dependent manner. Rats exposed to 200 mg Cd L⁻¹ had the highest eye weight. The differences in eye weight were significant among the test groups. Conversely, there was a dose dependent decrease in the body weight, feed intake, feed efficiency and water intake of rats exposed to cadmium in drinking water. Rats exposed to 200 mg Cd L⁻¹ had the least value for each of these parameters. The differences were significant (p<0.05) among the groups. The dry fecal output of rats was significantly (p<0.05) increased in rats exposed to 100 and 200 mg Cd L⁻¹ in drinking water relative to the other groups (Table 1). Cadmium also exhibited a dose dependent decrease in the activity

Table 1: Effects of oral cadmium on body weight gain, eye weight, dry fecal output, feed efficiency and feed and water intakes of rats

Parameters	Dose of cadmium administered in drinking water (mg L ⁻¹)			
	0	50	100	200
Weight of pair of eyes (g)	0.308±0.025 ^a	0.322±0.028 ^b	0.341±0.027 ^c	0.372±0.025 ^d
Body weight gain (g day ⁻¹ rat ⁻¹)	1.530±0.15 ^a	1.240±0.17 ^b	0.950±0.05 ^c	0.700±0.07 ^d
Feed intake (g day ⁻¹ rat ⁻¹)	21.680±0.21 ^a	18.060±0.15 ^b	15.120±0.17 ^c	12.350±0.20 ^d
Feed efficiency (g/body weight/g feed) × 10 ⁻²	7.050±0.12 ^a	6.860±0.16 ^b	6.280±0.13 ^c	5.660±0.14 ^d
water intake (mL)	55.300±1.60 ^a	41.710±1.70 ^b	35.410±1.6 ^c	30.650±1.10 ^d
Dry fecal output (g day ⁻¹ rat ⁻¹)	1.360±0.14 ^a	1.430±0.17 ^a	1.700±0.18 ^b	1.860±0.35 ^b

Results are expressed as Means±SEM (n = 10), Means of the same row followed by different letter(s) differ significantly (p<0.05)

Table 2: Effect of oral cadmium on activities of superoxide dismutases (SOD), catalase and level of lipid peroxidation in the eyes of rats

Parameters	Dose of cadmium administered in drinking water (mg L ⁻¹)			
	0	50	100	200
SOD (units g ⁻¹ tissue)	65.18±2.5 ^a	50.14±3.5 ^b	32.84±4.1 ^c	20.80±4.50 ^d
Catalase (units g ⁻¹ tissue)	2.34±0.3 ^a	2.61±0.5 ^a	2.53±0.3 ^a	2.90±0.40 ^a
TBARS (µmole MDA g ⁻¹ tissues)	220.25±10.5 ^a	248.70±12.30 ^b	278.00±13.01 ^c	369.00±12.08 ^d

Results are expressed as Means±SEM (n = 10), Means of the same row followed by different letter(s) differ significantly (p<0.05)

Table 3: Effect of oral cadmium on the activities of total ATPase, Mg²⁺-ATPase and Na⁺/K⁺ ATPase in the eyes of rats.

Parameters	Dose of cadmium administered in drinking water (mg L ⁻¹)			
	0	50	100	200
Total ATPase	25.8±1.9 ^a	28.5±2.2 ^b	39.5±2.4 ^c	42.6±2.6 ^d
Mg ²⁺ ATPase	21.3±2.1 ^a	22.9±2.6 ^a	24.3±2.7 ^a	23.5±2.4 ^a
Na ⁺ /K ⁺ ATPase	4.5±0.5 ^a	5.8±0.4 ^b	15.2±1.4 ^c	19.1±2.3 ^d

Results are expressed as Means±SEM (n = 10), Activities of ATPases are in µmole inorganic phosphate released per min per mg protein, Means of the same row followed by different letter(s) differ significantly (p<0.05)

of SOD with the maximum decrease observed at 200 mg Cd L⁻¹ dose level. Conversely, the results showed an increasing trend in TBARS concentration with increasing dose of cadmium. The differences observed in the values of SOD and TBARS were significant among the groups. However, no significant difference was observed in catalase activity among the groups (Table 2).

There was a dose dependent increase in Na⁺/K⁺-ATPase activity of rats with the maximum activity observed at the 200 mg Cd L⁻¹ dose level. The differences observed in the values were significant among the groups. A similar trend was reflected in the total ATPase activity of the rats. No significant difference was observed in the Mg²⁺-ATPase activity among the groups (Table 3).

DISCUSSION

The decreased weight gain of rats observed in this study is consistent with the reports of Horiguchi *et al.* (1996) and Asagba *et al.* (2002). Weight gain depends on availability of nutrients. Therefore the observed reduction in weight gain could have been due to the decrease in food intake of the rats (Table 1). Besides, the decrease in feed efficiency and increase in fecal out put of the cadmium exposed rats suggest that the metal decreases nutrient absorption and this may also have contributed to the decrease in weight gain of the rats. Thus, the present study is also in harmony with that of Elsenhans *et al.* (1999) which showed that cadmium decreases nutrient digestion and absorption.

Early effects of cadmium intoxication include the inhibition of various antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and depletion in the level of glutathione and this could lead to increased oxidative stress (Gupta *et al.*, 1991; Mates and Sanchez-Jimenez, 2000;

Liu *et al.*, 2000). One of the consequences of this is the enhancement of membrane lipid peroxidation. The inhibition of ocular superoxide dismutase activity observed in this study may be an indication of oxidative stress which is in accordance with the corresponding increase in ocular lipid peroxidation (Table 2). Lipid peroxidation has been reported to enhance tissue water content and permeability (Xiu-Mei and Earl, 1990) and this may account for the increase in the eye weights of cadmium exposed rats (Table 1). Despite the short period of exposure of the animals to oral cadmium, this study observed an inhibition of superoxide dismutase activity (Table 2). Thus the decrease in this enzyme activity may be an early event in the eye of rats exposed to cadmium orally. Superoxide dismutase is inducible and its activity increases as the need to protect an organ against oxidative damage increases (Trostler *et al.*, 1979). However, it should be noted that besides the antioxidant enzymes, the protein, metallothionein has also been linked with the protection of the eye in cadmium toxicity (Lu *et al.*, 2002). Metallothionein detoxifies cadmium by sequestering it and is also an efficient interceptor of free radicals (Gupta *et al.*, 1991; Klaassen and Liu, 1998). The lack of significant difference in catalase activity among the groups (Table 2) is not surprising, since reports in literature suggest that catalase does not represent the major route of hydrogen peroxide detoxification (Doroshov *et al.*, 1980; Aknes and Njaa, 1981).

When lipid peroxidation proceeds in any biomembrane, several fatty acids and lysophospholipids are released, leading to changes in biomembrane microviscosity and kinetic properties (Halliwell and Gutteridge, 1989; Ytrehus and Hegstad, 1991). This changes the ultrastructure and integrity of membrane causing loss of membrane bound enzymes. Na⁺/K⁺ ATPase is a membrane bound enzyme and its activity could reduce following the disruption of membrane integrity by cadmium. Several other mechanisms have been postulated for cadmium induced inhibition of Na⁺/K⁺ ATPase activity. One of these mechanisms is based on the ability of cadmium to uncouple oxidative phosphorylation (Yoshioka *et al.*, 1995). Since energy is required for the various ATPases, a decrease in ATP concentration should affect the activity of Na⁺/K⁺ ATPase. Inhibition of Na⁺/K⁺ ATPase has also been linked with interaction of cadmium with the thiol groups of critical proteins and enzymes (Timbrell, 1991). However, contrary to these findings, the present study demonstrates a significantly increased activity of Na⁺/K⁺ ATPase in the eyes of the rats exposed to cadmium relative to control rats (Table 3). The highest increase in the activity of the enzyme was observed in rats exposed to 200 mg Cd L⁻¹, followed by 100 mg Cd L⁻¹. The route of ingested cadmium is from intestine to liver and then kidney (WHO, 1992) and other organs are affected to a lesser extent. So the observed increase in Na⁺/K⁺ ATPase activity in the cadmium treated rats may be the result of an attempt of the tissues of the eye to try and adapt to a possible cadmium assault. Improved Na⁺/K⁺ ATPase activity would ensure improved cell viability as the resting membrane potential essential for proper function of the cell will be maintained (Voet and Voet, 1995). Thus, the observed increase in the activity of Na⁺/K⁺ ATPase in the eyes of cadmium exposed rats may be due to a compensatory rise in viable cells which may have been necessitated to reverse a possible inflow of water into the cells of the eyes. This may be an adaptive response in the eye to ensure that normal cellular functions are not completely compromised.

In conclusion, the results obtained in this study show that the eye is susceptible to oral cadmium induced oxidative stress and that Na⁺/K⁺ ATPase may be involved in the adaptation of this organ to oral cadmium exposure.

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