



Original Full Paper

Induction of Oxidative Stress by Subacute Oral Exposure of Cadmium Sulphate in Adult Poultry

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Abstract

Cadmium has been recognized as one of the most toxic environmental and industrial pollutants. In the present study, twenty-four adult poultry birds were divided into four groups. In groups II, III and IV cadmium sulphate was given in drinking water at the dose rate of 100, 200 and 400 mg/L, respectively for 20 days to assess the effect of cadmium on the antioxidant defence system and lipid peroxidation (LPO) of erythrocytes of adult poultry birds as compared to group I, which was maintained at basal diet and normal drinking water. It was observed that the activities of erythrocyte superoxide dismutase (SOD) and catalase (CAT) increased during subacute cadmium toxicity. The activity of glutathione-S-transferase (GST) was non-significantly ($P>0.05$) increased in group II and III; but, non-significantly decreased in group IV. The level of blood glutathione (GSH) was significantly decreased in cadmium exposed birds. However, the lipid peroxidation (MDA) was significantly increased in the cadmium exposed groups as compared to group I. On the basis of present study, it could be concluded that cadmium exposure altered the activities of antioxidant enzymes of erythrocytes and produce oxidative stress by disturbing the oxidative and antioxidative balance of the adult poultry birds.

Key Words: Cadmium, poultry, catalase, superoxide dismutase, blood glutathione, glutathione-s-transferase, lipid peroxidation.

Introduction

Cadmium (Cd) is an abundant, non-essential element that is widely used in electroplating and galvanizing, as a colour pigment in paints and in batteries. The increased release of cadmium from industrial process, waste disposal and cigarette smoke in the environment lead to a general concern for the potential toxic effects of cadmium. Cadmium contamination and toxicity have become a matter of concern in recent years and it has been recognized as one of the most toxic environmental and industrial pollutants. The extremely long biological half-life of cadmium essentially makes it a cumulative toxin, so

long past exposure could result in direct toxic effects of the residual metal (1). Exposure to this toxic metal can produce both acute and chronic tissue injury and can damage various organs including lung, liver, kidney, bone, testis and placentas depending on the dose, route and duration of exposure (14). Cadmium is redox stable metal, therefore, radical production by Cd must be mediated through some indirect mechanisms. Cadmium stimulates the formation of beta 2-microglobulin in urine which induces renal tubular dysfunction (19,32). Reactive oxygen species (ROS) are also produced inducing oxidative damage by disruption of the oxidative antioxidant defense system (21,4) in erythrocyte and various tissues. ROS are often

implicated in cadmium toxicology, either in a variety of cell culture systems (18) or in *in vivo* models through all routes of exposure (3). Although the effects of cadmium toxicity on oxidative stress in laboratory animals have been extensively reported, there are no studies focusing on poultry birds. Also the exact mechanism of oxidative stress is not clear. So, keeping these points in ~~our~~ mind, we planned to determine the effect of subacute exposure of different doses of cadmium sulphate on antioxidant defence system and lipid peroxidation of erythrocytes of adult poultry birds. The aim of our study was to get a better understanding of the role of oxidative stress caused by subacute exposure of different doses of cadmium sulphate and to determine the dose of cadmium sulphate to induce oxidative stress in adult poultry birds to develop experimental model.

Material and Methods

Chemicals

The chemicals used in the present study were hydrogen peroxide (H₂O₂), Potassium dihydrogen phosphate (KH₂PO₄), Disodium hydrogen phosphate (Na₂HPO₄), Pyragallol, Ethylene diamine tetra acetic acid (EDTA), Heparin, Thiobarbituric acid (TBA), Trichloro acetic acid (TCA), Tris buffer, DTNB (5,5'-Dithiobis-2-nitrobenzoic acid), Glacial metaphosphoric acid, Sodium chloride (NaCl), Sodium citrate, Reduced glutathione, CDNB (1-Chloro 2,4-dinitrobenzene) and Cadmium sulphate (3CdSO₄.8H₂O, M.W. 769.52). All the chemicals were of analytical grade and were purchased from S.d Fine Chem Ltd., India.

Animals and administration of cadmium

Adult poultry birds (Vencob strain) weighing 1.0 to 1.25 kg (40 days old) were used in the present study. The birds were kept at standard conditions in cages of poultry house of department of animal nutrition, COVAS, Palampur, India. They were allowed *ad libitum* access to basal diet and drinking water. The birds were divided in four groups. : Group I received normal basal diet and water (control); Group II, III and IV received cadmium sulphate in drinking water at the dose rate of 100, 200 and 400 mg/litre, respectively. The birds were submitted to the experimental treatments for 20 days. The experimental protocol was approved by institutional ethics committee.

Sample collection and Biochemical analysis

At the end of experiment, 24 hrs after the last dose, ~~the~~ blood samples were collected from the wing vein in ~~a~~-clean sterile glass tubes containing heparin as anticoagulant. The plasma was separated after centrifugation at 5000 g at 4 °C for 20 minutes in a high speed cooling centrifuge and was used to determine total protein. Washed erythrocytes were diluted in distilled water to make 1% haemolysate and used for the estimation of catalase (CAT), superoxide dismutase (SOD) and glutathione-S-transferase (GST), whereas 33% haemolysate in Phosphate buffer saline

(PBS, pH:7.4) of washed erythrocytes were used for lipid peroxidation [estimation of malonaldehyde (MDA)] . Whole blood was used for the estimation of blood glutathione (GSH).

The activities of SOD, CAT and GST were measured according to the methods described by Marklund and Marklund (24), Aebi (2) and Habig *et al.* (15), respectively. Lipid peroxidation was measured (MDA evaluation) by using thiobarbituric acid reactive substance as described by Ohkawa *et al.* (26). The GSH was measured by the method of Beulter (5) and total protein was determined by the method of Lowry *et al.* (23).

Statistical Analysis

Statistical analysis of the data was carried out by one way analysis of variance (ANOVA) followed by Dunnet's test. The values of all the cadmium exposed groups were compared to the control group and the values at P<0.05 and P<0.01 were considered as significant. The values were expressed as mean ± SE.

Results

The effect of subacute exposure of cadmium at different doses on antioxidant profile of erythrocytes in poultry birds is presented in Table 1. It was observed that the activities of erythrocyte SOD and catalase increased in all cadmium exposed groups as compared to control group. The increase in activities were in a dose dependent manner; but, a significant (P<0.01) increase was observed only in group IV, which was exposed to 400 mg/L of cadmium. The levels of blood glutathione in the body of cadmium exposed birds were significantly decreased as compared to control group. The activity of GST showed no significant difference between exposed and no exposed poultry.

The concentration of MDA, for the lipid peroxidation (LPO) parameter, was significantly increased at P<0.05 in group II and III, and at P<0.01 in group IV as compared to control group.

Discussion

Cadmium is one of the most toxic metal compounds released into the environment (16) and is one of the important environment pollutant that can be ingested or inhaled from a variety of industrial and dietary sources (17). It is dangerous because humans consume both plants and animals that absorb cadmium efficiently and concentrate it within their tissues. Cadmium is a ubiquitous toxic metal that may induce oxidative damage by disturbing the pro-oxidant and antioxidant balance in the tissue (25). The mechanism of cadmium induced oxidative stress is not fully clarified. SOD, catalase and glutathione peroxidase (GPx) are enzymes that provide cellular protection against the damage caused by free radicals and ROS. Measurement of these enzyme activities is an indirect and non-invasive method that could be used to assess oxidant stress (7).

Table 1. Effect of subacute oral exposure of different doses of Cadmium sulphate on different antioxidant enzymes, blood glutathione and lipid peroxidation in adult poultry birds. (n=6 and values are expressed as mean \pm SE)

Parameters	Control Group	Cadmium Exposed Groups		
	Group I	Group II (100mg/L)	Group III (200mg/L)	Group IV (400mg/L)
Superoxide dismutase (Units/ mg protein)	3.53 \pm 0.48	3.67 \pm 0.83	4.47 \pm 0.89	13.61** \pm 2.58
Catalase (μ M of H ₂ O ₂ decom/min/ mg protein)	20.99 \pm 2.05	27.95 \pm 5.65	32.94 \pm 4.01	52.78** \pm 11.27
Blood Glutathione (n moles/ml)	60.41 \pm 1.30	49.21* \pm 3.18	45.31** \pm 3.74	42.98** \pm 2.31
Glutathione-S-transferase (μ mole of GSH-CDNB conjugate formed/min/mg protein)	0.068 \pm 0.007	0.088 \pm 0.008	0.088 \pm 0.008	0.055 \pm 0.008
Lipid Peroxidation (nmol MDA formed/ml erythrocytes)	0.61 \pm 0.10	0.96* \pm 0.06	1.03* \pm 0.06	1.37** \pm 0.14

Note: The different values of cadmium exposed groups were compared to the Group I. The different superscript i.e. * and ** present level of significance at P<0.05 and P<0.001, respectively.

The results of the present study clearly revealed the ability of cadmium to induce oxidative stress in blood of adult poultry bird, as evidenced by increased lipid peroxidation and altered activities of antioxidant enzymes. It was observed that increased activities of erythrocyte SOD and catalase in all the cadmium exposed groups might be due to the activation of antioxidant defence system of the body. Antioxidants reduce oxidative radical-induced reactions and have protective effect on stabilization of metabolic processes in erythrocytes that prevent the development of oxidation stress and hypoxia (13).

Recent studies have reported that the activities of protecting antioxidant enzymes during cadmium toxicity vary with the dose, duration and route of cadmium exposure. Izgut-Uysal *et al.* (20) reported unchanged SOD activity in rats after exposure with 15 ppm of cadmium for 30 days. However, Hassan and Awad (17) have reported decreased activities of SOD and catalase in erythrocytes of rats after oral administration of 5 mg CdCl₂/kg for 30 days and his studies were further supported by Urchidia *et al.* (31). But, in the present study, activities of catalase and SOD were increased. This may be due to dose difference between the present study and above mentioned studies in rats. The low dose, used in earlier studies, might cause direct inhibitory effect on the activity of antioxidant enzymes. But, the low dose was unable to stimulate the antioxidant defence system of body to increase the production of these enzymes to cope up the free radicals, which was produced by high dose of Cd in present study.

GSH appears to be important in protecting the cell against Cd toxicity (29). The decreased levels of blood glutathione in cadmium exposed birds of present study are contrary to the observations of Rana and Verma (27), who observed increased GSH levels in tissues of rats after administration of 50 mg CdCl₂/kg on alternate days for 30 days. Also, Kamiyama *et al.* (22) observed increased GSH levels in liver and kidney after exposure of 0.228 mg CdCl₂/kg, 3 days/week. It appears that, at least in short-term exposure situations, there is good possibility that oxidative stress may be one of the mechanisms involved in Cd toxicity (28). Acute Cd toxicity may be due to exhaustion of GSH stores and the increase in oxidative stress (27). However, the activity of GST was not significantly altered in Cd exposed groups

It is well known that Cd interact with subcellular sites as mitochondria, peroxisomes and microsomes, resulting in generation of free radicals and LPO. in the membranous structure (9). Increased LPO indicates a decrease in the level of glutathione and changes in the activities of antioxidant enzyme (30,11,7). Excessive production of free radicals or ROS is mainly responsible for peroxidation of cell membrane lipids. Malondialdehyde (MDA) is a terminal product of the lipid peroxidation process and determination of MDA levels provides a good measure of lipid peroxidation, which is among the chief mechanisms of cell damage leading to necrosis or apoptosis (8). The increased levels of MDA in the present study were consistent with the finding of Hassan and Awad (17) (30 days oral exposure of cadmium chloride at 5 mg/kg), Izgut-Uysal *et al.* (20)

and El-Demerdash *et al.* (10) in rats erythrocytes and Erdogan *et al.* (12) in plasma of broiler. The increased MDA levels in different tissues during cadmium toxicity has also been reported. Increased lipid peroxidation has been observed in liver and kidney of adult female Sprague-Dawley rats after chronic exposure of Cd (subcutaneous injection with 5 micromol CdCl₂/kg/day, 5 times a week, for up to 22 weeks) (28). However, Kamiyama *et al.* (22) observed no increase in LPO in liver and kidney after chronic exposure of cadmium and El-Maraghy *et al.* (11) and Boujeblen *et al.* (6) observed decreased LPO after prolonged administration. Whereas, Djukic-cosic *et al.* (9) noticed that LPO increased in acute study and decreased in subacute study in liver of mice.

As to the assessment of oxidative status, results from our study show cadmium intake resulted in changes to all indicators of oxidative stress studied and the changed activities of SOD, catalase and GST and levels of blood glutathione and MDA is a reliable sign of imbalance between oxidative and antioxidative capacity of blood (20). So, the exposure to cadmium should be reduced and attention paid to sources of cadmium in food, water and other personal care products. Although, the levels used in the present study are very high and the natural contamination at these levels are rare, but low level exposure for long duration can be deleterious.

Conclusion

From the present study, it could be concluded that cadmium exposure produce oxidative stress by disturbing the oxidative and antioxidative balance of the adult poultry birds. The cadmium sulphate at 100 mg/L in drinking water can be used to induce oxidative stress in adult poultry birds. On the basis of our results and previous studies, it might be concluded that the activation or inactivation of antioxidant enzymes and production of oxidative stress vary with the type of exposure. The exact mechanism for production of oxidative stress by cadmium toxicity is not clear, so, further detail studies are needed to explore the exact mechanism so that diseases caused by oxidative stress can be minimized.

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