The Protective Role of Vitamin C Against Cerebral and Pulmonary Damage Induced by Cadmium Chloride in Male Adult Albino Rat

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Abstract: This study was designed to examine the protective role of vitamin C (VC) against oxidative stress and morphological changes induced by chronic exposure to cadmium chloride (CdCl2) in the lungs and brain. Male adult rats received CdCl2 (5 mg/kg body weight) daily for forty days. Vitamin C (VC) at a dose of 100 mg/kg body weight was given concomitantly with CdCl2 to the rats. Three animal groups were used in this experiment (control, CdCl2 and CdCl2+VC). The concentration of malondialdehyde (MDA), activity of superoxide dismutase (SOD) enzyme and concentration of glutathione (GSH) were measured in the lung and brain homogenates. Also, histopathological investigations were carried out in lung and brain tissues. CdCl2 administration significantly increased the levels of MDA and decreased the activity of SOD and GSH concentration in the lungs and brain versus those of control rats. Administration of vitamin C counteracted the changes of all measured parameters and appeared nearly like those of controls. Light microscopy revealed marked changes in the structure of the studied tissues of CdCl2 administered animals. Again, vitamin C restored the damage of tissues associated with CdCl2 administration. The present results suggest that vitamin C administration attenuated the oxidative damage and morphological changes induced by CdCl2 in the lungs and brain of rats.

Keywords: Cadmium, vitamin C, brain, lungs, lipid peroxidation, superoxide dismutase, glutathione.

1. INTRODUCTION

Cadmium (Cd) is a highly toxic element that is naturally present in all parts of the environment, including food, water, and soil [1]. It is extracted during the production of other metals, such as zinc, lead and copper and it is used in industrial and household products, mainly in batteries, pigments, metal coatings, plastics and some metal alloys [2,3]. The main source of toxic exposure of cadmium is by the inhalation route of cadmium particles or fumes during industrial operations [4,5]. It is also present in cigarette smoke, representing a significant source of exposure [6].

Cadmium accumulates and proves to cause severe damage to a variety of organs such as lung, brain, testis, kidney, liver, blood system and bone [7-9]. Pneumonitis, pulmonary emphysema, interstitial fibrosis together with cancer are a frequent response to cadmium exposure [10,11]. Because of its characteristics as a lung carcinogen, cadmium has been classified as a category 1 carcinogen (human carcinogen) by the International Agency for Research on Cancer and the National Toxicology Program of the USA [12].

Acute inhalation of sufficient exposure of cadmium can cause both a chemical pneumonitis and pulmonary edema from the toxic effect to the alveolar epithelium and endothelium [13]. Within 24 h of exposure, workers develop shortness of breath, fever, and fatigue, which can progress to pulmonary edema and death [14]. Chronic exposure to cadmium dusts and fumes has been suspected as a cause of emphysema, obstructive lung disease, pulmonary fibrosis, and lung cancer.

Exposure of adult rats to low or moderate doses of Cd induced lipid peroxidation (LPO) in all tissues, mainly lung and brain [7]. Monroe and Halvorsen [15] provided evidence that CdCl2 increases oxidative stress in nervous cells. The brain is particularly sensitive to oxidative damage because of its high rate of oxidative metabolism [16]. Cd also enhances the production of free radicals in the brain of adult rats and interferes with the antioxidant defense system that in turn leads to a Cd-induced alteration of the structural integrity of lipids [17]. In adult rats co-exposed to Cd, there was an increase of LPO in the corpus striatum and cerebral cortex [18]. Cd also induces a significant increase of free radicals production and LPO in brain of developing rats [19].

Cd(II) accumulates and is toxic in many organs, including kidneys, lungs and testis [20,21]. Nishimura et al. [22] reported that Cd(II) can enter into the brain parenchyma and neurons and causes neurological alterations in humans [23] and animal models [24], leading to lower attention, hypernociception, olfactory dysfunction and memory deficits. Moreover, Cd induced cerebral hemorrhage and cerebral edema in neonatal rats [25]. In experimental studies with animals, cadmium is able to induce neurotoxicity with a wide spectrum of clinical entities including neurological disturbance [26], changes in the normal neurochemistry of the brain [27].
Several authors have shown that antioxidants should be one of the important components of an effective treatment of cadmium poisoning [28-30]. Accordingly, to prevent undesired oxidative damage induced by reactive species, organisms are equipped with several lines of antioxidant defense. Vitamin C is an aqueous phase antioxidant has been established for many decades and has several important roles. It modulates intracellular redox status through maintaining sulphydryl compounds, including glutathione, in their reduced state. It has been suggested to repair protein hydroperoxides, through regeneration of parent amino acids by reduction. Also, it serves to maintain membrane alpha tocopherol and enzyme activities including hepatic mixed function oxidase activity [31]. Ascorbic acid, after being converted to dehydroascorbic acid by free radical reactions, is regenerated via the glutathione enzyme complex [32]. Gupta and Kar [33] reported that vitamin C can prevent increased lipid peroxidation levels resulting from cadmium toxicity. Vitamin C could serve as an effective antioxidant against restraint stress induced pro-oxidant status and increase the antioxidant enzyme activity in rat brain [34] and liver [35].

The present study has implicated the importance of antioxidants such as vitamin C in protecting living organism against the toxic effect of cadmium exposure.

2. MATERIALS AND METHODS

2.1. Experimental Animals

Thirty adult male Sprague-Dawley rats weighting 100-120 g were used in the present work. The animals were purchased from Aboraoash Joint Animal Breeding Unit. The animals were kept in a controlled light room with a cycle 12:12) at a temperature of 28±2 °C. All animals were given free access to standard laboratory chow and tap water.

2.2. Chemicals

Cadmium chloride (CdCl₂, 99% pure) was purchased from Aldrich Chemical Co. Cadmium chloride was dissolved in saline solution (0.9% NaCl). Ascorbic acid, dimethyl sulfoxide, Sodium dodecyl sulphate (SDS), Thiobarbituric acid (TBA) and epinephrine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ascorbic acid (Vitamin C) was dissolved in distilled water. All other chemicals were of the highest purity commercially available.

2.3. Experimental Design:

The rats were randomly divided into three groups, 10 rats each:

- **The first group**: designated Cd, was injected subcutaneously with cadmium chloride at a dose of 5 mg/kg body weight.
- **The second group**: designated Cd + VC, was injected subcutaneously with vitamin C at a dose of 100 mg/kg body weight. The administration of vitamin C was 30 min. before Cd injection and was given at 8 a.m.
- **The third group**: served as controls and the rats were injected with vehicles only.
- All the above injections were repeated daily for 40 days.

2.4. Tissue Preparations

The specimens from the selected organs (lungs and brain) were rapidly excised, and cut conveniently into small pieces, which were used for histology and histopathology. Another portions of the studied organs were frozen at −40 °C for measurement of lipid peroxidation (LPO) which is an oxidative stress indicator.

2.5. Measurement of Lipid Peroxidation

The method of measurement of oxidized lipids was based on that of Ohkawa et al. [36] using 1,1,3,3-tetramethoxy-propane as standard. A 10 (w/v) tissue homogenate from brain and lungs required for this assay (this homogenate contained 1% v/v dimethyl sulfoxide to prevent further oxidation). To 0.2 ml aliquots of tissue homogenate was added 0.2 ml 8.1 % w/v sodium dodecyl sulfate solution, 1.5 ml 20% v/v acetic acid solution (PH 3.5) and 1.5 ml 0.8% w/v thiobarbituric acid solution. The mixture was made up to 4.0 ml with distilled water and heated to 95 °C for 1 h. The samples were cooled and centrifuged at 2000 xg for 10 min and absorbance measured at 532 nm. Results were expressed as n mol malondialdehyde formation per g tissue.

2.6. Superoxide Dismutase (SOD) Activity

SOD activity was determined at room temperature according to the method of Misra and Fridovich [37]. Ten microliters of tissue extract was added to 970 ml (0.05 M, pH 10.2, 0.1 mM EDTA) sodium carbonate buffer. Twenty microliters of 30 mM epinephrine (dissolved in 0.05% acetic acid) was added to the mixture to start the reaction. SOD was measured at 480 nm for 4 min. Activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit, per mg protein.

2.7. Measurement of Glutathione (GSH) Concentration

GSH content was measured by a modification of the method of Beutler et al. [38]. Briefly, 500 μl of tissue extract was added to 2.0 ml of 0.2 M phosphate buffer and 0.25 ml 0.04% 5,5’dithio-bis 2-nitrobenzoic acid. Absorbance was read at 410 nm. GSH content was expressed as nanomoles of GSH per mg of protein. Protein concentrations were measured by the method of Bradford [39] using bovine albumin as standard.

2.8. Histological and Histopathological Examinations

For the histological and histopathological examinations, pieces of the organs were fixed in 10% of neutral buffered formalin (pH 7.2), dehydrated in ascending series of ethanol, cleared in methyl benzoate and embedded in paraffin wax. Paraffin sections of 5 microns in thickness were prepared and stained with Harris’s haematoxlin and eosin [40].

2.9. Statistical Analysis

Results of LPO were expressed as means ± S.E.M Differences between means were tested by the ANOVA followed by the Student-Newman-Keuls t-test. The percent of stimulation (S%) or inhibition (I%) in the mean values of LPO was calculated.
3. RESULTS

Quantitative results of cadmium oxidative stress induction in the lungs and brain of control and different treated animal groups were presented in Tables 1 and 2. As shown, administration of cadmium significantly increased (P<0.01) LPO levels (as indicated by the increase in malondialdehyde [MDA] levels) in the homogenates of the lungs and brain versus those of control rats by 53.6% and 68.8%, respectively. When vitamin C was given (30 minutes before cadmium) to cadmium-administered rats, it significantly inhibited (P<0.01) the increase of MDA by 47.4% and 45.8% in the lungs and brain, respectively.

The activity of SOD was severely inhibited (P<0.01) in the lungs and brain homogenates of cadmium-administered animals versus those of controls. The administration of vitamin C to rats which given cadmium markedly stimulated (P<0.01) the activity of SOD by 33.6% and 32.9% in both the lungs and brain.

Cadmium administration to the rats reduced total GSH concentrations (P<0.01) in the lungs and brain compared to those of controls by 51.5% and 49.4%, respectively. In contrast, vitamin C administration to cadmium-administered rats markedly restored (34.8% and 31.6%) the concentrations of GSH (P<0.01) in the lungs and brain, respectively.

Histopathological examination revealed marked destruction of both lungs and brain tissues. In the lungs, administration of Cd induced severe changes (Fig. 1B) versus those of controls (Fig. 1A). The changes include thickened-walled alveoli and alveolar septa, infiltration of a huge number of inflammatory cells and exudation in many places as shown in Fig. (1B). When vitamin C was given to Cd-administered rats, it restored these changes and the lung tissue appear nearly like those of control rats as shown in Fig. (1C). In the brain, Cd induced massive damage in the hippocampus and cerebral cortex (Figs. 2B and 3B) versus those of control animals (Figs. 2A and 3A) respectively. The most obvious changes are cellular atrophy, shrinkage, cellular necrosis, pyknosis, deeply stained and dark nuclei as shown in Figs. (2B) and (3B). Again, vitamin C administered rats exhibited more or less normal neurons in both hippocampus and cerebral cortex compared with those of Cd-group (Figs. 2C and 3C).

4. DISCUSSION

Cadmium (Cd) is known to produce a variety of health hazards in humans and experimental animals due to its ability to induce severe alterations in various organs and tissues including the nervous system, following either acute or chronic exposure [7]. Inhalation of Cd can cause pulmonary emphysema, fibrosis, and lung carcinomas in human and experimental models [41]. It promotes an early oxidative stress and afterward contributes to the development of serious pathological conditions [42]. The mechanism by which cadmium causes toxicity is far from understood, but it is believed to be related to oxidative stress.

### Table 1. Mean Values ± S.E.M of Lipid Peroxidation Product (MDA), Superoxide Dismutase (SOD), Glutathione (GSH) and Inhibition (I) and/or Stimulation (S) % in the Lungs Homogenates of Control and Different Treated Groups of Rats

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Groups</th>
<th>MDA (nmol/g Tissue)</th>
<th>SOD (Units/mg Protein)</th>
<th>GSH (μgm/mg Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>3.747 ± 0.172a</td>
<td>1.162 ± 0.067a</td>
<td>0.0181 ± 0.0007a</td>
<td></td>
</tr>
<tr>
<td>Cadmium (n=10)</td>
<td>8.079 ± 0.475b</td>
<td>0.714 ± 0.034b</td>
<td>0.0088 ± 0.0004b</td>
<td></td>
</tr>
<tr>
<td>Cd+Vitamin C (n=10)</td>
<td>4.247 ± 0.323c</td>
<td>1.075 ± 0.053c</td>
<td>0.0135 ± 0.0004c</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>I or S% vs control</th>
<th>S= 53.6</th>
<th>I= 38.6</th>
<th>I= 51.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>I or S% vs Cd</td>
<td>S= 47.4</td>
<td>S= 33.6</td>
<td>S= 34.8</td>
</tr>
</tbody>
</table>

S.E.M: standard error of the mean. Values in the same columns with unlike superscript letters significantly differ.

### Table 2. Mean Values ± S.E.M of Lipid Peroxidation Product (MDA), Superoxide Dismutase (SOD), Glutathione (GSH) and Inhibition (I) and/or Stimulation (S) % in the Brain Homogenates of Control and Different Treated Groups of Rats

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Groups</th>
<th>MDA (nmol/g Tissue)</th>
<th>SOD (Units/mg Protein)</th>
<th>GSH (μgm/mg Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>1.984 ± 0.161a</td>
<td>1.995 ± 0.057a</td>
<td>0.0214 ± 0.0001a</td>
<td></td>
</tr>
<tr>
<td>Cadmium (n=10)</td>
<td>6.233 ± 0.534b</td>
<td>1.033 ± 0.046b</td>
<td>0.0108 ± 0.0005b</td>
<td></td>
</tr>
<tr>
<td>Cd+Vitamin C (n=10)</td>
<td>3.377 ± 0.488c</td>
<td>1.539 ± 0.062c</td>
<td>0.0158 ± 0.0014c</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>I or S% vs control</th>
<th>I= 48.8</th>
<th>I= 49.4</th>
<th>I= 45.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>I or S% vs Cd</td>
<td>S= 45.8</td>
<td>S= 32.9</td>
<td>S= 31.6</td>
</tr>
</tbody>
</table>

S.E.M: standard error of the mean. Values in the same columns with unlike superscript letters significantly differ.
being completely understood, although lipid peroxidation (LPO) has long been considered to be the primary mechanism for cadmium toxicity [7]. Among the various effects induced by Cd in biological systems, the oxidative destruction of membrane polyunsaturated fatty acids, a phenomenon termed LPO, has been observed in numerous tissues both in vitro and in vivo [43]. The oxidative stress which contributes to the pathogenesis of cadmium toxicity is the consequence of either enhanced ROS production or attenuated ROS scavenging capacity resulting in tissue damage that is most easily assessed by measuring lipid peroxide levels [44,45].

In the present study, we found a significant increase in the levels of LPO and a significant decrease in both SOD activity and GSH concentration in the lungs and brain of cadmium-exposed rats compared to those of controls. Our results are in harmony with those of Luchese et al. [10] and El-Sokkary et al. [29,30] who found that cadmium induced oxidative damage which had been demonstrated by the previous parameters. Furthermore, several studies demonstrated that, LPO may be one of the important events responsible for pulmonary and cerebral toxicity of Cd [7,8]. The lung, because of its interface with the environment, is a major target organ for injury by exogenous oxidants such as environmental pollutants, cigarette smoke, drugs, chemotherapeutic agents and hyperoxia, as well as by endogenous ROS generated by inflammatory cells. Yang et al. [46] reported that Cd is able to cause oxidative cellular damage in lung fibroblasts, manifested by lipid peroxidation, elevated level of ROS formation, and mitochondrial membrane damage, which eventually leads to irreversible cell injury.

Stohs and Bagchi [47] demonstrated that cadmium is a potent cell poison and its toxicity was mediated by the oxidative damage of essential cellular macromolecules. Other reports demonstrate that Cd induces oxidative stress in blood and tissues by increasing lipid peroxidation and by altering the antioxidant status [48,49]. The brain, off all the organs, is thought to be vulnerable to oxidative damage due to its high oxygen consumption, presence of high concentrations of polyunsaturated fatty acids and nondegenerative nature of neurons, which may lead to various neurodegenerative diseases [50,51].

It has been proposed that Cd may initiate oxidative stress through the following two pathways: 1) depletion antioxidant enzymes [28,52] and 2) enhancing production of reactive oxygen species, both in vitro and in vivo [53,54].

The morphological investigations of the current study revealed different changes in the lungs and brain. Lung lesions are consisting of vascular severe inflammation in both alveoli and bronchioles with edema and congestion. These pathological changes are in agreement with the findings of Shin et al. [55] who reported that the lung is a primary target organ of systemic exposure to cadmium. Because cadmium is mainly absorbed through the inhalation of industrial pollution and tobacco smoke, the result is the accumulation of this metal in the lung. Also, Yamada et al. [56] noticed a dramatic increase in the number of alveolar neutrophilic leukocytes 6-48 h after intra-bronchial instillation of 1 mg cadmium chloride into lungs of dogs. Bell et al. [57] reported that Cd exposure was deleterious to the lung tissue causing mild to severe inflammation in the lung. However, inhalation of Cd has been implicated in the development of emphysema and pulmonary fibrosis [58]. McKenna et al. [5] reported that Cd-exposed lungs showed acute and more chronic pulmonary inflammation in both rats and mice with bronchiolar and alveolar lesions.

Administration of Cd induced morphopathological changes in the brain involved different types of neurons in...
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The examined regions (hippocampus and cerebral cortex). The neurons undergo either degenerative or apoptotic changes. The degenerative changes manifested by shrinkage and deeply stained neurons in both studied regions. These observations in harmony with experimental studies in the CNS of newborn rats and rabbits exposed to high doses of Cd, which showed extensive hemorrhage in the cerebral and cerebellar cortices, several pyramidal cells with pyknotic nuclei, neuroglial cells with cytolysis and altered purkinje cells [59,60]. It was reported that Cd has distinct neurotoxic effects in adults and newborn animals. In adult rats, high doses of Cd produce damage to the Gasserian and sensory spinal ganglia, but in newborn rats, high doses of Cd produce severe hemorrhagic lesions in cerebrum and cerebellum [59]. Also, Cd is a highly toxic metal; in particular, it was reported to produce neuropathological and neurochemical alterations in the central nervous system resulting in irritability and hyperactivity [61].

The necrotic and degenerative changes of brain may be attributed to the high susceptibility of rats to Cd toxicity and it was considered an important indication for neuro-toxic effect of Cd due to oxidative stress induction. This interpretation is confirmed by Williams [62] who reported that oxidative stress is one of the mechanisms that contribute to structural changes and it plays an important role in neuro-degeneration.

Recent experiments have implicated the importance of antioxidant such as vitamins in protecting living organism against the toxic effects of environmental chemicals [63]. Free radical scavengers and antioxidants, such as glutathione, vitamin E, vitamin C are capable of protecting against Cd toxicity [64]. Vitamin C is an important dietary antioxidant and in the general absence of metal ion-catalyzed reactions, it is qualitatively the single most important plasma antioxidant. Thus vitamin C has been used as a choice of antioxidants in vitro [65]. It has been known to protect all classes of lipids from oxidation under a number of relevant types of oxidant stress while other non-enzymatic antioxidants such as vitamin A, Vitamin E, glutathione, bilirubin, and urate merely lower the rate of oxidation or act in a more restricted [66]. Vitamin C is an important dietary antioxidant and significantly decreases the adverse effect of reactive species such as reactive oxygen that can cause oxidative damage to macromolecules such as lipids, DNA and proteins which are implicated in several diseases [67].

In the present investigation, vitamin C maintained the levels of LPO, SOD activity and GSH concentration produced in the lungs and brain nearly at control values. Accordingly, vitamin C inhibited the increase of LPO levels (by 47.4 % and 45.8%) in both lungs and brain at respectively and this inhibition was significant (p<0.05). The inhibiting effect of vitamin C on lipid peroxidation resulting from cadmium toxicity corresponds well to the finding of Gupta and Kar [33] and Gupta et al. [68] who proposed that Vitamin C supplementation helped to reduce MDA levels and at the same time increased the activity of antioxidant enzymes such as superoxide dismutase and glutathione peroxide in Cd-exposed rats. Also, Halliwell et al. [32] reported that vitamin C scavenges the aqueous ROS by very rapid electron transfer that inhibit LPO. In addition, studies of Rodriguez-Martinez et al. [69] and Muthuvel et al. [50] revealed that vitamin C acts against oxidative stress in central nervous system.

In the current study, vitamin C markedly reduced the histopathological changes induced by cadmium chloride in both lungs and brain (hippocampus and cerebral cortex) which had normal appearance on examination. These observations are in agreement with Oski [70] who recorded...
that highly unstable free radicals attack the polyunsaturated fatty acids of membrane phospholipids, and can damage both the structure and function of cell membranes in a chain reaction unless they are quenched by an antioxidant. Administration of a moderately large dose of vitamin C almost completely prevents protein damage, apoptosis and the lung injury [71]. Moreover, population surveys have linked a low dietary intake of vitamin C with worse lung function [72]. In addition, antioxidants have therapeutic importance in neurological disorders where oxidative stress is involved [73]. The antioxidant potential of ascorbic acid is not only attributed to its ability to quench reactive oxygen species, but also to its ability to generate other small molecule antioxidants, such as α-tocopherol, glutathione and B-carotene [74].

In conclusion, the results of the current study suggest that vitamin C is useful and effective antioxidant against the toxicity induced by CdCl₂ in lungs and brain of rats. Thus vitamin C can be used in co-treatment of cadmium poisoning, since it has the capability to alleviate many of the harmful effects of cadmium.

REFERENCES:


