

Effect of Acute Toxicity of Cadmium in Mice Kidney Cells

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ABSTRACT

Background: Cadmium is one of the most toxic heavy metals in our environment having a very strong ability to accumulate in body organs, especially in kidney. The present study was done to determine the genotoxicity and cytotoxicity in kidneys of rats exposed to cadmium.

Methods: Male rats (n=30), kept in standard conditions were used in this study. The animals were randomly divided into 2 groups (control and treatment). The treatment group was intraperitoneally injected with Cd (300µm/kg) at hours 0, 6, 12, 24, 48. Twenty four hours after the last injection, the rats were sacrificed and their kidneys were obtained. Then oxidative stress markers, malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase (SOD), were assayed in homogenized kidney for studying their cytotoxicity. For genotoxicity and DNA damage studies, Comet assay was run on isolated kidney cells. Data analysis was done by t-test and ANOVA using SPSS software version 15.

Results: MDA and GSH concentrations in normal and Cd exposed kidney cells were 287.01±37.30nmol/g.pr and 15.61±3.89µmol/g.pr and 609.24±87.87nmol/g.pr and 28.52±5.22µmol/g.pr, respectively. In addition, SOD activity in normal and Cd exposed kidney cells were 77.75±4.12 and 218.91±5.40 U/mg.pr, respectively. Comet assay results (content comet length, tail length, and head diameter) showed DNA breakage in the treatment group that was stimulated by Cd which was not seen in the control group.

Conclusion: The results demonstrated the genotoxicity effect of Cd on kidney cells as well as the ability of Cd to producing cytotoxicity.

Keywords: Cadmium, Comet Assay, DNA Damage, Kidney, Oxidative Stress.

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INTRODUCTION

Cadmium (Cd) is one of the most toxic heavy metals in environment. Cadmium and its salts, such as cadmium chloride, are one of the most important air and water pollutants because they have been used widely by lead, copper, and zinc smelter alkaline accumulator, paint, and plastic industries (1).

Their toxicity has been widely studied and reported. Cadmium exposure causes damage to living organs especially in human which leads to Itai-itai disease (2,3). Also, all Cd compounds have been classified as human carcinogenic elements (4).

The main sources of exposure to these metals are working with them through oral or dermal contact but it occurs

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mainly through inhalation (5), cigarette smoking, and alcoholic beverages (6).

Cadmium has a very strong ability to accumulate in kidney organs and this can be dangerous for kidney. Brzoska *et al.* (7) had shown that chronic exposure to cadmium compounds can damage the renal proximal tubular epithelial cells as a result of dysfunction proximal tubular manifested by low-molecular-weight proteinuria, glucosuria, aminoaciduria, and phosphaturia (7-9).

The molecular mechanism for describing the toxic effect of cadmium is not well-understood, but it is obvious that Cd itself is unable to generate damage and it has been shown that the relationship between Cd and free radicals is indirect (10). Several studies have shown relationship between Cd and oxidative stress since compounds with this metal can alter the antioxidant system in animals. This is mostly due to the reduced levels of glutathione and increased lipid peroxidation (11,12).

Studies on animals have shown that Cd is a stimulator for formation of reactive oxygen species (ROS) (13), hydrogen peroxide (14), and also hydroxyl radicals (15). These free radicals enhance lipid peroxidation, DNA damage, altered calcium and sulfhydryl homeostasis (16-18). These free radicals also affect cellular function by perturbing signal transduction, such as protein kinase C (PKC), mutagen activated protein kinase (MAPK), and cyclic AMP pathway; however, the mechanism is largely unknown (19,20).

Lipid peroxidation is the primary mechanism for Cd-induced toxicity (21). Through the Fenton reaction, oxidative stress produces hydroxyl radical species that are believed to initiate lipid peroxidation (10,22). Following this process, free radicals are produced and attached to any available molecule in intra-cellular environment or the extra one which eventually leads to cellular damage (10,22).

These damages are increased when the antioxidant defense systems, such as superoxide dismutase (SOD), catalase (CAT), or reduced GSH, have been suppressed by increased generation of ROS (23,24).

Since kidney is one of the main organs of Cd accumulation (7,25), this study was carried out to postulate kidney damage induced by cadmium, which is related to the oxidative damage and DNA breakage in kidney cells.

MATERIALS AND METHODS

Animals

In this study, 20 male rats, 5-6 weeks old with initial bodyweight of 30 ± 5 g, were obtained from the Laboratory Animal House of Baqiyatallah University of Medical Sciences, Tehran, Iran. The rats were kept under standard conditions (temperature 23 ± 2 °C, natural light-dark cycle). The rats were divided randomly into 2 groups (control and treatment) and housed in plastic cages, 5 rats in each cage, with free access to drinking water and a standard diet for one week. Then the rats received peritoneal injections of cadmium chloride at a dose of $300 \mu\text{m}/\text{kg}$ bodyweight in 0.2 ml distilled water for five times, at hours 0, 6, 12, 24, 48. The control group received 0.2 ml of 0.9% normal saline as placebo.

Tissue preparation

Twenty four hours after the latest injection, the animals were anaesthetized by chloroform and their kidneys were immediately obtained. These tissues were transferred to 3 ml ice-cold PBS for biochemical assay and comet assay.

Biochemical assay

The tissues which had been kept in PBS were divided to many parts and each part was homogenized according to the analytic assay protocol. Total glutathione (GSH) concentration was estimated by using CUSABIO BIOTECH CO, rat

glutathione peroxidase (GSH-PX) ELISA Kit Catalog No. CSB-E12146r (96T). Malondialdehyde (MDA) concentration was measured according to the method of OXitek TBARS Assay Kit ZMC Catalog: 0801192.

Super oxide dismutase (SOD) activity was determined following Kamiya Biomedical Company kit for the measurement of superoxide dismutase (SOD) inhibition activity (K-ASSAY, SOD Assay KT-219 (100 tests)). Total protein measured following the Brad-Ford method.

DNA damage assessment using comet assay

Single cell gel electrophoresis/comet assay were done for rapid genotoxicity assessment according to the following method:

1. Preparation of slides for the SCGE/comet assay:

1.0% agarose (500 mg per 50ml PBS), 0.5% LMPA (250 mg per 50 ml PBS), and 1.0% NMA (500 mg per 50 ml in Milli-Q water) were prepared using microwave or heat until near boiling and the agarose was dissolved. For LMPA, aliquot 5 mL samples were placed into scintillation vials and refrigerated until needed. When needed, agarose was briefly melted in the microwave. LMPA vials were placed in a 37°C water bath to warm and stabilize the temperature.

To remove the machine oil and dust, the slides were dipped in methanol and burned over a blue flame. While NMA agarose was hot, conventional slides were dipped up to one-third of the frosted area and gently removed. Underside of the slide was wiped to remove agarose and the slide was placed in a tray on a flat surface to dry. All prepared slides were placed in a cool and dry place.

In the next step, a suspension of 500-1000 isolated kidney cells was prepared in 100 microliters of 0.5% LMPA and mount it on upper side of the 1.0% agarose dipped

slide. Prepared slides were placed in refrigerator for 20 minutes to dry and were, then, covered by lysing solution (tris-base 10mM- pH=8, 1% SDS, 1% triton X100) for 2 hours at 4°C.

2. Electrophoresis of micro gel slides:

After at least 2 hours at ~4°C, the slides were gently removed from the lysing solution. The slides were placed side by side on the horizontal gel box near one end, sliding them as close together as possible. The buffer reservoirs were filled with freshly made pH>13 electrophoresis buffer until the liquid level completely covered the slides (bubbles were avoided over the agarose). The slides were allowed to sit in the alkaline buffer for 20 minutes to allow for unwinding of the DNA and the expression of alkali-labile damage. Power supply was turned to 24 volts (~0.74 V/cm) and the current was adjusted to 300 miliamperes by raising or lowering the buffer level and electrophoresis of the slides for 30 minutes. Then the power was turned off and the slides were gently lifted from the buffer and placed on a drain tray. The slides were dropped wise coat with neutralization buffer and it was allowed to sit for at least 5 minutes. The slides were drained and this process was repeated two more times.

The slides were stained with 80µL 1X ethidium bromide, left for 5 minutes, and then dipped in chilled distilled water to remove excess stain. The stained slides were viewed by fluorescent microscope (Nikon) to determine DNA breakage. The captured image was analyzed by comet score software to determine quantifiable DNA breaking index factors (tail length, % DNA in tail, head diameter, % DNA in head).

Statistical analysis

All data were expressed as mean \pm SD for 10 experiments in each group (n=10). Statistical analysis was performed with t-test. Also, the experimental groups were compared using a one-way analysis

of variance (ANOVA). SPSS software version 15.0 was used for statistical analysis and $P < 0.05$ was considered significant.

RESULTS

SOD activity and GSH and MDA concentrations

In the group exposed to the Cd, the results showed an increase in the activity of SOD in comparison with the control group ($P < 0.01$). In addition, the concentrations of MDA and GSH showed a significant increase in comparison with control group ($P < 0.03$) (Table1).

DNA breakage as DNA damage in comet assay

By using this method, the breakage of DNA of kidney cells exposed to cadmium happened and during the alkaline electrophoresis moved to a tail from nucleus (Figure1). Output results from the comet score software showed the potential of cadmium for DNA breakage (Figure2).

DISCUSSION

Kidney has been recognized as a critical target organ of Cd toxicity. The

present study was done to assess the oxidative status, DNA damage, and cytotoxicity of kidney after acute exposure to Cd. The mechanism of Cd-induced kidney damage is considered to be related to increased oxidative status. Increased oxygen of free radicals production seems to be induced by the interaction of Cd and mitochondrial structure (26).

For this purpose, concentration of MDA as an oxidative attack marker, an indicator of lipid peroxidation, and GSH for determined strength of antioxidant defense system and also the activity of antioxidant enzymes, such as SOD, were determined in kidney. A comet assay was applied for detecting DNA damage in kidney cells.

All data were expressed as mean \pm SD for the 10 experiments in each group ($n=10$). In the groups exposed to the Cd, the results showed an increase in the activity of SOD in comparison with the control group ($P < 0.01$). Moreover, MDA and GSH concentrations showed a significant increase in comparison with the control group ($P < 0.03$).

Table1. SOD activity and GSH and MDA concentrations in homogenized kidney

	GSH ($\mu\text{mol/g.pr}$)	MDA (nmol/g.pr)	SOD (U/mg.pr)
Control group	15.61 \pm 3.89	287.01 \pm 37.30	77.75 \pm 4.12
Treated groups	28.52 \pm 5.22*	609.24 \pm 87.87*	218.91 \pm 5.40**

*: significant with control group ($\rho < 0.03$)

** : significant with control group ($\rho < 0.01$)



Figure 1. Image from comet assay on kidney cells: (a) A normal kidney cell: no DNA breakage and movement and (b) kidney cells exposed to Cd: significant breakage and movement of DNA following the comet assay

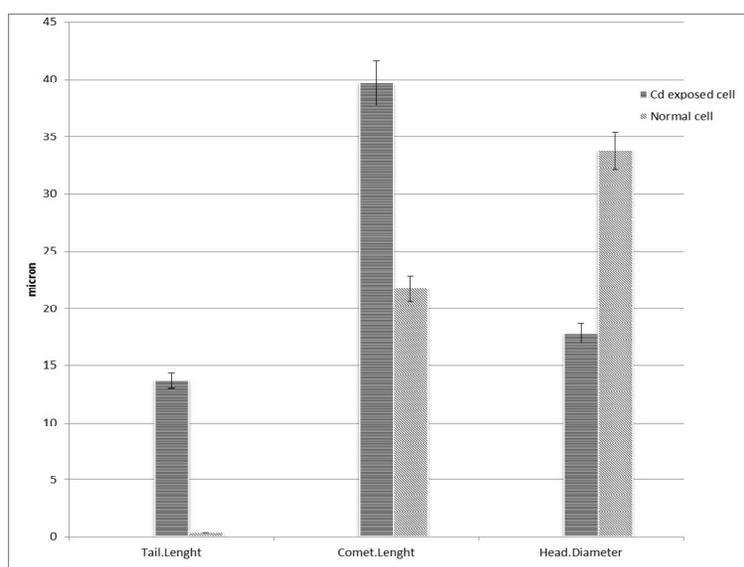


Figure 2. Analysis of comet picture with comet score software

Figure 2 shows DNA amounts in nucleus (DNA in head), DNA movement during the comet (comet length), and DNA amount in tail. The results showed significant changes in treated group in comparison with the control group.

A substantial alteration in GSH and MDA levels due to Cd treatment has been reported previously (26-28). Several studies have demonstrated that Cd exposure is associated with increased production of super oxide anions (29) and MDA (30) and decreased tissue levels of GSH (10). Thus the present study

attempted to probe significant relationships between kidney GSH, MDA, and SOD following acute peritoneal exposure to cadmium.

In the present study, MDA significantly increased in the rats exposed to Cd in comparison with the control group. These results suggest an increase in renal oxidative stress following acute exposure to Cd which had been reported previously by Babu *et al.* (30).

Based on the results of this study, the increase in kidney GSH could be explained by its stimulation to neutralize the

increased oxygen free radicals in an acute exposure condition. This is in contrast to the findings of Murugavel's study (10).

In the present study, the renal content of SOD increased which is not in agreement with the findings of the study by Bin Xu *et al.* (31).

This difference in SOD activity can be explained by two different causes. First, experimental condition of exposure; in the present study, acute form of exposure was used while Bin Xu *et al.* (2008) (31) used a chronic method of exposure. Second, in acute exposure to, Cd is not able to interact with Zn and Cu at the active site of SOD; however, in chronic exposure, Cd has an ability to interact with Zn and Cu; therefore, SOD activity (7,25,32,33) was inhibited although increased oxygen free radicals were associated with increased levels of antioxidant enzymes.

Cd-induced increased MDA concentration in kidney indicates an escalation of lipid peroxidation in this organ due to oxidative stress. Increased peroxidation of lipid intra- and extra-cellularly explains damage to the cells, tissues, and organs that may be due to inability of antioxidant defense systems.

CONCLUSION

The findings of comet assay on kidney cells showed an obvious DNA breakage in treatment rats that is not seen in the control group. This indicates that cadmium can act as a carcinogen and mutagen. Cd has the ability to accumulate in kidney cells and could be harmful for nucleus and cell organelles. Therefore, we suggest appropriate screening of individuals who are exposed to Cd, regular follow-up for any damage, and improving antioxidant defense system.

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