Original Article

Oxidative Stress and DNA Damage in Mice Kidneys Exposed to Cadmium Chloride

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Abstract:

Introduction: *Cadmium is one of the most toxic heavy metals in our environments having a very strong ability to accumulate in body organs especially in kidney. Our aim of present study was to determine the Genotoxicity and cytotoxicity in mice kidneys exposed to cadmium.*

Material and Method: *we sacrificed 30 male mice in this study that randomly divided into 2 different groups (1control& 1tratment) that every 5 mice in one cage and remained in standard conditions. After one week, we applied peritoneal injection to expose mice with Cd (300µm/Kg.b.wt) on 0, 6, 12, 24, 48 hrs. 24hr after latest injection the mice were killed and their kidney obtained, then Oxidative stress markers (malondialdehide (MDA), glutathione (GSH) and superoxide dismutase (SOD)) were assayed on homogenized kidney for studying cytotoxicity, and for Genotoxicity& DNA damage studies, we used Comet assay on separated kidney cells. Finally, to analyze statistical data, we used T-test and ANOVA using SPSS15 software.*

Results: *present results had shown that MDA and GSH concentration and also SOD ($\rho<0.05$)activity in treated group had a significantly increase in comparison with control group. The comet assay results obviously showed DNA breakage in treated group that was stimulated by Cd which was not seen in the control group.*

Keyword: *cadmium, kidney, oxidative stress, DNA damage, comet assay.*

Introduction:

Cadmium (Cd) is one of the most toxic heavy metals in our 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 and paint and plastic industries(1).

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

The main source of expos to these metals are in working with them as: oral or dermal contact but they occur mainly by inhalation (5),cigarette smoking and alcoholic beverages (6).

The kidneys are organs that cadmium has a very strong ability to accumulate in theirs 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 dysfunction proximal tubular manifested by Low-Molecular-Weight proteinuria, glucosuria, aminoaciduria and phosphaturia (7-9).

The molecular mechanism for described the toxic effect of cadmium is not well understood, but it is obvious that Cd, itself unable to generate damage and its registered that relationship between Cd and free radicals indirect(10). Many studies had shown communications of Cd and oxidative stress, since this metal compounds can alter the antioxidant system in animals. This causes may be occurred in levels of reduced glutathione and increase lipid per oxidation (11;12).

Studies on animals had shown that Cd is a stimulator for formation of reactive oxygen species (ROS) (13) hydrogen peroxide (14) and also hydroxyl radicals (15), that 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, but the mechanism is largely unknown(19;20).

Lipid peroxidation is primary mechanism for Cd induced toxicity (21). Through the Fenton reaction, oxidative stress produce hydroxyl radicals species that are believe to indicate lipid per oxidation (10;22). Following this process, free radicals are produced and attacked to any available molecule in intra cellular environment or the extra one, then its lead to cellular damage(10;22).

These damages are increased when the antioxidant defense systems such as super oxide dismutase (SOD), Catalase (CAT) or reduced GSH, been suppressed by increase generation of ROS(23;24).

As the kidney is one of the main organ of Cd accumulation (7;25) ; in this study we were postulate kidney damage induced by cadmium, which was related to the oxidative damage and DNA breakage in kidney cells.

Material and method:

Animals: in this study, we used 20 male mice, 5-6 week old and initially body weight 30±5 gram, obtained from the laboratory animal house of Baqiyatallah University Medical of Science. The mice were kept under standard condition (temperature 23±2 °C, natural light-dark cycle). The mice were divided randomly in to 2groups (1control& 1tratments); housed in plastic cage - each 5 mice in one cage - free access to drinking water and a standard diet for one week. Then the mice were exposed by peritoneal injection to cadmium chloride at a dose of 300µm/kg.b.wt in 0.2 ml distain water for five times, at times 0, 6,12, 24, 48 hrs. Respectively the control group received 0.2 ml of 0.9% normal saline as placebo.

Tissue preparation: 24hr after latest injection the animals were anaesthetized by chloroform and their kidneys were rapidly obtained. These tissues were transferred to 3ml ice-cold PBS for biochemical assay& comet assay.

Biochemical assay: The tissues which had been kept into the PBS were divided to many parts and each part 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)"

malondialdehide (MDA) concentration was measured according to the method of "OXItek TBARS Assay Kit ZMC Catalog: 0801192"

Super oxide dismutase (SOD) activity was determined following to the "Kamiya Biomedical Company kit for the measurement of Superoxide Dismutase (SOD) Inhibition Activity (K-ASSAY, SOD Assay KT-219 (100 tests))".

Total protein was measured following the Brad-Ford method.

DNA damage assessment using comet assay: single cell gel electrophoresis / comet assay had been doing for rapid genotoxicity assessment according to the following method:

Preparation of Slides for the SCGE/Comet Assay :

1. Prepared 1% (500 mg per 50ml PBS) and 0.5% LMPA (250 mg per 50 ml PBS) and 1.0% NMA (500 mg per 50 ml in Milli Q water). Microwaved or heated until near boiling and the agarose dissolved. For LMPA, aliquot 5 mL samples into scintillation vials (or other suitable containers) and refrigerated until needed. When needed, briefly melt agarose in microwave or by another appropriate method. Placed LMPA vial in a 37ºC dry/water bath to cool and stabilize the temperature.

2. Dipped the slides in methanol and burned over a blue flame to remove the machine oil and dust.

3. While NMA agarose was hot, dipped conventional slides up to one-third the frosted area and gently removed. Wiped underside of slide to removing agarose and laid the slide in a tray on a flat surface to dry.

Electrophoresis of Micro gel Slides

The procedure described is for electrophoresis under pH>13 alkaline conditions.

1. After at least 2hour at ~4ºC, gently removed slides from the Lysing Solution. Placed slides side by side on the horizontal gel box near one end, sliding them as close together as possible.

2. Filled the buffer reservoirs with freshly made pH>13 Electrophoresis Buffer until the liquid level completely covers the slides (avoid bubbles over the agarose).

3. Let slides sit in the alkaline buffer for 20 minutes to allow for unwinding of the DNA and the expression of alkali-labile damage.

4. Turned on power supply to 24 volts (~0.74 V/cm) and adjusted the current to 300 milli amperes by raising or lowering the buffer level. Depending on the purpose of the study and on the extent of migration in control samples, electrophorese the slides for 30 minutes.

5. Turned off the power. Gently lifted the slides from the buffer and placed on a drain tray. Dropped wise coat the slides with Neutralization Buffer, let sit for at least 5 minutes. Drained slides and repeat two more times.

6. Slides may be stained with 80µL 1X Ethidium Bromide, leave for 5 min and then dipped in chilled distilled water to remove excess stain. The coverslip was then placed over it and the slides are scored immediately or dried before staining as in step 7.

7. Drained slides; kept them for 20 min in cold 100% ethanol or cold 100% methanol for dehydration. Airs dries the slides and placed them in an oven at 500C for 30 min. Store in a dry area.

8. When convenient, rehydrated the slides with chilled distilled water for 30 min and stain with EtBr as in step 6 and covered with a fresh coverslip. Before viewing slides, blot away excess liquid on the back and edges. After scoring, remove coverslip, rinse in 100% alcohol to remove stain, let dry, and store for archival purposes if needed.

We say that, necessary, DNA breaking index factors determine quantifiable (Tail length, %DNA in Tail, Head diameter, %DNA in head) with comet score software.

Statistical analysis: all data were expressed as mean ± SD for number of 10 experiment in each group (n=10). Statistical analysis was performed with T-test. Also experimental groups were compared using a one-way analysis of variance (ANOVA). We used Statically Product and Service Solution (SPSS) version 15.0 for analysis which achieved data. In this study $\rho<0.05$ was considered significantly.

Results:

SOD activity and GSH& MDA concentrations:

In the groups that exposed to the Cd, our results showed an increase in the activity of SOD in comparison with the control group ($\rho<0.01$). And the concentrations of MDA and GSH also had shown a significant increase in comparison with control group ($\rho<0.03$). (Table1)

DNA breakage as DNA damage in comet assay:

By using of this method we saw that DNA of kidney cells which exposed to cadmium has been breakage and during the alkaline electrophorese moved to a tail from nucleus. (Fig1)

Output results from the comet score software, obviously shown that cadmium has a potential for DNA breakage. (diagram1).

Discussion:

The kidney has been recognized as a critical target organ of Cd toxicity. The present study was undertaken to assess the oxidative status, DNA damage and cytotoxicity of kidney after acute exposing to Cd. The mechanism of Cd-induced kidney damage is considered to be related to increased oxidative status. Increasing of oxygen of free radicals production seem to be induced by the interaction of Cd with 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.

A substantial alteration in GSH and MDA levels due to Cd treatment has been reported previously (26-28). Several studies have been demonstrated the Cd exposure is associated with increased production of super oxide anions (29) and MDA (30) and decreased tissue levels of GSH (10). Thus in the present study, we had endeavored to probe significant relationships between kidney GSH, MDA and SOD following acute peritoneal expose to cadmium.

In our study we have observed that MDA was significantly increased in mice which were exposed to Cd in comparison with control group. These results, suggest an increase renal oxidative stress following acute expose to Cd, that similar result had been reported by Babu et al (2006)(30) previously.

According our results, the increase of kidney GSH could be explained by its stimulating to neutralize the increased oxygen free radicals in an acute exposing condition. That is in contrast with Murugavel and Pari(2007)(10).

In agreement with these data, we have observed that renal content of SOD was increased that is against with Bin Xu et al(31).

This different result in SOD activity can be explained by two different causes: First, experimental condition of exposing, in this survey, we used an acute exposing but in Bin Xu et al(2008)(31), they used a chronic exposing method. Second, in acute exposing, Cd, it is not able to interact between Zn and Cu in active site of SOD, but in chronic exposing, Cd has an ability to interact with Zn and Cu and therefore inhibited the SOD activity (7;25;32;33). Although,

increased oxygen free radicals were associated to increase level of antioxidant enzyme in consist.

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

Results of our comet assay on the kidney cells, showed an obvious DNA breakage in treated mice that is not seen in control group. That is providing which cadmium can act as a carcinogen and mutagen material.

Our results in this study shown that Cd has ability in accumulation in kidneys cells and could harmful for nucleus and cell organelles. Therefore we sagest persons how work and expose with Cd, should follow up for any damage and also should improve antioxidant defense system.

Acknowledgment: we are thankful of professor Mehrani for technical help and Dr.Heydari for writing assistance. We also acknowledge Dr.Jafari.

Diagram1:

Analysis of comet picture with comet score software.

Table1:

SOD activity and GSH& MDA concentrations in homogenized kidney

	GSH(µmol/g.pr)	MDA(nmol/g.pr)	SOD(U/mg.pr)
Control group	15.61±3.89	287.01±37.30	77.75±4.12
Treated groups	28.52±5.22*	609.24±87.87*	218.91±5.40**

*: significant with control group($\rho<0.03$)
**: significant with control group($\rho<0.01$)

All data were expressed as mean ± SD for number of 10 experiments in each group (n=10). In the groups that exposed to the Cd, our results showed an increase in the activity of SOD in compare with the control group ($\rho<0.01$). And the concentrations of MDA and GSH also had shown a significant increase in compare with control group ($\rho<0.03$).

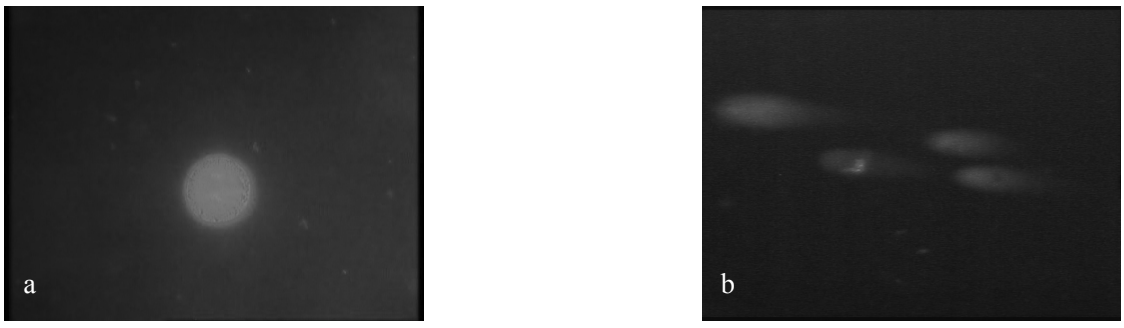
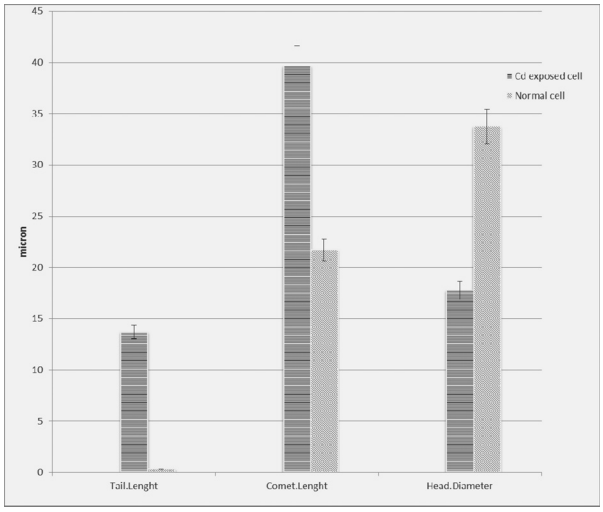


Figure1: picture from comet assay on kidney cells. (a) A normal kidney cell: this picture doesn't show any DNA breakage and movement. (b) Kidney cells which exposed to Cd: the DNA from these cells has a significant breakage and movement that showed following the comet assay.

This diagram showed DNA amount in nucleus (DNA in Head), DNA movement during the Comet (Comet Length) and DNA amount in tail. The result showed significant changes in treated group in compare with control group.



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