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Toxicology

Oral administration of cadmium depletes intratesticular and epididymal iron levels and inhibits lipid peroxidation in the testis and epididymis of adult rats

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ABSTRACT

Cadmium (Cd)-induced tissue injury depends on the accumulated Cd which differentially affects endogenous iron (Fe). To investigate this, adult rats were treated by oral gavage with Cd (50 mg/kg body wt.) once a week for 15, 30 and 60 days and sacrificed a day after last administration. After the 15th and 30th day of treatment, Cd had no effect on thiobarbituric acid reactive substances (TBARS) and endogenous Fe levels but exhibited anti-androgenic effects ($p < 0.05$) and caused histological damages. At day 60, Cd was accumulated by 156.30% and 364.77% above control values at concentrations that decreased endogenous Fe levels by 46.41% and 50.31% in the testis and epididymis respectively. The histological damages were characterized by decreased tubular diameter, damage to the epithelium leading to loss of tubular germ cells and absent of spermatozoa in the epididymal lumen. Although myeloperoxidase activities were increased, TBARS levels were found to decrease significantly at day 60 in the serum, testis and epididymis suggesting that the histological damages were not caused by lipid peroxidation. Furthermore, TBARS correlated negatively with Cd in the testis ($r = -0.251$, $p < 0.05$) and epididymis ($r = -0.286$, $p < 0.05$); Fe correlated positively with TBARS in the testis ($r = +0.217$, $p < 0.05$) and Cd correlated negatively with Fe in the testis ($r = -0.461$, $p < 0.05$) and epididymis ($r = -0.109$, $p < 0.05$). The antioxidant enzymes, superoxide dismutase and glutathione peroxidase were also decreased in the gonads after 60 days Cd treatment. Overall, anti-androgenic effects and histo-pathological changes are early indicators of direct effects of Cd and occur before decrease in TBARS which is secondarily related to the modifying of Fe contents.

1. Introduction

Cadmium is a widely distributed environmental contaminant that has toxic effects on several target tissues including the reproductive organs (testis, epididymis), and as well as having endocrine disrupting potentials [1–3]. It occurs naturally in the environment and as a pollutant from anthropogenic sources [4]. Its adverse effects on the biologic systems are thought to be related to its potential to accumulate in target tissues; an event that is influenced by several factors including age, gender, diet composition and nutritional status, but the most important is relationship between Cd and essential minerals, especially Fe [2,5]. Previous studies have shown that deficiency of Fe in animal models or humans enhances the rate of absorption and tissue accumulation of Cd [2,6,7]. The administration of Cd increases lipid peroxidation (LPO) in several tissues of Cd-injected animals *e.g.* rats, mice and inhibit antioxidant enzymes [3,8–13]. Some other studies have also reported that Cd administration decreases LPO in liver and kidney of the rodent, *Bank vole* [14] and in mouse liver [15]. These differential effects of Cd on LPO are essentially affected by minerals especially endogenous Fe concentrations in the tissues. However decreases in LPO as a result of Cd administration have not been reported previously in the gonads of rats and higher mammalian animal models, and it is also not known whether oral gavage of Cd to experimental rats decreases LPO in the reproductive organs. In studies were Cd increases LPO, the increased reactive oxygen species and oxidative stress were responsible for inactivating the antioxidant defensive enzymes and causing of oxidative damage [3,16–19] while in studies were Cd administration decreases LPO, the endogenous level of Fe and not Cd itself was responsible for the biochemical effects [14]. It therefore appears that decreased LPO in several tissues by Cd is indirectly through changes in Fe metabolism [14]. Therefore, Cd that accumulates in tissues regulates endogenous Fe level that drives susceptibility of target tissues to Cd-

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induced LPO [13,19,20].

Therefore, the main purpose of the present study was to evaluate Cd accumulation in the testis and epididymis after Cd oral gavage in rats, and its effects on LPO, antioxidant enzymes, histopathology of the gonads and steroidogenesis. Since endogenous Fe could modify the extent of Cd accumulation in tissues [2,14], the concentrations of tissue Fe were examined to establish if there be any relationship between accumulated Cd and endogenous Fe in the testis and epididymis of Cd-treated animals. Our data appears to suggest that long term Cd administration in rats inhibits endogenous Fe concentration and decreased *in vivo* LPO in the testis and epididymis, and that morphological and structural defects in the testis and epididymis and impaired steroidogenesis are earlier events than peroxidative changes in Cd-induced toxicity in the male gonads.

2. Materials and methods

2.1. Chemicals

Cadmium chloride and all other reagents used were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise stated. The $CdCl_2$ solution was prepared in normal saline (vehicle) and used throughout the study.

2.2. Animals and treatment and collection of testes, epididymis and serum

Forty adult male Wistar rats (*Rattus norvegicus*, weighing 113–120 g) were provided by the animal house of the Department of Biochemistry, University of Port Harcourt, and housed in four groups each containing ten animals. The animals were maintained at room temperature, with 12-hr light/12-hr dark cycle, and with free access to rat chow and water *ad libitum*. One week was allowed prior to the start of experiment for the animals to acclimatise to their surrounding environment and human contact. The control animals received normal saline throughout the study period. The treatment groups received 50 mg/kg body weight $CdCl_2$ based on our previous study [13] for 15, 30 and 60 days. All treatments were administered by oral gavage at least once per week, and the study terminated at 60 days to allow for the completion of spermatogenesis in rats [21]. All experimental procedures were in accordance with the principles and procedures of the National Institute of Health Guidelines for Animal Care and Use of Laboratory Animals. Animals in all groups were killed after last day of administration by cervical dislocation and blood was collected by cardiac puncture into plain bottles. The testis and epididymis were quickly removed and washed in 1.15% KCl (ice-cold) and pat-dried and testis weighed without removing the tunica albuginea. The blood was centrifuged at $4000 \times g$ at room temperature for 15 min and serum was collected and used for the determination of testosterone (T), luteinising hormone (LH), follicle stimulating hormone (FSH) and myeloperoxidase (MPO) activity and LPO. The right testes were fixed with Bouin's solution, sectioned and stained routinely with haematoxylin and eosin for microscopy and histological analysis while the left testes were used for measurement of biochemical parameters of oxidative stress. The final body weights of rats were taken prior to sacrifice.

2.3. Preparation of sample homogenate and assessment of oxidative stress

The left testes were weighed and homogenized in ice-cold 0.1 M Tris–HCl buffer (pH7.4) to produce 10% homogenate. The homogenate was centrifuged at 10,000 rpm (RPM), 4 °C for 15 min and the separated supernatant was used to measure 17β-hydroxysteroid dehydrogenase (17β-HSD), 3β-hydroxysteroid dehydrogenase (3β-HSD), MPO, catalase (CAT), glutathione peroxidase (GSH-Px), glutathione *S*-transferase (GST), and superoxide dismutase (SOD) activities and reduced glutathione (GSH) and LPO levels.

2.4. Determination of cadmium (Cd) and iron (Fe) levels in the testis and epididymis

Tissue slices for AAS were dried at 60 °C in an oven and then weighed. The dried weighed tissues were digested with a mixture of HNO_3 and HCl (3:1) at 120 °C. When fumes were white and the solution was completely clear, the samples were cooled to room temperature and the tubes were filled to 10 mL with distilled water and filtered. Samples were analysed to determine Cd and Fe using Solaar Thermo Elemental Atomic Absorption Spectrometer (AAS) model-SE-71906. Values less than 0.001 were below detectable limit. Samples were analysed in triplicate and the variation in coefficient was usually less than 10%. Concentrations of Cd and Fe were expressed as milligram per gram dry weight in testis

2.5. Enzyme immunoassay for testosterone, luteinising and follicle stimulating hormones

Competitive enzyme immunoassay was carried out using AccuBind™ testosterone kit according to the manufacturer's protocol (Monobind Inc. CA, USA). All samples were run in triplicate at 450 nm, and one set of testosterone (T) standard were included in each assay. The amount of testosterone present in the experimental sample was determined from a calibration curve (0–12 ng/mL). The within and between assay precision of the testosterone assay were 5.6% and 7.9% respectively. The cross-reactivity assay with other steroids tested was < 0.0001%. The minimum detection limit in the testosterone assay was 0.0576 ng/mL. Serum hormone levels for luteinizing hormone (LH) and follicle stimulating hormone (FSH) were determined using their respective Enzyme-linked immunosorbent assay kits (IBL Immuno-Biological Laboratories, Hamburg, Germany) according to the manufacturer's instructions. All samples were analysed in duplicate. The minimum detection limits in the LH and FSH assays were 1.27 ng/mL and 0.86 ng/mL, respectively. The cross-reactivity assay with other steroids tested showed < 0.01%. The intra-assay and inter-assay coefficients of variation (CVs) are 4.28% and 5.14% for LH, and 4.3% and 6.9% for FSH, respectively.

2.6. Determination of myeloperoxidase activity in the serum, testis and epididymis

Myeloperoxidase (MPO) activities in the serum, testis, and epididymis were determined as described previously [22]. Briefly, 110 μL TMB (2.9 mM) prepared in 14.5% DMSO/ sodium phosphate buffer (150 mM, pH 5.4) was mixed with 80 μL H_2O_2 (0.75 mM) and 10 μL sample in a 96-well plate. The mixture was incubated at 37 °C for 5 min following the addition of 50 μL H_2SO_4 (2 M) stop solution. The absorbance was read at 450 nm against a blank containing the mixtures except that sample was replaced with buffer using a BIOBASE-EL10A micro-plate Elisa Reader (BIOBASE Biotech Co., Ltd, Shandong, China) equipped with a SQLims PRO 2.0 SBS Software. The molar extinction for TMB of $5.9 \times 10^4\ mol^{-1}\ cm^{-1}$ was used to determine MPO activity and was expressed as units/min/mg protein.

2.7. Determination of lipid peroxidation and reduced glutathione levels

Thiobarbituric acid reactive substances (TBARS) were quantified as malondialdehyde (MDA) as determined previously [23]. The samples (500 μL) were precipitated with equal volume of 20% trichlorooacetic for 15 min at 10, 000 rpm, 4 °C. The supernatant was mixed with 0.075% thiobarbituric acid (0.5 mL) in 0.1 M hydrochloric acid and 1.5 mL Tris-KCl buffer (pH 7.4) and place in a boiling water bath for 1 h. The absorbance of the resulting pink chromogen was read at 532 nm in a BIOBASE BK-D560 Ver 2.0 spectrophotometer. The molar extinction for thiobarbituric acid-reactive substances (TBARS) of $1.56 \times 10^5\ mol^{-1}\ cm^{-1}$ was used to determine MDA concentration. To