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RENAL MORPHOMETRY OF FETUSES RATS TREATED WITH CADMIUM

MORFOMETRÍA RENAL DE FETOS DE RATA TRATADOS CON CADMIO

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SUMMARY: Cadmium is a heavy metal which is found in the soil, air and food. It is present in everyday life and currently it is being implicated as a toxic, teratogenic and carcinogenic agent. It causes lesions to several organs of humans and animals alike.

This work aims to investigate the toxic effects on fetuses of dams exposed to cadmium chloride treatment. Ten pregnant rats were divided into two groups. Five rats, the study group, received an intraperitoneal administration of cadmium chloride at 5 mg/Kg body weight on the 10th day of pregnancy and the other five, the study group, received a solution of 0.85% sodium chloride at the same dose and at the same application site.

The fetuses together with the placentas and umbilical cords were collected on the 20th day of pregnancy. The administration of cadmium chloride caused a significant reduction in the sizes of both the fetuses and the placenta. The lengths of the umbilical cords were also shorter in animals of the study group when compared with the control group.

This study also evaluated the morphological alterations caused in the proximal and distal convoluted renal tubules and collectors as well as the renal glomerular cells. Morphometry evidenced significant alterations in the proximal tubules and the glomerular cells and to a lesser degree in the distal tubules. The brush-border membrane cells were seen to be very sensitive and displayed some alterations. The glomeruli presented with edema and the Bowman's capsules were also affected.

KEY WORDS: 1. Cadmium; 2. Nephrotoxicity; 3. Karyometry.

INTRODUCTION

Cadmium exists in low concentrations in the earth's crust (Friberg *et al*., 1974; Bernard & Lauwerys, 1984; Departament of Health and Human Services, 1992 and Pinot *et al.,* 2000) and is generally associated with zinc, as a sulfite deposit. It is a malleable, soft, ductile, light silver-colored metal. It does not possess any essential function in the organism (Friberg *et al*.; Pinot *et al.*; Bernard *et al.*, 1979 and Swiergosz 2001) of any living system, but there is much evidence of its toxic effects. It can cause acute intoxication (Friberg *et al*.; Bernard & Lauwerys; Departament of Health and Human Services; Swiergosz and Roels *et al*., 1981) in workers directly exposed in their workplaces (Friberg *et al.*; Bernard & Lauwerys and Departament of Health and Human Services) and in populations in industrially polluted areas (Friberg *et al*.; Bernard & Lauwerys; Pinot *et al.* and Roels *et al*.). The anthropogenic sources are less innocuous than those resulting from the industrial production of batteries, (Adams *et al*., 1969) plastics, synthetic materials and alloys. It is re-encountered in the environment in the form of atmospheric emissions, (Demuynck *et al.,* 1976 and EPA-US 1991) liquid effluents and in liquid and solid forms of sewerage (Pinot *et al*.).

The use of phosphate fertilizers has had a great effect on the concentration of cadmium in the soil (Kumar *et al.*, 2000).

Cigarettes are an important source of exposure (Lewis *et al.*, 1972; Elinder *et al.*, 1976 and Piscator e*t al.*, 1976). Smokers are subjected to a greater exposure compared to non-smokers and this is reflected in elevated levels of cadmium by the human body (World 1992; Kido, 1995; ATSDR, 1999 and Ohta & Cherian 1991) it is slowly eliminated. In the kidney, this time interval is expressed in

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decades (Friberg *et al.* and Pinot *et al.*). This results in a continuous increase over the years in the body load, even in cases of low exposure and absorption levels (Friberg *et al.*; Bernar & Lauwerys and Pinot *et al.*).

On being absorbed it is transported to the liver and it stimulates the synthesis of methallotionein in the hepatocytes (Ohta & Cherian; Cherian & Shaikh, 1975 and Nordberg, 1978).

After oral or parenteral exposure or by inhalation in high concentrations, cadmium causes the formation of methallotionein in the liver (Cherian & Shaikh and Nordberg). This organ controls the release of cadmium to other tissues (Stohs *et al.*). Induction of methallotionein and the possibility of the production of other proteins, protect the structure of these cells from toxicity (Nordberg; Stohs *et al.*; Sato & Kondoh, 2002 and Squibb *et al.,* 1979). This induction is considered to be dependent on the balance between the new synthesis of methallotionein in the renal tubule cells and the release of cadmium ions from lysosomal cadmium-methallotionein removed from the tubular fluid (Stohs *et al.*; Squibb *et al.* and Squibb & Fowler, 1984).

The concentration of urinal cadmium is a useful indicator of exposure both in the workplace and environment as an increase in excretion in the urine is detected when renal tubular dysfunction occurs (Bernard & Hermans, 1997).

The aim of this work is to evaluate toxicity on fetal kidneys of rats caused by the administration of cadmium. This was achieved by performing an experimental study on rats to determine, utilizing karyometry, morphometric alterations induced in the proximal and distal convoluted tubules, the collector tubules and the glomeruli of kidneys of fetuses by treating pregnant dams with a single dose of cadmium chloride.

MATERIAL AND METHOD

Ten female and two male rats (*Rattus norvergicus albinus*, variety Wistar) were used and divided into two groups of five female and one male for breeding purposes. The virgin rats, on being selected, weighed an average of 227 grams and reached a mean weight of 330 grams on the 20th day of pregnancy, exactly at the moment preceding sacrifice.

Treatment with cadmium. The treatment of the rats occurred on the tenth day of pregnancy when a single inoculation was administered in the abdominal cavity of each female as follows:

Control group: injection of 1 milliliter of 0.85% sodium chloride solution

Treated group: injection of an aqueous cadmium chloride solution containing 5 milligrams per kilogram of body weight.

The pregnant dams were sacrificed after being duly anesthetized. A broad incision in the abdomen and uterus was necessary to expose the fetuses.

Histological procedures obeyed general. The nuclei were projected on paper and, taking care to only include elliptic cells, their outlines were traced using a black pencil. Subsequently, the greater and smaller axes of the nuclear images were measured using millimetered graph paper. The results were input in to the NUC computer program, elaborated by Dr. Geraldo Maia Campos from the Department of Odontology, University of São Paulo in Ribeirão Preto.

The following parameters were measured:

Greatest diameter (D), smallest diameter (d), mean diameter (MD), the ratio of the greatest and smallest diameters (D/d), volume (V), area (A), perimeter (P), the ratio of the volume and area (V/A), eccentricity (E), the shape coefficient (SC) and contour index (CI) (Sala *et al.*, 1980 and Gousset, 1908).

The diameters and the perimeters were measured in micrometers (μm), the volumes in cubic micrometers (μm^3) and the areas in square micrometers (μm^2).

The glomeruli were drawn in a similar manner to the nuclei of the renal tubules. The greatest and smallest diameters of the nuclei of both the Bowman's capsule and the glomeruli themselves were calculated.

Non-matched student t-test (Foulkes, 1978) was used to compare the morphological and karyometric parameters obtained in the two groups. The student t-test was applied using the Graph InStat computer program version 3.00 for Windows 95, San Diego California, USA. With this program the mean, median, standard deviation (SD), mean standard error (MSE) values of the superior and inferior confidence intervals (95%) the minimum and maximum values of the sample and the p- and t-values for each of the analyzed karyometric parameters were identified.

RESULTS

Morphological results. Table I demonstrates the mean results obtained using karyometry on the renal structures considered in the experiment.

The obtained means of the glomerular volumes were 46,076.86 μm^3 in the control group and 65,777.64 μm^3 in the treated group, giving a p-value of 0.0007 considered statistically significant (<0.05). In respect to the Bowman's capsule, the mean values were 101,836.90 μm^3 in the control group and 145,824.35 μm^3 in the treated group, giving a p-value = 0.0015 also considered statistically significant.

Table I: Mean greatest and smallest diameters, mean tubule diameter, greatest/smallest diameter ratio, volume, area, perimeter, volume/area ratio, eccentricity, shape coefficient and contour index of the nuclei of collector tubules and proximal and distal convoluted tubules of the fetal kidneys of the dams from the control and treated groups.

	Collector tubules		Proximal tubules		Distal tubules	
	Control	Treated	Control	Treated	Control	Treated
Greatest nuclear diameter (μm)	12.81	13.59	12.17	13.08	12.30	12.96
Smallest nuclear diameter (μm)	9.88	9.95	10.46	10.27	9.64	9.32
Mean tubular diameter (μm)	11.23	11.60	11.27	11.57	10.85	10.90
Greatest/smallest diameter ratio	1.31	1.38	1.17	1.29	1.29	1.41
Nuclear volume μm^3	663.45	721.51	717.02	738.20	617.30	609.26
Nuclear area μm^2	99.76	106.71	100.74	106.01	93.62	95.49
Nuclear perimeter μm	37.94	39.85	36.90	38.92	36.55	37.84
Volume/area ratio	6.59	6.64	6.97	6.85	6.43	6.21
Eccentricity	0.60	0.65	0.46	0.58	0.58	0.66
Nuclear shape coefficient	0.87	0.84	0.92	0.88	0.88	0.83
Contour index	3.81	3.88	3.70	3.80	3.80	3.90

DISCUSSION

Nephrotoxicity. The treatment with parenteral $CdCl^2$ in rats diminishes the mean glomerular filtration with a reduction in the filtration fraction. Moreover, continued exposure results in progressive sclerosis of t exposure at low levels or by oral exposure.

During chronic exposure, cadmium-metallothionein is liberated by the liver and transported to the kidneys (World Health Organization; Cherian & Shaikh; Nordberg). This complex if filtered by the glomeruli and reabsorbed by the cells of the proximal tubules by the transport mechanism of low-weight molecular proteins (Kido; Cherian & Shaikh; Nordberg and Foulkes). After entering into the lysosome, it undergoes stimulates the synthesis of more metalloprotein in the tubule cells and becomes linked to it (Cherian & Shaikh; Nordberg and Foulkes) but the intercellular metal, not bound to metallothionein, increases when its concentration exceeds the capacity of the proximal tubular cells to synthesize metallothionein (Shaikh, 1982). The low molecular weight allows the protein to be filtered through the glomerular filter and thus to be absorbed by the tubular liquid in the renal cells. This mechanism might explain the selective accumulation of cadmium in the renal cortex (Friberg *et al*.).

An increased excretion of cadmium in the urine in individuals with renal disease is caused by tubular reabsorption cadmium-metallothionein complex (Cherian & Goyer, 1976; Rodriguez-Barbero *et al*., 2000; Stinson *et al.*, 2003). Proximal tubular dysfunction in mammals, due to nephrotoxicity caused by cadmium, results in a reduced functional capacity of the brush-border membrane (Ahn Whan *et al*., 1999; Sabolic *et al.*, 2002). This damage is caused by diverse factors: 1) by direct inhibition by cadmium of the brush-border transporters; 2) by the shortening and reduced numbers of the microvilosities and 3) by the loss of specific brush-border transporters (Ahn Whan *et al*.).

The proximal tubules present with alterations in the greatest diameter (p-value =0.0185), in the ratio between the greatest and smallest diameters (p-value =0.0002), eccentricity (p-value <0.0001), the nuclear shape coefficient (p-value =0.0001), the contour indexes (p-value =0.0002). All of these were considered statistically significant. The value for eccentricity of the collector (p-value 0.092) and distal (p-value =0.006) tubules were respectively considered not significant and significant.

The following values for the collector tubules were considered to be statistically significant: greatest diameters (p-value =0.0392); the greatest/smallest diameter ratio (p-

value =0.0307); nuclear contour index (p-value =0.033); and the shape coefficient (p-value =0.045). For the distal tubules the following data were considered statistically significant: greatest diameters (p-value =0.043) the greatest/smallest diameter ratio (p-value =0.0067); eccentricity (p-value =0.006); nuclear contour index (p-value = 0.009); and the shape coefficient (p-value =0.008).

These findings demonstrate evidence of alterations in the shape with a tendency to rounding of the nuclei.

In relation to the glomeruli, (Jarup *et al*., 1995) when tubular damage occurs owing to cadmium, this leads to irreversible proteinuria and continuous exposure can cause glomerular damage with a reduction in the average filtration, even a long time after the end of exposure (Jarup *et al.*).

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RESUMEN: El cadmio es un metal pesado que se encuentra en la tierra, aire y alimentos. Está presente en forma coditiana todos los días de la vida y actualmente es catalogado como un tóxico, teratogénico y agente carcinogénico. El cadamio causa numerosas lesiones a los órganos humanos y animales.

Este trabajo tiene como objetivo investigar los efectos tóxicos en fetos de ratas expuestas a tratamiento con cloruro de cadmio. 10 ratas preñadas fueron divididas en dos grupos. Cinco ratas recibieron por administración intraperitoneal 5 mg/Kg de cloruro de cadmio por peso corporal, durante 10 días de preñez y otras cinco ratas recibieron bajo las mismas condiciones del otro grupo, una solución de cloruro de sodio al 0.85% .

Los fetos fueron extraídos junto con las placentas y funículos umbilicales a los 20 días de preñez. La administración del cloruro de cadmio causó una reducción significativa en los pesos de los fetos y placentas. La longitud del funículo umbilical fue más corta en el grupo experimental que en el grupo control.

El estudio evaluó las alteraciones morfológicas causadas en los túbulos proximal, distal, contorneados y colectores renales como también en las células del glomérulo renal. La morfometría evidenció alteraciones significativas en los túbulos proximales y en las células glomerulares y, en menor grado, en los túbulos distales. El borde en cepillo de la membrana celular se observó muy sensible y se visualizaron algunas alteraciones. Los glomérulos se presentaron con edema y la cápsula del glomérulo fue también afectada.

PALABRAS CLAVE: 1. Cadmio; 2. Nefrotoxicidad; 3. Cariometría.

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