A STUDY OF THE EFFECT OF CADMIUM TOXICITY ON SERUM PROTEINS AND IT'S RELATION TO PROTEINURIA IN MALE RATS

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SUMMARY: Male Wistar rats were injected daily for 40 days with varying doses of cadmium (0.25 to 2 mg/kg) as $CdCl_2$ i.p. At the time of experiments animals were anaesthetized and blood samples were withdrawn from their hearts. As the renal function tests serum urea and creatinine concentrations were determined. Administration of 0.25 or 2 mg of Cadmium lead to the elevation of serum urea by 194 or 316% and serum creatinine by 14 and 90% respectively. When 1 mg/kg of cadmium was administered daily for 75 days, it was found that serum urea and creatinine concentrations were elevated by 280 and 80% respectively, confirming renal damage by cadmium intoxification. Serum protein was decreased whereas urine proteins were elevated significantly in cadmium treated rats. Serum samples from both cadmium treated and untreated animals were electrophoresed on 10% SDS-polyacrylamide gel electrophoresis using discontinuous buffering system. It was observed that there was gradual disappearance of alpha-2 and beta 1 globulin bands from electrophoretic pattern. Instead a single sharp band was observed between beta 2 and gamma globulins only in the serum protein pattern of 2 mg/kg cadmium treated animals. The relationship between cadmium toxicity and proteinuria is discussed in some detail.

Key Words: Cadmium, kidney, proteinuria, protein pattern.

INTRODUCTION

Cadmium is a non-essential toxic element which enters body via a number of routes including food, water, air and by smoking of the cigarette (1). In the blood it is mainly accumulated in the red cells and binds to a low molecular weight protein. Metallothionein plays a major role in homeostasis of cadmium (2). More than 80% of cadmium is bound to metallothionein and found in liver and kidney (3). It is now widely thought that metallothionein is protective against cadmium toxicity and that intracellular cadmium bound to metallothionein is non-toxic (4). It has been reported that pre-treatment of experimental animals with small doses of cadmium prevent acute toxic effects of large doses of cadmium (5). Acute cadmium poisoning may produce degenerative changes in renal tubular cells (6). Cadmium was first recognized as a toxic element as the causative agent of Itai-Itai disease in Japan, a disorder mainly characterized by damage to the proximal renal tubule (7). Cadmium priminary affects tubular epithelium resulting in increased cadmium in urine, aminoaciduria, glucosuria and decreased renal tubular reabsorption of phosphate (6).

Cadmium has also been demonstrated to inhibit many enzymes (8) and competes with calcium metabolism (9) and alter phosphorylation patterns (10). The major aim of the present study was to investigate the relationship between cadmium toxicity and proteinuria and also to study the serum electrophoretic pattern changes following cadmium intoxification in rats.

MATERIALS AND METHODS

Male Wistar albino rats (initial body weight, 100 gram) were fed a standard rodent diet al libitum and water until reached desired weight (200-250 gram). Varying amounts of cadmium were injected daily over different period of times. On the day of

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Table 1: Effects of varying amounts of cadmium (0.25-2 mg/kg) on serum urea and creatinine concentration. Cadmium was injected daily for 40 days. Animals were killed and serum samples were collected as mentioned in methods. The data are expressed as the mean±SD. Each value represents the mean of five separate experiments.

mgCd/kg administered	0	0.25	0.5	1.0	2
Serum urea mg/100	31.94±0.36	37.83±0.19	49.34±0.38	77.44±0.39	133.12±0.7
Serum creatinine mg/100	1.59±0.08	1.88±0.03	1.95±0.05	2.23±0.04	2.94±0.09
Serum protein mg/100	4.68±0.08	4.52±0.09	4.43±0.06	4.04±0.11	2.25±0.17
Urine protein mg/100	3.26±0.01	4.29±0.4	17.7±0.08	28.3±0.1	98.8±0.15

experiments both cadmium treated and untreated controls were anaesthetized and blood samples were withdrawn directly from their hearts. Serum samples were collected by centrifugating at 2000 rpm. Serum urea, creatinine and protein concentrations were determined by routine laboratory methods. 24 hours urine samples were collected with the used of metabolic cages and concentrated prior to protein determination and also for electrophoresis study.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) technique was carried out with discontinuous buffering system as reported elsewhere (11).

All chemicals were obtained from Sigma Chemical Company (W. Germany). Deionized water used throughout this project to avoid metal contamination.

RESULTS

Initially, varying amounts of 0.25, 0.5, 1.0 and 2 mg cadmium as $CdCl_2$ per kg body weight were administered daily for 40 days as mentioned in methods. Serum protein, creatinine and urea concentrations were determined. The results obtained are presented in Table 1. Serum creatinine level was elevated by 14 or 90% in comparison to untreated cadmium group following injection of cadmium. Serum urea was elevated from 19 to 300%. Serum protein was decreased and urine protein level was elevated significantly following cadmium intoxication (Table 1).

In the next project the effects of cadmium on the same serum parameters was investigated following 75 days of Cd administration. To do this, cadmium 1 mg/kg was injected daily for 75 days. The data obtained were presented in Table 2. There is significant elevation of serum urea (280%) and Creatinine (80%) in comparison to the controls. Serum protein was reduced by 26% and urine protein was elevated significantly in comparison to controls (Figures 1a and 1b).

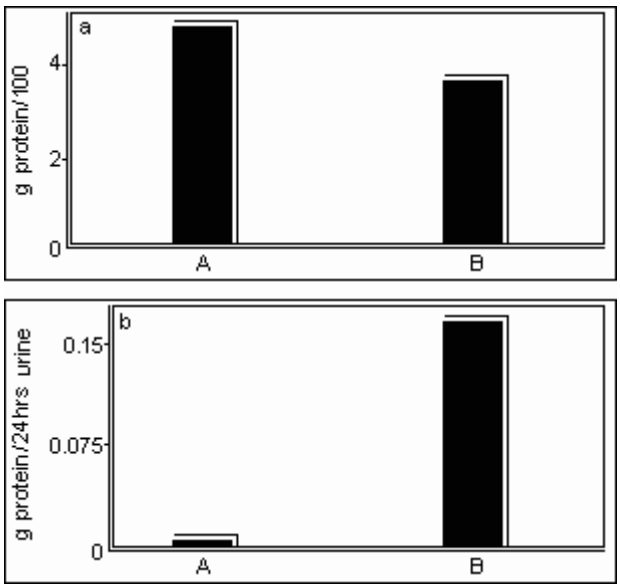

Figure 1: Serum (a) and urine protein (b) concentrations in normal (A) and 75 days cadmium treated (B) rats.

Lastly, the effects of cadmium on serum protein patterns was studied electrophoretically. To achieved this, 10 μl of diluted serum from both treated and untreated cadmium animals were applied to a 10% gel containing 1% SDS and electrophoresed with discontinuous buffering system for 3 hours. Comparison of the serum protein patterns from both cadmium treated and normal groups showed that there was a gradual decreased in alpha-2 globulin band in the pattern of serum Cd treated rat and also the appearance of a single sharp band between beta-2 and gamma globulins area. There was also significant changes in the pre-albumin fraction particularly in 2 mg/kg cadmium treated rats (Figure 2).

Table 2: Effects of 1 mg/kg cadmium on serum urea and creatinine concentration. Rats were injected with 1 mg/kg of cadmium daily for 75 days. They were killed on the day experiment and serum samples were collected as mentioned in methods. Data are expressed as the mean±SD. Each value is the mean of five experiments.

Serum parameters	Urea	Creatinine	Protein
Control	30.27±0.36	1.5±0.09	4.89±0.15
Cd-treated	117.18±1.31	2.73±0.17	3.56±0.28

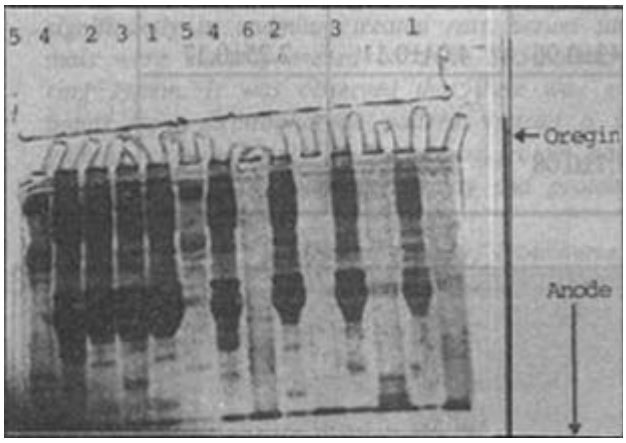

Figure 2: SDS-PAGE of rat serum protein. 1. Serum control, 2. Serum from 0.25 mg/kg Cd treated, 3. Serum from 0.5 mg/kg Cd treated, 4. Serum from 1 mg/kg Cd treated, 5. Red cell membrane, 6. Urine from 1 mg/kg Cd treated.

DISCUSSION

The present work was designed to assess short and long term effects of cadmium on the kidney function and it's connection with proteinuria. The data presented in this paper show that administration varying amounts of cadmium (0.25 to 2 mg/kg) daily for 40 days had significant effect on serum and creatinine concentrations (Table 1). When the effect of cadmium on the same parameters was studied after 75 days of cadmium administration same results were obtained. These data confirm the disability of kidney to excrete serum urea and creatinine following cadmium intoxification. Serum proteins concentrations were reduced and the elevation of urine proteins were observed. Gilrolami showed that administration of cadmium lead to the elevation urine creatinine and urea levels. No experimental data was available for serum urea or creatinine neither for the nature of the protein fractions which are entered urine (12).

Various hypothesis might be proposed to explain the pathogenesis of cadmium nephrotoxicity particularly the role of the metal binding metallothionein. This protein will be induced by a number of metals including cadmium (4, 14). Cadmium either in free form or bound to metallothionein could cause the renal disturbances (13). Using 10 % gel and SDS-PAGE we have found that administration of increasing amounts of cadmium lead to the gradual reduction of alpha-2 and beta-1 globulin bands (Figure 2). When the urine from both cadmium treated and also untreated controls were electrophoresed on SDS-PAGE two faint bands parallel to alpha-1 and beta-2 globulins were observed in urine protein pattern (Figure 2).

Interestingly, a single sharp band appeared between beta-2 and gamma globulins of serum protein pattern from cadmium treated animal in comparison to control group (Figure 2). Roels *et al.* (13) reported that beta-2 micro globulin was found in the urine of workers with renal cadmium above 216 μg/g as measured *in vivo* by neutron activation (15). It has also been reported that the urinary excretion of beta-2 micro globulin or alpha-1 micro globulin are the most widely used tests for the early detection of tubular damage caused by cadmium exposure (16). Inspite of body of literature concerning the cytotoxicity of cadmium and its effects on tubular cells reabsorption process we believe still further research should be done to elucidate the exact mechanism by which this toxic element causes proteinuria.

In summary serum urea and creatinine levels were elevated in cadmium intoxified rats. The elevation of serum urea and creatinine was dose dependent. Proteinuria was occurred in cadmium overload rats.

Alpha-2 and beta-1 globulins disappeared from the serum protein electrophoretic pattern of cadmium treated rats.

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