



SIMULTANEOUS EFFECT OF CADMIUM AND MERCURY ON SOME BIOCHEMICAL PARAMETERS OF TESTIS FUNCTION IN MALE RATS

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ABSTRACT

Cadmium chloride (100 mg/L) and/or mercuric chloride (25 mg/L) were given as drinking water to male Wistar rats for 10 weeks, to investigate their simultaneous effect on testes function. The administration of cadmium separately or in combination with mercury induces a decrease in serum testosterone levels, whereas mercury appeared to have no effect on this parameter. Results indicate that the administration of cadmium separately appeared to have no effect on malondialdehyde concentration in the testes, whereas in mercury and in combined metals treated groups this parameter shows a significant decrease compared to control group. The levels of glutathione (GSH) in the testis were increased significantly in all treated groups with a more remarked effect in mercury and in combined metals treated rats. In conclusion, from the above results, it is clear that there is not an additive effect between these two metals.

Key words: Cadmium, Mercury, Testicular toxicity, Rat.

INTRODUCTION

The pollution by heavy metals represents the biggest part of the attacks constantly undergone by the bodies¹. In all heavy metals, cadmium and mercury are known as extremely toxic. Cadmium (Cd) is a wide spread environmental pollutant, characterized by its toxicity in various organs². Cadmium is listed among the hazardous chemicals because it can enter the food chain³ and has a long biological half-life (about 30 years) in humans^{4,5}. The flow of Cd in ecological systems increases through major sources as mining, smelting and industrial use. Sources of human exposure to this metal include food, cigarette smoke and alcoholic beverages⁶. Mercury is one of the oldest toxicants known. There are three main chemical forms of mercury: (1) organic mercury, used as fungicides, herbicides, and wood preservatives; (2) inorganic mercury, for antiseptic and dermatological preparations; and (3) elemental mercury, used in the production of batteries, thermometers, and fluorescent tubes⁷. Mercuric chloride is one of the most toxic forms of mercury because it easily forms organomercury complexes with proteins⁸. The exposure routes of mercury include the ingestion of food such as fish and seafood, dermal absorption, and inhalation. In addition, amalgam tooth fillings were identified as the major source of mercury contributing to the body burden in humans without occupational exposure. For long time, studies in rodents have shown that testicular tissue is a sensitive target to Cd toxicity⁹. In experimental models, Cd exposure can affect testes weight and induce pathogenesis leading from reduced sperm counts and impaired sperm mobility to adverse effects on male fertility¹⁰⁻¹². Mercury compounds are known to affect testicular spermatogenic and steroidogenic functions in experimental

animals and men¹³. Oral exposure to mercuric chloride led to adverse effects on the reproductive performance of mice¹⁴.

Various mechanisms have been suggested to explain Cd and Hg induced cellular toxicity. Among these mechanisms, lipid peroxidation has been considered a primary initiating mechanism during Cd and Hg injuries^{12,15,16}.

In real life, the human population is exposed to complex mixtures of contaminants. So, the experimental work with combination of contaminants is more relevant on the human exposure than the work with a single substance. Therefore, the purpose of this experiment is to study of the combined effect of cadmium and mercury on some biochemical parameter in testis in male rat.

EXPERIMENTAL

Materials and methods

Animals and treatment

Male Wistar rats (126 ± 11 g) purchased from Siphat (Ben arous, Tunisia) were used in this study. Animals were housed individually and food and water were provided *ad libitum*. After a period of at later 1 week acclimatizing, animals were divided into four groups:

- (i) Control group (6 rats): Animals consumed distilled water as drinking water.
- (ii) Cadmium group (6 rats): Animals consumed a solution of cadmium chloride (100 mg/L) as drinking water.
- (iii) Mercury group (6 rats): Animals consumed a solution of mercury chloride (25 mg/L) as drinking water.
- (iv) Cadmium-mercury group (5 rats): Animals consumed a solution of cadmium chloride (100 mg/L) and mercury chloride (25 mg/L) as drinking water.

Metal solutions were prepared in distilled water.

After 10 weeks of treatment, rats were weighed and then euthanized with exsanguinations by severing the brachial artery after anaesthetizing with ether. Blood was collected and centrifuged, and the serum was conserved at -80°C . The testis were removed quickly from animals, washed in ice-cold physiological saline and conserved at -80°C for the assay of lipid peroxidation and glutathione.

Testosterone assay

Serum testosterone levels were measured by electrochemiluminescence by automat (Elecsys, RocheDiagnostics). The electrochemiluminescence is a form of chemiluminescence, which permits a high amplification of the signal. The reaction of chemiluminescence which entails the emission of the light was preceded by an electrochemical reaction. The actors of this reaction are magnetic microparticles papered of streptavidine, antibodies marked with the biotin, antibodies marked with ruthenium and tripropylamine.

Lipid peroxidation

This was performed using the method of thiobarbituric acid, which measures MDA-reactive products¹⁷ as described by Todorova et al.¹⁸ 0.5 mL Organ homogenates (10% w/v in phosphate-buffer 0.1 M, pH 7.4), 0.5 mL physiological solution and 0.5 mL 25% trichloroacetic acid were mixed and centrifuged at 2,000 rpm for 20 min. 1 mL of protein-free supernatant was mixed with 0.25 mL 0.5% thiobarbituric acid

and heated at 95°C for 1 h. After cooling, the intensity of pink color of the end fraction product was determined at 532 nm. The results are expressed in nmol MDA/g tissue.

Reduced glutathione

This was determined by spectrophotometric method, which was based on the use of Ellman's reagent¹⁹. 0.2 mL trichloroacetic acid was added to 0.4 mL homogenate (10% w/v in phosphate-buffer 0.1 M, pH 7.4). After centrifuging at 3000 rpm for 15 min., 0.4 mL supernatant was mixed to 2 mL of 0.3 N Na₂HPO₄ and 100 µL of 5,5-di-thiobis-(2-nitrobenzoate). The mixture was set to react for at least 5 min., and the absorbance was read at 412 nm. Reduced glutathione was used as the standard. Results are expressed in mg GSH/g tissue.

Statistics

Data are expressed as means ± SE. Statistical analysis was performed using One Way Analysis of Variance (ANOVA). Differences at $p \leq 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

As shown in Table 1, serum testosterone levels in cadmium and in combined metal groups were significantly decreased, whereas no change was observed in this parameter in mercury group as compared to control group. The serum testosterone levels in combined metal exposed rats were comparable to those in cadmium treated rats but significantly lower than those in mercury treated rats.

Results presented in Table 1 also indicate that there is no change in lipid peroxide level (measured as MDA) in cadmium group, whereas in mercury and in combined metal groups this parameter shows a significant decrease as compared to control group. The testes MDA concentrations in combined metal treated rats were comparable to those in mercury treated rats but significantly lower than those in cadmium treated rats.

Concerning the glutathione level, an increase in this parameter was noticed in all the treated rats as compared to control rats. The changes in glutathione level observed in the combined metal-exposed group were significantly higher than those induced by cadmium and comparable to those induced by mercury. The present study was undertaken to evaluate the function of testis in the condition of co-exposure to cadmium and mercury.

The serum testosterone levels were considered to be a useful indicator of testicular endocrine function²⁰. In the present study, it is interesting to note the significant decrease of the levels of serum testosterone in rats treated with cadmium. The decrease in this hormone suggests a decrease of its synthesis by the Leydig cells, since these cells are the main source of testosterone in rats. This means that the treatment with Cd inhibits the mechanism intervening in the process of the hormone synthesis in the Leydig cells. Previous studies have suggested that Cd reduces the serum testosterone levels in rodents^{21,22}. In contrast, Zeng et al.²³ have found an increase in plasma testosterone levels under the effect of cadmium. This contradiction between laboratories proves that Cd disrupts serum testosterone levels through different mechanisms, which depend on experimental conditions used. Further studies are necessary to define, how Cd disrupts plasma testosterone homeostasis? In mercury group, no change in serum testosterone levels has been noticed. Similar results have been reported in methyl mercury exposed monkeys²⁴, but others reports showed a decrease in testosterone level in rats exposed to methylmercuric chloride²⁵ or mercuric chloride²⁶. It is possible that these differences may be due to the concentration used and also to the duration of dosing and routes of delivery between different studies. On the other hand, in rats co-exposed to cadmium and mercury, serum testosterone levels was comparable to those in cadmium group and significantly lower than

those in mercury group. This means that there is not an additive effect between these two metals on testosterone synthesis.

Table 1: Effect of cadmium and mercury alone and in combination on serum testosterone and on testicular lipid peroxidation and glutathione levels in male rats

Groups	Parameters		
	Testosterone ($\mu\text{g/L}$)	MDA (nmol/g)	GSH (mg/g)
Control	5.93 ± 1.13	132.5 ± 3.9	0.03 ± 0.01
Cd	$1.92 \pm 0.47^{**}$	109.3 ± 17.2	$0.08 \pm 0.01^{**}$
Hg	8.88 ± 1.27	$25.9 \pm 10.1^{***}$	$0.67 \pm 0.06^{***}$
Cd + Hg	$2.57 \pm 0.61^{*bb}$	$27.7 \pm 9.1^{***aa}$	$0.58 \pm 0.06^{***aa}$

Data are means \pm SE; * $p \leq 0.05$ in comparison to control group; ** $p \leq 0.01$ in comparison to control group; *** $p \leq 0.001$ in comparison to control group; aa $p \leq 0.01$ in comparison to cadmium group; bb $p \leq 0.01$ in comparison to mercury group

The precise mechanism by which these two metals cause injury is unknown. However, one possible mechanism is by increasing lipid peroxidation, since previous studies have noted an increase in lipid peroxidation in rats treated with cadmium^{12,15} or with mercury²⁶. However, in the present study no sign of lipid peroxidation occurred in all treated rats. In fact, in cadmium group, the levels of MDA was comparable to those in control rats, and in mercury and in combined metals exposed rats a decrease in this parameter was noted. This shows that the peroxidative mechanism is still controversial. Therefore, another mechanism is probably involved. The MDA levels in rats co-exposed to cadmium and mercury was comparable to those noted in mercury group and significantly lowers than those in cadmium group. This means that there is not an additive effect between these two metals.

The results presented in this study show an increase in GSH levels in rat testis in all treated rats. This may be explained as a result of the transcription upregulation of γ -glutamylcysteine synthase, the enzyme that is responsible for GSH synthesis²⁷. The enhancement of glutathione levels induced by the combined treatment was comparable to that induced by mercury and significantly higher than that induced by cadmium. This means that there is not an additive effect between these two metals.

In summary, this study provides added information on the consequences of simultaneous exposure to cadmium and inorganic mercury on testicular function. From the above results, it is clear that there is not an additive effect between these two metals.

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