The Effect of Acute Cadmium Intoxication on Tissue Antioxidant Enzyme Activity in Rat Testis

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Abstract

Twenty-four male Wistar strain rats were injected 1mg/ml of cadmium chloride intraperitoneally for 21 days and 20 male rats were considered as controls. Postmortem examination was done 21 days after cadmium administration to determine antioxidant enzyme (superoxide dismutase, catalase and glutathion peroxidase) activity of testis and to observe histopathological changes in the testis. In the cadmium-treated group, the antioxidant enzyme activities of the testes were found to be increased when compared with control group. There was a statistically significant difference between two groups ($p<0.05$). In cadmium-treated group, histopathological changes which pointed out testicular damage were seen microscopically in testes. Our results suggested that increased antioxidant enzyme activity in the testis constitutes an adaptive response to the free radicals generated by testicular cells. Also this might be responsible for the biochemical mechanism of cadmium-induced testicular damage.

Key Words: *Cadmium, testicular damage, antioxidant enzyme activity.*

Akut Kadmiyum İntoksikasyonunun Rat Testisinde Doku Antioksidan Enzim Aktivitesine Etkisi

Özet

Yirmidört erkek rata 1 mg/ml kadmiyum klorid intraperitoneal olarak 21 gün süreyle verildi. Kontrol grubuna ise 20 erkek rat alındı. Yirmibir gün sonra tüm ratlar sakrifiye edildiler ve testiküler antioksidan enzim (süperoksit dismutaz, katalaz ve glutatyon peroksidaz) aktiviteleriyle histopatolojik testis bulguları kaydedildi. Kadmiyum uygulanan grupta antioksidan enzim aktiviteleri kontrol grubuna göre anlamlı derecede yüksek bulundu ($p < 0.05$). Ayrıca bu grupta testiküler hasara işaret eden mikroskopik bulgular gözlendi. Çalışmamızın sonuçlarına göre kadmiyum verilen ratlarda gözlenen yüksek testiküler antioksidan enzim aktivitelerinin kadmiyumun testiküler hücrelerde yol açtığı serbest radikal oluşumuna karşı gelişen adaptif bir reaksiyon olduğu ve bunun da kadmiyuma bağlı olarak gelişen testiküler disfonksiyonun fizyopatolojisinde önemli bir yeri olabileceği kanaatindeyiz.

Anahtar Kelimeler: *Kadmiyum, testiküler hasar, antioksidan enzim aktivitesi.*

The toxic effect of cadmium is well known and has been studied for many years (1-3). Toxicity of cadmium has been supposed that it can alter in enzymatic, histological and morphological patterns binding to certain mammalian cells. Cadmium is stored especially in testis which constitutes its target organ (4-6). But the biochemical mechanisms of cadmium-induced testicular damage have not been clarified yet.

It has been known that cadmium can be harmful for male reproductive functions (7). Acute toxicity may result from ingestion of food or beverages with high concentration of cadmium (8). Gouveia et al (4) have demonstrated that acute experimental cadmium intoxication caused testicular damage in the rats.

The antioxidant enzymes (AOE) such as superoxide dismutase (SOD), catalase (CA) and glutathion peroxidase (GP) are available in all aerobic mammalian cells. They protect the cells from the effect of free radicals. Free radicals contain one or more unpaired electrons. These molecules can undergo some reactions in a cell: reactions with lipids, proteins or DNA leading to

metabolic and structural modifications of cells which can ultimately cause cell death (9-10).

Prohaska et al (11) have shown that testicular toxicity of cadmium is accompanied by an elevation of the activity of GP which utilizes as substrate to inactive hydrogen peroxide and free radicals.

The aim of this study was to indicate the effect of acute cadmium intoxication on testicular antioxidant enzyme activities and to investigate the biochemical mechanism of cadmium-induced testicular damage.

Materials and Method

The study was performed on Wistar albino male rats weights ranging between 185-300 g. Experiments were placed in an environment maintained at 22.0±3.0 °C isolated from noise and with a 12-h light/dark cycle. Also the rats were group-housed at a density of 4 rats/cage: 20 rats were kept as controls and 24 rats were injected 1 mg/ml cadmium chloride intraperitoneally between 8: 00 and 9: 00 a. m. This dose proved to be LD_{50} in a pilot study done earlier. The control group received saline. All rats were given food and water *ad lib.*

The animals were decapitated 21 days after cadmium chloride injection under ketamine anesthesia and the testes were removed. One of the testes was used for AOE activity analysis and other for histopathological examination.

For histopathological examination, testis was fixed in formalin and embedded in paraffin. Specimens were stained with Hematoxyline-Eosin (H-E).

For testicular AOE activity analysis, testes were immediately perfused in situ with ice-cold saline and were frozen at -20° C until analyzed. The testes were homogenized in phosphate buffer (1:10 w/v) (0.01 M, pH=7.4) and then homogenates were diluted with buffer solution (1:100). The assays of AOE activities were immediately determined by standard spectrophotometry.

The catalase mediated decomposition of hydrogen peroxide was followed at 240 nm. One enzyme unit of catalase activity is defined as 1 nmole H_2O_2 decomposed per mg protein per minute. The activity of SOD was measured by inhibition of autoxidation of epinephrine at 480 nm. The level of enzyme that causes 50 % inhibition of ephinephrine autoxidation is defined as 1 unit. The activity of GP was determined by measuring disappearence of NADPH at 340 nm. An enzyme unit was defined as 1 nmole NADPH oxidized per mg protein per minute.

Testis protein was determined with Lowry method (12). All AOE activity measurements were given as units/mg protein.

The Mann-Whitney test was used to compare means between groups because normality could not be assumed. The threshold of significance was $p<0.05$.

Results

Sacrified animals which were given cadmium intraperitoneally showed higher mean AOE activities than the control group. SOD activity in cadmium-treated group was 35.8±2.3 U/mg protein and 12.3±1.2 U/mg protein in control group ($p<0.05$). CA activity was 47.4±5.1 U/mg protein in cadmium-treated group and 14.8±2.3 U/mg protein in control group ($p<0.05$). GP activity was 51.4±1.7 U/mg protein in cadmium-treated group and 27.4±3.1 U/mg protein in control group ($p<0.05$) (Table 1).

Table 1. Effect of cadmium on testicular antioxidant enzyme levels in cadmium-treated group and control group.

	Cadmium-treated	*Control*	*p*
SOD* (U/mg protein)	35.8±2.3	12.3±1.2	p <0.05
CA** (U/mg protein)	47.4±5.1	14.8±2.3	p <0.05
GP *** (U/mg protein)	51.4±1.7	27.4±3.1	p <0.05

* Superoxide dismutase ** Catalase *** Glutathion peroxidase

On histopathological examination, cadmium-treated group were observed that the testes were reduced in size and pale yellow, the cut surface was sligthly fatty and homogenous. H-E stained preparations showed atrophy of the testes with necrosis of the tubules, fibrosis of the intertstitium and vascular thrombosis. Some tubuli showed vascular hyperemia and hemorrhage in the intertubular area. The tunica albuginea was extremely thickened. In the control group there

was no any pathologic finding that pointed out testicular damage.

Discussion

It has been known that environmental or non-environmental exposure to cadmium effects the reproductive capacity of male animals and men (7,13,14). But the biochemical mechanism of cadmium-induced testicular damage has not been explained yet and it is open to investigation. In the present study, we tried to show the effect of acute cadmium intoxication on testicular AOE activity and to relate this to biochemical mechanism of cadmium-induced testicular damage.

Testis constitutes the target organ for cadmium toxicity (4-6). Its effect exerts on testis especially leading to decrease fertility capacity of sperm (13). On the other hand there is evidence suggesting that cadmium binds to the capillary endotelium of the testis and initiates cellular events which ultimately cause disruption of the blood-testis barrier (15).

Our results show that acute cadmium intoxication causes severe testicular damage and increases AOE activity markedly in the rats. As it is well known that antioxidant enzymes protect the cells from the toxic effect of free radicals. Also it has been repeatedly reported that hypoxic and hemorhagic tissues generate oxygen free radicals (16,17) which could cause cell death. Thus AOE activity has an important role for homeostatis of the body. Hypoxia and hemorrhagie can be resulted either from thrombosis or compression of peritubular vasculature in the testis after cadmium intoxication (4). Sugawara et al have shown that cadmium-induced hemorrhage in the testis is accompanied by an increase in lipid peroxidation in the organ (18). Maines et al have shown in the past that lipid peroxidation is promoted by the iron released in the course of degradation of endogenous heme in the cadmium-induced testicular dysfunction. This process is associated with the destruction of cellular membranes and their components (19). Our pathologic findings are in accordance with the presence of hypoxia such as necrosis of the tubules, vascular thrombosis, and fibrosis of the interstitium. We observed that there was the evidence of hemorrhage in the intertubular area. Our experiments showed that testis is susceptible to acute cadmium damage and we belive it, to be due to alterations in the microcirculation. Cadmium-induced vascular alterations are followed by ischemia and hemorrhage due to compression of the intratesticular vessels and ultimately necrosis (20,21).

The results of this study are in agreement with the study of Prohaska and Chung (11,22), in which they reported increased GP activity of testis in the rats after cadmium administration. In addition to this finding, we also demonstrated increased both SOD and CA activities. We believe that increased AOE activity in the testis is due to a protective response to the effects of free radicals produced by the testicular cells. In cadmium-treate rats, hypoxia and hemorrhage promote peroxidative damage in testicular cells.

In conclusion free radical damage plays an important role on cadmium-induced testicular damage and this may elucidate the physiopathology of cadmium damage to the testis.

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