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Zinc acetate pretreatment ameliorates cisplatin-induced Sertoli cell dysfunction in Sprague-Dawley rats

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Summary. The present study was undertaken to determine if prior administration of zinc acetate (ZnAc) or copper sulfate ($CuSO_4$) could prevent pituitary, Leydig, or Sertoli cell dysfunction subsequent to cisplatin administration in adult Sprague-Dawley rats. Animals were given cisplatin at a dose of 2 mg/kg daily for 5 days, with or without the i.p. administration of ZnAc (6 mg/kg per day) or $CuSO_4$ (5 mg/kg per day), beginning 5 days prior to and continuing through the administration of cisplatin. Control animals were given vehicle, $ZnAc_1$, or $CuSO_4$. Animals were sacrificed 1 week after the initial cisplatin injection. Cisplatin administration resulted in suppressed serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels as well as a 77% reduction in serum testosterone and an 82% reduction in testicular testosterone. The concomitant administration of either ZnAc or $CuSO_4$ did not result in a significant difference relative to animals receiving cisplatin alone, although administration of both cations alone significantly reduced testicular testosterone content. Serum androgen-binding protein (ABP) was not significantly lowered in any treatment group. There was a marked reduction of 57% in testicular ABP content relative to control values subsequent to cisplatin administration. This reduction was partially prevented by ZnAc treatment; the testicular ABP concentration was only 15% lower than that in controls (not significant). Since the cisplatin-induced reduction in serum FSH was not altered by ZnAc pretreatment, we conclude that the near normalization of testicular ABP content may be evidence of improved Sertoli cell function. In contrast, cisplatin-induced decreases in the serum gonadotropins and testicular androgens were not lessened by pretreatment with either cation. Further studies may be warranted to determine whether ZnAc pretreatment has a beneficial effect on spermatogenesis during cisplatin treatment.

Introduction

cis-Diamminedichloroplatinum (II) (DDP, cisplatin) is a heavy-metal coordination compound with antineoplastic effects. Since the discovery of the cytostatic action of platinum by Rosenberg in 1965 [18], this compound has become an important agent in clinical oncology and is part of the potentially curative regimen for germinal cell tumors [17].

Clinically, the administration of cisplatin-based chemotherapy has been associated with severe but potentially reversible damage to the germinal epithelium [10]. In addition, subclinical hypergonadotropic hypogonadism, consistent with Leydig cell failure, has been noted [1]. We [14] and others [11] have reported that the administration of cisplatin to adult male Sprague-Dawley rats is associated with an acute onset of hypoandrogenism, which is dose-related. In addition, we have reported that cisplatin administration results in biochemical and morphologic evidence of Sertoli cell dysfunction, including leakage of the blood-testis barrier within 1 week of the initial dose [15].

It has been sugested that cisplatin binds to metallothioneins in the kidney [19] and that preadministration of zinc or copper may lessen both renal and system toxicity due to cisplatin administration in mice [13]. The present study was undertaken to determine whether the preadministration of zinc acetate (ZnAc) or copper sulfate ($CuSO_4$) could ameliorate pituitary, Leydig cell, or Sertoli cell injury resulting from cisplatin administration in adult Sprague-Dawley rats.

Materials and methods

Animals. Mature male Sprague-Dawley rats (300–350 g) were obtained from Charles River Laboratories (Wilmington, Mass). They were maintained in an air-conditioned, light/dark-controlled animal room and were given lab chow and water ad libitum.

Experimental protocol. Animals were given cisplatin (Bristol-Myers Corp., Syracuse, NY) intraperitoneally (i.p.) at a dose of 2 mg/kg daily for 5 days. All animals received daily i.p. hydration with 20 ml normal saline, with or without the i.p. administration of ZnAc (6 mg/kg per day) or $CuSO_4$ (5 mg/kg per day), beginning 5 days prior to and continuing through the administration of cisplatin. Control animals received either vehicle, ZnAc, or $CuSO_4$. Animals were sacrificed by decapitation at 1 week after the initial injection of cisplatin. Trunk blood samples were collected and sera were stored at −30° C. Following sacrifice, the testes and epididymides were removed and weighed. One-half of one testis was fixed in Bouin's solution and processed for regular histology; 4-μm-thick sec-

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tions were stained with periodic acid Schiff's reagent and counterstained with hematoxylin [16]. The other half of the testis was stored at −30° C for the measurement of androgen-binding protein (ABP) and testosterone. The remaining testis was placed in plastic vials capped with paraffin and stored at −30° C prior to evaluation of zinc and copper content by atomic absorption spectrophotometry [21]. The protocol was approved by the animal safety subcommittee of the East Orange Veterans Administration Medical Center.

Hormone measurement. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and ABP concentrations were determined by double-antibody RIA using reagents provided by the National Institute of Diabetes, Digestive and Kidney Diseases (NIADDK). NIADDK rat FSH (rFSH) RP-2, rFSH-I-5, and anti-rFSH S-7 and rat LH (rLH) RP-2, rLH I-5, and anti-rLH S-5 were used for FSH and LH assays, respectively. The details of these assays have previously been described [8]. To avoid interassay variation, FSH and LH were measured in one respective assay for all samples. The intra-assay coefficient of variation was approximately 8% for both hormones. The sensitivity of the assays was 1.0 ng for FSH and 0.5 ng for LH/ml serum. Testosterone concentrations were determined in serum and testicular extracts without chromatography by RIA using antibody provided by Radioassay Systems Laboratory (Carson, Calif). The cross-reactivity of this antibody with dihydrotestosterone was 18.7%; the intra-assay coefficients of variation was about 7%. Testicular ABP concentrations were determined in tissue extracts according to the method of Gunsalus et al. [6]; the intra-assay coefficient of variation was 5.9%.

Analysis of data. All values are expressed as the mean ± SEM. One-way analysis of variance was used to compare the means of data obtained from different dose regimens. When significance was obtained, Duncan's multiple range test was used to determine difference among groups at the $P < 0.05$ level.

Results

Body, testis, and epididymal weights

Body weights were reduced by 18% ($P < 0.05$) in animals receiving cisplatin (Table 1). ZnAc or $CuSO_4$ pretreatment alone did not alter body weight or prevent body weight loss in the cisplatin-treated animals. Testicular and epididymal weights were significantly decreased in every group of animals except those receiving ZnAc ($P < 0.05$).

Serum and tissue hormone concentration

Serum LH values were suppressed to undetectable levels by cisplatin, with or without concomitant ZnAc or $CuSO_4$ administration (Table 2). $CuSO_4$ alone, but not ZnAc, suppressed serum LH. Serum FSH values were significantly

Table 1. Body, testis, and epididymal weights

Weight (g)	Treatment group[a,b]					
	Control	ZnAc	$CuSO_4$	CIS	CIS-ZnAc	CIS-$CuSO_4$
Body	414±6	408±6	394±12	338±7*	344±14*	308±10*
Testis	1.69±0.06	1.70±0.04	1.54± 0.05*	1.50±0.04*	1.42± 0.02*	1.46± 0.03*
Epididymis	0.63±0.03	0.63±0.02	0.55± 0.03*	0.49±0.03*	0.51± 0.02*	0.49± 0.02*

[a] $n = 5$ animals per treatment group
[b] All values expressed as X ± SEM
* $P < 0.05$ of control values
CIS, cisplatin

Table 2. Hormonal indices following cisplatin administration

	Treatment group[a,b]					
	Control	ZnAc	$CuSO_4$	CIS	CIS-ZnAc	CIS-$CuSO_4$
Serum:						
FSH (ng/ml)[c]	19.3± 1.8	7.4± 2.2*	8.6± 2.4*	8.7± 1.7*	7.3± 1.4*	6.0± 0.7*
LH (ng/ml)[d]	1.3± 0.4	1.4± 0.4	<0.5	<0.5	<0.5	<0.5
ABP (μleq/ml)[e]	510±118	422±29	398±42	363±48	383±55	371±65
Testosterone (ng/ml)	5.2± 1.2	5.3± 0.5	1.8± 0.4*	1.2± 0.7*	0.5± 0.2*	0.4± 0.2*
Testis:						
Testosterone (ng/testis)	272±67	117±26*	74± 8*	50±12*	48± 4*	39± 5*
Testosterone (ng/g per testis)	166±46	68±15*	48± 7*	34± 9*	30± 3*	26± 3*

[a] $n = 5$ animals per treatment group
[b] All values expressed as X ± SEM
[c] Standard NIADDK rFSHRP-2
[d] Standard NIADDK rLHRP-2
[e] Standard NIADDK rABPGMB-E-1
* $P < 0.05$ of control values
CIS, cisplatin

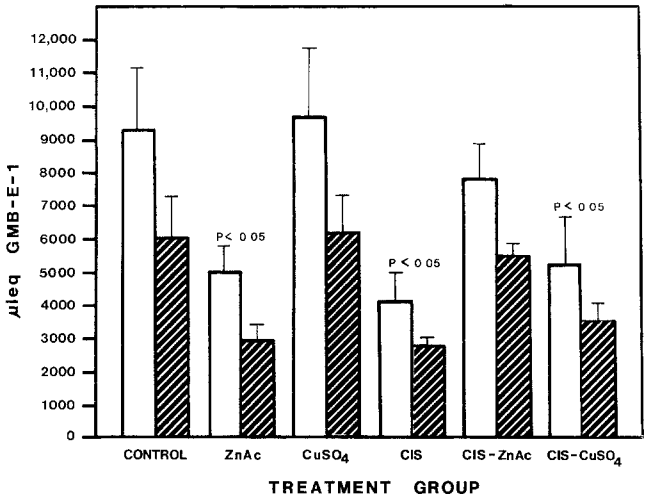

Fig. 1. Testicular ABP. □ µleq/testis; ▨ µleq/testis

lowered by cisplatin, ZnAc, and $CuSO_4$. Once again, however, the concomitant administration of ZnAc or $CuSO_4$ did not prevent the cisplatin-induced decrease.

Compared to control levels, serum testosterone concentration was lowered by 77% ($P < 0.05$) in animals receiving cisplatin and by 65% ($P < 0.05$) in those receiving $CuSO_4$ alone. ZnAc pretreatment alone had no effect on serum testosterone levels. The group of animals treated concomitantly with ZnAc or $CuSO_4$ in addition to cisplatin had serum testosterone concentrations that were not significantly different from those in the group of animals treated with cisplatin alone.

Testicular testosterone concentration, expressed either as ng/testis or ng/g per testis, followed a similar pattern. Compared to control levels, cisplatin treatment lowered testicular content by 82% ($P < 0.05$), whereas $CuSO_4$ treatment alone resulted in a 35% ($P < 0.05$) reduction. The administration of ZnAc resulted in a 57% ($P < 0.05$) reduction in testicular testosterone content. The concomitant administration of either ZnAc or $CuSO_4$ with cisplatin failed to prevent the marked reduction in testicular testosterone observed with cisplatin treatment alone.

ABP

Serum ABP concentration was not significantly lowered in any treatment group (Table 2). There was a marked reduction of 57% ($P < 0.05$) in testicular ABP content relative to control values subsequent to cisplatin administration (Fig. 1). This reduction was partially prevented by ZnAc treatment; the testicular ABP concentration was only 15% lower (not significant) than that in controls. On the other hand, $CuSO_4$ alone did not have any effect testicular ABP content, nor did it significantly lessen the reduction after cisplatin administration.

Heavy metal analysis

There were no significant differences in testicular copper or zinc concentration among the treatment groups (data not shown).

Discussion

Clinically, men who are treated for testicular carcinoma with cisplatin-based chemotherapy sustain severe but reversible damage to the germinal epithelium [10] as well as subclinical hypergonadotropic hypogonadism characterized by elevated serum LH values and normal serum testosterone concentrations, which is consistent with Leydig cell failure [1].

We [14] and others [11] have noted that the administration of cisplatin to rats markedly depressed both serum and testicular testosterone levels. Our results suggest that the suppression of LH levels may be a mechanism in the initiation of the dose-dependent hypoandrogenism, whereas Maines et al. [11] have reported that cisplatin resulted in the suppression of cytochrome P-450 and microsomal heme concentrations. In addition, we [15] previously reported that cisplatin administration to rats results in both acute and chronic Sertoli cell dysfunction as characterized by leakage of the Sertoli-Sertoli tight junction, alterations in seminiferous tubular fluid composition, and decreased testicular ABP.

Whether or not these testicular lesions can be prevented has not previously been investigated. In contrast, there is a large body of clinical and experimental literature on the prevention of cisplatin-induced renal tubular damage. Cisplatin is an inorganic complex formed by a central atom of the heavy metal platinum, surrounded by chlorine atoms and ammonia groups in the *cis* relationship [3]. Chellman et al. [2] have suggested that the nephrotoxicity of cisplatin would thus be similar to that of other heavy metals, involving a depletion of intracellular glutathione or attachment to sulfhydryl groups of protein necessary for enzymatic function. Cisplatin has also been shown in vitro to bind to metallothioneins, which are inducible, sulfhydryl-rich intracellular proteins believed to play an important role in providing a protective effect against the toxic effect of many heavy metals [9].

In Wistar rats that were treated with cisplatin and pretreated with either ZnAc or vehicle, the renal tissue and subcellular platinum levels were significantly lower in the ZnAc-pretreated group than in untreated animals [20]. In mice subcutaneously inoculated with Ehrlich tumor cells, the preadministration of $CuSO_4$ prior to cisplatin depressed the renal and systemic toxicity of the drug without altering its antitumor efficacy [12].

Although the uptake of cisplatin by testes is known to be small but significant in both humans [4] and rats [22], subcellular localization of the compound in the testis has not been characterized. In the present experiment, we sought to determine whether prior administration of ZnAc and $CuSO_4$ could prevent the short-term deleterious effects of cisplatin on Leydig and Sertoli cell function.

Our results confirm that cisplatin administration to rats induces significant hypoandrogenism that is unaltered by the concomitant administration of ZnAc or $CuSO_4$. Interestingly, the administration of either $CuSO_4$ or ZnAc alone significantly lowered total testicular testosterone content below control values. $CuSO_4$ administration did not significantly lower testicular zinc levels, thus excluding relative zinc deficiency as an explanation for this observation. On the other hand, serum LH levels were suppressed by $CuSO_4$ to undetectable levels, suggesting hypopituitarism as a possible explanation for the observed hypoandrogenism. Although prior reports indicate that Leydig cells contain copper in quantitites that fluctuate markedly [23], there are no reports that excess levels of copper are deleterious to the testis. This observation was in contrast to the reduction in testicular testosterone seen after ZnAc

administration without a concomitant fall in serum LH levels, suggesting a deleterious effect of zinc on Leydig cell function.

In rodents, ABP is synthesized only by the Sertoli cells and is the most commonly used marker for Sertoli cell function [7]. The fall in total testicular ABP content observed after cisplatin administration was partially prevented by concomitant ZnAc administration. Zinc is prominently localized in the seminiferous tubules [5]. Whereas the zinc concentration of Sertoli cells varies, being correlated with the stage of development of the nourishing spermatids [23], the function of zinc in Sertoli cells is unknown. A direct benefit of zinc on Sertoli cell function is excluded by the decrease in testicular ABP content in animals receiving ZnAc alone, which may be due to a reduction in serum FSH. Although testicular zinc concentrations were not different in the cisplatin-treated animals and controls, this does not discount the hypothesis that concomitant ZnAc administration prevents a significant reduction in testicular zinc content due to cisplatin that would be evident at an earlier time point, or that ZnAc acetate can competitively inhibit cisplatin transport into Sertoli cells. Since serum FSH values were no higher in the cisplatin-treated animals given ZnAc than in those given cisplatin alone, the ability of ZnAc pretreatment to prevent a reduction in testicular ABP content is unlikely to be due to a pituitary effect, although an alteration in the bioactivity of FSH cannot be totally excluded.

In summary, we conclude that the deleterious effect of cisplatin on Sertoli cell function, as determined by testicular ABP content, can be partially prevented by ZnAc administration. Further studies will be necessary to determine whether ZnAc can prevent leakage of the Sertoli-Sertoli tight junction and whether any effect is noted on the restoration of spermatogenesis.

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