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ORIGINAL PAPER

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Effects of Cisplatin on the Sperm Morphology of *Rattus Orvegicus*

Abstract: The mutagenicity and genotoxicity of chemotherapeutic drugs used as chemical antibiotics in most tumours as anticarcinogenic agents were studied. Cis-diammine-dichloroplatinum (cisplatin) is a commonly used chemotherapeutic agent in the treatment of ovarian, testicular and bladder cancer. It is used intravenously, intraarterially and intraperitoneally. Group I adult male rats were injected with a single dose at a level of $^1/_2$ LD_{50} (l6 mg/kg body weight (a half carcinogenic dose). Group II rats were treated with 9 mg/kg body weight (recommended dose) intraperitoneally injected. The abnormally shaped sperms were recorded after 1, 2, 3, 4 and 5 weeks post-injection. The frequency of abnormally shaped sperms was noted within the first three weeks post-injection, and highly significant abnormalities were recorded at the end of second week (181.4 ± 24.9) and (145.1 ± 28.6) in Groups I and II, respectively. Recovery from the genotoxicity of cisplatin was achieved at the end of the 5th week.

Key words: Cisplatin, Sperm morphology, *Rattus orvegicus*.

Introduction

Cisplatin is of value in the treatment of metastatic tumours of the testis, usually as a major component of combination chemotherapy regimens, and particularly in combination with bleomycin and etoposide. It is also used in metastatic ovarian tumours and advanced bladder cancer, and has been reported to be active against a wide range of other solid tumours (Shernian *et al.* 1985).

The cytotoxic effect of cisplatin is thought to result from the covalent binding of the cisplatin to DNA. The resultant adducts are cross-links of three

تأثير السيزبلاتين على أشكال الحيوانات المنوية للجرذان البالغة (راتس نورفيجيكس)

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المستخلص:إستهدف هذا البحث تقنين الآثار الجانبية للعقار الكيميائى سيزبلاتين على أشكال الحيوانات المنوية للجرذان البالغة. استخدم فى هذا البحث 60 جرذ ذكر بالغ متوسط وزنة من 100- 120 جرام وقسمت إلى مجموعتين أساسيتين ـ 30 جرذ فى كل مجموعة . وقسمت كل مجموعة إلى 6 مجموعات صغيرة (خمسة جرذان لكل مجموعة). أعتبرت المجموعة الفرعية الأولى فى كل مجموعة كبيرة مجموعة ضابطة عوملت بمحلول فسيولوجى، بالحقن فى التجويف البريتونى. عوملت جرذان المجموعة الأولى بجرعة نصف مسرطنة تعادل نصف الجرعة نصف المميتة LD_{50} (16مليجرام لكل كيلو جرام من وزن الجسم) بالحقن مرة واحدة فى التجويف البريتونى ذبحت الجرذان بعد 1 , 2 , 3 , 4 , 5 أسابيع على التوالى. بينما عوملت جرذان المجموعة الثانية بالجرعة الأمنة (٩ مليجرام لكل كيلو جرام من وزن الجسم) وتم ذبح الجرذان بعد 1 , 2 , 3 , 4 , 5 أسابيع على التوالى. تم أخذ الخصية وتشريحها ووضع البربخ العلوى فى محلول ملحى ثم تم عمل مسحة منوية على شرائح نظيفة وصبغت الشرائح بصبغة الفولجين النووية. وتم الفحص بالميكروسكوب الضوئى بعدسة عينة قوتها 10x وعدسة زينية قوتها 100x بمعدل 1000 حيوان منوى لكل جرذ.

وقد تبين من الدراسة :أن المجموعات المعالجة بواسطة الجرعة الأمنة والجرعة نصف المسرطنة أدت الى زيادة ذات دلالة إحصائية فى عدد الحيوانات المنوية المشوهة خلال الأسابيع الثلاثة الأولى بعد الحقن. وكان أقصى معدل للتشوه الشكلى للحيوانات المنوية بعد الأسبوع الثانى من الحقن بمعدل 148.2% ، 180.4% لكل من الحيوانات المعالجة بالجرعتين المختلفتين على التوالى إذا ما قورنت بالمجموعة الضابطة 48.9%. وتم الأستشفاء من الآثار الجانبية للسيزبلاتين فى الأسبوع الرابع والخامس. من هذا البحث يلاحظ أن أستحدام السيزبلاتين فى جرعتة الأمنة يؤدى الى أعراض جانبية ممثلة فى تشوهات فى الحيونات المنوية مما يقلل من درجة الخصوبة.

كلمات مدخلية: السيزبلاتين، الحيوانات المنوية، الجرذان البالغة

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types: intrastrand, interstrand and DNA-protein (Sherman & Lippard, 1987). The most common adduct is the intrastrand cross-link bridging adjacent guanines at the N-7-position (Pinto & Lippard, 1985 and Eastman, 1987). It also inhibits DNA synthesis and replication by inactivating the template.

Other observations, related to the genotoxic effects of cisplatin therapy include damage to inicrotubule formation (Jnoue *et al.* 1985) and G2 arrest (Andrews & Howell, 1990). Most studies of the genotoxicity of cisplatin on germ cells have been done in males. However, male and female germ cells differ in their susceptibility to mutation induction. Thus, genotoxic data extrapolated to female germ cells must be treated with caution. (Jekunen *et al.* 1994). The present study is an attempt to explore if cisplatin therapy may or may not affect sperm shape of male rats.

Material and Methods

Cis-diamnuno dichloro platinum II (cisplatin):

Cisplatin is an inorganic platinum-containing complex. It is thought to act as an alkylating agent which is potentially carcinogenic. Its antineoplastic action is cell cycle nonspecific (Ogawa, 1985). Cisplatin was synthesized by Marceils and Reedijk (1985). It is a yellow powder, slightly soluble in water and in 0.9 sodium chloride solution (1 mg/1 ml of saline solution) and must be stored in airtight containers and protected from light. Cisplatin is administered intraperitoneally and the therapeutic dose for humans is 100 mg/Ms day as a single dose monthly (Vermorken, *et al.* 1984). The recommended dose for rats is 9 mg/kg body weight as a single dose (Adler, & El-Tarras, 1990), and $^1/_2$ LD_{50} of cisplatin in male rats is 16mg/kg body weight, which is half a carcinogenic dose (Connors, *et al.* 1982).

In the present work 60 adult male rats (*Rattus norvegicuis*) were obtained from the farm of the Faculty of Veterinary Medicine, Zagazig University, weighing an average of 100 ± 20 gm. The specimens were classified into 2 main groups, each of 30 rats. Each group was subdivided into 6 subgroups each of 5 rats. In both groups, rats of the first subgroup were given 1.0 ml saline solution and served as a control group. Subgroups 2-6 represented rats of weeks 1-5 respectively.

Group I

Each rat in the 5 subgroups received an evening intraperitoneal injection of a single dose of $^1/_2$ LD_{50} cisplatin (1.6 mg/rat/day). Five rats were sacrificed after 1, 2, 3, 4 and 5 weeks and sperm smears were taken from the caudal epididymis of the testes.

Group II

Each rat from 25 intact rats was intraperitoneally injected with the recommended dose of 0.9 mg/rat/day as a single dose in the evening. After 1, 2, 3, 4 and 5 weeks, five rats were sacrificed and sperm smears were taken from the caudal epididymis of the testes. Sperm smears of 60 rats were stained by Feulgen nuclear stain (Feulgen & Rossenbeck. 1924), and 1000 sperms were microscopically examined for each rat. A binocular microscope, with x10 eye piece and x100 oil immersion objective lenses was used. Abnormally shaped sperms were recorded. Micrographs were taken whenever necessary. Statistical analysis for each treatment was evaluated by a dispersion test based on χ^2 (Snedecor & Cochran, 1976).

Results

The rat sperm consists of two main parts, the head and the tail. The head is triangular in shape, having a broad base and pointed tip. The pointed tip has a characteristic curvature. The head region is mostly occupied by the nucleus. The tail is several times the length of the head. It begins as a cylindrical part at the base of the head region and then the diameter decreases progressively and ends in a filamentous form (see Fig. 3).

In the present study, the frequency of normal sperm shape reached 951.1 ± 9.3 /1000 sperms. Occasionally, some sperm cells may be present with rather an unusual shape. However, such cells appear in limited numbers with an average of 48.9 ± 9.3 /1000 sperms. Cisplatin in a dose of 0.9 mg/rat/day intraperitoneally injected into rats resulted in an obvious effect on the sperm shape when compared with the control group. The abnormally shaped sperms were significantly increased after the first and second weeks post-injection. This increase persisted for 4 weeks post-injection with a peak after the second week (Tables 1 & 2).

Thereafter, there was a gradual decline in frequency that continued until the end of the experiment (5 weeks). However, there was always a variation in the response between rats treated with the different doses of cisplatin. The higher response

was reported after the second week, reaching 148.2 ± 27.6 and 180.4 ± 24.9 1 in rats treated with the recommended dose and rats treated with $^1/_2$ the carcinogenic dose of cisplatin, respectively. By the end of the fourth week, the average number of deformed sperms reached 67.7 ± 14.8 and 53.4 ± 11.5 in both groups of treated rats, respectively, which was within the control values. However, the abnormally shaped sperms remained higher in number than the control level at the end of the 5th week. After the 5th week, the frequency returned to its normality.

The sperm shape abnormalities involved either the head or the tail. In many cases, both the head and tail were deformed (Tables 1 & 2).

The majority of deformed sperm abnormalities involved the head morphology, which reached a peak in week two post-injection, reaching 4.6% and 7.4% in both groups of treated rats respectively compared with the control of 2.4%, as in Table (3) and Fig. (2).

Table 1: Effect of cisplatin given intraperitoneally in a dose of (0.9 mg/rat/day) on the sperm morphology of rats.

Weeks	Normal sperms /1000 M ± SD	Deformed sperms / 1000 M±SD			Total abnormal sperms/1000 M±SD
		Head	Tail	Head and tail	
Control	951.1 ± 9.3	24.1 ± 4.7	10.3 ± 2.3	14.5 ± 2.6	48.9 ± 9.3
1	860.7 ± 24.1	33.7 ± 2.6	16.3 ± 7.3	89.3 ± 14.2***	139.3 ± 24.1***
2	851.8 ± 27.6	49.5 ± 10.1**	38.4 ± 8.0**	60.3 ± 9.5**	148.2 ± 27.6***
3	866.2 ± 28.7	61.5 ± 16.2**	24.2 ± 7.1*	48.1 ± 5.4**	133.8 ± 28.7***
4	932 ± 14.8	23.8 ± 5.2	21.0 ± 6.3	22.2 ± 3.3*	67 ± 14.8
5	950.1 ± 11.8	20.5 ± 3.2	14.5 ± 2.5	14.9 ± 6.1	49.9 ± 11.8

* P<0.05, ** P < 0.01, *** P < 0.001

Table 2: Effect of intraperitoneal injection of cisplatin (1.6 mg/rat/day) on the sperm morphology of rats.

Weeks	Normal sperms /1000 M ± SD	Deformed sperms / 1000 M±SD			Total abnormal sperms/1000 M±SD
		Head	Tail	Head and tail	
Control	951.1 ± 9.3	24.1 ± 4.4	10.3 ± 2.3	14.5 ± 2.6	48.9 ± 9.3
1	840.3 ± 25.5	85.3 ± 14.3**	20.2 ± 3.1**	54.4 ± 8.1**	159.7 ± 25.5***
2	819.6 ± 24.9	90.4 ± 14.5**	21.3 ± 2.1**	68.7 ± 8.3***	180.4 ± 24.9***
3	866.2 ± 28.7	71.5 ± 16.2**	14.1 ± 5.3	48.2 ± 7.2**	133.8 ± 28.7***
4	946.6 ± 11.5	29.0 ± 6.7	5.3 ± 0.9	19.1 ± 3.9	53.4 ± 11.5
5	960.5 ± 12.5	20.3 ± 8.2	5.0 ± 1.3	14.2 ± 3.0	39.5 ± 12.5

* P<0.05, ** P < 0.01, *** P < 0.001

Table 3: Comparison of % abnormality of sperm heads in the rats treated with both doses of cisplatin.

Weeks	Deformed head / 6000 sperms											
	Rats treated with 0.9 mg/rat/day						Rats treated with $^1/_2$ LD_{50} 1.6 mg/rat/day					
	Hooks		Unusual head		Total		Hooks		Unusual head		Total	
	N	%	N	%	N	%	N	%	N	%	N	%
Control	58	1.0	86	1.4	144	2.4	-	-	-	-	-	-
1	61	1.2	141	2.4	202	3.4	130	2.1	281	4.7	411	6.8
2	65	1.2	211	3.5	276	4.6	192	3.2	250	4.2	442	7.4
3	48	0.8	138	2.3	186	3.1	137	2.3	192	3.2	329	5.5
4	43	0.8	95	1.7	138	2.5	95	1.7	39	1.6	134	2.3
5	41	0.7	85	1.4	126	2.1	39	1.3	81	1.7	120	2.0

N = number of abnormal heads

The head deformities are presented in various forms. Sometimes the apical part showed an unusual curvature, or was abnormally straight and in many cases the size of the head was within the normal limits. In other cases, the size was abnormally large as it had an irregular outline which was rather difficult to describe, as in Fig. (3).

Abnormalities in the tail region varied between being duplicated at its junction with the head and other deformities in the tail, as in Figure (3). All of these deformities were mostly seen within three weeks post-injection of cisplatin, and also during the recovery from the side effects of cisplatin, mostly in rats treated with the recommended dose but less than with those rats treated with the 1/2 carcinogenic dose.

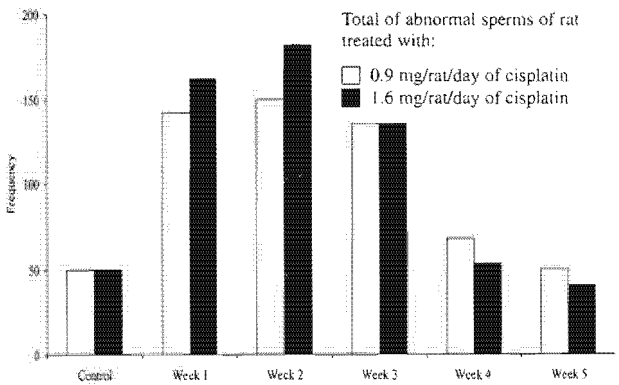

Fig. 1: The frequency of abnormally shaped sperms of the rats treated with both doses of cisplatin.

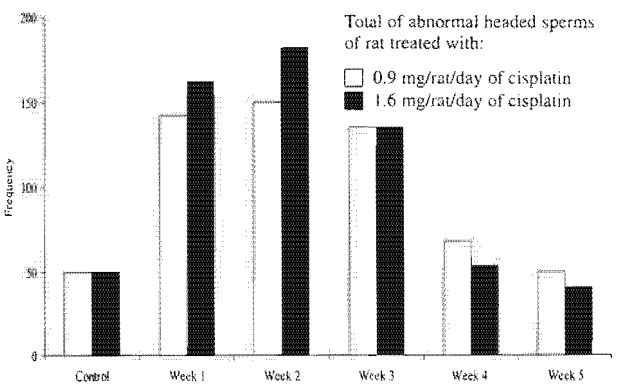

Fig. 2: Comparison between the percentage of abnormal heads of sperms of rats treated with both doses of cisplatin.

Discussion

In an attempt to find out the way in which cisplatin affects germ cell development, it was found that the therapeutic dose (9 mg/kg body weight) resulted in an abundance of abnormally shaped sperms. The peak of abnormality was observed in the second week post-injection. Abnormalities in shape affected the head region, tail region or both.

Deformities of the tail most probably resulted in improper motility, which in turn limited the chance for such a sperm to fuse with an ovum. In our opinion the deformities of the tail region indicated that the cytotoxicity of cisplatin has a high level of adduct formation with mitochondrial DNA, where the tail formation and motility of sperms depend on the energy provided from mitochondria. This notion is in agreement with the results recorded by Adrie *et al.* (1985), Plooy *et al.* (1985), Andrews *et al.* (1988) and Sabet *et al.* (1990). They attributed increased sensitivity of germ cells to cisplatin and high toxicity of cisplatin to intra-lysomal storage or metabolites. This means that lethality should be due to cytoplasmic effects of the drug and high level of adduct formation with mitochondrial DNA, which could occur and cause dysfunction of the cells. The head shape abnormalities are markedly increased. One type of head shape abnormality affected its tapering anterior part; such sperms with untapered anterior region might have been also unable to fuse with ovum for zygote formation. In addition, there are various head shapes that lead to an irregular form which was rather difficult to describe.

The present results point to the sensitivity of the component of head region to cisplatin. In most cases the nucleus occupies all the head region, and the genotoxicity of cisplatin has formed intrastrand cross-links with nuclear DNA or with defective repair. This suggestion is supported by the observations of many previous authors (Roberts *et al.* 1982) and (Plooy *et al.* 1984). It is postulated that potentially lethal platinum DNA adduct appears to be the intrastrand cross-link. This is because it amounts to more than 1% of the total amount of platinum that had reacted with nuclear DNA, and because of high penetration of cisplatin into the nucleus and the tendency of nuclear germ cells to accumulate platinum. Other authors, such as Roberts and Friedlas, (1987) Adler and El-Tarras (1989 & 1990) and Zamble *et al.* (1996) have found that cisplatin produces a clastogenic effect on differentiated mouse spermatogenic cells with a number of aberrations per cell that increase in a dose-dependent manner. Cisplatin also induced genetic aberration during the pre-meiotic phase of DNA synthesis (Adler & EI-Tarras 1990) and (Kenneth *et al.* 1999).

More sperms became affected at the end of the second week post-injection with cisplatin. We suggest that late spematids seem to be the most sensitive cells in the spermatogenic series to cisplatin with longer time needed for recovery from cisplatin genotoxicity, indicating that other cell

stages in spermatogensis can also be affected by cisplatin. This assumption is supported by many authors. Meistrich *et al.* (1982) found that differentiated murine spermatogonia, spermatocytes and spermatids were all sensitive to cellular death after cisplatin therapy while stem cells were relatively resistant. Genesca *et al.* (1990) noted an increase in chromosome breaks and univalents in all male germ cell tested with cisplatin treatment. Drasga *et al.* (1983), Johnson *et al.* (1984), Brenner *et al.* (1985) and Fossa *et al.* (1985) revealed that most patients with testicular malignant tumors are rendered azospermic by cisplatin therapy and restoration of spermatogenesis occurs in many patients after cessation of therapy, but not all patients will demonstrate return of fertility.

In the present study, the recovery from side effects of platinum extended for 5 weeks or more in male rats treated with cisplatin until it reached the control limit, showing the tendency of platinum to form DNA adduct strands and accumulate with nuclear DNA. Our results coincide with the data obtained by Rothmann and Weick (1981) and Guichun *et al.* (1999). They reported that most patients with testicular cancer recover by day 39 post treatment with cisplatin. Schwartz & Vidone (1981), Ward *et al.* (1982) and Gershenson *et al.* (1983) suggested that the incidence of germ cell failure is related to the age and period at which the patients received cisplatin therapy.

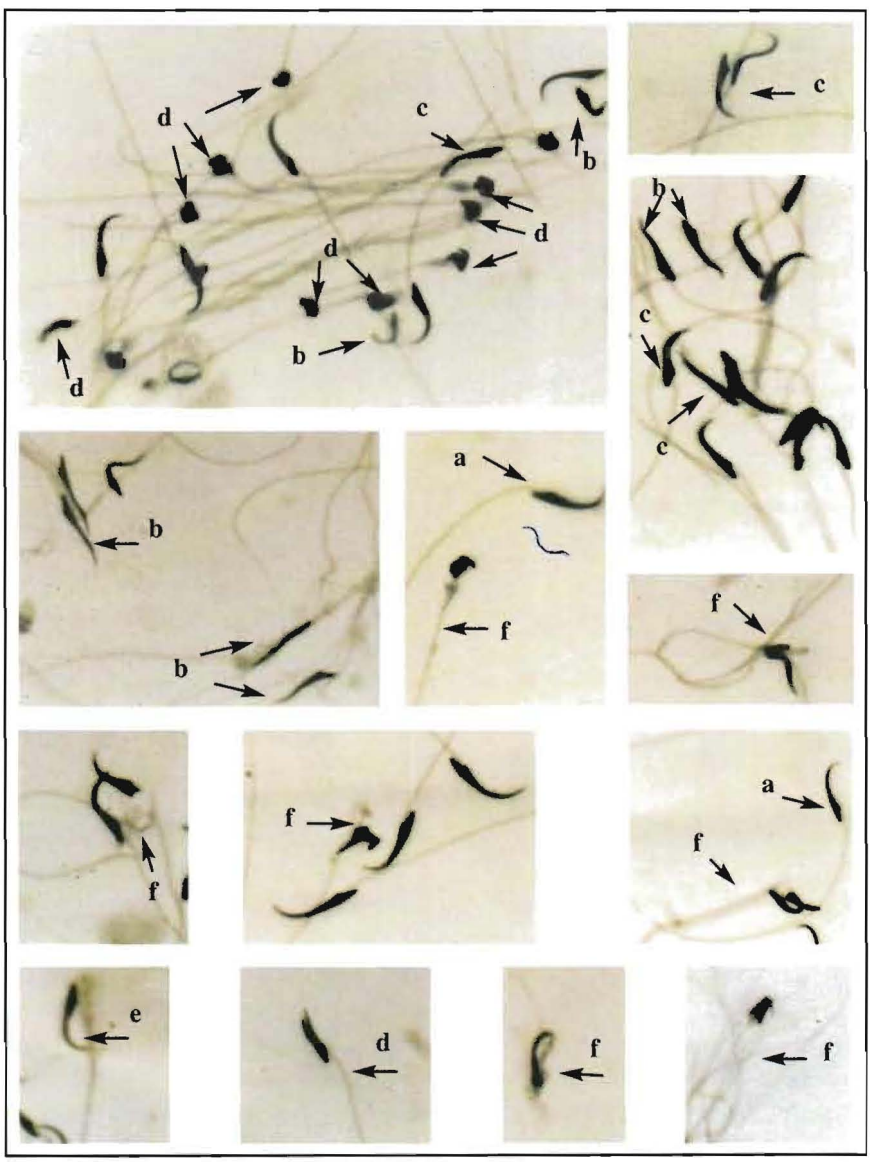

Fig. 3: Sperm cells showing:
a) Normal sperm, b) Sperms showing unusual acute curvature, c) Sperms with unusual hooks
d) Sperms with unusual heads, e) Sperms with unusual tails, and f) Sperms with unusual heads and tails

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