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Dose dependent alterations in epididymal sperm counts of cisplatin or carboplatin treated male wistar rats

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Abstract
Infertility is a major concern for young men of reproductive age under-going chemotherapy. Unfortunately chemotherapeutic treatment for neoplastic diseases commonly impairs fertility either temporally or permanently. In general, chemotherapeutic agents target rapidly dividing germ cells, and it is therefore results in impairment of spermatogenesis leading to depletion in sperm counts in cancer survivors. Though chemotherapeutic agents can cause gonadal injury, the nature and extent of the damage is drug-specific and dose-related. Hence the present study was designed to examine the dose-dependent alterations in epididymal sperm counts of cisplatin or carboplatin treated male wistar rats.

Keywords: Cisplatin Carboplatin, Spermatogenesis, Epididymal sperm count, Dose-dependent alterations, Rats.

Introduction:

Recent studies on secular trends in male reproductive health have provided conflicting evidence with some investigations suggesting that sperm counts have declined significantly during the past 50 years. A number of possible causes including exposure to chemoherapeutic drugs have been suggested by some of these investigations. Moreover many chemotherapeutic drugs often can cause severe alterations in spermatogenesis (Bahadur 2000).

The testis is a known target organ for injury resulting from exposure to both chemotherapeutic and toxic environmental agents. Chemotherapy induced physiological damage to male germ cells in the testis has been associated with fertility, which is monitored by parameters of semen quality. Exposure to more than 100 chemicals individually or as mixtures including chemotherapeutic are known to induce detrimental effects on semen quality (Boekelheide 2005).

Platinum-derived drugs are playing an increasing important role in the treatment of a variety of neoplasms. Much understanding of the present platinum drugs has come from the studies with cisplatin. The introduction of cisplatin based chemotherapy has greatly improved the survival rate of patients from testicular germ cell tumors. Eventhough a number of platinum coordination compounds exhibit antiviral and antitumor activities, cisplatin and its direct analog carboplatin are effective anticancer drugs currently approved for the treatment of several human carcinomas (Lynch *et al*. 2005).

But unfortunately the use of cisplatin however is limited by significant dose related toxicity notably nephrotoxicity, ototoxicity and peripheral neuropathy. To improve the therapeutic index of platinum compounds, new analogs have been developed. The second-generation platinum compound i.e., carboplatin entered the clinical trails in 1981 and showed a very similar activity profile to that of cisplatin, with a good response in ovarian, lung, head and neck and testicular cancers. It is currently the second most widely used platinum anticancer drug in the world (Martindale 2002).

Despite its effectiveness in the suppression of cancer cells, the administration of carboplatin is also associated with a variety of side effects which include myelo-suppression, alopecia, embryotoxic and teratogenic effects (Chung *et al.*, 1998). However many reports available on cisplatin or carboplatin caused toxicity in various tissues, but very limited studies demonstrated the antifertility effects caused by cisplatin and carboplatin anti-cancerous drugs. Hence, an attempt has been made in the present study to investigate the effect of platinum-based anticancer drugs on epididymal sperm count in male wistar rats.

Materials & Methods:

Animals

Healthy adult male wistar rats of same age group (70±5 Days) were selected for the present study. Animals were housed in an air conditioned animal house facility at 26±1^{o} C, with a relative humidity of 75%, under a controlled 12 h light/dark cycle. The rats were reared on a standard pellet diet (HLL Animal Feed, Bangalore, India) and tap water adlibitum.

Test chemicals

Cisplatin was purchased from Sigma chemicals, St.Louis Co., MO, USA. This compound was dissolved in 0.9% normal saline to obtain the final concentration of the 1, 1.5, 3, 4.5, and 6 mg/kg body wt. of the animal respectively.

Carboplatin was purchased from Sigma chemicals, St.Louis Co., MO, USA. This compound was dissolved in 0.9% normal saline to obtain the final concentration of the 1, 5, 10, 15, and 20 mg/kg body wt. of the animal respectively.

Experimental Design

The rats were divided into thirteen groups each consisting eight animals for the experiment. The rats in the first group were served as control and received 0.9% of normal saline only. Whereas the rats in the group II-XI were injected with graded doses of cisplatin (1, 1.5, 3, 4.5, and 6 mg/kg body weight) or carboplatin (1, 5, 10, 15, and 20 mg/kg body weight) respectively. Injections were given intra-peritoneally to rats on 1^{st,} 3rd and 5th day of experimentation. On 45th day of experiment, animals were sacrificed by cervical dislocation.

Collection of epididymal sperm

The epididymal sperm were collected by cutting epididymis into small pieces and flushing the sperm in normal saline. The sperm collected was centrifuged at 225 × g for 10 min. The pellet was resuspended in 2.0 ml of normal saline. An aliquot of sperm suspension was homogenized for few seconds, centrifuged at 800 × g for 10 min and used for analysis.

Sperm Count

The epididymal sperm was obtained as described above and incubated at 37^{o}C. The epididymal fluid was then diluted to a volume of 5.0 ml of pre-warmed (37^{o}C) normal saline. The epididymal fluid was subjected to sperm count using Neubauer haemocytometer as described by Belsey *et al.* (1980).

The epididymal fluid was drawn up to the 0.5 mark of WBC pipette (White Blood Cell pipette) and the semen diluting fluid (sodium bicarbonate 5 g, formalin 1 ml, distilled water 99.0 ml) was drawn up to '11' mark, and subsequently mixed well. One drop was added to the haemocytometer chamber and allowed the sperms to settle by keeping haemocytometer in humid place (wet chamber) for 1 h. After incubation the number of spermatozoa in the appropriate squares of the haemocytometer was counted under the Olympus microscope (model No. 018) at 100X or 400X.The sperm concentration refers to the number of spermatozoa / ml fluid, and calculated using the following formula.

$$\text{Sperm count} = \frac{\text{No. of spermatozoa counted x dilution factor x depth factor}}{\text{No. of areas counted}}$$

Results:

The average sperm count in cauda epididymal plasma was found to be 60.98 ± 4.24 million/ml in control rats. As shown in Table 1, a moderate but not significant decrease in sperm count was observed in experimental rats treated with 1 or 1.5 mg/kg body weight of cisplatin and the average sperm count was found to be 60.11 ± 9.01 million/ml and 59.55 ± 2.73 million/ml respectively in these groups (Fig.1).

A significant decrease ($P<0.001$) in the sperm count (-33.84%) was observed in rats exposed to 3 mg/kg body weight of cisplatin when compared with that of the control rats (Table 1; Fig.1).

Table 1: Effect of different doses of cisplatin on sperm count in rats.

	Cisplatin (mg/kg body weight)					
Control	1	1.5	3	4.5	6	ANOVA
60.98±4.24	60.11^{ns}± 3.01 (-1.42)	59.55^{ns}± 2.73 (-2.34)	40.34*± 2.40 (-33.84)	33.87*± 2.21 (-44.45)	22.17*± 1.27 (-63.64)	$F_{5,42}$= 278.05 P < 0.0001

Values are mean ± S.D of eight animals. Values in the parentheses are percent change from control. Values are significantly different from control at *p<0.001, ns = not significant.

Table 2: Effect of different doses of carboplatin on sperm count in rats.

	Carboplatin (mg/kg body weight)					
Control	1	5	10	15	20	ANOVA
60.98±4.24	59.02^{ns}±5.70 (-3.21)	58.85^{ns}±3.75 (-3.49)	30.84*±1.17 (-49.42)	25.92*±1.86 (-57.49)	19.55*±1.09 (-67.94)	$F_{5,42}$= 247.57 p< 0.0001

Values are mean ± S.D of eight animals. Values in the parentheses are percent change from control. Values are significantly different from control at *p<0.0001, ns = not significant.

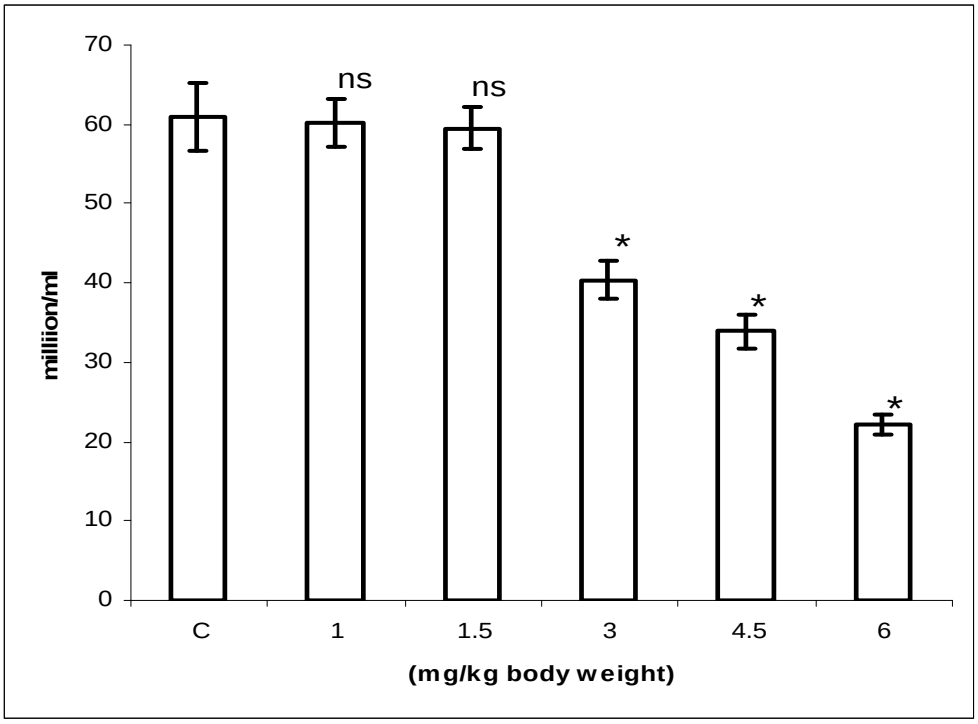

Figure 1: Effect of exposure to graded doses of cisplatin on sperm count in rats.

Values are mean ± S.D of eight individuals. Values are significantly different from control at *p<0.001, ns = not significant.

These decreases were more in 4.5 mg/kg body weight (-44.45%) and 6 mg/kg body weight (-63.64%) cisplatin injected rats (Table 1; Fig.1).

Sperm count was not significantly decreased in rats injected with 1 or 5 mg/kg body weight of carboplatin (Table 2). The average sperm count was found to be 59.02 ± 5.70 and 58.85 ± 3.75 million/ml in these groups respectively. A significant decrease (P<0.001) in the sperm count (-49.42%) was observed in rats exposed to 10 mg/kg body weight of carboplatin when compared with that of

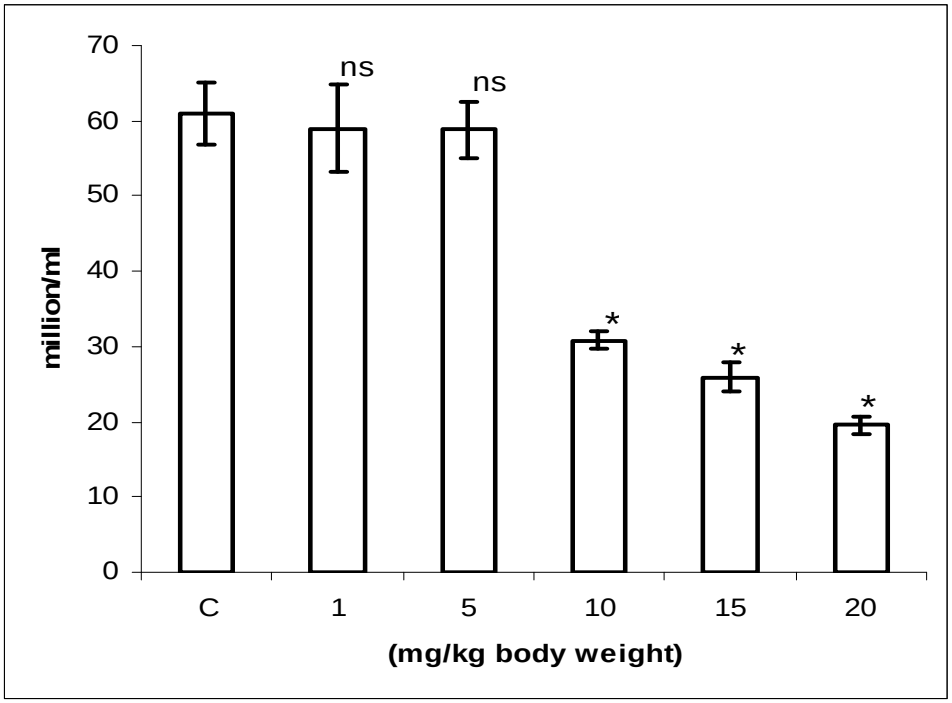

Figure 2: Effect of exposure to graded doses of carboplatin on sperm count in rats.
Values are mean ± S.D of eight individuals. Values are significantly different from control at *p<0.001, ns = not significant.

the control rats (Table 2; Fig.2). The decrease was more in 15 (-57.49%) and 20 mg/kg body weight (-67.94%) carboplatin exposed rats (Table 2; Fig.2).

Discussion:

In the present study a dose-dependent decrease in the cauda epididymal sperm count was observed in rats exposed to cisplatin or carboplatin and indicates decreased spermatogenesis. It is well known that, cytotoxic drugs depress spermatogenesis in mammals (Wyrobek *et al*. 1983) by causing death of developing germ cells in the seminiferous tubules. This results in the elimination of active cells of spermatogenesis and thereby results in reduction in daily sperm production (Lu and Meistrich 1979).

Spermatogenesis is a highly regulated differentiating system, both temporally and spatially. Germ cells, in particular, differentiating spermatozoa are extremely susceptible to cytotoxic agents because of their rapid proliferation. The non-proliferate leydig cells and sertoli cells survive most cytotoxic therapies but could suffer functional damages (Wang *et al*. 1998).

The present investigation clearly indicates that cisplatin treatment at a dose of 3 mg/kg body weight or above and carboplatin at a dose of 10 mg/kg or above results in significant reduction in epididymal sperm count in experimental rats when compared with controls. The results suggests that the decreased testosterone levels might be responsible for the reduction in decreasing sperm count in rats injected with cisplatin or carboplatin.

The dose dependence decrease in sperm count in experimental rats in the present study is in agreement with earlier reports. It was observed that the activities of 3β-hydroxy steroid dehydrogenase and 17β-hydroxy steroid dehydrogenase were decreased significantly in the leydig cells cultured with different concentrations of cisplatin in mice (Malarvizhi and Mathur 1996). Recent studies on carboplatin also

demonstrated a dose dependent decrease in sperm density and inferior semen quality in mice (Oshio *et al*. 1990) and in patients treated with PEB i.e., combination chemotherapy of cisplatin, etoposide, bleomycin (Petersen *et al*. 1994).

Within the testis, the main target cells for toxicants that disrupt spermatogenesis are the somatic cells, (leydig and sertoli cells) and the germ cells. In animal models, each of these cell types can be selectively targeted by specific toxicants, resulting in apoptosis. Detailed investigations in animal models on the testis indicate that platinum compopunds have broad activity, targeting leydig cells, sertoli cells and germ cells (Boekelheide 2005).

Germinal epithelial damage leading to oligospermia or azoospermia has long been recognized as a consequence of treatment with chemotherapeutic agents, and there is also evidence of leydig cell impairment following treatment. Chemotherapy may have a direct toxic effect on the leydig cells. Histological examinations in testes further indicate significant damage to sertoli cells, leydig cells and germ cell populations induced by cisplatin (Cherry *et al*. 2004). Cisplatin based chemotherapy (Cisplatin, vinblastine, and bleomycin) led to persistent impairment of fertility and leydig cell function in the majority of patients with testicular cancer accompanied with significant reduction in sperm production (Hansen *et al*. 1990).

Conclusion:

In the present study, a significant dose-dependent reduction was observed in the cauda epididymal sperm count of male rats exposed to cisplatin or carboplatin. The result indicate that exposure of rats to platinum based anticancer drugs affects the spermatogenesis which may be due to the decreased intratesticular concentrations of testosterone by showing their toxicity on leydig cells. The observed reduction in cauda epididymal sperm reserves of the exposed rats suggests depression of spermatogenic activity, which probably indicates a decrease in the number of developing sperm.

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