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Testosterone mediated partial recovery of carboplatin induced reproductive toxicity in male wistar rats

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Abstract:

The present study aimed to investigate the possible interference of carboplatin on spermatogenisis and to investigate the protective role of testosterone against carboplatin mediated impairment of spermatogenisis. Rats were divided into three groups consisting of eight animals in each group. The rats in the first group were served as control and received 0.9% of normal saline only. The rats in the second group were received carboplatin (10 mg/kg body wt) only. The rats in the third group were received carboplatin (10 mg/kg body wt) and testosterone (4.16 mg/kg body wt) 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. Epididymal sperm counts and evaluation of sperm motility were done by the method of Belsey (1980). Sperm viability was determined by the method of Talbot and Chacon (1981) and the percent of sperm coiling was determined by the method of Jeyendran (1984). 3β-hydroxy steroid dehydrogenase (3β-HSD; EC.1.1.1.51) and 17β-hydroxy steroid dehydrogenase (17β-HSD; EC.1.1.1.64) were assayed by the method of Berg Meyer (1974). The study shows that carboplatin treatment has led to many reproductive abnormalities. Testosterone administration along with carboplatin, caused partial restoration of reproductive abnormalities in experimental rats.

Keywords: Carboplatin, Spermatogenisis, Steroidogenic marker enzymes, Testosterone, Reproductive abnormalities, Rats.

Introduction:

Platinum-derived drugs are playing an increasing important role in the treatment of a variety of neoplasms. Thousands of platinum compounds have been synthesized in an attempt to overcome toxicity of cisplatin. Surprisingly none of these have been able to substitute cisplatin in routine chemotherapy treatments, except the most successful of the second-generation platinum compound i.e., cis-diamine-1, 1-cyclobutane-diacarboxylate platinum (II) which is also known as carboplatin.[1]

Carboplatin is a platinum co-ordinated compound, which is formed by replacing the chloride groups of cisplatin with 1, 1-cyclobutane-dicarboxylate ligand. The mechanism of action of carboplatin is very similar to that of cisplatin, forming preferential cross links with guanine in DNA, thus eventually causing cell death.[2]

Carboplatin readily crosses the cell membrane and inside the cell, the ring structure is hydroxylated by water to form the active moiety. In the active form, it forms irreversible covalent bonds with DNA and inhibits DNA replication, RNA transcription and protein synthesis. Intra-stand cross links at the N-7 position of guanine are predominant binding sites of carboplatin. Carboplatin causes cell cycle arrest in the G2-phase and then induces programmed cell death or apoptosis.[3]

Despite its effectiveness in the suppression of cancer cells, the administration of carboplatin is associated with a variety of side effects alopecia, rash, neuro-cytopenia and thrombo-cytopenia. In addition myelosuppression, neurotoxicity, ototoxicity, embryo toxicity, teratogenicity has also been evaluated in carboplatin administered experimental animals such as guinea pigs, chinchillas and rats (Kai *et al.*,

1988; Chung *et al.*, 1998).[4,5] Carboplatin treatment also demonstrated oxidative renal injury, enhanced lipid peroxidation, platinum content, plasma creatinine and blood urea nitrogen levels in rats.[6]

Androgens play a vital role in initiating and maintenance of male reproductive function or testicular function which includes spermatozoa production. The main testicular androgen i.e., testosterone is produced by leydig cells under the stimulation of pituitary LH, which is essential for spermatogenesis, fertility and maintenance of the male phenotype. Spermatogenesis depends on the action of testosterone which is produced by leydig cells in the testis.[7] Spermatogenic failure has been a recognized consequence of treatment with chemotherapeutic agents.[8]

Many reports available on carboplatin caused toxicity in various tissues, but very limited studies demonstrated its antifertility effects. So, an attempt has been made in the present study to investigate the effect of carboplatin on testicular functions in male rats. In addition studies were also extended to analyze the male reproductive functions after co-administration of testosterone to rats exposed to carboplatin.

Materials and 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° 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

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 10 mg/kg body wt. of the animal. Testosterone was obtained from the local drug suppliers.

Experimental Design

The rats were divided into three groups consisting of eight animals in each group. The rats in the first group were served as control and received 0.9% of normal saline only. The rats in the second group were received carboplatin (10 mg/kg body wt) only. The rats in the third group were received carboplatin (10 mg/kg body wt) and testosterone (4.16 mg/kg body wt). 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.

Epididymal sperm count, motility and viability

Epididymal sperm counts and evaluation of motility of epididymal sperm were done by the method of Belsey.[9] The epididymal sperm was obtained as described above and incubated at 37°C. The epididymal fluid was then diluted to a volume of 5.0 ml of pre-warmed (37°C) normal saline. An aliquot of this solution was placed in Neubaeur chamber and motile sperm were counted by using microscope.

Sperm motility was expressed as a percent of motile sperm of the total sperm counted. Non-motile sperm numbers were first determined followed by counting of total sperm. The ratio of live and dead spermatozoa was determined using 1% trypan blue by the method of Talbot and Chacon.[10]

HOS test

The hypo osmotic swelling test (HOS test) for investigating the functional integrity of sperm membrane has been introduced as a useful assay in the diagnosis of infertile semen. The principle of HOS assay is based on fluid transport across the sperm tail membrane under hypo osmotic conditions until equilibrium is reached. Due to influx of fluid, the tail coils, considered as hypo osmotic response, which can be readily identified under phase-contrast microscope. The sperms were exposed to hypo osmotic medium and observed for coiled tails under the microscope and the percent of coiling was determined by the method of Jeyendran.[11, 12]

Assay o f test icular stero idogenic mar ker enzymes

The testicular tissue was homogenized in ice-cold Tris-HCl buffer (pH 6.8). The microsomal fraction was separated and used as enzyme source. The activity levels of 3β-hydroxy steroid dehydrogenase (3β-HSD; EC.1.1.1.51) and 17β-hydroxy steroid dehydrogenase (17β-HSD; EC.1.1.1.64) were assayed by the method of Berg Meyer.[13] The enzyme assays were made under the conditions following zero order kinetics after preliminary standardization regarding linearity with respect to time of incubation and enzyme concentration.

The reaction mixture in a volume of 2.0 ml contained: 100 μ moles of sodium pyro phosphate buffer (pH 9.0), 0.5 μ moles of co-factor (NAD for 3β-HSD and NADPH for 17β-HSD), 0.08 μ moles of substrate (dehydro epi andro sterone for 3β-HSD and androstenedione for 17β-HSD) and 20 mg equivalent of microsomal protein as enzyme source.

The reactions were carried out in a quartz cuvette of 1.0 cm path at 23±1° C. The absorbance at 340 nm was measured at 20s intervals for 5 min using UV-spectrophotometer (Hitachi U-2001). Protein content in the enzyme source was estimated by the method of Lowry[14] using bovine serum albumin as standard. The enzyme activities were expressed in micromoles of NAD converted to NADH mg/ protein/min for 3β-HSD or micromoles of NADPH converted to NADP mg/ protein/min for 17β-HSD.

Statistical analysis

The data were presented as mean ± SD. Statistical analysis was performed using analysis of variance (ANOVA) followed by Dunnett's test using SPSS 10.0.

Results:

No mortalities were observed in control or experimental groups. No behavioral abnormalities were observed in experimental animals. A significant decrease in sperm motility and viability was observed with reduction in average sperm counts in cauda epididymal tissue of carboplatin treated rats. Sperm coiling percentage was also decreased significantly in these rats. The average percentages of above mentioned sperm parameters were significantly increased in carboplatin + testosterone treated rats when compared with carboplatin alone treated rats (Tab-1).

The results presented in (Fig.1) indicate that a significant decrease in activity levels of 3β-HSD (A) and 17β-HSD (B) observed in carboplatin treated rats when compared with control rats. But carboplatin + testosterone treated rats showed significant increase in activity level of 3β-HSD (A) and 17β-HSD (B) when compared with carboplatin alone treated rats. In addition many abnormal sperms were observed in carboplatin treated rats (Fig.2). But any abnormal sperms were not observed in control and carboplatin + testosterone treated rats.

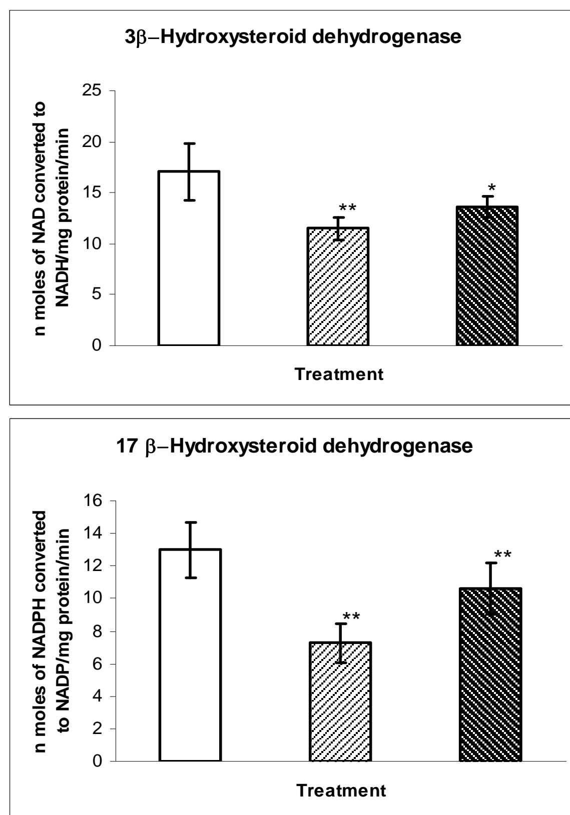
Discussion:

The present study demonstrates the adverse effect of carboplatin on spermatogenesis and steroidogenesis and protection caused by testosterone co-administration.

Table 1: Effect of carboplatin and carboplatin + testosterone on sperm parameters in rats

Parameters	Control	Carboplatin	Carboplatin + Testosterone	ANOVA
Sperm count (million/ml)	60.98±4.24	30.84**±1.17 (-49.42)	41.17**±1.62 (+33.49)	$F_{2,21}$= 256.26 p< 0.0001
Viable sperm (%)	72.65±2.19	53.83**±1.51 (-25.80)	68.18**±3.19 (+26.65)	$F_{2,21}$=134.50 p< 0.0001
Motile sperm (%)	66.74±2.84	51.47**±2.50 (-22.87)	59.32**±2.48 (+15.25)	$F_{2,21}$= 68.377 p< 0.0001
HOS coiled Sperm (%)	60.29±2.14	40.86**±4.89 (-32.22)	49.36*±2.74 (+20.80)	$F_{2,21}$=63.250 p< 0.0001

Values are mean ± S.D of eight individuals. Values in the parentheses are percent change from control. Values are significantly different from control at *p<0.001, ** p<0.0001.
For calculation of % change and 'p' for carboplatin + testosterone injected rats, carboplatin injected rats served as controls.



☐ Control; ▨ Carboplatin; ▩ Carboplatin + Testosterone

Fig 1: Effect of carboplatin or carboplatin+testosterone on the activity levels of 3β-HSD and 17β-HSD in the testis of rats.

In the present study it was observed that the exposure to platinum-based anticancer drugs caused significant decrease in sperm count, sperm motility, sperm viability and HOS sperm tail coiling in rats. So an attempt has been made to observe whether these changes could be restored in rats exposed to platinum compounds after testosterone treatment. In view of this carboplatin was administered alone and in combination with testosterone to male rats.

Determination of sperm volume, sperm motility, sperm viability and sperm functional test were performed to assess the quality and functional status of sperm which play an important role in male fertility determination. As an index for male reproduction the quality and quantity of sperm was evaluated. Sperm motility can be selectively affected by chemotherapeutic drugs that reduce fertility. Studies have examined that rat sperm motility as a reproductive end point and sperm motility assessments are an integral part of reproductive toxicity test guidelines. A physiological hypo-osmotic swelling test was also performed to determine the functional capacity of sperm. [15]

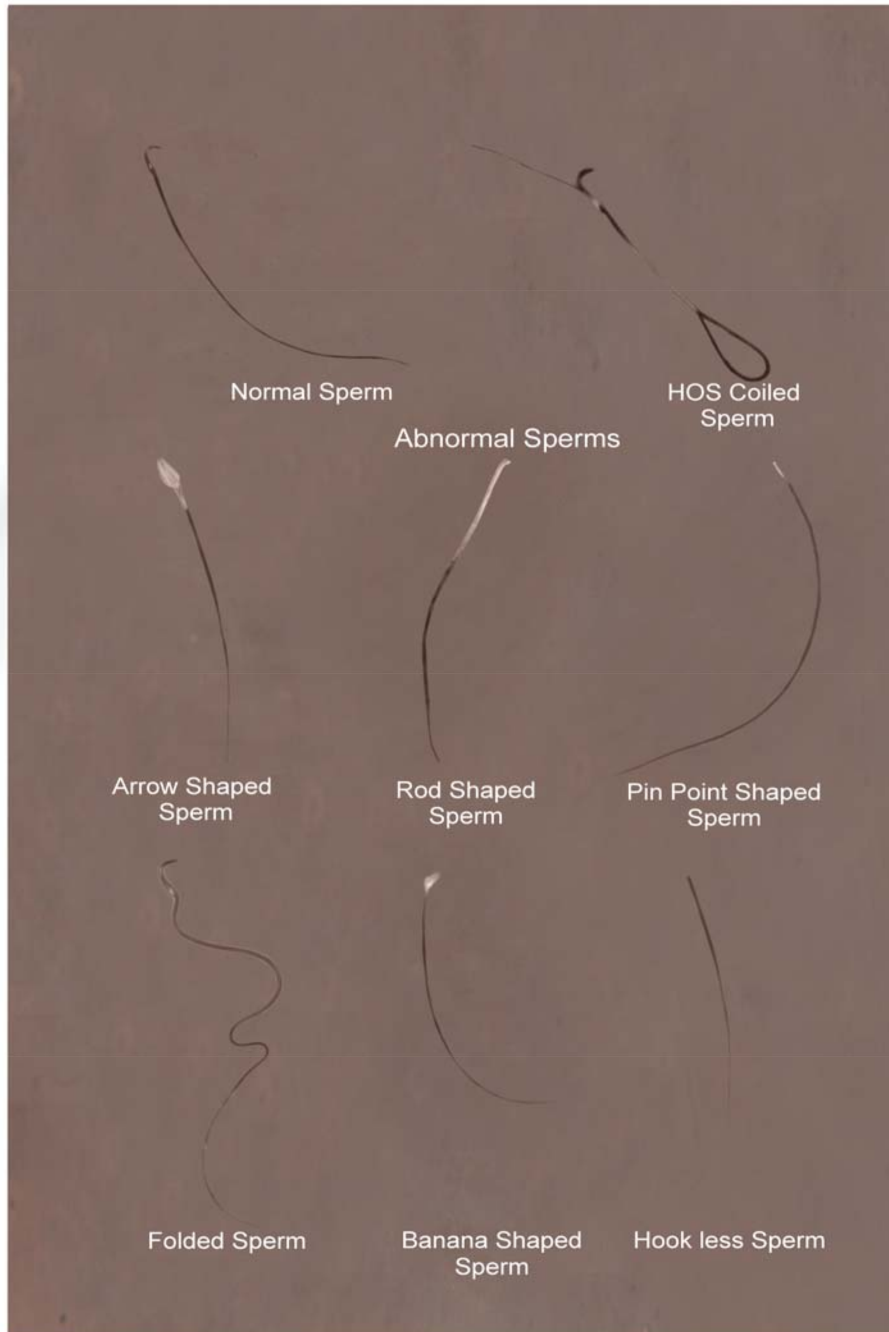


Fig. 2: Normal and abnormal sperms (after exposure to carboplatin) observed in rats.

Spermatogenesis is a well regulated process starting with spermatogoneal stem cells and ending with differentiated, motile spermatozoa. The significant decrease in sperm count, sperm motility, sperm viability and sperm function in carbopatin injected rats indicates an inhibitory effect of the same on spermatogenesis. The present findings are in agreement with the earlier reports such as decrease in sperm motility in rats and in humans treated with platinum-based anticancer drugs such as cisplatin.[16, 17]
Exogenous administration of testosterone caused restoration of sperm quality and quantity in experimental rats exposed to carboplatin. Since, spermatogenesis is well known to be dependent on androgen levels and testosterone is also thought to be the predominant androgen involved in spermatogenesis. Low levels of exogenous testosterone can partially or fully maintain spermatogenesis in rats treated with chemotherapeutic agents.[18] Furthermore, all features of testosterone deficiency should be reversible with appropriate testosterone replacement therapy.[19]
The two key enzymes involved in the biosynthetic pathway of testosterone are 3β-HSD and 17β-HSD. The activity levels of 3β-HSD and 17β-HSD have been used to study the testicular steroidogenesis of rats in different experimental conditions. These two enzymes are having regulatory functions in the maintenance of steroidogenesis and also involves in the synthesis of testosterone. In carboplatin treated rats, a significant decrease in the activity levels of 3β-HSD and 17β-HSD was observed which clearly indicates the impairment of steroidogenesis.
The decreased steroidogenic enzyme activity levels indicate decreased androgen production in experimental rats which in turn lead to decreased reproductive activities in male rats. It seems carboplatin acts on leydig cells and inhibits the testosterone production which was evident by decrease in the activity levels of 3β-HSD and 17β-HSD enzymes in the testes of experimental rats. Since, the enzyme 3β-HSD is localized exclusively within the leydig cells in the testes. Previous studies also have been demonstrated the decreased activity levels of 3β-HSD and 17β-HSD in the leydig cells cultured with platinum compounds.[20]
The activity levels of 3β-HSD and 17β-HSD were significantly increased in carboplatin + testosterone treated rats when compared with carboplatin treated rats. This increase in 3β-HSD and 17β-HSD activities levels in testis indicates the restoration of steroidogenesis and leads to normal fertility in carboplatin + testosterone treated rats (Fig-1). Injection of testosterone significantly increased the steroidogenic marker enzyme (3β-HSD and 17β-HSD) activity levels in the testis of carboplatin treated rats. This may result in increased androgen production which in turn enhances the male reproductive efficiency.
In addition many abnormal sperms were observed in carboplatin treated rats, when compared with controls and carboplatin + testosterone treated rats. Morphology of head of several sperms in experimental rats were with rod shape, arrow shape, banana shape, and pin point shape head instead of its normal hook shape (Fig.2).

Conclusion:

Exposure to platinum compounds affected the male reproduction in many ways, namely reducing the sperm count, sperm motility and function, increase in abnormal sperms and decrease in the testicular 3β-HSD and 17β-HSD activity levels. This indicates that platinum compounds treatment affects male reproduction. When testosterone was administered to carboplatin injected animals, the above reproductive abnormalities were partially reversed. This observation clearly indicates that decrease in male reproductive functions caused through

platinum compounds, can restore by co-administration of testosterone. So the present study suggests that patients under carboplatin treatment may be prescribed with testosterone during treatment period to maintain their reproductive health. Of course, the present data cannot be extrapolated to humans. It needs further studies to ascertain whether similar situation exists in humans. Studies in this direction would indicate, testosterone supplementation to the patients treated with platinum-based anticancer drugs which gives protection against reproductive toxicities.

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