

# Research Paper: Cisplatin Induce Changes of Cell Surface Glycoconjugates in Germinal Epithelium of Seminiferous Tubules

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## ABSTRACT

**Introduction:** Cisplatin is a platinum-based drug widely used for the treatment of different cancers. Cell surface glycoconjugates play an important role in cell-cell interactions. The present investigation was carried out to study the toxic effects of double dose injection of cisplatin on cell surface glycoconjugates in rat as an experimental model.

**Methods:** In this experimental study, 45 adult male Sprague Dawley rats were used. Experimental group E1 and experimental group E2 received two repeated dose of 2.5 mg/kg and 5 mg/kg of cisplatin, respectively in the beginning of the first and fifth week of the experiment. After 8 weeks of injection, rats were killed. Tissue samples were removed and prepared sections were stained with H&E, PNA (Peanut agglutinin), and UEA (Ulex europaeus agglutinin) methods. Prepared microscopic slides were utilized for both histopathological and morphometrical studies. The obtained data were analyzed by ANOVA and Tukey tests using SPSS.

**Results:** Cisplatin administration induced a significant decrease in internal and external diameters of seminiferous tubules in the experimental groups compared to the control one ( $P < 0.0001$ ). There was also significant difference between control and experimental groups with regard to germinal epithelial thickness of seminiferous tubules ( $P < 0.0001$ ). Moreover, there was a significant difference between control and experimental groups with regard to spermatogenesis index ( $P < 0.004$ ). Also a significant elevation was seen in staining intensity of germinal epithelium to PNA and UEA in experimental groups, compared to control group ( $P < 0.0001$ ).

**Conclusion:** Cisplatin induces a dose-dependent morphological changes of germinal epithelium and extensive changes in distribution pattern of fucose- and Gal/GalNac-containing glycoconjugates in seminiferous epithelium in rats.

## Key Words:

Cisplatin, Seminiferous tubules, Glycoconjugates, Spermatogenesis, Rat

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## 1. Introduction

**C**isplatin (CP) is a platinum-based chemotherapeutic drug that effectively used for treatment of different cancers [1] such as ovarian, testes, cervical, bladder, breast, and lung carcinomas [2, 3]. The main mechanism of antineoplastic effect of CP is interfering with purine base of DNA and cessation of cell cycle [4]. Cisplatin create crosslink with cellular DNA of healthy cells of the reproductive, nervous and auditory systems which restricts its use in the treatment of cancer [3, 5].

CP is not a classical DNA-directed alkylating agent but a natural alkylating factor and induce biochemical and tissue changes in testis particularly in germinal epithelium [6]. Testicular injury of CP has been attributed to the direct damage of spermatogenic cell lines [7, 8], Sertoli cells [9], and Leydig cells [10, 11]. Although there are many possible manifestations of toxic damages to spermatogenesis, long-term result of CP administration is sequential testicular atrophy and consequent infertility [9]. Impairment of spermatogenesis [11], chromosomal abnormalities of spermatozoa [12], and transient or permanent azoospermia are the main consequences of CP-based chemotherapy for testicular cancers [13]. Radiotherapy and chemotherapy alter spermatogenesis process. Chemotherapeutic drugs inhibit rapid division of cancer cells as well as other normal cells [14]. Cell surface glycoconjugates as a class of glycolipids or glycoproteins are known to play an important role in cell-cell and cell-extracellular matrix interactions, cell-cell recognition, communication, differentiation, embryogenesis, tumorigenesis, tumor progression, and metastasis [15, 16].

Carbohydrate components of glycoconjugates explain their chemical, physical, structural variety, and biological properties [17]. Most carbohydrates of glycoconjugates are oligo- or poly-saccharides sugars with linear or branched chains, including mannose (Man), fucose (Fuc), galactose (Gal), glucose (Glc), N-acetylgalactosamine (GalNAc), N-acetylglucosamine (GlcNAc) and sialic acid (NeuAc). These monosaccharides in different linear or branched compositions constitute a high diversity in glycoconjugates families, and thus to a high number of possible modifications of proteins and lipids with different properties and functions. Normal structure and function of the cell reflects normal glycosylation pattern of posttranslational modification of cell surface glycoconjugates [18].

Any changes in these terminal sugars determine cells' behavior in normal and pathologic states such as neoplas-

tic process or metastatic potential of cancer cells [19]. Lectins as group of non-immunogenic biological compounds with plant or animal origin are a valuable marker for determination of terminal sugars of glycoconjugates with high specificity and sensitivity. Lectins detect any changes of glycoconjugates and shows that these molecular modifications are responsible for potential abilities of cancer cells for metastasis and invasion [20, 21].

Glycoconjugates have many important roles in spermatogenesis of mammals. According to Blackmore study glycoconjugates with fucose as a terminal sugar are responsible for vesicular transport from Golgi apparatus to acrosome. It seems that this property may have important roles in cytodifferentiation process of spermiogenesis [22]. Turk et al. reported that CP induces loss of weight of testis, epididymis and seminal vesicle in rats [23]. Bar-Shira et al. showed that CP induces disruption of spermatogenesis by different mechanisms, including apoptosis and break in DNA chains [24]. Seaman et al. believed that toxic effect of CP is mediated by Sertoli cells injury [6]. This study aimed to evaluate the changes of cell surface glycoconjugates in spermatogenic cell lines after cisplatin injection in rats.

## 2. Material and Methods

In this experimental study, 45 adult male Sprague-Dawley rats weighting  $200 \pm 30$  g were randomly divided into experimental ( $n=30$ ) and control groups ( $n=15$ ). Then, they were kept under identical conditions one per cage (12:12 h light: dark cycle,  $22^\circ\text{C} \pm 2^\circ\text{C}$  and 45%-50% humidity) and fed with standard animal food and water. In the next step, rats of the experimental group were randomly divided into 2 subgroups. Subgroup I (E1) were received 2 consecutive dose of 2.5 mg/kg of cisplatin (MYLAN Co., USA) and experimental subgroup II (E2) received 5 mg/kg of cisplatin. CP was injected intraperitoneally at the beginning of the first and fifth week of the experiment. At the end of the eight week, rats of control and experimental groups were given ether anesthesia and killed, and then their testes were removed, weighted and fixed in 10% formalin saline solution. Tissue samples were processed routinely and 7  $\mu\text{m}$  paraffin sections were stained with H&E method. The sections were examined using light microscope to investigate the spermatogenesis process. After calibration of the light microscope (Motic), histological sections were evaluated for internal and external diameters of seminiferous tubules, epithelial thickness and spermiogenesis index in at least 100 seminiferous tubules in each rat of experimental and control groups.

### Lectin histochemistry

Lectin histochemistry was carried out according to the Spicer method [25]. Prepared sections were deparaffinized and hydrated according to routine procedures. Thereafter, the HRP conjugated lectin (PNA [Peanut agglutinin] or UEA [Ulex europaeus agglutinin]) were used, purchased from Sigma Company (USA). For this purpose, the lectins were first diluted with PBS up to 10 µg/mL. Next, the sections were incubated with diluted lectin for two hours in humidified chamber. After this stage, tissue samples were washed with buffer and placed in 0.03% DAB (Diaminobenzidine) as the chromogen solution which contain 0.1% H<sub>2</sub>O<sub>2</sub>. The sections were washed, dehydrated, cleared, and mounted. The samples were blindly and separately ranked from 0 (no reaction) to 4 positive (very severe reaction) according to staining intensity.

### Statistical analysis

The morphometric data were subjected to parametric methods of analysis of ANOVA followed by Tukey post hoc test (using SPSS version 17.0). Results are presented as mean and standard deviation values. A value of P<0.05 was considered as statistically significant.

### 3. Results

Statistical data for measured variables showed a significant difference for testis weight between experimental groups and control one (P<0.031) (Table 1). There were also significant decrease in internal and external diameters between experimental groups and control one (P<0.0001) (Table 1). Statistical analysis also demonstrated a significant decrease in epithelial thickness of

seminiferous tubules between control and experimental subgroups (P<0.0001) (Table 1). Furthermore, there was a significant decrease in spermiogenesis index in experimental group, compared to the control group (P<0.004, Table 1). Statistical analyses for staining intensity of germinal epithelium to PNA and UEA confirmed a significant difference between control and experimental group (P<0.0001) (Table 1).

### Histological findings

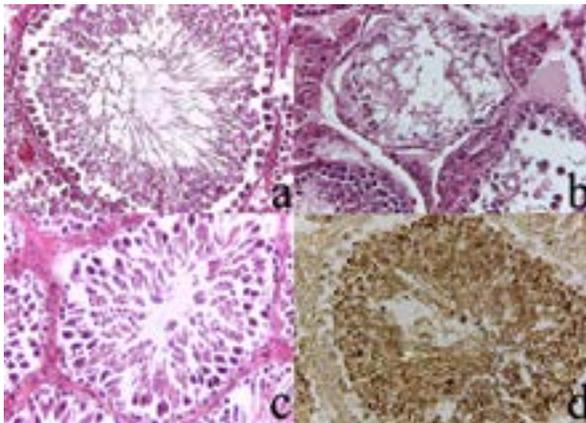
Histological study of the prepared slides showed so many structural changes in the experimental groups compared to the control group. These histological changes are loss of intercellular adhesion between Sertoli and spermatogenic cell lines and a decrease in thickness of germinal epithelium, tubular shrinkage, germ cell degeneration and interstitial edema. Maturation arrest in spermatogenic cell lines and perivascular fibrosis were the most prominent findings in the experimental groups (Figures 1, 2).

### 4. Discussion

The present study results showed the staining intensity of germinal epithelium to PNA lectin which demonstrate terminal sugar of Gal/GalNac containing glycoconjugates increase from basal to apical compartment in experimental group compared with the control. This glycoconjugates may be very important for cell-cell and cell-extracellular matrix interaction and cytodifferentiation [15, 16]. In germinal epithelium, the proper organization of spermatogenic cell lines is very important for normal spermatogenesis. Our study showed a reverse gradient of staining intensity of germinal epithelium to UEA compared to PNA lectin. Spermatogonia have

**Table 1.** Comparing the obtained data after injection of cisplatin between control and experimental group

Groups Variables	Mean±SD			P
	Control Group	Group E1	Group E2	
Internal diameter (µm)	4.36±40.85	3.43±30.11	3.78±28.72	0.0001
External diameter (µm)	5.98±109.87	6.24±84.8	6.9±77.88	0.0001
Epithelial thickness (µm)	3.21±34.54	2±27.47	1.86±24.72	0.0001
Testis weight (g)	1.33±0.22	1.17±0.15	1.17±0.14	0.031
Spermatogenesis Index	27.93±3.75	23.86±4.76	22.60±4.25	0.004
Staining intensity of PNA	2.43±0.42	3.06±0.14	3.16±0.19	0.0001
Staining intensity of UEA	1.05±0.32	2.15±0.1	2.21±0.1	0.0001

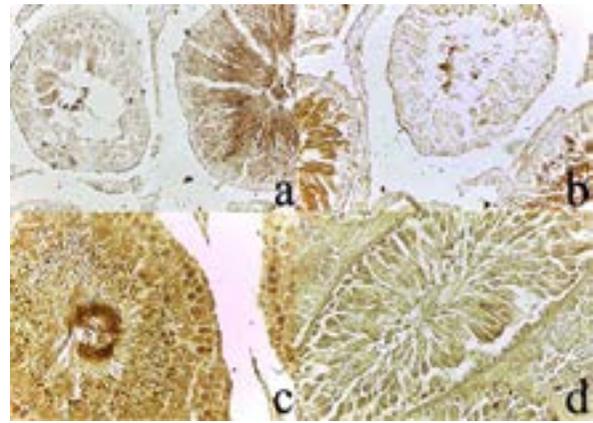


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**Figure 1.** Normal arrangement of spermatogenic cell lines and Sertoli cells of seminiferous tubule in the control group (a), affected tubule with disorganized architecture in spermatogenic cell lines and cellular changes in interstitial cells in Group E1 (b) (a, b H&E×40), Cellular changes of spermatogonia and spermatocytes in group E2 (c) (H&E×40), Severe staining intensity of germinal epithelium to PNA lectin for demonstration of Gal/GalNac containing glycoconjugates from basal (no reactivity) to apical (severe reactivity) compartment in control group (d) (PNA ×40)

no reaction to PNA; spermatozoa's tail have a severe staining intensity to this lectin. The study of Sato et al. showed similar results for germinal epithelium and also confirmed lamina propria have a positive reaction to PNA lectin [26]. Furthermore, results of the present study showed the staining intensity of germinal epithelium to UEA lectin which demonstrate terminal sugar of fucose-containing glycoconjugates increases from apical to basal compartment in the experimental groups compared with the control group. Spermatogonia have a high affinity to UEA lectin, but spermatozoa's tail has a mild reaction. The study of Blackmore et al. showed that fucose-containing glycoconjugates facilitate cell-cell interactions [22] suggesting the main role for this fucosylated carbohydrates in spermiogenesis in cancer patients. Degeneration of germinal epithelium of seminiferous tubules may induce changes in expression pattern of glycoconjugates after cisplatin injection. This changes including germ cells apoptosis and loss of junction between spermatogenic cell lines and sertoli cells [6, 20].

The present study shows that cisplatin is able to change different parameters in testis such as decrease in testis weight between experimental and control groups. The study of Cherry et al. showed that cisplatin injection induces decrease in testis weight, sperm count and loss of germ cells in germinal epithelium [11] which is accordance with our results. Thus, the decrease in testis weight could be a manifestation of degeneration, ne-



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**Figure 2.** Negative reaction of the affected tubule to PNA lectin in Group E1, (a), decrease in thickness of seminiferous epithelium, and tubular shrinkage in group E2 (b), (a, b PNA×40). Positive reaction of spermatogonia to UEA demonstrating fucose-containing glycoconjugates in group E1, (c), reverse reaction of germinal epithelium to UEA lectin in the experimental group (group E2) compared to PNA lectin (d) (c, d UEA ×40)

crosis, and decrease of epithelial thickness in germinal epithelium [27].

The present study results showed significant decrease in internal and external diameters of seminiferous tubules after cisplatin injection which agrees with the study of Leandra and associates [2]. Loss of cell junctions may be a manifestation of spermatogenesis impairment [28]. The junctional complex between Sertoli and spermatogenic cell lines is necessary for preservation of morphology and volume of tubules [29]. The study of He et al. showed that vimentin plays an important role in cell junctions between adjacent Sertoli cells [30], which its expression decreases after cisplatin injection [31], a possible mechanism for changes in diameters of seminiferous tubules. Thus, the diameter of seminiferous tubules is one of most important parameters for evaluation of spermatogenesis [32]. On the other hand, the thickness of germinal epithelium is another good marker for the evaluation of spermatogenesis. Chemotherapeutic drugs induce damage to germinal epithelium which may lead to oligospermia or azospermia in cancer patients [11].

Statistical analysis of the present study showed decrease in epithelial thickness of seminiferous tubules between experimental and control groups. Also qualitative result of the present study showed tubular shrinkage and interstitial edema in experimental group which are in accordance with Beytur et al. study results [33]. Furthermore, our results showed spermiogenesis index signifi-

cantly decreased in experimental groups compared with the control group. In accordance with our results, Rezvanfar et al. study showed that sperm count, spermiogenesis index and differentiated germ cells decreased after cisplatin injection [34]. It seems that cisplatin induces many quantitative and qualitative changes in rats' testes in a dose dependent manner, including extensive changes in distribution pattern of fucose- and Gal/GalNac-containing glycoconjugates in seminiferous epithelium, loss of intercellular junction between Sertoli cells, decrease in the height of germinal epithelium, tubular shrinkage, germ cell degeneration, interstitial edema, perivascular fibrosis, decrease in internal and external diameters of seminiferous tubule, decrease in germinal epithelial thickness and spermiogenesis index.

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### Conflict of Interest

The authors declared no conflicts of interest.

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