THE PROTECTIVE EFFECT OF IRBESARTAN AGAINST BLEOMYCIN-INDUCED MALE RATS REPRODUCTIVE TOXICITY

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ABSTRACT

Bleomycin (BL) is a glycopeptide antibiotic derived from the bacterium Streptomyces verticillus which is routinely utilized for treatment of human cancers. The study aims to investigate the protective effect of irbesartan administration against bleomycin-induced reproductive toxicity in adult male rats. Rats were divided into 4 equal groups (10 each): (1) control, received normal saline 0.5ml/animal/daily i.p. for 8 wks. (2) received bleomycin 15mg/kg/i.p. three times weekly for 8 weeks, (3) received Irbesartan 10mg/kg/day by gavage for 8 weeks, (4) received bleomycin and irbesartan at the same mentioned doses and for the same period. After the end of the treatment period, blood samples were taken for serum hormonal analysis (testosterone, FSH and LH), head of epididymis was minced for sperm count, and finally testes samples were taken for histopathological study. Bleomycin induced male rat reproductive toxicity represented by significant decline in the serum testosterone level, significant decrease in the relative sperm content in the head of epididymis (sperm/ mg of epididymis head) and many adverse histological changes. All these toxic effects were alleviated by irbesartan. However, it improved these changes but didn’t bring them to the control limits. The present study clearly indicates that irbesartan (20mg/kg/day) can attenuated the male reproductive toxicity of bleomycin.

Keywords: Bleomycin, Irbesartan, Reproductive, Toxicity, Male, Rats

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Introduction

Chemotherapy comprises the use of chemical agents to discontinue the growth and exclude cancer cells even at distant sites from the origin of primary tumor. However, it does also remove not only the fast-growing cancer cells but also other fast-growing cells in the body[1]. Treatment of males with anticaner drugs, such as cyclophosphamide, bleomycin, etoposide and cisplatin, doxorubicin, methotrexate and many other cytotoxic drugs, caused a range of reverse consequences including micronuclei in 2-cell embryos, pre-implantation and post implantation loss, growth default, congenital malformation and postnatal death[2-8]. DNA- damaging agents are among the most effective anticancer agents in clinical use, these include, anti-metabolites as 5-fluorouracil and bleomycin…etc[9]. Where more of these drugs cause DNA damage, inducing cross-links besides single and double strand breaks[9-10]. Many studies showed that many cytotoxic drugs affected semen quality in males[11], which correlated with infertility.

In animal models, acute or chronic administration of the chemotherapeutic drugs such as cisplatin, etoposide and bleomycin induced various male reproductive adverse effects shortly after exposure[12]. Bleomycin when used in anticancer treatment, it caused noxious effects on spermatogenic function, the treated male patients showed azoospermic or oligozoospermic, depending on the dose and period of treatment[11]. The reproductive systems are targets for toxicity of many drug groups included antimicrobial, anticaner, cardiovascular, CNS, anti-inflammatory and many other drug groups[13-14]. Many mechanisms were proposed to explain the adverse effects of cytotoxic drugs on the male reproductive system including inhibition of hypothalamic-pituitary- gonad axis, reduced testosterone production, reduced sperm production and interference with the transport of sperm through the duct system or its delivery into the female genital tract[15]. The current study was designed to investigate the male rat reproductive toxicity of bleomycin, and to verify the preventive effects of an angiotensin receptor blocker (irbesartan) against the reproductive toxicity of bleomycin.
Materials and methods
Forty male Wister rats (150-200 g, 12 week old) were used in this study, they were obtained from animal center of Thi-Qar University, College of science. Rats were housed in a well-ventilated animal house (22±2°C and 12h light/12h darkness). Diet and water were given ad libitum. Animals were divided into 4 groups (10 each), the first group was given normal saline (the vehicle) 0.5ml/animal/daily i.p. for 8 wks, to serve as control. The second group was given bleomycin15mg/kg i.p. three times weekly for 8 weeks. The third group received irbesartan 10mg/kg/day, orally for 8 weeks, while the fourth group was given a combination of bleomycin and irbesartan at the same mentioned doses and for the same period[16-18]. At the end of the treatment period, all animal were killed by neck dislocation after light anesthesia. Blood samples were taken by cardiac puncture. Serum testosterone, LH and FSH levels were determined by Enzyme linked immunosorbent assay kits[19]. Sperm count was determined in the head of epididymis according to previous methods[17, 19]. Sperm viability was detected using 0.05% Eosin and Nigrosin stains. Sperm motility was estimated according to method recommended by WHO[20]. The testes were removed fixed in 10% formalin for 24, washed, dehydrated, cleared and embedded in paraffin, thick paraffin sections about 4μm thickness were stained with Hematoxylin and Eosin[21-22].

Statistical analysis
The significances among groups were determined by student-t-test using SPSS.

Results
As shown in table 1, bleomycin didn’t affected serum levels of LH and FSH as compared with control, but it significantly (p<0.01) decreased serum testosterone level. Irbesartan alone also showed no effect on LH, FSH and testosterone levels, but when used in combination with bleomycin, it significantly (p<0.01) elevated the declined level of testosterone up to normal limit. Sperm count, viable sperm%, sperm motility% were significantly declined (p<0.01, p<0.01, And p<0.05, respectively), while sperm deformity % was increased (p<0.001) in bleomycin treated group. Using of irbesartan alone didn’t significantly affected all sperm parameters, while when irbesartan was used in combination with bleomycin, it attenuated all the adverse effects of bleomycin on semen properties. It significantly increase sperm count, viable sperm% and sperm motility% p<0.05, for all), and significantly decrease sperm malformation% p<0.05 in comparison with bleomycin, but it didn’t bring these parameters to the normal limits (table 2).

Histopathological study revealed that testis sections of bleomycin group showed smaller seminiferous tubules with vaculation, detachment of basal membrane, absent of mature spermatozoa, decreased number of spermatogonium and spermatocytes, lower numbers of sperms in the seminiferous tubule lumen and degeneration of interstitial cell with edema, compared with control. Using of irbesartan with bleomycin led to improving of all the structural changes associated with bleomycin treatment (Fig 1: A, B and C).

Table 1: Effect of bleomycin, irbesartan and a combination of both drugs on serum testosterone, LH and FSH levels.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Testosterone nmol/l</th>
<th>LH MIU/ml</th>
<th>FSH MIU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.98± 0.07*</td>
<td>4.19± 0.09*</td>
<td>4.62±0.22*</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>0.58 ± 0.32b</td>
<td>4.09±0.01a</td>
<td>4.32±0.20a</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>1.87± 0.09*</td>
<td>3.99±0.02b</td>
<td>4.54±1.4b</td>
</tr>
<tr>
<td>Bleomycin-Irbesartan</td>
<td>0.83± 0.52c</td>
<td>4.10±0.02*</td>
<td>4.20±0.06*</td>
</tr>
</tbody>
</table>

Vertically, similar letter means not significant

Table 2: Effect of bleomycin, irbesartan and a combination of both drugs on sperm parameters.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sperm count (×10⁶/mgepididymal head)</th>
<th>Sperm Viability %</th>
<th>Sperm Motility %</th>
<th>Spermatozoa deformity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>87.42±3.76*</td>
<td>91.03 ± 1.01*</td>
<td>66.62± 2.02*</td>
<td>17.82±1.87*</td>
</tr>
</tbody>
</table>
**Figure 1:** Histological section of testis of bleomycin group shows vaculation of seminiferous tubules, detachment of basal membrane, decreased number of spermatogonium and spermatocytes, decrease the amount of sperms in the seminiferous tubules lumen and degeneration of interstitial cell (B) compared with control (A). Irbesartan ameliorates all the structural in the testis changes appeared in bleomycin group (C) (40X).

**Discussion**

Rats and mice models are mimicking human being response to cytotoxic drugs. In the present study, revealed that bleomycin induced testicular damage characterized by significant decrease of serum testosterone level, while it did not induced significant changes in the serum level of LH and FSH compared with untreated control animals. These results were further confirmed by histological investigations. These results clearly indicated that bleomycin did not possess central endocrine effect. However, it appeared that it induced direct testicular effects, which revealed by decline of testosterone level. This effect could be attributed to the degeneration of interstitial (leydig) cell noted in histological examination. The decline of testosterone participated in decreasing sperm content of epididymis because the
spermatogenesis from spermatogonium to spermatid was controlled by testosterone, in addition to direct toxicity of bleomycin on spermatogonia, primary and secondary spermatocytes\(^\text{[23]}\). Germin tissue of the testis was sensitive to damage from anticancer drugs. All cells that underwent rapid division were susceptible to the toxic effects of chemotherapy, and this included cells involved in spermatogenesis\(^\text{[24]}\).

As in this study, anti-cancers were associated with increased DNA damage and decreased chromatin quality reflected by an increase in the sperm malformation percent. DNA damage was noted after in vivo treatment with many anticancer drugs. Men treated with chemotherapy for cancer showed increased and persistent DNA damage in sperm\(^\text{[25]}\). DNA damage of the anticancer drugs could be fatal and decreased viable sperm percent\(^\text{[17]}\). It appeared that toxic effects of anticancer drugs on androgenesis and spermatogenesis was mediated by generation of free radicals. Using of antioxidants can protect from the reproductive toxicity of anticancers\(^\text{[8]}\). The selenium nano-particles (Nano-Se) as an established strong antioxidant with good bioavailability were proven in protection against toxicity of cisplatin. The same results were achieved with the using of flavonoids. The antioxidant; coenzyme-Q10 also protected testis from the toxicity of doxorubicin\(^\text{[8, 26-27]}\).

Both angiotensin converting enzyme inhibitors and angiotensin receptor blockers decreased the inflammation, fibrosis, and degenerative changes in cardiotoxicity associated with the using of cytotoxic drugs\(^\text{[27-28]}\). Irbesartan also possessed anti-inflammatory and antioxidative effects\(^\text{[29, 30]}\). Therefore the protective action of irbesartan against male reproductive toxicity of bleomycin could be attributed to the the antioxidant, anti-inflammatory effects and to its inhibitory effects against degeneration associated with cytotoxic drugs.

**Conclusion**

According to the results, the present study clearly indicates that bleomycin (15mg/kg) possessed deleterious effects on testicular structure of rats. Furthermore, irbesartan (20mg/kg/day) can attenuate the male reproductive toxicity of bleomycin.

**References:**