VALSARTAN ATTENUATED BLEOMYCIN-INDUCED MALE RAT REPRODUCTIVE TOXICITY

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ABSTRACT

Bleomycin induced male rat reproductive toxicity represented by significant decline in the serum testosterone level, significant decrease in the relative sperm content of the head of epididymis (sperm/ mg of epididymis head) and many adverse histological changes. All these toxic effects were ameliorated by valsartan, however, it improve these changes but didn’t bring them to the normal limit in the control group.

KEYWORDS: valsartan, bleomycin, reproductive toxicity, rats.

INTRODUCTION

The functional diversity of the male reproductive system and the complexity of its hormonal regulation provide a potential site for the drug effects. Injury may result in inhibition of hypothalamic-pituitary-gonad axis, reduced testosterone secretion, sperm production and interference with the transport of sperm through the duct system or its delivery into the female genital tract. The quality of the sperm available for fertilization may also be impaired.[1-2]

However, the male reproductive system is a target for toxicity of many drug groups and environmental agents including radiation, solvents, anesthetic gases, heavy metals, herbicides, pesticides, anticancer, cardiovascular, antibiotics, anti-inflammatory and other drugs [5-8], many medicinal plants [10-40], also affected reproductive system functions, including Achillea santolina, Ailanthus altissima, Alhagi maurorum, Allium cepa, Althaea rosea, Ammannia baccifera, Anthemis nobelis, Anethum graveolens, Arachis hypogaea, Arctium lappa, Asclepias curassavica, Asplenium trichomanes, Avena sativa, Bacopa monniera,
Bryophyllum calycinum, Caesalpinia crista, Calendula officinalis, Calotropis procera, Carum carvi, Capsella bursa-pastoris, Carthamus tinctorius, Chenopodium album and Date palm, affected the physiology of male reproductive system.

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. Any substance that interferes with the function of the Leydig cell which responsible for steroidogenesis, will produce hormonal disturbances. There are also many other potential sites for interference with sex-hormone balance, including interference with androgen or gonadotropin receptor binding, alteration in circulating gonadotropin levels, and alterations in the metabolism or clearance of androgens.\(^2\)

The epididymis is frequently overlooked as a potential site for toxicity. It is much more than a transport conduit for sperm, and it has a number of complex functions which are affected by changing of its structure throughout its length.\(^3\)\(^-\)\(^4\)

The main problem in cancer chemotherapy is the lack of selectivity toxicity. They killed many rapidly dividing normal cells (bone marrow, gut epithelium, spermatogenic cells, lymphoid tissue, ovarian and uterine tissues and fetus. Many anticancer drugs such as cyclophosphomide, bleomycin, etoposide, cisplatin, camptothecin, doxorubicin, trimetrexate, methotrexate and many other cytotoxic drugs induced male reproductive toxicity.\(^41\)\(^-\)\(^45\)

This study was designed to investigate the male reproductive toxicity of bleomycin and the efficacy of valsartan to reverse this toxicity.

**MATERIALS AND METHODS**

Forty male Wistar rats (12 week old) were used in this study. They were housed in a well-ventilated animal house, at a temperature of 22±2°C and exposed to 12 h light and 12 h dark/day, standard food 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 bleomycin 15mg/kg /i.p. three times weekly for 8 weeks. The third group was received valsartan 10mg/kg/ day by gavage for 8 weeks. While the fourth group was given a combination of bleomycin and valsartan at the same mentioned doses and for the same period. The model, dose levels and schedule were
based on previous studies. At the end of the treatment period, all animal were killed by neck dislocation after light anesthesia. Blood samples were taken for hormonal analysis. Epididymis were taken for sperm count and testes were taken for histological study. Serum testosterone, LH and FSH levels were determined by Enzyme Linked Immunosorbent Assay kits. The epididymal sperm suspension is prepared in 1 ml of phosphate buffered saline (PBS) at pH 7.2. The sperm count was determined in a hemocytometer. An aliquot from the suspension (1 ml) was diluted 1:40 with PBS. A sample of the diluted suspension is charged into a counting chamber (Neubauer's chamber). The total sperm count in eight squares (Except the central erythrocyte area) of 1 mm² each was determined and multiplied by $5 \times 10^4$ to get the total count. The testes were removed and fixed in Bouin's fluid for 24 h. After excessive washing in 70% alcohol, the tissue was processed for paraffin embedding and 5 μ thick paraffin sections were stained with hematoxylin and eosin.

Results were analyzed by one-way Analysis of Variance (ANOVA). Values of $P<0.05$ were considered statistically significant.

**RESULTS**

As shown in table 1, bleomycin was significantly decreased serum testosterone level ($p<0.01$) and relative sperm content of epididymis (sperm/ mg of epididymis head) ($<0.001$) in comparison with the control group. However, bleomycin didn’t induced significant changes in the serum levels of LH and FSH. On the other hand, valsartan didn’t induced significant changes in all parameters when used alone in comparison with control, but when valsartan used in combination with bleomycin, it attenuated the adverse changes on the testosterone level and relative sperm content of epididymis (sperm/ mg of epididymis head), but it didn’t bring them to the control limits.

**Table 1: Effect of bleomycin on serum testosterone, LH, FSH and relative sperm content of epididymis (sperm/ mg of epididymis head) and the attenuating effect of valsartan.**

<table>
<thead>
<tr>
<th>groups</th>
<th>Testosterone nmol/l</th>
<th>LH MIU/ml</th>
<th>FSH MIU/ml</th>
<th>Relative sperm content of epididymis (sperm/ mg of epididymis head)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.8± 0.08</td>
<td>5.3± 1.1</td>
<td>5.6±1.0</td>
<td>99.32 ± 2.45</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>1.37± 0.02 $p&lt;0.01$</td>
<td>5.0±0.9 NS</td>
<td>5.4±1.2 NS</td>
<td>72.56 ± 3.24 $p&lt;0.001$</td>
</tr>
<tr>
<td>Valsartan</td>
<td>2.6± 0.09 NS</td>
<td>4.92±1.3 NS</td>
<td>5.2±1.4 NS</td>
<td>98.82 ± 3.22 NS</td>
</tr>
<tr>
<td>Bleomycin and valsartan</td>
<td>2.2± 0.11 $p&lt;0.05$</td>
<td>5.14±1.2 NS</td>
<td>5.5±1.6 NS</td>
<td>88.63 ± 2.87 $p&lt;0.05$</td>
</tr>
</tbody>
</table>

All groups were compared with the control group, NS: not significant
Histological study showed that testis sections 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). Valsartan treated group showed no structural changes (C), but using of valsartan with bleomycin ameliorates all the structural changes in the testis appeared in bleomycin treated group (D).

**DISCUSSION**

Rat and mouse models are models mimicking human being response to cytotoxic drugs. Our results showed that bleomycin decrease sperm content of epidydimis. Germ cells, in
particular, differentiating spermatozoa are extremely susceptible to cytotoxic agents because of their rapid proliferation. However, leydig and sertoli cells resist the toxicity of cytotoxic drugs, but could suffer functional damages.\textsuperscript{[54]}

The study showed that bleomycin didn’t affect ganadotropine secretion, which clearly indicated that the high specialized neural and endocrine cells resist the cytotoxicity of anticancer drugs. However, it appeared that bleomycin induced direct testicular effects represented by degeneration of interstitial (leydig) cell which subsequently reflected by decline of serum testosterone level. This effect participated in decreasing sperm content of epihydmis because the spermatogenesis from spermatogonium to spermatid was control by testosterone.\textsuperscript{[53, 55]}

Anticancers were known as a male reproductive tract toxicant. In anticancer treated rats, a decrease in sperm quality was associated with increased DNA damage and decreased chromatin quality. It appeared that anticancers toxic effects on androgenesis and spermatogenesis is mediated by free radicals. Many studies showed that antioxidants can protect from the reproductive toxicity of anticancers.\textsuperscript{[56]}

Accordingly, antioxidant can protect from the male reproductive toxicity of anticancers such as cisplatin and doxorubicin. The selenium nano-particles (Nano-Se) as an established strong antioxidant with more bioavailability and less toxicity was 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.\textsuperscript{[56-58]}

The protective effect of valsartan could be attributed to its antioxidant effect. The pro-inflammatory factors including endoplasmic reticulum (ER) stress chaperones and inhibitory κBβ (IkBβ) contribute to testis damage in hypogonadism in rats produced by a high-fat diet (HFD) and low dose streptozotocin (STZ). These effects were attenuated by valsartan. On the other hand, Leydig cells cultured with high glucose showed upregulated IkBβ, ER stress sensor PERK (PKR-like ER kinase) and p-Akt/Akt in vitro. These changes which occur due to a component of inflammation linked to activated NADPH oxidase, can be significantly alleviated by valsartan.\textsuperscript{[59]}

According to the results of this study, we can concluded that valsartan can attenuated the male reproductive toxicity of bleomycin.
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