The Effect of Busulfan on Body Weight, Testis Weight and MDA Enzymes in Male Rats

Nasibeh Hosseini Ahar1, Arash Khaki2*, Ghasem Akbari3, Marefat Ghaffari Novin4

Abstract
Objectives: Busulfan is one of the anti-cancer drugs, despite of the tremendous usefulness in biotechnology and therapy of chronic diseases such as leukemia, lymphoma, ovarian cancer and it can lead to impaired spermatogenesis. The aim of this study was to seen body and testicular weight and serum Malondialdehyde (MDA) enzyme in rat was performed following the use of busulfan.

Material and Methods: This study was conducted on 20 adult male rats ranging in age from 6 to 8 weeks. Animals in the two group includes a control group and the experimental group that received 50 mg/kg busulfan as a single intra peritoneal injection and after 8 weeks, body and testis weight and serum MDA levels were measured. Then Data were analyzed with SPSS 16. P<0.05 was considered significant, and results were compared between the two groups.

Results: single dose of busulfan, induced its effects on body, testis weights and serum MDA levels. Body weight in experimental and control groups, was respectively, 297.40 gr and 301.00 gr. Body weight of rats in the experimental group than the control group was decreased, but the difference was not significant (P>0.05). Testicular weight in both control and experimental groups, respectively, were 1.45 gr and gr 0.960. Difference between the two groups was significant decreased (P>0.05). Comparison of serum MDA of control and experimental groups, were showed 3.81±1.5 and 6.9±1.1 nmol/lit. No difference between the two groups was significant increased (P>0.05).

Conclusion: It can be concluded that the use of busulfan can reduced body weight and testicular weight, and increased serum MDA and could be side effects in reproduction process.

Keywords: Busulfan, Serum MDA, Testis Weight, Body Weight

Introduction
Several factors have effect on very sensitive and complex process named spermatogenesis, and Lead to infertility or reduced fertility (1). One of the most important factors affecting spermatogenesis is chemotherapy that by creating adverse effects on the cell division process will eventually lead to azoospermia (2). Busulfan is alkylating chemotherapeutic agents that used in order to treat chronic leukemia, ovarian cancer, lymphoma and Milo Proliferative Disorders and also used before bone marrow transplantation in cancer patients (3). It is a class of medications called alkylating agents.
This drug with non-specific binding to DNA strand, acts on the cell cycle and inhibit DNA activity. In fact, its cytotoxic effect is by interfering in DNA replication and RNA transcription (4). After one or two intraperitoneal injection, it can destroy high amount of spermatogenesis (5).
Busulfan has side effects on various organs including the liver, skin, bladder, nervous system and gonadal function, and is potentially carcinogenic and mutagenic (6). Despite of the tremendous usefulness in biotechnology and therapy of chronic diseases, its exact effects on the testis structure and parameters of epididymal-spermatozoa has not yet been well studied. Thus, the present study examined body weight, testicular weight and serum MDA levels in rats was performed following the use of busulfan.

Material and Methods

Animals
Twenty Wistar male rats ranging in age from 6 to 8 weeks (200±20 g) were used. Animals Were randomly allocated into two groups, control group (n=10) and experimental group (n=10) that received a single intraperitoneal injection of busulfan (50 mg/kg body weight) diluted in sesame oil as previously described (19,20). animals were housed in wire cages at 22±1 °C under a 12-h light-dark cycle with 70% humidity and fed a standard diet and water. Animals were maintained and experiments were conducted in accordance with the Principles of Laboratory Animal Care of Tabriz University of Medical Sciences, Iran.

Drugs preparation
Preparation of busulfan solution: Busulfan (Sigma, USA) was first dissolved in DMSO (dimethyl sulfoxide), (Sigma,
USA), then an equal volume of sterile water was added to obtain a final busulfan concentration of 5 mg/ml.

**Surgical procedure**
At the end of the treatment period, (56th day) the rats were weighed and anesthetized by using intraperitoneal Pentobarbital sodium (40 mg/kg), then killed; the peritoneal cavity was opened through a lower transverse abdominal incision. As well as, testes in control and experimental groups were immediately removed and fixed by perfusion with 4% formaldehyde in buffered solution for 20 minutes and after wards their left testes were taken out and weighed. (A&D GF600, Germany)

**Measurement of MDA**
Before perfusion, in order to measure the plasma MDA levels, blood samples were immediately collected by cardiac puncture and the plasma separated from the blood cells by centrifugation (2500 rpm for 30 min). All blood samples were then immediately stored at -20 °C until further analyses. MDA levels were determined with TBA assay, according to the method as described by Rao et al. (7).

**Data analysis**
All experimental data are presented as means±SD. Each experiment was performed at least three times and subjected to statistical analysis. Representative experiments are presented in the Figure. For statistical analysis, analysis of variance (Mann–Whitney) was performed to determine whether there were differences among two groups (P<0.05). A P value less than 0.05 was considered significant.

**Results**

**Body and testis weight**
The obtained results in this study were illustrated in Table 1. There was no significant difference in body weights between two groups (P<0.69). Testis weight was reduced significantly in experimental group in comparison with the control group (P<0.008; Figures 1 and 2).

**MDA Assay**
MDA levels of plasma of rats in experimental group were significantly higher than in control group (P<0.032; Table 1; Figure 3).

**Discussion**
The administration of busulfan to male patients with malignant diseases may cause temporary or permanent sterility (8). Estimation of testis parameters such as body and testis weight and MDA levels of plasma by stereological methods lead to better evaluation of the spermatogenesis process.

In the present study, we have determined the extent of changes in mouse testis weight, body weight and MDA levels following Busulfan administration. Male rats were subdivided into two groups, control and experimental group. The chosen period of time (6 and 8 weeks) provided sufficient time to monitor the changes of spermatogenesis factor in testes in busulfan treated animals. Also, according to Nagano et al. (9) experimental group receiving Busulfan showed decreased in body weight, testicular weight and increased adjusting blood MDA enzyme in comparison to control group. This result could be due to cytotoxic effects of Busulfan with Transferring the alkyl group(s) to various
Table 1. Body weight, Testis weight and MDA levels of control and experimental groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Body weight (g)</th>
<th>Testis weight (g)</th>
<th>MDA level (nm/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>301±22.47</td>
<td>1.45±0.1</td>
<td>3.81±1.5</td>
</tr>
<tr>
<td>Experimental</td>
<td>5</td>
<td>297.4±7.98</td>
<td>0.96±0.07*</td>
<td>6.9±1.1*</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE.

*Significant different at P<0.05 level (compared with the control group).

cellular. However, DNA alkylation events may constitute major incitements leading to cell death (10). There are several types of chemotherapeutic drugs which show cell damage stage-specific cytotoxicity to spermatogonia (11). Busulfan partially eliminates stem cells because of its alkylating nature (12) and kills cells by producing free radicals (13). Therefore, it seems busulfan inhibit the spermatogenesis process, especially by oxidative damage. Other mechanism suggested that busulfan increased the level of ck18, a surface marker on sertoli cell. The elevation of this marker caused spermatogenesis disorder and infertility (14). In this study, busulfan decreases body weight and testes weight. Zheng Wei et al. showed that there was a direct relationship between testis weight and germinal cells number in primates (15). Bucci et al. showed that busulfan caused chromosomal abnormalities and dominant lethal mutations in sperm (16).

This study also showed that busulfan increased MDA levels of plasma. Malondialdehyde can be used as a marker of oxidative stress and a potential marker for predicting assisted reproductive techniques (ART) outcomes (17). Hosseinzadeh Colagar et al. in 2013 observed MDA concentration in oligozoospermic and azoospermic men was significantly higher than normozoospermic. And represented evaluation of plasma MDA could be beneficial diagnostic tool for defining sperm fertilization potential (18). Therefore, it is suggested that, these parameters (MDA level, body weight, and testis weight) could help in distinction and treatment of male infertility especially in idiopathic cases.

Conclusion
The present study provides detailed information on the effects of busulfan on testis weight, body weight and MDA levels. The information has the potential to modify and improve the administration of busulfan to increase its efficiency to make a reliable infertile recipient animal for Future studies, in one hand, and also to decrease its numerous side-effects in the clinical application of the drug, on the other hand.

Ethical issues
The local ethics committee approved the study.

Conflict of interests
Authors declare that there is no any conflict of interests.

Acknowledgments
This research was supported by Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. The code of this research protocol was 9217.

References
12. Ray S. Evaluations of antiperoxidative potential of ascorbic acid on busulfan-induced lipid peroxidation


