ASCORBIC ACID;
PROTECTIVE EFFECT OF ASCORBIC ACID ON ANTICANCER INDUCED CHANGE IN VOLUME OF TESTES

Aisha Abdul Haq¹, Soofia Nigar², Sarwat Jabeen³

ABSTRACT... Objectives: To demonstrate the volume changes that are produced by anthracycline Doxorubicin DOX on the testes of mice and designed to study volumetric changes in DOX affected testes with co-administration of antioxidant Vitamin C. Design: An experimental study. Setting: Institute of Basic Medical Sciences. Dow University of Health Sciences. Period: September 2011 to May 2012. Methods: Experimental study using thirty male mice of five weeks old were separated into 3 groups (A, B & C). Each group had ten mice and was treated with normal saline 1 ml intraperitoneal (IP) in group A, DOX alone (0.003 mg in 0.03ml /gm body weight IP for 3 doses on 6th, 8th and 10th day of study) in group B and DOX (0.003 mg in 0.03ml /gm body weight IP for 3 doses on 6th, 8th and 10th day of study) + Vitamin C (0.5 mg in 0.01ml/gm body weight per orumdaily) in group C. After completion of study, animals were sacrificed and the testes were kept in Bouin’s fluid and volume was measured. Results: By using SPSSS version 16, statistical analysis was done, using ANOVA test to evaluate the significance of concerned parameter among different groups studied. The administration of DOX induced significant reduction (P <0.001) of volume of testes when compared to controls. However co-administration of Vitamin C with DOX significantly increase (P <0.001) the volume as compared to DOX group. Conclusion: This study suggested that the antioxidant Vitamin C has a significant role in ameliorating the damage of testes induced by DOX, showing improvement in the morphology and morphometry of testes.

Key words: Doxorubicin, Vitamin C, Mice, Antioxidant, Oxidative Damage, Sperms.

INTRODUCTION
Anthracyclines are very efficient chemotherapeutic agents, derivatives of streptomycespeucetius a pigment producing bacteria. Among them Doxorubicin (DOX) is widely used quinone containing antitumor antibiotic drug from this family for broad range of neoplasms. It acts by intercalating within DNA double helix between nucleotide base pairs thereby inhibiting DNA and RNA polymerization. Pathogenic mechanism of this chemical proves to be the formation of reactive oxygen species, mediators of tissue injury.

Doxorubicin is broadly used to treat Hodgkin disease, childhood leukemias, neoplasms of lung, breast, thyroid, gallbladder, testis and others. Ideally a chemotherapeutic agent should selectively cause insult to tumour cells without having any toxic effect on healthy tissue but this is unfortunately not the case.

Use of doxorubicin in clinical practice is hindered by emergence of wide variety of its side effects on non targeted tissues leading to organopathies and causes toxicity to both somatic and germ cell in reproductive tissue resulting in testicular atrophy and aspermatogenesis. Testes are paired male reproductive organ in scrotal sac for production of spermatozoa, histologically consist of seminiferous tubules and interstitial spaces having leydig cells. Tubules have two types of cell, sertoli and germ cells.

On the basis of the fact that many adverse effects of DOX occur through the formation of free radicals, anti oxidant therapies have been considered and have proven to be effective in assuaging...
effects of DOX on testis. The potential role of dietary antioxidants as tocopherol, ascorbic acid etc to reduce the activity of free radical-induced reactions has drawn increasing attention. Despite the fact that chemotherapy-induced formation of free radicals is well demonstrated, both in vitro and animal studies have shown that the co-administration of antioxidants did not reduce the antitumor effect of cytotoxic agents such as Doxorubicin. Furthermore, the survivals of animals co-administered with antioxidants were increased compared to the survival of animals that received chemotherapy alone. Common and easily available natural antioxidant is the water soluble glucose derivative Vitamin C (C₆H₈O₆). It is a well known antioxidant and a known free radical scavenger, present abundantly in tropical diet rich in citrus fruits and vegetables. Vitamin C has been reported to be effective as a protectant against oxidative damage caused by various compounds. Aim is to investigate the effects of chemotherapeutic agent Doxorubicin and the potential antioxidant properties of Vitamin C when acting in combination with Doxorubicin in the mammalian somatic and germ cell. Important goal is to reduce the cytotoxic effects of Doxorubicin to non targeted tissues. Oxidative damage to the testicular germinal epithelium is a potential side effect of cancer therapy and is of particular concern in case of men of reproductive age, having cancers with high cure rates; therefore present study is designed to evaluate the protective role of Vitamin C as an antioxidant on DOX induced testicular damage in mice by using volume analysis. It is thereby beneficial in decreasing the incidence of reproductive side effects from chemotherapy in our clinical setup.

Prognosis of survival may be improved by antioxidants through improving the therapeutic index of co-administered chemotherapeutic drugs, i.e. enhancing the capability to tolerate high doses of chemotherapy with uninterrupted treatment schedules.

METHODS
This study was carried at the Institute of Basic Medical Sciences (IBMS), Dow University of Health Sciences (DUHS) Karachi with collaboration of animal house and with assistance of Dow diagnostic research and reference lab (DDRRL) DUHS from September 2011 to May 2012.

The mice used in the experiment were acquired from Charles River Breeding Laboratories, Brooklyn, Massachusetts, USA. Their cross breeding was done at the animal house of OJHA campus, DUHS Karachi. The animals were separately held in tagged plastic cages in an experimental room of animal house, where they were provided the balanced laboratory diet.

Thirty, healthy male NMRI mice of 5 ± 1 week old were obtained from animal house of OJHA campus, DUHS. They were housed in tagged cages with five mice in each cage, under normal circadian rhythm of 12 hours light and 12 hours dark with unrestricted food and water ad libitum.

Grouping Protocol
The animals were divided into A, B and C groups. Each group had 10 animals.

Group A (Control):
Control group contained 10 male mice and received normal saline 1 ml IP on 6th, 8th and 10th day of the study.

Group B (DOX):
Group B had 10 animals and received DOX in dose of 0.003 mg/gm or 0.003 mg in 0.03 ml/gm body weight IP, up to 3 doses on 6th, 8th and 10th day of study (total cumulated dose 0.009 mg/gm).

Group C: (DOX + Vitamin C)
Group C comprised of 10 animals and received DOX in a dose of 0.003 mg/gm or 0.003 mg in 0.03 ml/gm body weight IP and Vitamin C in dose of 0.5 mg/gm or 0.5 mg in 0.01ml/gm body weight P.O. DOX on 6th, 8th and 10th day of experiment and Vitamin C was given daily.

After dissection and identification, testes were freed from surrounding tissues, the dimensions for volume were measured with vernier caliper and formula \(\text{Volume} = \frac{4}{3} \times \pi \times \text{length} \times \text{breadth} \times \text{depth} \times \frac{1}{2} \) (cubic cm) was then used to estimate the volume of testes. After fixation in Bouin’s fluid
for 24 hours, longitudinal sections of testes were taken and were placed in properly marked and labelled cassettes for further processing. After passing through running water they were kept in solution of lithium carbonate in 70% alcohol to wash out extra fixative. Later on tissues were dehydrated using strength of alcohol from 70 to 100%. After dehydration and infiltration, embedding with paraffin was performed and tissue blocks were made.

**RESULTS**

Comparison of volume of testes between the control group (A) and DOX group (B).

The mean ± S.D of volume in control group was 0.0087 ± 0.0041 mg and in DOX group was 0.00063 ± 0.00029 cm³. The comparison of mean volume of these two groups showed the P value 0.001 at 95% C.I as shown in table and graph.

The significant decrease in testicular volume was observed in the DOX group.

Comparison of volume of testes between the DOX group (B) and DOX + Vitamin C group (C). The mean ± S.D of volume in DOX group was 0.00063 ± 0.00029 cm³ and in DOX + Vitamin C group was 0.0060 ± 0.0059 cm³. The comparison of mean volume of these two groups showed the P value 0.019 at 95% C.I as shown in table and graph.

The significant increase in testicular volume was observed in the DOX + Vitamin C group.

Comparison of volume of testes between the control group (A) and DOX + Vitamin C group (C). The mean ± S.D of volume in control group was 0.0087 ± 0.0041 cm³ and in DOX + Vitamin C group was 0.0060 ± 0.0059 cm³. The comparison of mean volume of these two groups showed the P value 0.332 at 95% C.I as shown in table and graph.

A non significant decrease in testicular volume was observed in the DOX + Vitamin C group.

Table Comparison of Mean ± SD of volume (cm³) of testes in different groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
<th>P value among groups</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (A)</td>
<td>0.0087 ± 0.0041</td>
<td>A vs B &lt; 0.001*</td>
<td>0.0034 0.0127</td>
</tr>
<tr>
<td>DOX (B)</td>
<td>0.00063 ± 0.00029</td>
<td>B vs C 0.019*</td>
<td>0.0007 0.0100</td>
</tr>
<tr>
<td>DOX + Vitamin C (C)</td>
<td>0.0060 ± 0.0059</td>
<td>A vs C 0.332*</td>
<td>-0.0019 0.0073</td>
</tr>
</tbody>
</table>

* Tukey HSD Post Hoc Multiple comparisons test

**DISCUSSION**

Most of the drugs for cancer therapy are known to cause toxic side effects in various viscera including testis. A plan of action to lessen the side effects of anticancer drugs with retaining their chemotherapeutic efficacy is indispensable. Scanty numbers of studies are available on the role of Vitamin C as an antioxidant on the effects of DOX and the interpretable data is sparse.

The volume of testes in DOX group, when compared to the control group showed the P value of < 0.001 whereas in the DOX group this P value turned out to be 0.019 when DOX group was compared with DOX + Vitamin C treated group. This P value was 0.332 between control and DOX + Vitamin C group. This indicated the significant
change of volume in DOX and non significant change in DOX + Vitamin C group when both the groups were in comparison to the control group.

The decrease in testicular volume suggests damage of the testis, is also associated with maturation arrest. In addition, parameters such as tubular diameter, seminiferous epithelium thickness, number of tubules and arrangement of germinal cells can also give information about the testicular damage. Decrease in testicular volume in DOX group in current study was also observed by Salu in 2009. Under light microscope general damage in testes treated with DOX was seen. A significant reduction of the number of tubules occurred in this group in present study. Similar findings were seen in studies in 2009, indicating atrophic changes in tubules of testis and showing replacement of tubules by interstitial cells.

The precise mechanism of its protective effect on testes needs to be further explored; however, because Vitamin C did not seem to affect the antitumor effect of DOX, the combined treatment of DOX and Vitamin C holds an assurance as a safe and effective chemotherapeutic regimen.

CONCLUSION
Since DOX is in demand for treatment of various cancers, the resultant damage induced by drug is unavoidable. Therefore our results raise the hope that co-administration of Vitamin C with DOX may be a reassuring answer to the very serious side effects of DOX in patients undergoing chemotherapy.

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REFERENCES


Kindness is the language which the deaf can hear and the blind can see.

– Mark Twain –