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ORIGINAL ARTICLE

Testicular Fibrosis, A Setback of Chemotherapy and Ameliorating Role of Ascorbic Acid

AISHA ABDUL HAQ[1], SADIA IQBAL[2], UZMA HAMEED[3], AISHA HASSAN BROHI[4], AMNA MUGHAL[5], DURESHAWAR REHMAN[6]
[1]*Associate Professor Anatomy Department Dow Medical College DUHS, Karachi, Pakistan*
[2]*Assistant Professor Anatomy Department Dow Medical College DUHS, Karachi, Pakistan*
[3]*Assistant Professor DIDC DUHS, Karachi, Pakistan*
[4,5]*Assistant Professor Anatomy Department Dow Medical College DUHS, Karachi, Pakistan*
[6]*Lecturer, King Saud bin Abdul-Aziz University for Health Sciences, Riyadh, KSA,*
Corresponding author: Aisha Abdul Haq, Email: aisha.haq@duhs.edu.pk, Cell: +92 335-2333326

ABSTRACT

Statement of Problem: The struggle for surviving cancer individuals who have undergone chemotherapy is still a dilemma. Chemotherapeutic agent, Doxorubicin (DOX), causing testicular damage, has been a concern of oncologists for many years. As DOX causes oxidative damage on non-tumorous tissues by the foundation of free radicals, the challenges of protection with various antioxidant therapies have been attempted. The foremost objective was to reduce the cytotoxic properties of DOX to healthy cells. Vitamin C, a water-soluble vitamin, based on its antioxidant property, was applied to study its potential protection to the testicular tissues after oxidative damage by DOX.

Objectives: To demonstrate fibrotic changes in the interstitium of testes generated by anthracycline DOX on the mice and designed to study the morphometric changes in DOX affected testes with co-administration of antioxidant Vitamin C.

Methodolgy: An Experimental study using male mice were separated into 3 groups (A, B & C). A was treated with normal saline 1 ml intraperitoneal (IP), B was given DOX alone (0.003 mg in 0.03ml /gm body weight IP for 3 doses on 6th, 8th and 10th day of study) 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 orum daily) was delivered to group C. Animals were sacrificed on completion and organs were kept in Bouin's fluid and later processing and staining of the tissue was done.

Result: Statistical analysis was performed, using ANOVA test to evaluate the significance of parameter among three groups. Widening of interstitium with significant interstitial fibrosis and intra tubular empty spaces were evident as compared to controls. However, co-administration of Vitamin C with DOX significantly reduced ($P < 0.001$) fibrosis indicating protective role of Vitamin C against DOX induced testicular damage.

Conclusion: Experimental study suggested that the antioxidant Vitamin C has a potential role in improving the parameter damaged by DOX.

Keywords: Doxorubicin, Vitamin C, Mice, Antioxidant, Oxidative damage, Interstitial, Fibrosis

INTRODUCTION

Chemotherapy treatment is a Foundation of anticancer regimens, considerably adding to the recent increase in childhood cancer survival rates. This Established cancer therapy targets not only malignant but also normal healthy cells resulting in side effects including infertility.

DOX appears to be the family of anthracyclines, which are the best potent anti-cancerous treatment ever made. This class of anthracycline was basically formed from Streptomyces peucetius in 1960, a pigment generating Streptomyces. Even with advances in oncology, improved anthracyclines are yet to be produced consequently DOX is still in need in preclinical and clinical research; with the aim of discovering better options for its best efficacy in patients with tumour.[1,2.]

The target of treatment with DOX is not what was thought because of its adverse effect on non-targeted tissue like testes. It ensued not only in the damage of sperms cell, but also in its diminished making.[3] Its administration leads to reduction in testicular weight, reduced sperms count and germ cells apoptosis. Reduced testicular weight expresses atrophic changes in the seminiferous tubules.[4]

Extreme drop in diameter of seminiferous tubules with sloughing, vacuolation, decrease sperms count, widening of interstitium and atrophy were manifested by other studies.[5,6] Increased number and size of interstitial Leydig cells were observed as a compensatory comeback to degenerated epithelium.[7]

Insufficient studies have been done to display reversing effect of antioxidant on DOX induced oxidative damage in testis. The anti-cancer action of DOX is due to its competence to interpose in double helix of DNA. DOX intercalated in DNA in the nucleus of tumor cell, cannot be reduced and only reduced form of DOX, that is semiquinone leads to free radical formation.[8]

Clinical trial by Prasad (2002) [9] on patients with advanced non-small cell carcinoma of lung, revealed 33% survival rate for one year with chemotherapy alone while it was up to 54% in those who took vitamin A, E and C along with the chemotherapy.[9]

Vitamin C (Ascorbic acid) fits in to the free radical scavenging class of antioxidants. It is water soluble and rescues the oxidative damage to the tissues by lowering free radicals. [10,11]

It has greater effects than other antioxidants and stimulates the growth of collagen. This improves in walling off the tumour.[12]

Noticeable reduction in levels of numerous antioxidants comprising Vitamin C was perceived with the treatment of DOX in breast cancer and thus increasing the sensitivity of healthy tissues to radical damage, thus indicating the need to use antioxidants along with chemotherapy.[13]

Potential of Vitamin C in clinical use is evident by many studies. Its advantage to health may be ascribed not only to its antioxidant property but also to its anti-atherogenic and immunomodulator activity.[14]

Rationale was to show that Vitamin C as an antioxidant could be a modest and cost-effective treatment to enhance therapeutic effectiveness and to lower lethal side effects of DOX in clinical chemotherapy.

METHODS AND MATERIAL

Animals: Thirty NMRI mice of 5 ± 1 week old from animal house of OJHA were housed in tagged cages with unrestricted food and water ad libitum.

Chemicals: In this experiment drugs used include Doxorubicin hydrochloride, Vitamin C and normal saline.

Grouping protocol: The animals were divided into A, B and C groups with ten mice each.

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

Group B (DOX): Group B received DOX in dose of 0.003 mg/g or 0.003 mg in 0.03 ml /gm body weight IP, [15] 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 received DOX in a dose of 0.003 mg/gm or 0.003 mg in 0.03 ml /gm body weight IP [15]and

Vitamin C in dose of 0.5 mg/gm or 0.5 mg in 0.01ml/gm body weight P.O.[16] DOX on 6th, 8th and 10th day of experiment and Vitamin C was given daily. Animals were anaesthetized by deep ether anesthesia and dissected through midline abdominal incision.

Histological processing of testicular tissue: After fixation longitudinal sections of testes were taken for further processing. Later on tissue blocks were made. Five µm thick sections were obtained from rotary microtome and were mounted on marked glass slides.

Slides stained with Masson's trichome were used for observation of interstitial space for fibrosis under 40x objective and 10x ocular lenses.

The sections were viewed and photographed by using Nikon light microscope (Nikon eclipse 0121824, 50 i, Japan) with an attached photograph machine (Nikon digital sight DS-L1, 218299, Japan)

All information was analyzed by SPSS software version 16. To find the statistical difference between the groups, One Way Analysis of Variance (ANOVA) or Kruskal Wallis test was used. In case of significant result, Tukey – multiple Comparisons post Hoc test was applied to check the pair wise comparison at 5% level of significance (95% confidence interval C.I).

RESULT

Interstitial Space: On special staining (Masson's trichrome), slides were observed for fibrosis and comparison was done between control and drugs administered groups. In control, less widening of interstitial space with no fibrosis between tubules was seen. Widening of interstitial spaces with fibrosis was seen in animals exposed with DOX. Improvement was seen with co-administration of Vitamin C with DOX as shown in figure 1-3.

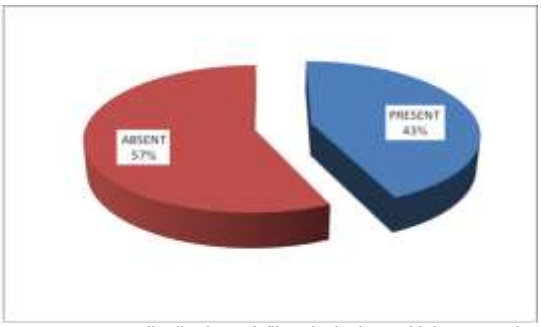
Presence of fibrosis in interstitial space: More than fifty percent of mice (57%) have no fibrosis in their interstitial space whereas 43 % have fibrosis in interstitial space as shown in graph-1.

Comparison of fibrosis between the control group (A) and DOX group (B): The mean frequency of fibrosis in interstitial space in the control group was 0% and in DOX group was 100%. Fibrosis in interstitial spaces of DOX group was seen in all animals in this group (100%) as shown in table-1, figures 1 and 2.

Comparison of fibrosis between the DOX group (B) and DOX + Vitamin C group (C): The mean frequency of fibrosis in interstitial space in DOX group was 100% and in DOX + Vitamin C group was 30%. Absence of fibrosis in interstitial spaces of DOX + Vitamin C group was seen in 70% of animals, as shown in table-1, figures 2 and 3.

Table-1: Frequency distribution of fibrosis in interstitial spaces of testes in different groups

Groups	Control (A)		DOX (B)		DOX + Vitamin C (C)	
Fibrosis in interstitial spaces	n	%	n	%	n	%
Absent	10	100	0	0	7	70
Present	0	0	10	100	3	30
n = number of mice						



Graph-1: Percentage distribution of fibrosis in interstitial spaces between seminiferous tubules of teste

Comparison of presence of fibrosis between the control group (A) and DOX+ Vitamin C group (C): The mean frequency of fibrosis in interstitial space in control group was 0 % and in DOX + Vitamin C group was 30%. Fibrosis in interstitial spaces of DOX + Vitamin C group was seen in 30% of animals, as shown in table-1, figures 1 and 3.

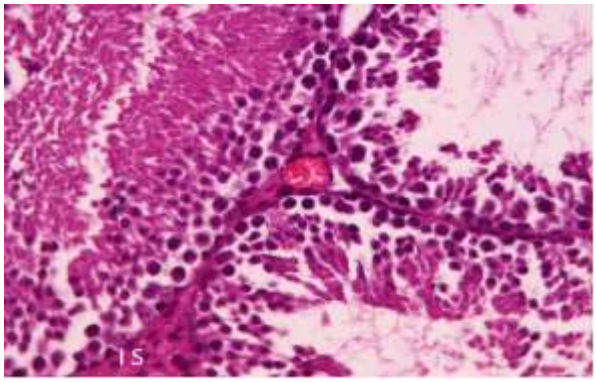


Figure-1: Photomicrograph of Masson's trichome stained 5 µm thick section of testis showing interstitial space IS with no fibrosis in control mouse at x400.

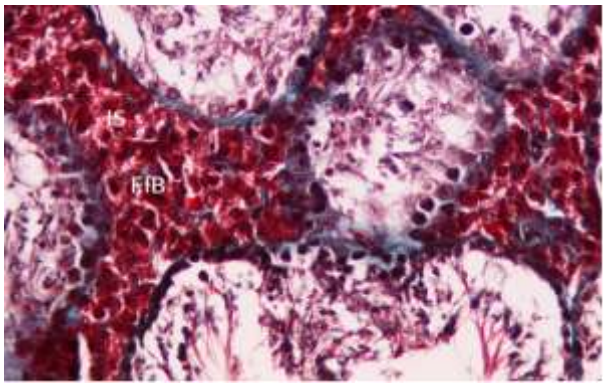


Figure-2: Photomicrograph of Masson's trichome stained 5 µm thick section of testis showing interstitial spaces IS with fibrosis FIB in DOX exposed mouse at x400.

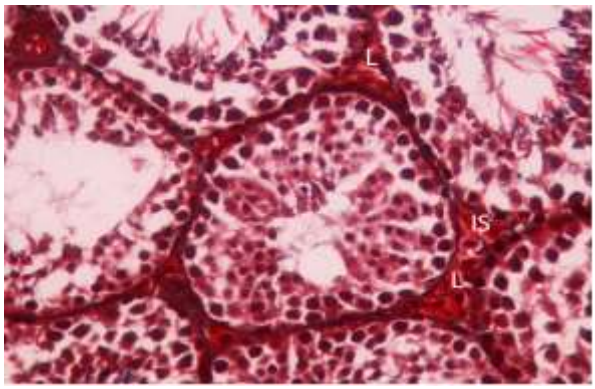


Figure-3: Photomicrograph of Masson's trichome stained 5 µm thick section of testis showing interstitial space IS with no fibrosis in mouse given DOX + Vitamin C at x400.

DISCUSSION

For fibrosis in interstitial space, mean frequencies were 0%, 100% and 30% in control, DOX group and DOX + Vitamin C group respectively.

Fibrosis was seen to fill the interstitial spaces of animals treated with DOX; this is in agreement with findings by kang in

2002, who observed disappearance of almost all germinal cells in seminiferous tubules with reduction of the diameter of tubules and their replacement by fibrinoid debris in interstitial space. [17]

Decreased levels of vitamin C seem to occur in several diseases which are associated with increased oxidative damage, like diabetes mellitus and cancer.

In reproductive system of male, Vitamin C has vital role as far as spermatogenesis is considered.[18] Its deficiency results in disturbance of spermatogenesis; thus, its defensive action against oxidative damage imparts a crucial role in maintaining spermatogenesis.[19]

A distinctive reduction was noted in seminiferous epithelial damage in the mice treated with Vitamin C + DOX, in comparison to those solely receiving DOX. Previous research indicated protective activity of Vitamin C due to its antioxidant action. On the other hand, absence of fibrosis was also noted.

This confirmed the protective role of Vitamin C against compounds that caused damage by free radical formation.[20] Indeed, Vitamin C seemed to reduce the damage caused by the DOX treatment and this can be confirmed when the present data is compared to the sperm's parameters obtained from studies using other anthracycline and drugs with the same mechanism of action as in DOX.[16,21.]

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.[21, 22]

No detailed morphological studies have been done, regarding the possible protection carried by Vitamin C to the integrity of seminiferous tubules affected by DOX, opening new grounds for further research.

CONCLUSION

In conclusion, the study provides a substantial proof that DOX chemotherapy damaged the testicular tissues and significantly produces fibrosis in interstitial tissue while Vitamin C co-administration effectively improved the deteriorated condition of testes by using chemotherapy.

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