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Effect of Cyclophosphamide on Micro Anatomy of Testes of Male Albino Rats

Javed Ahmad Khan	Assistant Professor,Department of Anatomy, Govt. Medical College Srinagar,J&K,India.
Suhail Ahmad Gilkar	Assistant Professor,Department of Physiology, Govt. Medical College Srinagar,J&K,India.* Corresponding Author
Showkat Ahmad	Post graduate student,Department of Anatomy, Govt. Medical College Srinagar,J&K,India.

ABSTRACT

Cyclophosphamide an alkylating agent widely used in the treatment of various neoplastic diseases and diseases associated with immune disorders. It is known to cause many side effects and the target organs being liver, lungs, heart and reproductive organs. The present study was aimed to see the effects of this drug in the testes of male albino rats. After approval from the animal ethical committee, Govt. Medical College Srinagar, 68 male albino rats were used for the study. The animals were divided into three groups viz. Group A, Group B and Group C. Group A served as a control group while Group B and Group C served as the drug treated groups(low dose and high dose respectively).The animals were kept in uniform animal husbandry conditions. The animals were sacrificed at the intervals of 3, 6, 9 and 12 weeks. Tissue sections of 5 to 7 microns of the testes were made, stained with Eosin and Hematoxylin and studied under light microscope. It was observed that there occurred changes like absence of spermatozoa, decrease in number of spermatocytes,atrophy of seminiferous tubules and increase in the thickness of the inter tubular septae in the drug treated animals.

Thus it was concluded that Cyclophosphamide affects the testes of male albino rats at microscopic level and the changes are dose and duration dependent.

KEYWORDS Cyclophosphamide, albino rat,seminiferous tubules,hematoxylin,inter tubular septae.

INTRODUCTION.

Cyclophosphamide also known as cytophosphane is an alkylating agent of nitrogen mustard specifically the oxazophosphorine group. In 1959 it became the eighth anticancer agent to be approved by FDA[1]. Cyclophosphamide is used either as a single agent or in combination with other chemotherapeutic agents in the treatment of various neoplastic diseases like lymphomas, chronic lymphocytic leukemia, breast cancers and solid tumorsc[2,3]. Because of its immunosuppressive properties, Cyclophosphamide is used in the treatment of certain autoimmune diseases either as a sole agent or in combination with glucocorticoids. Cyclophosphamide is given orally or parenterally. Orally it is given in the form of tablets or solutions while parenterally IV route is preferred. Cyclophosphamide is metabolized in liver by hepatic cytochrome 450 to an active metabolite 4-hydroxycyclophosphamide which has a tautomer aldophosphamide. These two metabolites are in steady state with each other[4]. Aldophosphamide cleaves spontaneously to generate phosphoramide mustard and acrolein. The action of cyclophosphamide is due to phosphoramide mustard. It acts by forming DNA cross links both between and within DNA strands at guanine N-7 position. This is irreversible and leads to cell apotosis[5].

Cyclophosphamide causes many side effects like hemorrhagic cystitis, hepatic veno-oclusive syndrome, pulmonary fibrosis, gastrointestinal bleeding, irreversible azoospermia, alopecia etc[6]. Metabolites of cyclophosphamide are excreted primarily in urine in an unchanged form, so drug dosage needs to be appropriately adjusted in the setting of renal dysfunction[7]. Because of its wide use in the treatment of various neoplastic diseases and diseases associated with altered immunity, cyclophosphamide is known to cause many side effects and the target organs being liver, urinary bladder, lungs and male reproductive organs. Because of its adverse effects on male reproductive organs the present study is aimed to see the effects of cyclophosphamide on the microanatomy of testes of albino rats and to correlate these findings in human beings.

METHODS

Sixty eight albino rats weighing on average 100 grams were taken from the animal house of our college after approval from institutional animal ethics committee. The animals were divided in three groups: Group A (control group) of 20 rats were fed with routine diet and water, Group B (low dose group) of 24 albino rats were given cyclophosphamide at the dose of 0.5mg/100gms of weight of rat besides the routine diet and Group C (high dose group) of 24 albino rats were given high dose of cyclophosphamide at the dose of 0.7mg/100gms of weight of rat besides the routine normal diet. The drug was given by mixing it with pellets of flour. The animals were kept in different cages labeled as (A), (B) and (C) under uniform husbandry conditions.

Dose of the drug: The dose of the drug for the rats was calculated from the daily therapeutic dose for human beings which is 5mg/kg body weight. The dose of the drug for albino rats was calculated as 0.5mg/100gms weight of albino rats as low dose group and 0.7mg/100gms weight of albino rats as high dose and the process of drug administration continued for 12 weeks.

The animals were sacrificed in intervals 3, 6, 9 and 12 weeks to study the effects of the drug. 5 rats from group A and 6 rats each from group B and C were taken in each sitting. After anaesthetizing the animals with chloroform, a midline scrotal incision was given. Testes were identified and removed and were put in between blocking papers. The tissues were processed by using standard histological techniques. 5 t0 7 micrometer thick sections of the tissues were made, stained with hematoxylin and eosin and observed under compound light microscope. The observations were recorded.

RESULTS

During 3 week no change in the histology of testes of group B animals was seen as compared to the control group and sperm whorls were seen in the seminiferous tubules however the sperm whorls were absent in the seminiferous tubules of group C animals during the same period (fig 1). During 6 to 9 weeks there was decrease in spermatocytes in seminiferous tubules of group C animals while in group B animals the testes depicted normal histology but sperm whorls were absent. The changes seen during 9 to 12 weeks were in the form of atrophic changes in seminiferous tubules and basement hyperplasia while the changes seen in group animals during same duration were not much pronounced instead there was decrease in spermatid number and absent sperm whorls in the seminiferous tubules (fig 2, 3).

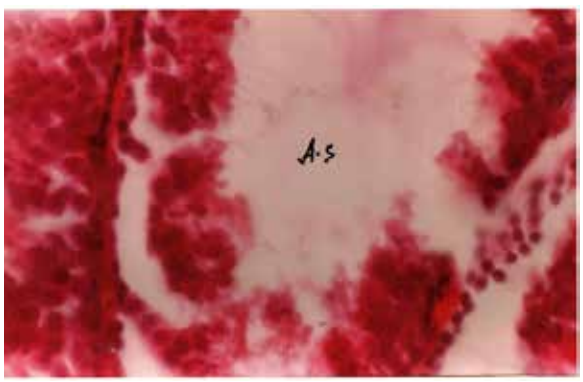

Fig 1: Photomicrograph of group C animals during 3 to 6 weeks showing decreased sperm whorls in the seminiferous tubules.

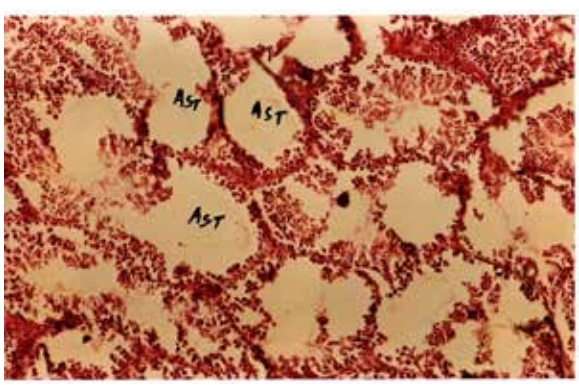

Fig 2: photomicrograph of testis of group C animals at 9 weeks showing atrophic tubules (AST).

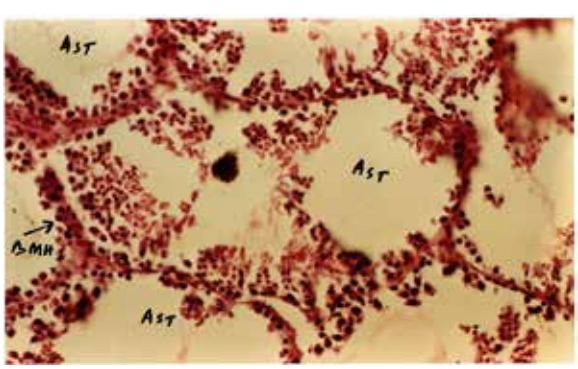

Fig 3: Photomicrograph of testes of group C animals showing atophic somniferous tubules (AST) and basement hyperplasia (BHM).

DISCUSSION

Cyclophosphamide is routinely used for treatment of various neoplastic diseases and diseases associated with altered immunity. A lot of work has been done on the effect of cyclophosphamide on the reproductive organs and outcome of pregnancy of the animals treated with cyclophosphamide. In the present study histological changes were seen in the testis of cyclophosphamide treated rats during 3 to 12 weeks of drug administration. The changes were gradual and doze and duration dependent. The changes were more pronounced in the group C animals. In the initial weeks of treatment, that is during first 3 weeks there were no changes in the histological structure of testes of group B animals however there was decrease in sperm whorls in the seminiferous tubules of group C animals. As with duration of treatment, the changes became more apparent particularly in group C animals. The changes were in the form of arrest of spermatogenesis at various stages in the form of decreased number of spermatocytes and spermatids. At 9 to 12 weeks of drug administration the seminiferous tubules became atrophic and there was hyperplasia of the basement membrane. KF Fairley et al (1972)[9] while studying the effects of cyclophosphamide on testis of the patients receiving the drug observed that most of the patients developed azoospermia .There was atrophy of seminiferous tubules .In the present study the observation of arrest of spermatogenesis at various stages with atrophy of seminiferous tubules correlate with the observations made by the earlier workers. Velez de la, Calle JF et al (1997)[10] while observing the reproductive effects of cyclophosphamide in male rats at different stages found that the affected tubules exhibited atrophy, exfoliation and a decrease in the number of spermatogonium,primary spermatocytes and spermatids .In the present study observations like tubular atrophy and decrease in the number of spermatogonia and primary spermatocytes correlate with the observations made by the earlier workers .Gosh D et al (2002)[8] observed that cyclophosphamide inhibits testicular gametogenic and steroidogenic activity in rats. These observations correlate with the observations made in the present study.

SUMMARY AND CONCLUSION

From the present study we observed that Cyclophosphamide inhibits spermatogenesis by arresting the process at various stages like the stage of meiosis and spermiogenesis.It also causes the tubules to atrophy. Thus it is concluded that this drug be judiciously used in young males and the individuals must be routinely monitored for any change in the number of spermatozoa and other testicular changes.

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