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RESEARCH ARTICLE

GROSS EXAMINATION OF TESTIS – TREATED WITH RNASE A AND METOSARTAN

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ABSTRACT

Histological examination of rat testis treated with RNase A and drug metosartan by staining with eosin Y has proven to be one of the challenging things because it reveals the exact histology associated with it. Treatment of testis with RNase has resulted in necrosis of tissue, where as with metosartan, leydig and sertoli cells are wiped off. However treatment of tissue with both RNase and drug has resulted in inter linkage of sperms and loss of clear demarcation as cells when observed under compound microscope.

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INTRODUCTION

Testis consists of seminiferous tubules which surrounds and form the lobule of testis in the scrotal sac. Spermatogonia forms the basal layer and also acts as stem cells to produce sperms. Each sperm contains head in which nucleus is present, mid piece having so many mitochondriae and tail. Sertoli cells acts as nourishing material for the developing sperms and these are non divisable in number so any damage in the cells leads to permanent deterioration of cells. Leydig cells produce leutinizing harmone in response to stimulus from the pituitary gland and acts on testosterone secretion. RNase A is a endoribonuclease that cleaves the RNA in to ribonucleotides. In testis ribonucleolytic activity has been found in seminal vesicles and in monkey caudal epididymal region repressed by testosterone (Sandrin Castella *et al*., 2004; Gupta and Setty, 1995). RNase A was found to be present in epididymis and whether there is any homology in testis that has to be find out. It was found that RNases are involved in processing of tRNAs, RNA-DNA hybrids and rRNAs (Kristine *et al*., 2002; Regnier and Grunberg-Manago, 1990). Metosartan is used to cure cardiac arrhythmias' and many other problems. It contains salts metoprolol 38.46% and telmisartan 61.54% as an ingredient. It was used as an apoptotic drug in the present study and its effect on sperm and testis was studied. Our present aim is to study about the effects of these two associated with sperms and histology of testis when treated with RNase, drug and also in combination of both RNase and the drug.

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Methodology: Isoaltion of testis and invitro treatment studies

Rat was anesthetized and cut in the lower abdomen using the scissors and foreceps, the testis was collected and treated with 1. RNase A (100µl) purchased from bros scientifics tirupati and PBS (10ml) 2. RNase A (100µl) + metosartan(100µl) purchased from royal pharmacy near v.v mahal road tirupati + PBS (10ml) 3. Metosartan (100µl) and PBS(10ml) 4. Control only PBS (10ml) for 25 min, separately, and minced and thin smear was prepared.

Fixation and dehydration of the tissue

The smear of testis were spread evenly on slides and were fixed in 10% formalin for 2hr 30min. The slides were dehydrated by the using. alcohol 70% for 30 min followed by alcohol 96% for 30 min and with, alcohol 100% for 30 min. Later using absolute alcohol (100%) for 1hr each for twice and finally for 30 min. the slides were dehydrated.

Staining

After dehydration, the fixed smear was stained in eosin solution for 5 min and washed in 95% alcohol for twice each for 5 min. The slides were observed under microscope at 1000X (10xX100x).

RESULTS

Histological examination of testis

The results obtained on microscopic analysis has revealed the following

The control testis smear consists of sperms, sertoli and leydig cells and dividing primary spermatogonia and the sperm nucleus stained with eosin Y observed under microscopic examination. The sperm heads are intact and no damage to the cells but eosin passes only through the membrane of dead tissue. Hypo osmotic changes in the sperm due to incubation in PBS has resulted in vigorous changes in the tail regions indicate swelling of them in 30min.

Histological examination of Testis treated with RNase A:

Testis treated with RNase A allows tissue and cells to become necrotic due to expectation of breakdown of the RNA of testicular cells and sperm cell. The shrunk cells were observed due to the treatment of RNase A. RNase A enters cells through endocytosis (Chao and Raines, 2011) by binding to cell surface proteoglycans containing like heparin sulphate (Chao *et al*., 2010) where as in case of sperms the RNaseA enters through the nutrient supplementation material of sertoli cells. Sertoli and leydig cells were undergone deterioration. Gaint multinucleated cells were found in the tissue. Macrophages were absent to engulf the necrotic tissue. Treatment with RNase A has resulted in protection of sperms from osmotic effect which was observed in Figure 2. So, RNase can be used to protect sperm from osmotic damage.

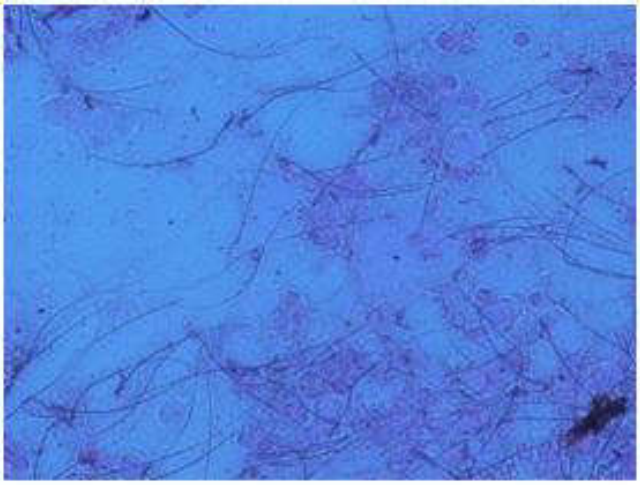

Figure 1. Testis was decapsulated and minced, then slides were prepared as thin smear and followed with histology staining using eosin yellow. The arrows indicate leydig cells on top and primary spermatogonia and looped tails on bottom (10X X 100X)

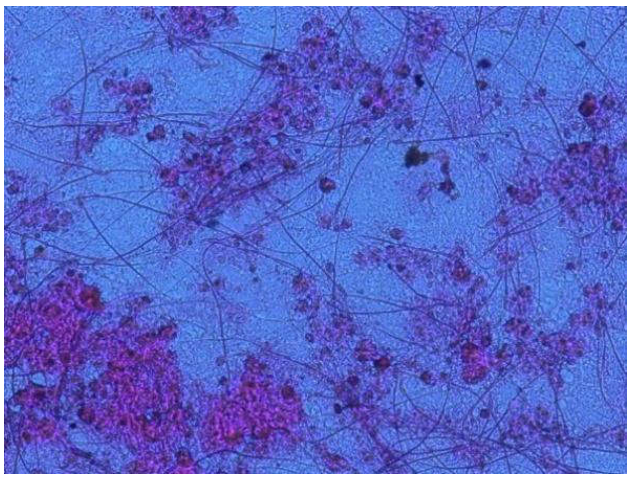

Figure 2. Histological examination of testis smear treated with RNaseA : The testis was treated with RNase A for 25 min and slides were prepared and stained with eosin Y. (10X X 100X)

Histological examination of testis treated with drug metosartan

Treatment of testis with the drug has resulted in apoptosis of the total tissue and there was found separation of heads from the sperms, and the leydig cells and sertoli cells were found to be totally disintegrated. The sperm number was low at some places of the sample. There was looping of sperm tails similar to both control and RNase treated one. Gaint multinucleated cells were found to be absent.

DISCUSSION

The transport of substances across the membrane is not only required for sperm motility but also for maintainance of sperm function. It is important to see whether the drug interferes with that of sperm motility, function and in penetration in to ovum. Sperm head consists of small volume of fluid than tail so there will be no fatal effect to head due to influx of water (Yazdanian *et al*., 1998) Motility not only effected by swelling and also on microtubular action of sperm tail. Our results on histology studies of testis has revealed that sperms and the cells associated with it. Sperm consists of head, middle piece with numerous mitochondria and tail. In control there is distibution of leydig cells, sertoli cells and also primary spermatogonia cells from which sperms are produced after maturation. In RNase A treated sample whole cells are undergoing modification later wiped off. Eosin staining has proven to be that which stains cytoplasm (Jeyendran *et al*., 1984). Future aspects includewhether apoptosis occurs in whole parts or in testis alone and also to be proved that which type of pathway comes in to play in both apoptosis and necrosis.

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