International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Histological Examination of Testis Treated with Drug Mesosartan and RNase

Eswari Beeram

Ph.D. Sri Venkateswara University, Tirupati, Andhrapradesh, India- 517502

Abstract: ***Histological examination of testis is one of the major application in detecting the problems of infertility. Treatment with RNase A alone lead to disintegration of extracellular matrix and degradation of extracellular matrix proteins synthesising RNA. When treated along with metosartan resulted in rearrangement and filling of extracellular matrix space and cell migration. Future perceptives includes finding whether the drug inhibits RNase or not and whether it interacts with signalling pathways or not.***

Keywords: histology, RNase A, metosartan

1. Introduction

Seminiferous tubules of testis are about 200 µm and consists of several lobules. In this Spermatogonia a kind of stem cells that form the basal layer of the Epithelium and involved in the Production of sperms. RNase A has known to the homology to that of RNase 10 which is found on the Chromosome 14, in which the Glycosylated isoforms has been Found by the two dimensional Electrophoresis which Constituted about 87% of the protein in porcine epididymis [(1).]Elastin fibres are composed of protein elastin that fills up the Extracellular space produced by the fibroblast cells and smooth Fibres [(2)]. Collagen is also one of the components of extracellular space [(3, 4)] and fills up the space. RNase A is a protein of 124 Amino acids, without any carbohydrate Moiety. It consists of 19 of 20 Amino acids, in which tryptophan is absent. The three dimensions Structure of the enzyme depends on the amino acid composition and folding in three dimensional form. Metosartan is a class of Drugs that bind to angiotensin receptors II beta. It is used to treat high blood pressure when heart is unable to pump the blood. It is also used for kidney failure patients with diabetes.

2. Results

It is clear from the figure that elastin fibres and collagen bundles are distributed throughout the area and the bundles are connected to each other with disulphide bonds and in some places individual fibres are also present. When compared with control the treated one has ruptured fibroblasts as observed in figure 1.

Histology of testis without any treatment:
Control was kept in deep freezer for 12 days and the result was obtained. It also contained the elastin and collagen fibres but those were disappeared due to after 4 days of picturisation. The fibroblasts are clearly arranged which is clear from the figure2.

Histology of testis treated with RNase:
The elastin and collagen bundles has been separated as disulphide bonds are broken and the fibroblasts are ruptured in some
Places which are clear from the figure 3 and the cells have disintegrated.

Histology of testis treated with

Drug mesosartan and RNase:
The bundles and fibres of collagen and elastin are rearranged and the space between the fibres are filled with Extracellular matrix and cell migration has Beenseen.

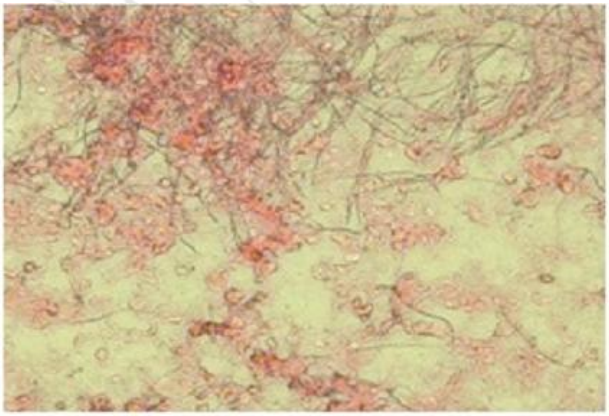

Figure 1: Histology of testis treated with drug metosartan. Testis treated with drug metosartan in PBS for 12 days and kept it in deep freezer and picturised the result after 2 days.

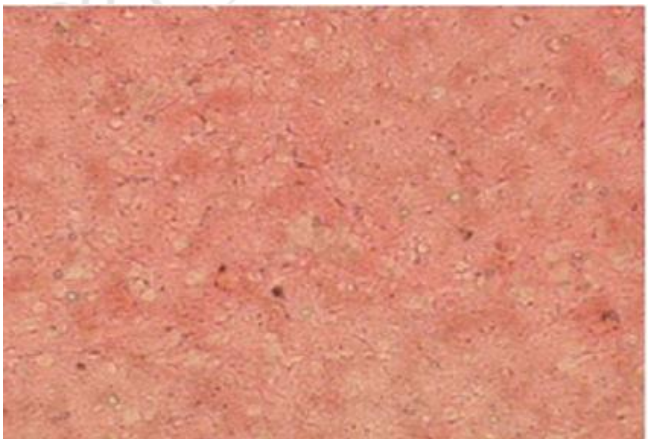

Figure 2: Control: histology of testis. It was picturised after 2 days of slide preparation after incubation in deep freezer for 12 days

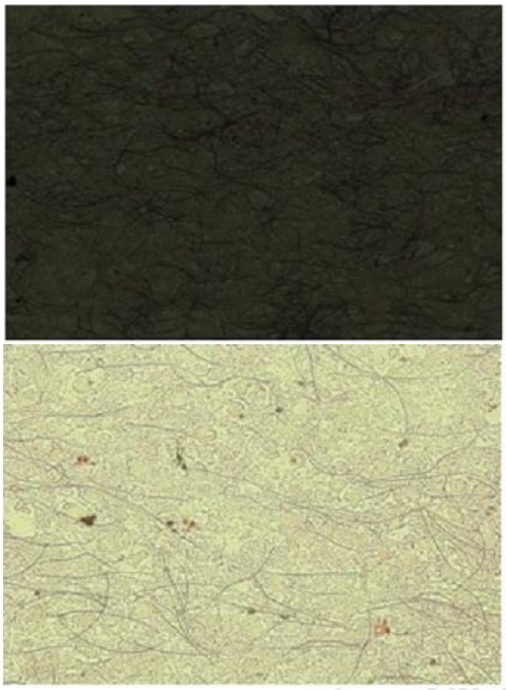

Figure 3: Histology of testis after treatment with RNase: testis was collected and incubated with RNase in PBS and kept in deep freezer for 12 days. Fibroblasts are ruptured and the elastin fibers, collagen bundles are separated.

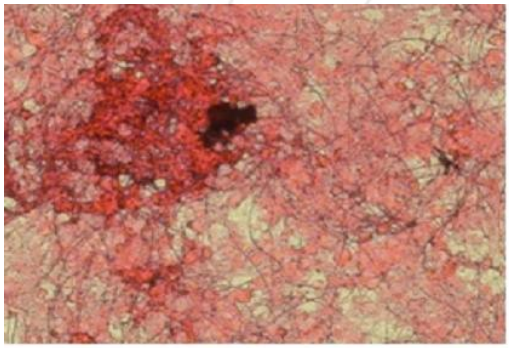

Figure 4: Histology of testis treated with drug metosartan and RNase A: tissue was treated with drug metosartan and RNase A in PBS which is kept in deep freezer for 12 days.

3. Discussion

Histological examination of testis is one of the major applications in the infertility treatment. Fibroblast arrangement, elastin fibre and Collagen fibre arrangement is Clearly shown from the data and the Treatment with RNase A resulted in the clearance of extracellular Matrix due to degradation of the Extracellular matrix proteins Synthesising RNA. Treatment with Drug and RNase a resulted in Filling of the extracellular matrix and cell migration which clearly Indicates the inhibition of RNase A by the drug. Metosartan belongs to angiotensin II beta family drugs.

It is used for treating high blood pressure and in patients with kidney failure those having diabetes. Future perceptives include to find whether the drug inhibits RNase A or not and also to find whether the drug interacts with signalling pathways or not.

4. Material and Methods

Drug preparation:
Metosartan (mesoprololtartarate (25mg) and telmisartan (50mg)) 1 tablet dissolved in 50ml of distilled water.

RNase preparation:
20mg RNase in 5ml PBS

Study design:
1) Deep freezer control with PBS
2) Treatment with drug alone (100µl) and PBS (10ml)
3) Treatment with RNase alone(200µl)
4) Treatment with RNase (200µl) and drug (100µl) And PBS (10ml)

Histology slide preparation:

Slides has been dehydrated in the following way. Formalin 10% -
1) 0-3hr, Alcohol 70% - 30 min ,
2) Alcohol 96% - 30 min, alcohol
3) 100% - 30 min, alcohol 100%-
4) 1hr, alcohol 100% - 1hr, alcohol
5) 100% - 1hr, alcohol100% -30 min

Slide preparation:
Animal was sacrificed and testis was collected, treated with various means as per the study design and kept in deep freezer for 12 days and allowed to attain room temperature, made in to slurry by mincing testis and thin smear was prepared.

Staining:

Stain the slides in eosin solution for 10 min Wash in tap water for 1-5 min. Dehydrate in 95% . Alcohol for two times of 5min each and observed in bright field Microscope.

References

[1] Anton Krutskikh, Ariel Poliandri,Victoria Cabrera-Sharp, Jean Louis Dacheux, Matti Poutanen, and Ilpo Huhtaniemi Epididymal protein Rnase10 is required for post-testicular sperm maturation and male fertility.FASEB J. 2012 Oct; 26(10): 4198–4209
[2] Liu X, Zhao Y, Gao J; et al. (February 2004). "Elastic fiber homeostasis requires lysyl oxidase-like 1 protein". Nat.. Genet. **36** (2): 178–82. doi:10.1038/ng1297. PMID 14745449
[3] Di Lullo, Gloria A.; Sweeney, Shawn M.; Körkkö, Jarmo; Ala-Kokko, Leena & San Antonio, James D. (2002). "Mapping the Ligand-binding Sites and Disease- associated Mutations on the Most Abundant Protein in the Human, Type I Collagen". *J. Biol. Chem.***277** (6): 4223–4231. *doi*:*10.1074/jbc.M110709200*. *PMID* *11704682*.
[4] Sherman, Vincent R. (2015). "The materials science of collagen". Journal of the Mechanical Behavior of Biomedical Materials **52**: 22–50. *doi*:*10.1016/j.jmbbm.2015.05.023*