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Research Article

LONG TERM STUDY ON EFFECTS OF DRUG METOSARTAN ON TESTES AND SPERMS OF MALE WISTAR RATS

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A R T I C L E I N F O

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A B S T R A C T

Weight of testes and epididymis was found to be increased in R Nase A+ metosartan treated group than compared to control. Qbanding of testes samples treated with metosartan resulted in collapse of chromosome chain formation where as in remaining groups staining of chromatin is not seen when compared with control. Flow cytometric analysis proved that the drug induces profound apoptosis in both testes and sperm samples. Inspite of this, the drug does not affect histology of rat testes. In sperm sample of rats treated with RNase A + Aspirin showed profound apoptosis compared to testes of same group. Sperm count was restored to some extent in long treatment of normal rats treated with drug metosartan but lower than the remaining groups.

INTRODUCTION

Metosartan is an antihypertensive drug used for regulation of blood pressure and in treatment of angia pectoris. From previous reports of metosartan, the drug was found to induce azoospermia in male wistar rats in hypertensive condition(1) and decrease in sperm count was observed in short term treatment of rats invivo and found to induce apoptosis in both testes and sperm through release of cyt C (2) . So, long term study of drug is necessary and resulted in restoration of sperm count to some extent compared to short term study. Up to now, there are so many reports on telmisartan and metoprolyl but the update on background of metosartan is uncertain. So, long term treatment of drug on rats proven to be useful to study the effects of metosartan in normal patients as a system to use the drug for treatment of cancerEffect of metosartan on gonad weight is not studied up to now. However. There is interesting change in body weight observed with long term treatment of drug. RNase A is an endonuclease that causes apoptosis in cancerous cells.

In this case the enzyme is used in combination of metosartan and Interesting thing is it resulted in normal distribution of cells in all phases of cell cycle where as aspirin is well known from previous reports for its antiapoptotic property (3) and used in this study to know its effect on the enzyme. Cell cycle analysis of cells after drug treatment is commonly attributed to the cancer to know the ploidy and maximum number of dividing cells. In this study we used flowcytometry to analyse the affect of drug on reproductive potential of rats.

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Many drugs does not affect the histology of the tissue and same was found with metosartan also (4). So, this study is mainly focussed on the solution to the azoospermia induced by the drug in male reproductive organs.

RESULTS

Histopathology of testes of rats after long term treatment with metosartan, RNase A + Metosartan, RNase A+ Aspirin and control:

Histology of testes was unaffected in rats treated with drug, RNase A and Aspirin. It also found that histology of rats was unaffected by most of the drugs like resperidone and fluoxetine (1).

The testicular cell membrane was found to be intact and there is no infiltration of cells and shrinking of tissue is absent and no observable damage inside the tissue except the elongation of the seminiferous RNase + Aspirin treated group than compared to control. Whereas such elongation of tubules was not seen incase of RNaseA+ Metosartan as proven with flowcytometry results also. Sertoli cells, Leydig cells were found to be normal and there is no damage to stem cells also.

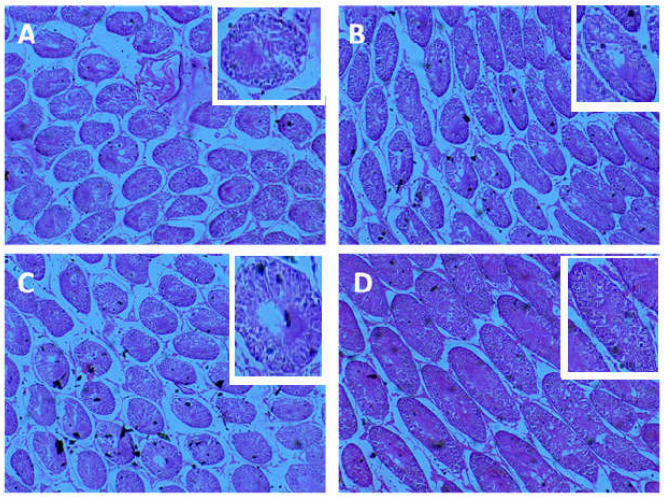

Figure 1 Histopathology of testes. Treated with distilled water (A), Metosartan (B), RNase A+ Metosartan (C) and RNase+ and aspirin with 1mg/kg body weight (D). Metosartan, RNase A and aspirin was given with dosage of 833µg/kg body weight. Each group consists of six animals and the weight of animals was calculated at day0 and the dose was given to them accordingly, whereas control rats were treated with distilled water for 30days and the animals are dissected at 31st day. When compared with control, there is no damage to the testes tissue in remaining treated groups

Body weight of Male rats on day0 and Weight of Gonads and Epididymis of Rats After 30 days of Treatment

The dosage of rats was calculated according to the body weight of the rats at day 0. The weight of testes and epididymis was recorded after dissection of animals at 31st day. Weight of testes was drastically increased in RNaseA+ metosartan group compared to control group where as weight of epididymis was also decreased in same group compared to control. The reason for decrease in weight is uncertain. Decrease in weight of epididymis was seen in R+A group which is due to apoptosis in both control and R+A group as aspirin is a antiapoptotic drug.

Table 1 Body weight at day 0, weight of Testes and Epididymis of rats treated with metosartan, RNase A and Aspirin

S.No	Body weight at day 0 (g)	Dose administered (µg)		Weight of testes after 30 days of treatment (g)	Weight of epididymis after 30 days treatment (g)
1	269.65±14.68	0.269ml of distilled water	**Control**	1.1131±0.04	3.1309±0.10
2	288.81± 11.8	260µg metosartan	**Metosartan treated group**	1.155± 0.02	3.0125± 0.11
3	290.44 ± 10.4	260µg RNase+260mg Metosartan	**RNaseA+ Metosartan group**	1.2020±0.03	3.0853±0.20
4.	282.84±12.9	260µg RNase A+0.31mg Aspirin	**RNaseA+ Aspirin**	1.1375±0.09	3.1082±0.16

Sperm count in control and treated groups:

Table 2 Sperm count of rats treated with metosartan, Aspirin and RNase A

S.No		Sperm count in epididymis/ml	Sperm count in testes/ml
1.	**control**	35 x10^5	14x10^4
2.	**Metosartan**	166x 10^4	2x10^5
3.	**RNase A+ Metosartan**	2x10^6	26x10^4
4.	**RNase A+ Aspirin**	181x10^4	32x10^4

From table 2 the highest sperm count is found in control and next to control RNase A + metosartan group and lowest with metosartan due to profound apoptosis similar to sperm count in testicular sample also and control of epididymis have highest sperm count compared to remaining groups as apoptosis occurs due to profound rate of division in cells proven by flowcytometry results. Viability of sperms are not tested in this study so, the dead sperms were also counted but according to flowcytometry results the control of testis found to be normal with low number of apoptotic cells.

Flowcytometry analysis of testes and sperm suspensions of rats treated with metosartan, RNase A, Aspirin and control tissue

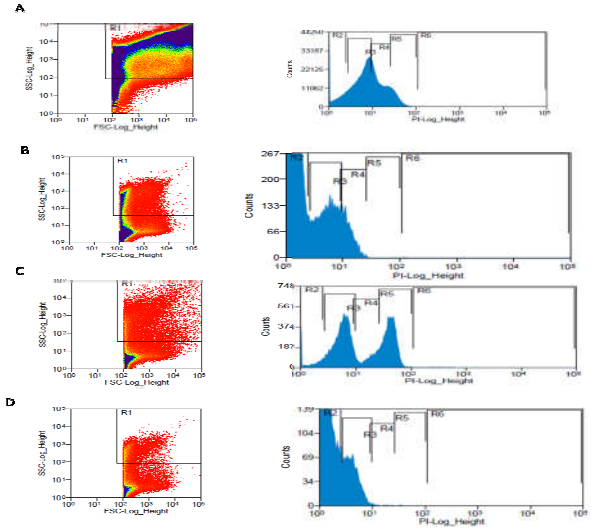

Figure 2 Flow cytometry of testes of rats treated with RNase A, Metosartan and Aspirin. Gating of single cell population was carried with FSC Vs SSC. The resulting population was analysed for cell cycle with number of counts Vs PI- Log _ Height. The single cell population percentage present after gating was taken as 100%. (A) Cell cycle plot of control testes. (B) testes tissue treated with metosartan. (c) testes tissue treated with RNase A+ Metosartan. (D) testes tissue treated with RNase A+ Aspirin.

From figure 2 the control cells undergoing apoptosis is minimal with16% and with minimal M phase cells and aneuploid cells. Where as in testes treated with metosartan resulted in profound apoptosis of cells as per previous data of (Eswari beeram and Thyagaraju kedam 2018). In case of RNase A+ Metosartan treated rats the count is normal with distribution of cells in all phases of cell cycle but with some amount of G1 phase arrest. Whereas rats treated with RNase A+ Aspirin showed profound apoptosis with minimum number of cells in remainimg phases of cell cycle.

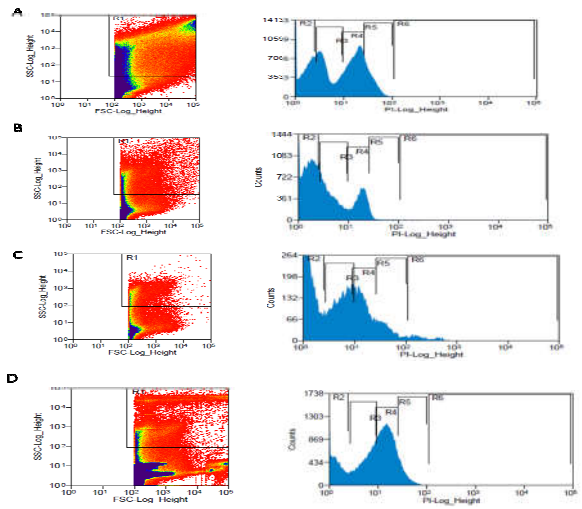

Figure 3 Flow cytometry of Sperm samples of rats treated with RNase A, Metosartan and Aspirin. Epididymis was collected after 30 days of treatment and sperm suspension was collected and diluted with water and analysis of cell cycle was performed with flowcytometry after PI staining. (A) Control of rats treated with distilled water. (B) rats treated with metosartan. (c) rats treated with RNase A+ Aspirin. (D) Rats treated with RNase A+ metosartan.

In control rats the sperm population is showing maximum apoptotic cells along with maximum dividing cells. Most of the cells undergoing apoptotic cells are contributed by S-phase cells. In metosartan treated rats, sperm cells are undergoing maximum apoptosis when compared with control by decreasing cells entering the S- phase. Rats treated with RNase A + metosartan showed maximum distribution of cells in M- phase along with apoptosis. In case of RNase A+ Aspirin treated rats, sperm cell suspension showed maximum apoptosis and minimal number of dividing cells. As per previous data aspirin induces apoptosis in cells at concentration of 1mg/kg body weight. In case of RNase A + metosartan treated rats the number of cells undergoing apoptosis is approximately similar to number of dividing cells.

Q – banding of testes samples of rats treated with metosartan RNase A and aspirin in combination

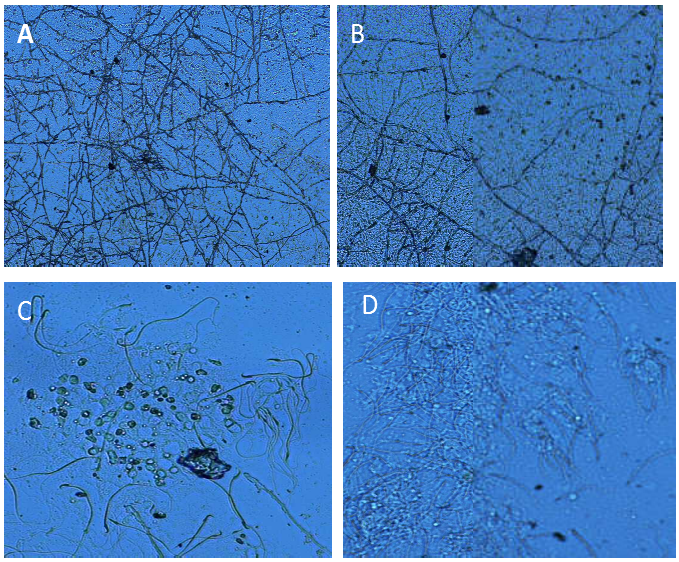

Figure 4 Q – banding of testes samples of rats treated with metosartan, RNase A and Aspirin. (A) Control treated with distilled water. (B) Treated with metosartan (C) Treated with RNase A+ metosartan. (D) Treated with RNase A+ Aspirin.

From the figure 4 Q- banding control tissue showed highly organised chromatin with arms of chromosomes united to form long chains where as in metosartan collapse of chromosome chains up to some extent, where as in remaining treated groups chromatin is not stained and Q- banding of these samples resulted in staining of sperms.

MATERIAL AND METHODS

Dosage of drug and RNase A

Dosage of drug, RNase A was calculated according to the body weight of the rats and as a total 833µg//kg body weight was administered to each rat orally for 30 days and on 31st day rats were dissected and testes, epididymis were collected and stored in dry ice for 1 day and slides were prepared on next day. Control rats were administered with distilled water similarly to the treated groups.

Dosage of aspirin

Aspirin was given in combination with RNase A orally with concentration of 1mg/ kg body weight.

Flowcytometry

Tunica albuginea was removed and the testes was cut in to pieces in citrate buffer (5) (250mM sucrose and 40mM trisodium citrate) and homogenised in mortar and pestle. The resulting solution is allowed to pass through the nylon mesh of 50microns pore size and the resulting solution was diluted with buffer in 1:3 ratio and stored at -80^{0}c up to analysis of sample by flow cytometry. Similarly sperms were collected from epididymis in buffer and the resulting suspension was diluted with buffer in 1: 3 ratio. 10mg of PI was dissolved in 10ml of water and 1.25 ml of PI solution was added to each sample separately and stored at 4^{0}C up to usage of sample by flowcytometry. Before the flowcytometry passage, the tissue suspension is allowed to pass through cell strainer of 40µm separately for each sample.

Histopathology

Dissected testes were fixed in formalin for 3 hrs and after that they were stored in absolute ethanol up to usage of tissue for histopathology staining.

Q- Banding

Quinacrine hydrochloride stain was prepared in 0.1N acetic acid one day before use. Testes smears were prepared before 3 days of staining and air dried. Quinacrine hydrochloride solution was taken separately for each sample and slides were stained individually. After staining the slides were washed in acidified water (0.1 N CH3COOH) for two times and stored in dark as the dye is light sensitive.

Sperm count

Sperm count of the treated samples was performed as per Eswari Beeram (2019)

DISCUSSION

Weight of the testes was increased in metosartan+ RNase A group compared to control and decrease in weight was observed with RNase A+ Aspirin group compared to control epididymis. This is may be due to apoptosis by combined effect of drug aspirin and enzyme used. Metosartan known to induce toroids in testes tissue subjected to invitro treatment. Toroids are the association of protamines with DNA at 50kb interval. So, in order to form ring structure the chromatin should be cleaved first and there are previous reports at metosartan induces ds breaks in DNA which may results in catenation of DNA. From this study metosartan induces collapse or decrease in long chains of chromatin with joined arms of chromosomes.

Cell cycle analysis, histopathology and Q- banding supports use of RNase A along with metosartan in order to release drug slowly and reduce the side effects of the drug like apoptosis and ds break induction. Sperm count restoration was observed with long term usage in normal rats but in hypertensive rats it induces azoospermia at physiological concentration (1). As the drug induces apoptosis in cancer cells (7) RNase A can be used as a carrier for metosartan but working on efficient drug targeting we can acheive the anticancer effect of the drug. Where as in Hypertensive rats use of RNase A + metosartan system to restore reproductive potential is required.

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