Metosartan induces endometrial carcinoma in rat testicles – A study by histology

Eswari beeram

Department of biochemistry, Sri Venkateswara University, Tirupati, Andhra pradesh, India.

Abstract: Background: Antihypertensive drug metosartan is well known for its action against hypertension in humans but recent research findings has shown that metosartan also induces endometrial carcinoma mainly leading to germ cell tumors. The individual components of metosartan namely telmisartan and Metoprolol are individually known for its proangiogenic properties in coronary tissue of animals and humans. Method: male wistar rats of 3 months are grouped accordingly and dissected under hygiene condition and testes were dissected and fat tissue were removed and washed with distilled water and slides were prepared and stained with eosin Y and air dried for scanning electron microscope. Results: In this study the drug induce angiogenesis in testicles of men and suppose to cause tumor growth and metastasis. RNase A is well known for its anticarcinogenic activity and in this report it inhibited angiogenesis up to some extent when given in combinational treatment with metosartan and induces apoptosis of testis tissue when treated with RNase A alone. Conclusion: Metosartan induces tumors in testicles of male rats by inhibiting the RNase A.

Key words: Metosartan, Telmisartan, Metoprolol, Endometrial carcinoma, VEGF, RNase A, Angiogenesis, Yolk sac tumor, Germ cell tumor, Sertoli cells, Leydig cells.

Introduction: Most of the drugs intake results in adverse effects on reproductive system of humans leading to infertility. If the effects are permanent it may cause sterility also. As sperms contribute to the 50% of the chromosomes in offspring studies on testes and epididymis is necessary for treatment of infertility. In most of the cases the drug results in positive effects towards treatment. Telmisartan is generally used in combination therapies for treatment of hypertension in adults. In normal rats treatment with metosartan a combination of telmisartan and Metoprolol resulted in inhibition of testes RNase [1] and acts with positive cooperitivity towards binding with RNase A. However the study on effects of metosartan on histological aspects of testes and epididymis was not up to the mark till date. In case of lung tissue telmisartan reported positive effects on the rat lung tissue [2] where as treatment with testosterone esters resulted in decrease of testes and epididymis weight and caused leukocyte infiltration [3]. Similarly carvedilol doesn’t alter the histology [4]. Majorly telmisartan acts through blocking the androgen type I receptor but it also decreases SBP by increasing the load of sodium in urine and also pulse pressure [5]. Telmisartan was unable to attenuate the damage caused due to irradiation [6] and from the results of Eswari beeram (2018) telmisartan as combinational dosage therapy causes serious deleterious effects on fertility in BP induced rats [7].

Telmisartan effects can be reduced by sodium chloride intake which ameliorates plasma rennin levels in type II diabetes [8]. Telmisartan show proangiogenic properties so it usage and dosage should be reduced in cancer patients as it may lead to metastasis. In addition to antihypertensive drugs, anticancer agents like adiramycin affects stem cell count of the spermatocytes. Some of the drugs affect at cell cycle [9] also.

Angiogenesis is the growth, division and proliferation of endothelial cells from the stage of embryo to and during adult stages. Two types of angiogenesis namely sprouting angiogenesis and intussusceptive angiogenesis takes place in tissues. Sprouting involves synthesis of new vessels directed by Vascular Endothelial Growth Factor (VEGF–A) secretion. The main stimulator of VEGF-A is the hypoxia condition. Tip of the vessels filopodia consists of receptors for VEGF-A and stimulates Notch signalling in the stalk cells and filopodia attach the substratum and migrates in the direction of VEGF-A. Where as in
intussusceptive involves bifurcation of vessels in the tissue in which they are already present. Although exact mechanism of intussusceptive angiogenesis is unknown, this type of angiogenesis also require VEGF-A for the process. Cancer cells produce VEGF-A to induce tumor angiogenesis so treatment of ischaemia, coronary heart diseases and gene therapy is not possible. Muthukkaruppan 1982 and his colleagues was the first to study the relation between angiogenesis and tumor survivability. Various angiogenetic factors like metalloproteases, growth factors like VEGF and Tumour necrosis factor (TNF), fibroblast growth factors, granulocyte colony stimulating factors are known to induce angiogenesis [10]. Upto now drugs that target Matrix metallo proteinases (MMPs), endothelial matrix proteins and Extra cellular matrix (ECM) signalling components like integrin was proven to be useful. Study of histology is one of the known methods to study about VEGFRs distribution and germ cells at various stages of development in the endometrial carcinoma.

Results:

Figure 2.1 showed loss of sertoli cells, leydig cells, primary spermatocytes and secondary spermatocytes and broken basement membrane. From these results it is clear that control rat is as [11,12] but some parts of the testes is intact with normal histology so there is no problem with the slide preparation. Sperm production is found to be normal and the morphology of sperms also appears to be normal. Staining of the tissue is even throughout the slide and excessive staining of cytoplasm is not seen.

Figure 2.2 Histology of rat testis treated with antihypertensive drug metosartan. (A) Showed excessive angiogenesis in testicles with (B) Abnormal sperms with looped tails shown with left side arrows and abnormal head shown with right side arrows (C) showed endometrial carcinoma with yolk sac tumor represented with arrow.

From the figure 2.2 treatment with metosartan resulted in excessive angiogenesis in the tissue. where as figure 2.2 B showed abnormal sperms with looped tails and abnormal sperm heads. Endometrial carcinoma with germ cell tumors associated with yolk sac occurs at early stage with either metastasis or with absence of metastasis but treatment with metosartan induced angiogenic properties as it contain telmisartan and Metoprolol as components. Yolk sac germ cell tumors generally show positive for serum alpha feto protein [13]. In this case serum AFP levels are not studied. Yolk sac tumor in figure 2.2C showed positive staining of keratin.

In invivo condition there was decrease in sperm count when treated with metosartan proved by the studies of Eswari Beeram (2018). As the drug is promoting angiogenesis [figure 2.2A] we can expect metastasis. Mostly affected tissues include liver, pelvis[14] and diaphragm[15]. So, further prognosis on rat testicles treated with metosartan is needed. Figure 2.2A showed excessive staining of cytoplasm and figure 2.2B showed moderate staining of cytoplasm of rat testicular tissue.
Metosartan binds to RNase A with positive cooperitivity and is a negative modulator of enzyme [1] and inhibits RNase A [17]and the inhibition is relieved after that. So, some of the tissue may show angiogenic properties after treatment with both metosartan and RNase A.

Figure : 2.5 Scanning electron microscopic examination of histology sample treated with RNase A: (A) Histology of testis control. (B) Histology of testis tissue treated with RNase A shows apoptosis of the tissue. positive results with previous literature.

From figure 2.5A seminiferous tubules of testes are clearly visible with minimum amount of disorganisation where as 2.5B shows the apoptosis of testes cells treated with RNase A contains antineoplastic activity.

Discussion:
There are so many cancer causing agents but few was listed as potential agents of carcinogenesis and termed as carcinogens by NIH. In them six factors are listed as carcinogens by NIH. In them hepatitis B and Hepatitis c is known to cause liver cancer and human papilloma virus known to cause cervix cancer in women. Non biological agents listed as human carcinogens include X-rays gamma rays, Naphthalene, diazoaminobenzene, Nitrobenzene [18] etc., also induce carcinogenesis.

Yolk sac tumor is one associated with endometrium of testes and also referred as germ cell tumor in both women and men. So many chemicals listed above cause cancer but new report about metosartan in this study is that it induces germ cell tumors as it can cross testes-blood barrier and show its effects. It also promotes angiogenesis in testicles whether the type of angiogenesis is sprouting or intussusceptive is uncertain. As angiogenesis requires VEGF, may be the drug acts as agonist and phosphorylates VEGFR [19].Alpha fetoprotein in serum is one of the biomarker [13] which is not studied and require further justification for study.
RNase A is well known for its cytotoxic activity [20] as normal cells synthesise Ribonuclease inhibitor (RI) which binds to RNaseA where as in cancer cells angiogenin acts as scavenger [21] so apoptosis of cancer cells is possible. However it is not effective in invivo condition as cancer cells synthesise miRNAs in high concentration. Metoprolol which is a beta blocker and telmisartan angiotensin receptor blocker was known individually for their angiogenic property [22]. In case of telmisartan there were previous reports that it induces angiogenesis in VEGF dependent manner through activation of PI3K/eNOS/NO pathway [23]. Phosphotidyl inositide 3 kinase (PI3K) phosphorylates AKt which phosphorylates endothelial Nitric oxide synthase (eNOS) which is downstream regulator of PI3K/AKt as inhibitors of this pathway decreased the levels of eNOS which synthesise NO. NO concentration is necessary for this pathway decreased the levels of eNOS which synthesise NO. NO concentration is necessary for angiogenesis.

Metosartan is proved as inhibitor of RNase found in testicles so the cytotoxicity and anti apoptotic pathway and anti inflammatory pathways are compromised in germ cells which may be the main reason for endometrial carcinoma in testicles in invitro condition.

**Material and methods:**

**Preparation of drug and RNase A:**
Metosartan was purchased from Royal pharmacy Tirupati. Drug was dissolved in water with prior to treatment. RNase A was also dissolved with water

**Experimental design for invitro studies:**
3months old male wistar rats were purchased from Raghavendra Enterprises, Bangalore and maintained at ambient temperature and conditions. Rats were sacrificed and the testes collected was treated with distilled water which acts as control and other tissues with metosartan, RNase A and both metosartan and RNase A containing 10ml distilled water and 1mg/ml of metosartan and RNase A for 30 min and slides were prepared after the incubation period.

**Preparation of slides:**
Testicles treated were minced in sterile distilled water and thin smear was prepared and allowed to air dried.

**Histology of rat testes:**
Slides prepared were subjected to dehydration as follows. The slides were treated with 10% formalin for 3hrs and 70% alcohol for 30 min followed by 96% alcohol for 30 min and 100% alcohol for 30min, followed by incubation with 100% alcohol for 30min replacing the fresh alcohol for every 1 hr up to 3hrs followed by 30 min incubation with 100% alcohol and allowed to air dry. After drying, the slides were stained with eosin Y for 10min and washed in tap water for 5 min. the slides were dehydrated for two times each with 5 min with 95% alcohol and observed under 20X objective lens of Olympus bright field microscope.

**Scanning electron microscope:**
Slides stained with eosin Y solution were shade dried for 2 months and observed under zeiss Scanning electron microscope.

**References:**
Eswari Beeram / Metosartan induces endometrial carcinoma in rat testicles – A study by histology


[23]. Ana Costa, Joana Afonso, Catarina Osório, Ana L Gomes, Francisco Caiado, Joana Valente, Sandra I Aguiar, Francisco Pinto, Mário Ramirez and Sérgio Dias (2013). miR-363-5p regulates endothelial cell properties and their communication with hematopoietic precursor cells. Journal of Hematology& Oncology 2013, 6:8