Review

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Review on Effects of Metosartan on Testes Tissue

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Abstract

Metosartan induces so many deleterious effects in male Wistar rats treated with drug and RNase. It causes pre maturation of sperms and reduces the sperm count as well as reduces viable sperm count. It induces the x-chromosome desynapsis leading to testicular carcinoma. The effects are so profound in case of drug than compared to the enzyme RNase. Metosartan induces apoptosis in testicular tissue mainly through intrinsic pathway which is mainly due to genotoxic agents. The drug mainly inhibits RNase A in rat testes and has positive effect on Mitosis.

Keywords: Metosartan; RNase A; Desynapsis; Netosis; Sperm count; Pre maturation; Apoptosis

Introduction

Metosartan is one of the antihypertensive drugs taken for study to assess reproductive potential in male rats. It mainly consists of two components metoprolol succinate and telmisartan which induces ds breaks in DNA. Metoprolyl known to affect sperm motility so, metosartan was taken to assess reproductive potential and to study drug interactions with DNA. The collapse of chromatin is observed during apoptosis which requires fragmentation of genome in the cells [1]. DNase digestion of chromatin leads to degradation of linker DNA leaving the nucleosomes. As RNA is also a component of chromatin along with DNA and protein, and RNase a treatment also causes disruption of chromatin [2].

Ion exchange chromatography is one of the column chromatographic techniques that aid in separation of proteins based on their charge. Cation exchange chromatography is used to separate cationic proteins and anion exchange chromatography to separate anionic proteins. In my study DEAE and carboxy methylcellulose were used for separation of RNase A from rat testes [3]. Cytochrome C is a water-soluble protein of molecular weight 12 KDa. It exists mainly in a reduced form which prevents apoptosis and acts as electron carrier in electron transport chain. NADPH regulates the reduced form of Cyt C through regeneration of glutathione. Nucleotides like NADPH binds to Cyt C and prevent its binding to APAF1 inhibiting apoptosis. Cyt C is a heme protein that gives maxima between 390-600 nm in oxidized form and metosartan was known to cause apoptosis by the release of Cyt C from testes and sperm recorded using UV Visible spectrophotometry [4].

Effects of Metosartan in Testes

Metosartan induces double-stranded breaks in testicular DNA which are minimized by RNase A treatment to some extent. After treatment with metosartan using aniline blue staining resulted in pre maturation in sperms compared to RNase A. Histological examination in deep freeze condition and fresh examined tissue showed the development of yolk sac tumor in testis tissue whereas RNase treatment resulted in suppression of tumor compared to combined effect of both RNase A and metosartan as the drug reversibly inhibits metosartan through both competitive and non-competitive inhibition [5].

The drug maximally inhibits RNase A for 3 hrs. and not more than 4 hrs. The i/ki ratio is about-1.6 which indicates inhibition of RNase A by metosartan. From the recent reports of mine RNase A in rats was successfully isolated from the testis with molecular weight of 24 kDa. The isolated enzyme was subjected to agarose gel electrophoresis followed by metosartan treatment resulted in no catalytic activity of the enzyme with regard to cleavage of substrate RNA. Telmisartan is the component of metosartan responsible for inhibition of RNase A whereas Metoprolol is responsible for cytotoxic activity of metosartan associated with testes [6].

The drug-induced the nuclear pore enlargement in testes through association with epigenetic regulation of chromatin dynamics. The metosartan has reducing property of disulfide bonds which may lead to collapse or disassembly of chromatin of mature sperms leading to male infertility. In hypertensive patients, the damage is minimal with regarding to ds breaks and DNA fragmentation but the presence of apoptosis is observed with agarose gel electrophoresis as a measure of DNA fragmentation index. The drug also caused aspermia in hypertensive rats treated with metosartan.

Metosartan induced apoptosis in testes tissue treated *in-vivo* with the drug through oral gavage and also caused desynapsis of the x-chromosome in sperms leading to genetic aberrations in the offspring [7]. Long term analysis of drug showed no histological aberrations in the testes tissue compared to *in-vitro* which caused the adenocarcinoma of the testes tissue. The reason may be due to direct exposure to the drug at higher concentrations. RNase caused apoptosis of the cells but the profound effect was seen in drug-treated rats compared to normal rats. Isolation of enzyme was done by DEAE and Carboxymethyl cellulose resins prepared in the laboratory using buffers. As DEAE cellulose exchanges the anions which result in separation of RNase of Testes and to elute first itself as it is a basic protein. MALDI-TOF mascot results showed expression of Cyt c and death protein-6 in the testes [8].

RNase A-induced Netosis in testes tissue which is inhibited by treatment with metosartan which is due to non-availability of RNase A. The induction of Netosis with RNase A is due to immune responsive reactions seen in rats as the enzyme is from bovine pancreas. The neutrophils in-filtered form the extracellular traps seen in Netosis induced by the RNase A. The drug may prevent the immunogenic reactions by sequestering the antigen in rats. SEM examination of testes tissue helps in identification of Netosis induced by RNase A [9].

RNase A showed peak activity in two fractions isolated by ion-exchange chromatography. HPLC identification and qualitative analysis showed and identified RNase A is present in first fraction. The number of viable sperms was decreased with metosartan compared to RNase and metosartan. The fractions isolated showed contamination in the form of RNA which is degraded after treatment with pure RNase A. The cytotoxic effect of metosartan is mainly due to its direct effect on DNA of sperms as sperm cells lack DNA repair mechanisms to counteract the damage induced by the drug [10].

Discussion and Conclusion

RNase A is found to be expressed in pancreas and secreted into digestive track to degrade the RNA present in the food. There are reports regarding protection of chromatin collapse by RNase H2. So, identification of RNase present in testes is a crucial thing as the drug intake disturbs chromatin integrity. Epididymis consists of RNase 10 which belongs to RNase A superfamily. So, experiments were conducted in our lab for isolation, identification and characterization of RNase present in testes. Rat testis RNase A is isolated by ion-exchange chromatography and further resolved by HPLC into two different Ribonucleases and MALDI- TOF MS was used for confirmation and to know the expression of proteins in the fraction separated by ion-exchange chromatography. SDS-PAGE and Immunofluorescence have confirmed the presence of RNase A in testis and its location are intracellular in testis and extracellular in pancreas.

In addition to the confirmation of RNase A the protein profile from MALDI showed the expression of Cyt C which is of both somatic and germ cell origin. Programmed cell death protein 6 was another protein present in the fraction that is specific to Rattus mitochondria. As treatment of testes in combination with RNase A and metosartan showed less chromatin cleavage compared with RNase A treated sample indicates maybe the drug inhibits the RNase A. So, studies based on Enzyme kinetics and Enzyme inhibition by U.V spectroscopy it was confirmed that enzyme was inhibited by the substrate Metosartan and was found to be negative modulator of RNase A and shows positive cooperatively.

In-vivo analysis of drug metosartan on enzyme RNase A is necessary as *in-vivo* involves complex environment compared to the *in-vitro* analysis as many metabolic reactions are going simultaneously and detoxification of the drug in the liver also to be considered while performing the study. The half-life of drug telmisartan was found to be 24 hrs. and for Metoprolol it was found to be 3-4 hrs in the body. So, *in-vitro* experiments can be considered along with *in-vivo* studies. Metosartan treatment of adult rats showed reduced sperm count, sperm viability and mitochondrial viability also. In addition to these quality parameters, analysis of chromatin integrity with aniline blue staining showed extensive DNA fragmentation in metosartan treated group compared to the combined treatment of drug metosartan and RNase A.

Comet assay and agarose gel electrophoresis further confirmed the DNA fragmentation by presence of cleaved fragment in agarose gel electrophoresis and the presence of DNA ds breaks by comet assay. Analysis of release of Cyt C from mitochondria showed profound apoptosis in drug-treated rats but release of Cyt C was not seen in RNase A+metosartan treated rats. Release of Cyt C was high in both testes and sperm of rat mitochondria compared to normal rats and its release causes activation of intrinsic pathway of apoptosis by activating caspase 9 and formation of apoptosome complex which finally leads to death of the tissue. So, *in-vivo* analysis has shown drug treatment results in apoptosis in both testes tissue and sperm cells.

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