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Research Article

INHIBITION OF RNase BY DRUG METOSARTAN IN TESTIS TISSUE

Eswari beeram

Department of Biochemistry, Sri Venkateswara University, Tirupati,
Andhra Pradesh, India-517502

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ABSTRACT

RNase is the model for studying inhibition by metosartan as it states the reliability of the enzyme inhibition by certain drugs. The drug inhibits RNase for 1-3hrs and the samples 4 and 5 have show fullfledged activity of RNase.

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INTRODUCTION

RNase is used as one of the model to study drug inhibition because less is known about metosartan the drug of β -receptor blocker on heart that is used to treat cardiac arrhythmias. So it is important to do studies on this drug as previous reports of mine reported apoptosis in germinal tissues, and to find that whether it inhibits RNase A in vitro or not was one of the task that was to be determined by this study. As we have found out that as it inhibits RNase A, protein synthesis is also effected but not to that extent as the drug is active only for 2-3 hrs. It is important to study whether it arrest RNase A only in testis or globally. RNaseA an endonuclease that cuts the RNA from middle of it by cleaving after cytidine^(1,2) and also the uridine bases as fast as possible⁽³⁾. Agarose gel electrophoresis is one of the technique used for the study to know if the drug inhibits RNase or not by simply monitoring through degradation of RNA.

MATERIALS AND METHODS

Agarose gel electrophoresis

1% agarose gel acts as the medium for this study. Proteinase cocktail was useful to stop the reaction after prescribed time period as it inhibits nucleases. Loading buffer was prepared with 4g sucrose, 25mg bromophenol blue and 10ml of distilled water and the experimental setup was as follows. 100 μ lRNA sample (10mg/ml), 100 μ l RNase (5mg/5ml PBS), 100 μ l drug metosartan (25mg/50ml) and incubated for 1- 5 hrs in each aliquot as 1hr, 2hr, 3hr,4hr and 5 hrs and for figure B it is similar to figure A conditions except the RNA was 50mg

/50ml from which 1ml is used and R+R is included with 100 μ l RNase A and 1ml RNA and the study is only for 3hrs. After the time interval it was treated with proteinase K cocktail (5mg/ ml) of which diluted to 50 μ g/ml and it also contains 1mM EDTA and 0.5% SDS and for figure 2 b-mercaptoethanol which inhibits RNase A was used. The samples were kept aside in deep freezer for 1 day and the experiment was performed on the next day for second set up on the same day. 1X TAE buffer was made from which 20ml was taken and make up to 500ml. Samples of 20 μ l were loaded after keeping the gel cast in electrophoresis tank with buffer. The electrophoresis was carried for 2hrs and after that, the gel was put on the UV transilluminator and images were Captured.

RESULTS

From the figure B the bands were respectively seen for only at 1hr,2hr and 3hr samples which means that the drug inhibits RNase and where as drug is active for only 3 hrs invitro and the RNase will become active in the 4 th and 5th hrs from the UV- Visible studies. So the protein synthesis for that particular RNA molecules that has to be degraded by the cell was not proper as the enzyme is inhibited which is a wastage of energy by the cell if it inhibits globally and also there will be more useless effects such as not degradation of regulatory RNAs which has kept in mind.

The experiment also states that the time required for inhibition of RNA as observed as comet on the gel which indicates some amount of RNA was degraded with RNase before incubation with proteinase K cocktail.

*Corresponding author: **Eswari beeram**

Department of Biochemistry, Sri Venkateswara University, Tirupati, Andhra Pradesh, India-517502

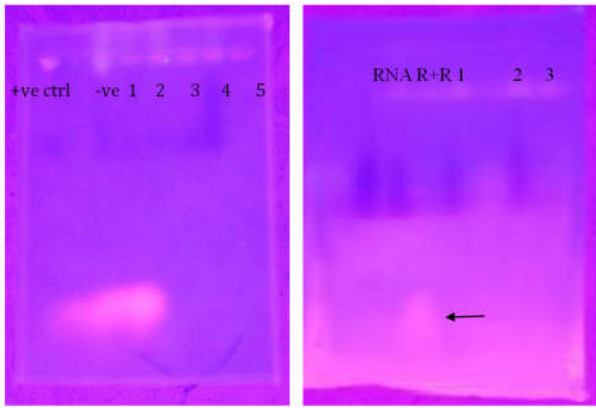


Figure A

Figure B

The positive control was loaded by taking some amount of RNA powder and dissolved in distilled water from which 10 μ l was taken and loaded. If you observe carefully there is fluorescence present in the well itself, Which indicates that the sample was not moved due to high concentration and in the wells 1,3,5 are moving in opposite direction which indicates hydrodynamic interaction between different parts of RNA where as in second set up 1ml of RNA solution was taken.

In the figure A +ve ctrl indicates positive control i.e., RNA, -ve indicates no Sample and 1,2,3,4,5 indicates hrs of incubation. In figure B, R+R indicates RNA+ RNaseA, 1,2,3 are incubation in hrs with RNA+RNase A+ drug metosartan. Arrow indicates degraded RNA. From the figure it is clearly understood that the drug inhibits RNase and the future aspect include to find whether it competitively inhibit the enzyme or not.

DISCUSSION

Agarose gel electrophoresis normally used for molecular biology studies to know about RNA and DNA detection, degradation, purity. But here a new approach by us to use it for inhibition studies of RNaseA by drug metosartan. It was also known from this that b- mercaptoethanol is effective in inhibiting the reaction than the Proteinase k.

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