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Evaluation of cytotoxicity of Atenolol in Allium cepa L.

Madhavi Jangala, Santoshi Manche, Suman Mudigonda*, Meghanadh Koralla Raja, Bapu Rao Sangras, Vaidyanath Konagurtu**

Department of Genetics and Molecular Medicine, MAA E.N.T. Institute, Somajiguda, Hyderabad, India.
*Department of Biotechnology, University College of Science, Osmania University, Saifabad, Hyderabad, India.
**Corresponding Author Email: konagurtuv@yahoo.co.in

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Abstract

Atenolol, an adrenergic beta blocker, commonly employed in the management of cardiovascular diseases, has been evaluated for its cytotoxicity using Allium cepa L assay system. Four different treatments, 2 spurt treatments (T_1 & T_3) of 2h duration each varying at time points of cell division cycle (6AM to 8AM and 11AM to 1PM), T_2 of 3h duration (8AM to 11AM), and T_4 of 17h duration (approximately for one cell cycle), were given with 10µg/ml Atenolol to the root meristems of A. cepa and were observed for their recovery for 72h with 24h intervals. All the treatments induced wide range of aberrations including disturbed meta-anaphases, chromosome breaks, bridges, and stickiness. The 0h of T_2, 48h of T_1, 72h of T_1 and T_4 showed significant decrease in cell division. Differential response to the drug toxicity and significant decrease in cell division indicates the possibility of Atenolol affecting mechanisms that ensure DNA stability and the cell cycle progression. In view of the widespread use of the drug in the management of cardiovascular diseases and chronic hypertension, more intensive studies employing mammalian and submammalian assay systems are needed.

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Key words: Mitotic Index, Cell Division Cycle, Genotoxicity.

1. INTRODUCTION

Atenolol, a beta blocker is a replacement of propranolol, generally employed by the physicians for the management of hypertension, angina, tachycardia, and acute myocardial infarction [1]. The dose levels are between 50 to 100mg/day of oral administration with a half life of about 10h. It is rapidly absorbed from the gut and attains a peak concentration in the serum within 2-3h [2]. Its metabolism is minimal due to its hydrophilic nature and almost 50% of the drug is always available in the circulatory system [3]. Adverse side effects viz., hepatotoxicity, lupus erythematosus, septal panniculitis, memory impairment, breast pain and swelling are known to be induced by Atenolol [4-9]. It is also associated with fetal growth retardation when given in pregnancy [10, 11]. Moreover, chronic exposure to Atenolol resulted mainly in the induction of chromosome loss in in vitro and in vivo studies [2]. No teratogenicity of Atenolol is reported till date. However, some studies have shown mutagenic potential of other widely used beta blockers [1, 12-15].

The published data on cytotoxicity of Atenolol affecting the cell division and repair mechanisms is not available. In view of this, the present study was conducted to evaluate the effect of Atenolol on cell division cycle (CDC) and maintenance of the check points. It is assumed that in short time frame most cells will be in similar phase and consequently a similar number of cell divisions will takes place. The hypothesis evaluated is whether there is a difference between times of exposure to the drug vis a vis dynamics of cell cycle in inflicting genetic damage. The preliminary results are presented in this paper.

2. MATERIALS AND METHODS

Onion bulbs were purchased from a farmer’s market (Rythu Bazar) situated at Mehadipatnam, Hyderabad. Uniform sized...
Table S1: The effect of Atenolol on Percent frequency of chromosomal aberrations, Mitotic and Phase Indices

<table>
<thead>
<tr>
<th>Time</th>
<th>System/ Treatment</th>
<th>No of Cells examined</th>
<th>No of dividing cells</th>
<th>Mean ± SE</th>
<th>Mitotic Index</th>
<th>Total Aberrant cells</th>
<th>% Frequency of Abnormal cell</th>
<th>Phase Index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td>Total cells</td>
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<tr>
<td>72Hrs</td>
<td>Control</td>
<td>300</td>
<td>300</td>
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<td>0</td>
<td>93.97</td>
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<td>Control</td>
<td>300</td>
<td>243</td>
<td>48.6±14.1</td>
<td>8.1</td>
<td>68</td>
<td>7.28†</td>
<td>28.09</td>
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<tr>
<td></td>
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<td>300</td>
<td>216</td>
<td>43.2±9.4</td>
<td>7.2</td>
<td>3</td>
<td>68.78±13.0</td>
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<td>263</td>
<td>52.8±7.4</td>
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<td>56</td>
<td>1.87†</td>
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<tr>
<td>48hrs</td>
<td>Control</td>
<td>300</td>
<td>254</td>
<td>50.8±5.1</td>
<td>8.5</td>
<td>18</td>
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<td>7.15†</td>
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<td>0.52</td>
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<td>479</td>
<td>95.8±6.02</td>
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<td>116</td>
<td>3.80†</td>
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<td>6.3</td>
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<td>16</td>
<td>116</td>
<td>3.80†</td>
<td>24.26</td>
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</table>

* p < 0.05, ** p < 0.01, *** p < 0.005 in One Way ANOVA, ‡‡ p < 0.01 level (2-tailed) and ‡ p < 0.05 ,Correlation significant level (2-tailed),† test Significant, †< 0.05 Chi square significant
Figure 1a-d: Spectrum and frequency of chromosomal aberrations induced by Atenolol.
bulbs were selected and the yellow shallows of dry bottom plate covering the root primordial is carefully removed prior to the immersion of the root zone into the water. Healthy bulbs with growing roots of 1-2cm long were used in the experiment. Atenolol tablet were purchased from the local chemists and druggist shop and aqueous solution of 10µg/ml Atenolol was poured into 100ml corning glass beakers, over which onion bulbs were placed for the treatment in such a way that growing roots are well immersed in the solution. Four different treatments, T₂, T₄ of 3h (8AM to 11AM) and 17 h duration respectively and two spurt treatment T₁ and T₃ of 2h duration at different time point (6AM to 8AM and 11AM to 1PM) were given. Appropriate controls were maintained. Each treatment was replicated 4-5 times and the recovery was observed at the end of 24h, 48h and 72h.

The root tips were fixed in a Carnoy’s fixative (ethyl alcohol: acetic acid in 3:1 ratio) for 24h and further processed by Haemotoxylin squash technique of Subramanyam and Subramanyam (1970). Briefly, the root-tips were hydrolyzed in 1N HCl for eight minutes at 60°C, rinsed with distilled water for 2-5 minutes and then transferred to 4% Ferric alum (mordent) for 20 minutes followed by rinsing with water. 1-2mm long root tips were stained using Haematoxylin and squashed in a drop of 45% acetic acid after smearing the cover slip with Meyer’s albumin and flaming it. Observations were made under Olympus microscope for various cell division stages. Approximately, 500 cells of 6 meristems were screened to determine mitotic index, phase index and frequency of specific chromosomal aberration. The percent frequency of aberrations was computed based on the total number of cells scored and on the number of cells in division.

Statistical analyses were performed on mitotic index and chromosomal aberration frequency using SPSS vs 18 (Chicago). The one way ANOVA, Chi Square and Z test was performed to determine the significant differences between the cell divisions, mitotic phases and chromosomal

---

**Figure 2:** Different Chromosomal Aberrations induced by Atenolol in root tips of Allium cepa a) Late prophase with ring Chromosome b) Late Prophase with fragments c) Disturbed Metaphase with fragments d) Sticky Metaphase with ring chromosome e) Metaphase with ring chromosome and breaks and gaps f) Disturbed Metaphase with breaks and fragments g) Star Anaphase h) Anaphase with breaks and vagrants i) Anaphase with bridges j) Late Anaphase with breaks and adjacent cell with micronucleus k) Disturbed metaphase in a polyploid cell l) micronucleus in a bivalent cell
aberrations of treated and their respective controls. Pearson’s
Correlation was performed to find the correlation between
cell division and chromosomal abnormalities.

3. RESULTS AND DISCUSSION

The cell division cycle can be delineated into major
phases G1, S, G2 and M phase. Cell division manifests in a
periodic manner during 24h period [26-31]. A double rhythm
was found in roots of Allium cepa grown under room
condition in moist sawdust [32]. In our study, the cell division
was shown to be maximum between 8AM to 11AM (Table
S1). Treatments before and after the cell division maxima are
expected to be in G2 phase and in G1 phase respectively. By
treating with drugs specifically for the duration of each phase
and allowing the recovery period of three cycles of cell
division (24, 48 and 72 hours) one can evaluate the effect of
the drugs on each check point and their role in maintaining
DNA and/or chromosomal stability during the cell division
cycle.

DNA damage checkpoints are essential for the
survival of cell and organism. Several genes control the
ability of cells to arrest the cell cycle in response to DNA
damage, providing chance to repair [16-18]. The genome data
of Arabidopsis pointed out that the DNA repair is conserved
highly between plants and mammals than within the animal
kingdom [19, 20]. Allium cepa assay system was evaluated by
several workers to assess genotoxicity [21-25]. In the present
study, Allium cepa assay was employed to evaluate the
cytotoxicity of Atenolol in relation to cell division cycle.

The specific aberration induced depends on the time
at which the interphase nucleus is exposed to a clastogen
[33]. Interesting results were obtained when mitotic index
was considered as a function of damage afflicted on the
genetic material. The data on mitotic index and different
phase indices are presented in Table S1. The mitotic index
results (one way ANOVA) showed significant difference
between the treated and respective controls at 48h and 72h
recovery of T1, 0h recovery of T2, and 72h of T4 (Table S1).
Moreover, significant differences in mitotic phase indices
(chi-square) were found at different recovery periods when
compared to their respective controls of different timepoint
treatments. (Table S2)

When treatment was given before the cell division
maxima (T1), the MI was enhanced and declined
subsequently, when compared to the control at the end of
second and third recovery periods respectively. The G2
checkpoint arrests damaged cells in G2, delaying entry into
mitosis until the damage gets repaired [34]. Possibly, the G2
check point arrested the cells entering into the mitosis until
48h. The unrepaird cells entering into mitosis may account
for the significant enhancement and decline of MI.
Significant decrease in prophase (72h) and telophase (48h
and 72h) and increase in metaphase (0h and 72h) and
anaphase (48h and 72h) indicates accumulation of the cells at
meta-anaphase.

Significant decline in MI was seen even at 72h
recovery period of T4 (one cell cycle treatment) indicating
cell death due to damage. The phase index varied
significantly at the telophase (0h, 24h and 48h). Furthermore,
the fact that the chromosomal aberrations prevailed even
after three cycles of recovery indicates that the damage
caused by the chronic exposure to the drug failed to get
repaired, and eventually resulting in the cell death.

When the treatment is given exclusively after the
cell division maxima (T4), there was cyclic increase and
decrease in the MI and chromosomal aberrations as compared
to the control. The Prophase, Anaphase and Telophase index
differed significantly at different recovery periods. Exposure
in the G1 phase results in damage of the entire chromosome.
Probably, the aberrations seen at the 48h recovery period is
due to the lesions becoming homozygous. No significant
difference could be seen in the mitotic index; however,
chromosomal aberration decreased considerably at the end of
72h. This implies that the repair mechanisms after cell
division maxima were efficiently correcting the errors.

T3 treatment showed high sensitivity and the
chromosomal aberrations persisted in all the recovery
periods. Significant decrease in the MI immediately after the
treatment indicates the arrest of the cells entering into the
mitosis. The increase in chromosomal aberration frequency at
the first recovery cycle possibly be due to the release of
arrested cells or/and aberration caused earlier could have
resulted in more aberration in the next generation. Further,
significant correlation was observed between the cell division
and chromosomal aberrations at the recovery periods of 48h
of T1, 24h of T2, 0h and 48h of T3, 0h of T4 recovery periods
(Table S1) indicating that the aberrations seen in these
treatments are dependent, perhaps due to arrest at the mitotic
division. However, no significant difference was seen in the
phase indices suspecting that the arrest of the cells was before
the cell division maxima probably at the G2 phase.

Frequency and the spectrum of chromosomal
abnormalities elicited by Atenolol following the treatment of
onion root meristems during different timepoints of cell cycle
viz; T1, T2, T3 and T4 are given in Fig S1 and S2. The
frequency of aberrant cells observed was significantly higher
in the treatments than that of controls.

The data on the distribution of chromosomal
aberrations (Fig S1 and S2), indicate that Atenolol provoked
a wide spectrum of cytological abnormalities; disturbed meta
and anaphases, chromosome breaks, stickiness, bridges,
fragments at ana-telophase, chromosomes lagging and
condensation, micronucleus and polyplody. The metaphase
and anaphase perturbations were observed more frequently.
The clastogenic action of Atenolol was evident from the
appearance of breaks and gaps. Chromosome breakages are the result of unfinished repair or misrepair of DNA which can result in cell death or a wide variety of genetic alteration [35-37]. A sticky chromosome can also lead to death of the cells [38]. The presence of the micronucleus in different cells leads to permanent chromosomal loss [2]. Polyploid cells indicate that the cells might have entered into endoreduplication followed by cell division arrest [39].

4. CONCLUSION: - It is quite possible that the Atenolol provoke disturbances in the cell division cycle affecting the MI, phase index and chromotoxicity. The present work is the first of its kind to monitor the possible genotoxic potential of antihypertensive drug Atenolol, making use of exclusive treatments during different phases of CDC and evaluating the consequences in toto. The results obtained in the study by using different end points are quite interesting. The preliminary results suggest that there is a significant damage caused by the drug. More in-depth studies are warranted.

5. ACKNOWLEDGEMENTS
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6. REFERENCES


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