Amlodipine Exhibits Cardio Protective Effect on Doxorubicin Induced Cardiotoxicity in Rats

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Abstract

The protective effects of amlodipine and carvedilol on DOX induced cardiotoxicity were investigated in albino wistar rats by measuring the enzymatic, non-enzymatic antioxidant levels, serum enzyme levels and study of ECG alteration.

Cardio toxicity was induced on 7th day (DOX 50 mg/kg ip) for different groups of animals. The rats were divided in to 4 groups (n=6), Group-I normal control, group-II DOX (50 mg/kg ip), group-III Amlodipine (10 mg/kg oral) + doxorubicin (50 mg/kg ip) and group-IV carvedilol (3mg/kg oral) + doxorubicin (50 mg/kg ip) for 10 days. On 11th day rats were anaesthetised (ketamine100mg/kg ip), and ECG was measured using power lab software. Blood samples were collected by retro orbital plexus and the obtained serum was used for the estimation of CK, CK-MB, LDH, calcium. The rats will be sacrificed by ketamine overdose and the heart tissue was isolated and PMS was prepared from its portion. From the PMS SOD, catalase, GSH, LPO were estimated. The remaining portion was used for histopathology study.

The results of this study reveals that there are increased level serum and tissue biomarkers in DOX amlodipine and carvedilol treated rats and tissue biomarkers level had decreased in DOX induced cardio toxicity. The animals treated with Amlodipine and Carvedilol showed decrease level of serum biomarkers. The tissue antioxidant level has increased. Further, ECG and Histopathological study showed significant improvement in amlodipine and carvedilol when compared to DOX treated rats. From the results of the study it is concluded that amlodipine and carvedilol has showed cardio protective effect on DOX induced cardiotoxicity in rats.

Keywords: Amlodipine; Carvedilol; Cardio protective; DOX (Doxorubicin); ECG (Electrocardiogram)

Introduction

Cardiovascular diseases (CVDs) are the major health problem of advanced as well as developing countries of the world. Hypertension is the common cardiac disease followed by Ischemic Heart Disease (IHD). CVD include Coronary Heart Disease (CHD, heart attacks), rheumatic heart disease, peripheral artery diseases, congenital heart disease and heart failure [1] Doxorubicin is broad spectrum anticancer drug. It is a potent anthracycline antineoplastic agent and it is a chemotherapeutic drug. It is used in the different types of cancer like solid tumors (breast and ovarian cancer) and haematological tumors (leukemia and lymphomas) and lever cancer. The main cause of DOX is cardiotoxicity occasionally associated with heart failure in severe cases [2] Mechanism of DOX-induced cardiotoxicity involves the generation of Reactive Oxygen Species (ROS), especially superoxide anion and hydrogen peroxide. The main cellular damage caused by ROS includes lipid peroxidation, protein cross-linking, and DNA fragmentation. These may lead to cardiac dysfunction, apoptosis, and development of cardiomyopathy [3-6].
Materials and Methods

Experimental animals

Albino wistar rats were procured from Venkateshwara Enterprises Bengaluru. The animals were kept in animal house of PES College of pharmacy Bengaluru for experimental use. Animals were acclimatized for 7 days under standard husbandry conditions i.e.; room temperature of 27 ± 10 °C; relative humidity 45- 55% and a 12:12h light/ dark cycle. The animals had free access to the standard rat pellet with purified water. Each experimental group had separate set of 6 animals. The approval of Institutional Animal Ethics Committee (IAEC) of PES College of Pharmacy was taken prior to the animal experiments (Reference NO. - PESCP/ IAEC/02/13-12-2014). All the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Study of Cardio protective activity of Amlodipine and Carvedilol in drug induced cardio toxicity by using animal model experiment.

Model 1: Study of Cardio protective activity of Amlodipine and Carvedilol in Doxorubicin induced cardio toxicity Experimental design [7,8].

The animals were randomly divided into 4 groups consists of 6 animals each.

Wistar rats weighting (180-200g) either sex was selected under healthy conditions for experimental purpose.

Group 1: Normal control received normal water (vehicle) for 10 days

Group 2: Received Doxorubicin 5 mg/kg ip at single dose on 7th day. Vehicle was administered for 10 days except 7th day.

Group 3: Received Amlodipine 10mg/kg orally for 10 days and Doxorubicin 5mg/kg ip on 7th day

Group 4: Received Carvedilol 3mg/kg orally for 10 days and Doxorubicin 5mg/kg ip on 7th day

After 24 h (11th day) rats were anaesthetized electrocardiograph was recorded using power lab system. Blood samples were collected by retro orbital puncture and serum was separated and used for the estimations of serum biomarkers Creatinine, Creatinine kinase, Creatinine kinase –MB, LDH. Following animals were sacrificed by administration of over anaesthesia heart specimen was collected and used for histopathology and also used for tissue biochemical estimations SOD, LPO, catalase and GSH.

Recording of ECG [9]

1. The animal was restrained by ketamine 30 mg/kg ip and rested down to the surgical table.

2. Then the positive electrode was attached to the surface of the left limb, the negative electrode was attached to the surface right limb and the earth electrode was attached to the surface right limb and the earth electrode was attached to the surface of the posterior limb of the animal.

3. The electrode was then attached to the transducer which was connected to the data acquisition system.

4. The ECG was recorded in the system with the help of lab tutor softer.

Collection of blood samples and separation of serum [10]

The animals were subjected to light ether anaesthesia. The blood was collected from retro orbital plexus in each rat and collected in ependorff’s tubes and allowed to coagulate for 30 min at 37° C. The coagulated blood was centrifuged at micro centrifuge at 2500 rpm for 10 min the freshly prepared serum is used for assay or stored the samples in aliquot at •20ºC which was further used for biochemical estimations; avoid repeated freeze/thaw cycles.

Preparation of tissue homogenate [11]

The isolated heart was rinsed with ice cold normal saline to remove excess of blood and 10%w/v homogenate was prepared in 10% chilled phosphate buffer (PH 7.0). The tissue was chopped and minced with Teflon homogenator at 3000 rpm for 15 min at 4°C to separate the debris. The collected supernatant was again centrifuged at 5000 rpm for 20 min at 4°C to further break the cell membranes. The obtained supernatant was used for assay or stored at ≤20ºC (94).

Biochemical analysis [12]

At the end of the experimental period, overnight fasted animals were sacrificed by cervical decapitation under light ether anesthesia and blood was collected, serum was separated by centrifuging at 3,000 rpm for 10 min. The serum was used for assay of biochemical parameters such as CK-MB, CK, LDH and Calcium. After biochemical estimation animals were euthanized by overdose of Ketamine anesthesia and heart was used for tissue parameters such as SOD, GSH, LPO and Catalase, remaining heart tissue was used for histopathology.

Histopathology studies.

The heart of different groups was perfusion-fixed with 4% paraformaldehyde in 0.1 M phosphate buffer. The heart was removed and postfixed in the same fixative overnight at 48°C. The heart was then routinely embedded in paraffin and stained with Hematoxylin- Eosin.
Statistical analysis

Values were expressed as Mean±SEM from 6 animals. The results were subjected to statistical analysis by using one-way ANOVA followed by Bartlett’s test for to calculate significance. P<0.05 was considered as significant.

Results

Doxorubicin induced cardiotoxicity

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>P wave (sec)</th>
<th>QRS complex (sec)</th>
<th>QT interval (sec)</th>
<th>RR interval (sec)</th>
<th>HR beats/ min</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>0.024±0.0021</td>
<td>0.026±0.0019</td>
<td>0.049±0.0041</td>
<td>0.30±0.020</td>
<td>200.8±11.</td>
</tr>
<tr>
<td>II</td>
<td>Cyclophosphamide</td>
<td>0.17±0.042</td>
<td>0.049±0.0075</td>
<td>0.193±0.04</td>
<td>0.58±0.09</td>
<td>109.9±17.10</td>
</tr>
<tr>
<td>III</td>
<td>Amlodipine+ Cyclophosphamide</td>
<td>0.023±0.0016</td>
<td>0.016±0.003</td>
<td>0.036±0.0064</td>
<td>0.27±0.01</td>
<td>202.3±2243</td>
</tr>
<tr>
<td>IV</td>
<td>Curvedole + Cyclophosphamide</td>
<td>0.025±0.0028</td>
<td>0.021±0.001</td>
<td>0.032±0.0031</td>
<td>0.31±0.017</td>
<td>194.7±9.3</td>
</tr>
</tbody>
</table>

Table 1: ECG Mean±SEM in each group.

ECG was expressed in second in every group and each value is expressed as Mean ± SEM for groups of 6 animals done by One Way ANOVA followed by Bartlett’s test for equal variances. Where **P<0.01, ***P<0.001; compared with normal control group.

**P<0.01, ***P<0.001; compared to toxic group. Normal rats showed normal ECG wave patterns whereas animals treated with Doxorubicin alone showed significant alterations in QRS complex, ST segment and RR interval.

Figures 1-4: Group-I, Group-III and Group-IV have shown significant decrease in the P, QRS, QT and RR interval when compared with Group-II (Toxic Group).
Figure 5: Group-I, Group-III and Group-IV have shown significant increase in the heart rate when compared with Group-II (Toxic Group).
Table 2: Effects on CK-MB, CK, LDH and Calcium in normal, DOX, amlodipine and carvedilol treated rats.

Each value is expressed as Mean ± SEM for groups of 6 animals done by One Way ANOVA followed by Bartlett’s test for equal variances. Where "***a P<0.001; compared with normal control group, "**b P<0.01, "***b P<0.001; compared to toxic group.

Figures 10-13: Group-I, Group-III and Group-IV have shown significant decrease in the level of CK, Calcium, CK-MB, LDH when compared with Group-II (Toxic Group).
Table 3: Effect on Catalase GSH, LPO and SOD in normal, DOX, amlodipine and carvedilol treated rats.

Each value is expressed as Mean ± SEM for groups of 6 animals done by One Way ANOVA followed by Bartlett’s test for equal variances. Where ***a P<0.001; compared with normal control group **b P<0.01, ***b P<0.001; compared to toxic group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Drug treatment</th>
<th>Catalase µMole of H₂O₂ / min</th>
<th>GSH Units/mg of protein</th>
<th>LPO nmoles/ MDA/min/mg of protein</th>
<th>SOD Units/mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Normal control</td>
<td>153.8 ± 3.478</td>
<td>173.3 ± 17.83</td>
<td>7.433 ± 1.366</td>
<td>29.83 ± 1.72</td>
</tr>
<tr>
<td>Group-II</td>
<td>Doxorubicin treated</td>
<td>31.93S±6.12***a</td>
<td>24.88± 3.32***a</td>
<td>14.50± 0.528***b</td>
<td>4.527±0.827***a</td>
</tr>
<tr>
<td>Group- III</td>
<td>Amlodipine+ Doxorubicin</td>
<td>89.36± 0.74***b</td>
<td>130.6±25.4***b</td>
<td>6.442 ± 0.94***b</td>
<td>19.84±1.38***b</td>
</tr>
<tr>
<td>Group- IV</td>
<td>Carvedilol+ Doxorubicin</td>
<td>53.67± 1.174***b</td>
<td>109.3 ±11.35***b</td>
<td>7.810±1.290***b</td>
<td>20.23±2.09***b</td>
</tr>
</tbody>
</table>

Figure 14: Group-I, Group-III and Group-IV have shown significant decrease in the level of GSH when compared with Group-II (Toxic Group).

Figure 15
Figures 15-17: Group-I, Group-III and Group-IV have shown significant increase in the level of GSH, catalase, LPO when compared with Group-II (Toxic Group).

Histopathological studies of the heart in DOX induced cardiotoxicity.

Figure 16

Figure 17

Figure 18: Normal: Myocardium shows intact arrangement of the cardiac muscle. The interstitial space appears intact. [H & E 10x].

Figure 19: DOX Treated Cardiac muscle fibers show loss of integrity of myocardial cell membrane, myofibrillar structure with loss of striations and loss of continuity with adjacent. myofibrils. [H & E 10x].
Figure 20: Amlo+Dox: Myocardium shows intact arrangement of the cardiac muscle fibers. Some of the cardiac muscle fibers show loss of integrity of myocardial cell membrane, myofibrillar structure with loss of striations (arrow). [H & E 10x].

Figure 21: Carve+Dox: Myocardium shows intact arrangement of the cardiac muscle fibers, few of the cardiac muscle fibers show loss of integrity of myocardial cell membrane, myofibrillar structure with loss of striations. [H & E 10x].

Discussion

Electrocardiograph

ECG studies showed significant alterations in group-II rats (DOX treated) rats as compared to Group-I (normal rats). There is a significant increase in the P wave duration indicating atrial hypertrophy and atrial arrhythmias, QT interval indicates myocardial infarction, T wave indicating hyperkalemia, increased RR interval indicating ventricular tachycardia and prolongation of QRS complex causing conduction impairment in ventricles.

Rats treated with Amlodipine (10 mg/kg oral) + Doxorubicin (50 mg/kg ip) and Carvedilol (3 mg/kg oral) + Doxorubicin (50 mg/kg ip) and showed significant changes in the P wave, QRS complex, reduction in QT interval, RR interval and normal heart rate when compared to DOX and CYP group. This indicates the normal heart condition. This may support as cardioprotective [13].

Doxorubicin model

Heart tissue parameters such as SOD, GSH, Catalase and LPO. Rats treated with DOX (50 mg/kg ip) showed decrease in the level of SOD, GSH and Catalase when compared to the normal rats. This is due to Generation of free radicals decreases in antioxidant mechanism and there by damage cellular constituent. And also due to the high affinity of doxorubicin towards phospholipids component of the mitochondrial membrane in the myocardial cells, thus leads to the accumulation of doxorubicin in the heart tissue. Rats treated with Amlodipine (10 mg/kg oral) + Doxorubicin (50 mg/kg ip) and Carvedilol (3 mg/kg oral) + Doxorubicin (50 mg/kg ip) showed a significant increase in levels of SOD, GSH and catalase when compared with Doxorubicin treated group.

Rats treated with DOX (50 mg/kg ip) showed increase in the level of LPO when compared to the normal rats. Lipid peroxides which are the break products of lipids are measured in the tissues as an indicator of the lipid peroxidation. In this experiment, MDA levels were significantly elevated after a single dose of DOX and supported the hypothesis that a major role is played by free radicals in DOX cardiotoxicity. Rats treated with Amlodipine (10 mg/kg oral) + Doxorubicin (50 mg/kg ip) and Carvedilol (3 mg/kg oral) + Doxorubicin (50 mg/kg ip) showed a significant decrease in levels of LPO when compared with Doxorubicin treated group [14] Serum parameters such as CK, CK-MB, LDH and Calcium. Decrease in the concentration of these enzymes in the heart may cause myocardial damage to the cells. In the presented study DOX treated groups showed increase in the level of these enzymes. Because myocardial cells which are damaged due to incomplete oxygen supply or glucose, the cell membrane becomes permeable or may rupture which results in leakage of enzymes. Rats treated with Amlodipine (10 mg/kg oral) + Doxorubicin (50 mg/kg ip) and Carvedilol (3 mg/kg oral) + Doxorubicin (50 mg/kg ip) showed a significant decrease in levels of these enzymes when compared with Doxorubicin treated group [15].

Conclusion

From the result of the study it is concluded that amlodipine and carvedilol has cardio protective activity against doxorubicin induced cardio toxicity in rats. It is also concluded that doxorubicin...
and cyclophosphamide administered rats with amlodipine and carvedilol have shown significant attenuation of the oxidative stress and free radical scavenging. The ECG of amlodipine and carvedilol i.e. group-III and group-IV has significant effect on P wave, QRS complex, QT interval and heart rate remaining group also had direct effect on each parameter of ECG.

Cardioprotects of amlodipine and carvedilol significantly reduced the elevated levels of serum biomarkers like CK, CK-MB, LDH, calcium when compare to DOX induced cardiotoxicity.

Amlodipine and carvedilol has shown significant increase in the levels of tissue biomarkers such as SOD, GSH, catalase. Considering improvement in the serum biomarker levels and tissue biomarker levels amlodipine and carvedilol showed cardio protective activity.

Finally, it concluded that amlodipine and carvedilol has cardio protective effect on doxorubicin induced rats.

References