Effects of Ivabradine on Cardiotoxicity Induced by Doxorubicin Treatment in Rats

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Abstract

The aim of this study was to investigate the effects of ivabradine against doxorubicin (DOX)-induced cardiotoxicity in rats.

Material and Methods: A total of 28 female rats were randomly classified into 4 groups: (A) control (n=6 rats), (B) doxorubicin (DOX-treated group) (n=7 rats) received single dose of DOX (20mg/kg, i.p), (C) DOX+ivabradin treated group (n=8 rats) (10mg/kg p.o.) ivabradine lh before the DOX treatment continued for 10 days and ivabradine group (n=7 rats) received ivabradine (10mg/kg/day p.o.) for 10 days. Electrocardiogram, heart rate, blood pressure, biochemical markers of oxidative stress, serum creatine kinase level and histopathological changes were measured before and 10 days after the beginning of drugs.

Results: Of the present work showed that ivabradine decreased heart rate; attenuated doxorubicin-induced elevation of oxidative stress and histopathological change.

Conclusion: Ivabradine showed protective effect against doxorubicin induced cardiomyopathy. This may be partly through heart rate lowering effect of ivabradine, and decreased oxidative stress.

Key Words: Ivabradine — Doxorubicin — Cardiomyopathy — I 09 current.

Introduction

DOXORUBICIN (DOX), a member of the quinine-containing anthracycline class of cytostatic antibiotics, is a potent and broad spectrum chemotherapeutic agent Hi. Its clinical use is limited seriously by its cardiotoxicity [2]. The drug's toxicity is known to be closely related to the generation of reactive oxygen free radicals, lipid peroxidation, and decreased glutathione (GSH) levels [3]. Increased oxidative stress and antioxidant deficits have been suggested to play a major role in DOX-induced cardiotoxicity [4]. It has been showed that doxorubicin treatment caused an increase in mean blood pressure in an experimental model [5]. This important finding may play a role to the pathogenesis and therapy of DOX-induced cardiotoxicity.

This hypothesis is also consistent with the findings of the studies, suggesting that elevated heart rate represents a risk factor for cardiovascular morbidity and mortality both in primary prevention and in patients with hypertension, coronary artery disease, and myocardial infarction [6]. HR variability has been shown to be associated with coronary plaque ruptures and subclinical inflammation in healthy middle-aged and elderly patients [7,8].

Ivabradine, a HR-lowering agent, acts in the sinus node by selective and specific inhibition of the cardiac pacemaker If current that controls the spontaneous diastolic depolarization in the sinus node with no effect on myocardial infarction [6]. It has been reported that HR reduction by ivabradine reduced oxidative damage and thereby improved endothelial dysfunction [6]. Furthermore, Heusch [10], declared that ivabradine exerted pleitrophic action beyond HR reduction. Many studies and clinical applications of ivabradine belong to the cardiology area and are particularly related to the symptomatic treatment of stable angina pectoris in patients with normal sinus rhythm who have contraindication to or intolerance to beta blockers and heart failure due to left ventricular systolic dysfunction for its pure pharmacodynamic properties.

The aim of the present study was to investigate the possible effects of ivabradine against DOX-induced cardiotoxicity in rats using biochemical markers of oxidative stress both in serum and in tissue specimens.
Material and Methods

Animals:
A total of 28 female albino strain rats of 10-12 weeks of age and weighing 250-300 g were brought from Experimental Animal Breeding Farm, Helwan, Cairo and placed in a temperature of 21±2°C in which a 12:12-h light: Dark cycle was maintained. The rats were fed with a standard chow pellet diet with tap water ad libitum. All experiments in this study were done in pharmacology department of Benha College of Medicine and approved by the committee of Benha University.

Experimental protocol:
The rats were randomly assigned to four groups as follows:

Group (I): Control group: (n=6 rats).

Group (II): Doxorubicin (DOX) (20mg/kg i.p.) group: (n=7 rats) injected intraperitoneally (i.p.) with DOX (DOXO-Teva®, Med-Ilac, Istanbul, Turkey) 20mg/kg in a single dose.

Group (III): DOX+ ivabradine- treated group: (n=8 rats) injected i.p. In a single dose of 20mg/kg DOX with 10mg/kg p.o. Ivabradine 1 h before the DOX treatment and continued for 10 day.

Group (IV): Ivabradine group: (n=7 rats) received ivabradine 10mg/kg/day p.o. For 10 days.

Ivabradine (Coralan®, Servier Corp, Istanbul, Turkey) was dissolved in 0.5ml saline (0.09% NaCl) solution and the control group received the same amount of saline by the same route.

The applied dosage of DOX was chosen according to the related literature from our research team’s previous research III. In these studies, ivabradine was dissolved in saline to obtain a final concentration of 10mg/ml, which has been reported to cause marked antioxidative and endothelial improving effects [6,7].

Electrophysiological parameter:
• Electrocardiogram.

• Measurement of blood pressure by rat tail method (Ullian et al. IIII);

Blood pressure of rats in each group was measured at the end of the study by rat tail method: Systolic and mean blood pressures were monitored with rat tail plethysmography using a pneumatic pulse transducer. Rats were anaesthetized with urethane 25% in a dose of 0.6ml/100gm body weight, and then rats placed in the restrainers for 15 to 30 minutes prior to taking readings. The pneumatic cuff fits over the rat’s tail, then inflated to occlude the pulse and allowed to deflate slowly until the pulse pressure are observed on the pulse channel of the recorder. The pulse sign should be monitored to see when the pulse signal begins to become detectable and reach the maximum pulse height. The start of pulsation is viewed on the tracing and is referenced to the pressure curve signal at that point, this reading is analogous to systolic blood pressure, while, the mean blood pressure measured at stability of pulsation and referenced to the pressure curve [12]. Rats of systolic blood pressure of 140mmHg or more were considered hypertensive [13].

• Tissue and blood sampling:
Measurement of CPK serum level:
The blood samples (2ml each) were allowed to clot at room temperature, centrifuged at 3000 rotation/minute and the sera were separated. Samples were stored at —20°C in dark containers.

Measurement of malondialdehyde:
Malondialdehyde (MDA), an end-product of peroxidation of cell membrane lipids caused by oxygen-derived free radicals, is considered a reliable marker of oxidative stress and was determined by measurement of the chromogen obtained from the reaction of malondialdehyde with 2-thiobarbituric acid, according to Aruoma et al. [14]. The MDA values are expressed as (umol/mg).

• Histopathological examination of the cardiac sections:

After records were successfully completed in all 28 rats, the animals were scarified. The hearts were quickly removed and divided equally into two longitudinal sections. One of these parts was placed in formaldehyde solution for a routine histopathological examination by the light microscopy. The other half of the cardiac tissue was frozen in the liquid nitrogen and then stored at —20°C until assayed for thiobarbituric acid reactive substances (TBARS) a lipid peroxidation product. Trunk blood was collected from abdominal aorta to determine the serum levels of CK-enzymes.

• In vitro effect of ivabradine on isolated rabbit heart: (Mc Colak et al. 1151).

Apparatus:
Modified langendorfs method (Langendorff, 1895). The apparatus permits delivery of oxygenated Ringer’s solution at constant rate and temperature of 37°C.
Procedure:
Rabbits weighing 1.0-1.5Kg were used. The animal was sacrificed by cutting the throat. The chest was opened, the heart quickly excised, and transported to a dish containing oxygenated Ringer-lock solution. The heart was thoroughly squeezed to expel any blood from the chambers of the heart. The tissue was cleaned and fixed through the aorta to the cannula of the langendorff, s apparatus. The temperature was kept constant by means of an electric automatic thermostat; a thermometer was inserted through one side of the cannula to ensure a constant temperature of the heart at 37°C throughout the experiment. A hook was attached to the ventricular wall of the heart and was attached by a thread to a side way lever which recorded the contractions of the ventricle on slowly moving drum. Ivabradine was added to the cannula in a dose response curve starting by 10ug/ml up to 1000ug/ml.

Statistical analysis:
Data are presented as mean±SD. Multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by Turkeys’ test as a post-hoc test. The 0.05 level of probability was used as the criterion for significance.

Results
1- Doxorubicin induced changes in rats:
Doxorubicin administration (10mg/kg per day i.p) significantly increased heart rate, lipid peroxidation (MDA), creatine kinase activity and histopathological changes in myocardial tissues (Table 1). On the other hand, doxorubicin did not change SBP.

2- Effect of ivabradine administration on Doxorubicin induced changes:
Prophylactic doses of ivabradine significantly reduced the effect of doxorubicin on heart rate, creatine kinase enzyme (Table 1, Fig. 1).

3- Effect of ivabradine on systolic blood pressure:
Administration of ivabradine (10mg/kg/day p.o.) did not change in systolic blood pressure when compared with control group (Fig. 2).

4- Effect of ivabradine on lipid peroxidation (MDA):
Administration of ivabradine (10mg/kg/day p.o) significantly reduced lipid peroxidation (MDA) in compared with control group (Table 1).

5- Effect of ivabradine on myocardium histopathological structure:
Administration of ivabradine (10mg/kg/day p.o.) significantly reduced the effects of doxorubicin on myocardial structure when compared with control group (Fig. 3).

6- Effect of ivabradine on isolated perfused rabbit heart:
It was observed that ivabradine produced no change in the amplitude of contraction of isolated rabbit heart (Fig. 4).

Table (1): Effects of ivabradine (10mg/kg/day p.o.) on mean heart rate ±SD mean systolic blood pressure ±SD and mean MDA±SD and creatine kinase (CK) (U/L) in doxorubicin induced changes in rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Heart rate (beat/min.) Mean±SD</th>
<th>SBP (mmHg) Mean±SD</th>
<th>MDA (umol/mg tissue) Mean±SD</th>
<th>CK (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>346.55±20.14</td>
<td>120.65±4.80</td>
<td>26.11±5.57</td>
<td>240.01±28</td>
</tr>
<tr>
<td>Doxorubicin group (20mg/kg i.p.)</td>
<td>390.2±1.99a</td>
<td>120.11±6.61</td>
<td>37.62±6.25a</td>
<td>990.00±61 a</td>
</tr>
<tr>
<td>Ivabradin+DOX group</td>
<td>280.34±30.05b</td>
<td>120.13±7.82</td>
<td>28.48±3.75b</td>
<td>291.00±13b</td>
</tr>
<tr>
<td>Ivabradine group (10mg/kg p.o.)</td>
<td>260.1±23.44a9b</td>
<td>123.42±6.34</td>
<td>29.21±6.14</td>
<td>245.11±29</td>
</tr>
</tbody>
</table>

a Significant difference as compared with control group at p<0.005.
b Significant difference as compared with doxorubicin group p<0.005.
Fig. (3): Effect of ivabradine on doxorubicin induced histopathological changes in rats.
A- A photomicrograph of a cut section in the heart of a control rat (group I) Showing interlacing (a) bundles of cardiomyocytes with (b) spindle shaped nucleus with abundant eosinophilic cytoplasm (H & Ex40).
B- A photomicrograph of a cut section in the heart of a doxorubicin rat (group II) Showing (a) normal appearing cardiomyocytes with (b) scattered foci of necrotic areas (H & Ex40).
C- A photomicrograph of a cut section in the heart of a DOX.+ ivabradin rat (group III) Showing interlacing (a) bundles of cardiomyocytes with (b) spindle shaped nucleus with abundant eosinophilic cytoplasm (H & Ex40).
D- A photomicrograph of a cut section in the heart of a ivabradin rat (group VI) Showing interlacing (a) bundles of cardiomyocytes with (b) spindle shaped nucleus with abundant eosinophilic cytoplasm (H & Ex40).

Fig. (4): A record demonstrating the effect of gradually increasing doses of ivabradine on the isolated perfused rabbit’s heart contractions.

Discussion

The current study showed that doxorubicin treatment resulted in significant heart rate elevation as compared with that of control group. These findings were on line with those of Rabelo et al. [16] who reported that basal tachycardia was observed in DOX-treated rats as compared to that of control group. In addition, Venkatesan [17] declared that animals receiving DOX had an increase in HR compared to control groups.