Advances and Prospects of Nicardipine Effects in Attenuation of Hydroxy-Daunorubicin Induced Acute Cardiotoxicity in rats.

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Abstract

Hydroxy daunorubicin is a cytotoxic antibiotic which is being used for a wide range of tumors but its prolonged use has lead to cardiomyopathy in numerous cases. Present study was designed to investigate the role of nicardipine - L-type calcium channel blocker, used for the management of hypertension and angina pectoris against the hydroxydaunorubicin induced cardiotoxicity. Sixty Wale-Sprague male rats were used in this study, ranging from 300gm-350gm in weight. The animals were randomly divided into three groups, twenty rats in each group. Group I: (control): received physiological saline (4ml/kg/day), orally for ten consecutive days. Group II (Hydroxydaunorubicin group): received a single dose hydroxydaunorubicin (20mg/kg) intraperitoneally. Group III (pretreatment group): received nicardipine (0.1mg/kg) intraperitoneally daily, starting six days before hydroxydaunorubicin (20mg/kg) and continued for four consecutive days. At the end of the experiment, blood samples were collected for cardiac biomarker determination. The animals were dissected and hearts were taken for histopathological examination. Hydroxydaunorubicin has shown to increase cardiac Troponin I, LDH, MDA, and interleukin-17 significantly \( (p<0.05) \) compared to the control group while as pretreatment with nicardipine showed a significant reduction in cardiac biomarkers significantly \( p<0.05 \).

Keywords: Hydroxydaunorubicin, nicardipine, cardio-toxicity.

Introduction

Hydroxydaunorubicin is a cytotoxic antibiotic which is used for a wide range of tumors [1]. Prolonged use of hydroxydaunorubicin leads to cardiomyopathy [2]. Mechanism of hydroxydaunorubicin induced cardio-toxicity includes free radical formations and induction of immunogenic reactions [3, 4].

The risk factors intended in the progress of cardio-toxicity are age, total cumulative doses, underlying disease, previous thoracic irradiation and concomitant drugs administrations, the common hydroxydaunorubicin dose ranged from 60-80mg/m²/3weeks, but more than a cumulative dose of 550mg/m² the risk of cardiomyopathy will recurrently increases [5].

Additionally, left chest irradiations increase risk of cardio-toxicity by 2.6%, but right side chest irradiation in combination with hydroxydaunorubicin increases it to only 0.3% [6]. The major and attractive theory of hydroxydaunorubicin-induced cardio-toxicity has been calcium homeostasis since hydroxydaunorubicin induces
calcium discharge and liberate from cardiac sarcoplasmic reticulum, verapamil has shown cardio-protective effects from hydroxydaunorubicin- induced cardiotoxicity in rats, through suppression of intracellular calcium over-loading which leads to attenuation of hydroxydaunorubicin-induced mitochondrial injury [7].

Nicardipine is an L-type calcium channel blocker, used for the management of hypertension and angina pectoris; it improves myocardial perfusion and is regarded as a coronary vasodilator with minimal systemic side effects [8, 9].

Therefore, the aim of the present study was to investigate the cardio-protective effects of nicardipine in hydroxydaunorubicin induced cardio-toxicity in the experimental rats.

**Animals and Methods**

The experimental work was performed in Department of Clinical Pharmacology and Therapeutic, College of Medicine, Al-Mustansiriyia University, Baghdad-Iraq. The study was agreed and approved by Scientific committees of Medical Board Juries and accepted by Ethical committees of animal experimentation.

**Experimental design**

Sixty Wale-Sprague male rats were used in this study ranging from 300-350gm in body weight the rats were housed in cages and kept at 27°C with artificial light-dark cycles. They had free access to drinking water and libitum. The animals were randomly divided into three groups, twenty rats in each group. **Group I**: (control): received physiological saline (4ml/kg/day), orally for ten consecutive days. **Group II**: (hydroxydaunorubicin): received a single dose hydroxydaunorubicin (20mg/kg) intraperitoneally. **Group III**: (pretreatment group): received nicardipine (0.1mg/kg) intraperitoneally daily, starting six days before hydroxydaunorubicin (20mg/kg) and continued for four consecutive days. On day 10, the rats were decapitated, blood samples were collected into test tubes, and sera were separated through centrifugation at 3000 pm for 15minutes at 4°C for determination of cardiac biomarkers. The hearts were collected, cleaned, rinsed with physiological solutions and fixed in 10% buffered formalin for histopathological examination.

**Drugs and kits**

Hydroxydaunorubicin 50mg/vial (Adriblastina, Pfizer) and Nicardipine 2.5mg/ml (Cardene, Italy) were obtained from private pharmacies.

**Cardiac biomarkers estimations**

- Estimation of serum lactate dehydrogenase (LDH): Serum LDH activity was estimated by LDH kit (Linear Chemical, S.L., China).
- Estimation of serum cardiac troponin I: It was done by commercially available calorimetric ELISA KIT Catalog NO: E-EL-R1253 pg. /Ml (Linear Chemical, S.L., China).
- Estimation of serum malondialdehyde (MDA): It was estimated via calorimetric ELISA KIT Catalog NO: E-EL-0060 ng/ml (Linear Chemical, S.L., China).
- Estimation of serum IL-17: It was determined via commercially available Interleukin 17.ELISA KIT catalog NO: E-EL-Ro566 pg/ml (Linear Chemical, S.L., China).

All kit methods were done according to the instruction of manufacture.

**Histopathological evaluation**

The animal hearts was dissected and fixed in 10% formalin for one day. Three-micrometer thick paraffin heart sections were stained with haematoxylin for light microscope examination [10]. A bare, minimum of eight fields for each heart section was examined and consigned to the severity of alterations.

The characteristic of present study regarding the animal types and gender, chemical kits, drugs used in this experimental study and all methods used in this study are summarized in a table (1).

**Statistical analysis**

Data were presented as mean ± S.E and the statistical significance of difference was determined via one-way analysis of variance (ANOVA), followed by an unpaired t-test. A probability value of $p <0.05$ indicates a significant difference.
Table 1: Characteristics of the study

<table>
<thead>
<tr>
<th>Number of animals</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of animals</td>
<td>Wale–Sprague male rats</td>
</tr>
<tr>
<td>Groups</td>
<td></td>
</tr>
<tr>
<td>Doxorubicin group</td>
<td>20 rats</td>
</tr>
<tr>
<td>Control group</td>
<td>20 rats</td>
</tr>
<tr>
<td>Nicardipine+ doxorubicin group</td>
<td>20 rats</td>
</tr>
<tr>
<td>Kits</td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>ELISA KIT catalog NO:E-EL-0060 ng/ml</td>
</tr>
<tr>
<td>Troponin I</td>
<td>ELISA KIT catalog NO:E-EL-R1253 pg/ml</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate Dehydrogenase (LDH) pmol/ml</td>
</tr>
<tr>
<td>IL-17</td>
<td>ELISA KIT catalog NO:E-EL-Ro566 pg/ml</td>
</tr>
<tr>
<td>Drugs</td>
<td></td>
</tr>
<tr>
<td>Hydroxydaunorubicin</td>
<td>50mg/vial (Adriblastina, Pfizer)</td>
</tr>
<tr>
<td>Nicardipine</td>
<td>2.5mg/ml (Cardene, Italy)</td>
</tr>
<tr>
<td>Rout of administration</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>Duration of experiment</td>
<td>10 days</td>
</tr>
<tr>
<td>Chemicals</td>
<td>10% formalin, haematoxylin and Eosine</td>
</tr>
</tbody>
</table>

Results

No mortality was seen in any treated groups, heart/body weight ratio founded to be debated in hydroxydaunorubicin treated group, this outcome reflected the structural changes, but not reached to the level of significance, heart: bodyweight ratio x (10^-3) was 2.007±0.067, 1.998±0.008 and 1.9866±0.005 for the control group, hydroxydaunorubicin group and nicardipine+hydroxydaunorubicin respectively figure (1).

Cardiac biomarker changes

Hydroxydaunorubicin increases cardiac Troponin I, LDH, MDA, and interleukin-17 significantly p<0.05 compared to the control group. In hydroxydaunorubicin group, pretreated with nicardipine, significant reduction in cardiac biomarkers was found<0.05, (table2).

In hydroxydaunorubicin treated group, MDA serum levels were correlated with cardiac troponin-I serum level increments, (R^2 value was 0.415), (Figure 2).

Moreover, there was a strong correlation among other cardiac biomarkers LDH with cardiac Troponin I, cardiac Troponin I with IL-17, IL-17 with MDA, IL-17 with LDH figures (3, 4, 5, and 6).

Cardiac tissue changes in hydroxydaunorubicin induced cardio-toxicity

Hydroxydaunorubicin-induced-cardiotoxicity leads to significant changes in myocardial tissue architecture including cytoplasm vacuoles, myofibrillar disorganization, decreased numbers of nuclei, loss of muscle fibers striation and fragmentation with necrosis. In control and nicardipine pretreated groups, sections show normal rat myocardial tissues morphology characterized by peripherally located normal oval nuclei, with branching striated muscle fibers. Pretreatments with nicardipine produced significant attenuations in myocardial lesions induced by hydroxydaunorubicin, figure (7).
Table 2: Effects of hydroxydaunorubicin and nicardipine+hydroxydaunorubicin on serum cardiac biomarkers in acute hydroxydaunorubicin-induced cardiotoxicity.

<table>
<thead>
<tr>
<th>Cardiac biomarkers</th>
<th>Control (N=20)</th>
<th>Hydroxydaunorubicin (N=20)</th>
<th>Nicardipine+ Hydroxydaunorubicin (N=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum troponin I pg/ml</td>
<td>29.041±0.52</td>
<td>210.361±1.072*</td>
<td>66.859±1.084&quot;</td>
</tr>
<tr>
<td>Serum MDA ng/ml</td>
<td>33.966±0.8</td>
<td>290.726±1.453*</td>
<td>118.175±0.617&quot;</td>
</tr>
<tr>
<td>Serum LDH pmol/ml</td>
<td>81.764±1.05</td>
<td>299.104±2.529*</td>
<td>166.075±0.207&quot;</td>
</tr>
<tr>
<td>Serum IL-17 pg/ml</td>
<td>55.012±1.08</td>
<td>286.682±1.684*</td>
<td>201.305±1.349&quot;</td>
</tr>
</tbody>
</table>

Data expressed as mean±SE, *p<0.001 compared with control, "p<0.05 compared with hydroxydaunorubicin.

MDA: (malondialdehyde); LDH: (lactate dehydrogenase); IL-17: (Interleukin 17).

Figure 2: Correlations between MDA serum levels and cardiac troponin I serum levels in hydroxydaunorubicin-induced cardiotoxicity.

Figure 3: Correlations between LDH serum levels and cardiac troponin I serum levels in hydroxydaunorubicin-induced cardio-toxicity.
Figure 4: Correlations between IL-17 serum levels and cardiac troponin I serum levels in hydroxydaunorubicin-induced cardio-toxicity.

Figure 5: Correlations between IL-17 serum levels and MDA serum levels in hydroxydaunorubicin-induced cardio-toxicity.

Figure 6: Correlations between IL-17 serum levels and LDH serum levels in hydroxydaunorubicin-induced cardio-toxicity.
Discussion

Hydroxydaunorubicin-induced cardio-toxicity is attributed to the various mechanisms including oxidative stress, intracellular calcium dys-regulation, mitochondrial damage, and apoptosis [11]. Hydroxydaunorubicin produced significant elevations in cardiac biomarkers with significant cardiac tissue changes. In the present study, intra-peritoneal dose of hydroxydaunorubicin produced significant cardiac damage that reflected by increasing in the serum level of cardiac biomarkers.

It is well-known that cardiac troponin-I is standard biomarker for myocardial injury and cardio-toxicity [12]. Cardiac troponin-I releases into the plasma when cardiac myocytes are injured so, a rise in the cardiac troponin-I serum levels during treatment with hydroxydaunorubicin reflect the occurrences of acute cardiotoxicity thus, there was an elevation of cardiac troponin-I serum levels rapidly after a high dose of hydroxydaunorubicin which accurately predicts the development of cardiac dysfunction, therefore cardiac troponin-I is considered as susceptible & dependable marker for myocardial damage to appropriate clinical and prognostic implications In addition troponin-I serum levels were correlated with disease severity and may anticipate the onset of cardiac damage [13, 14]. Pretreatment with nicardipine significantly decreased the cardiac troponin-I serum levels since the threshold levels of the cardiac biomarker in cardio-toxicity is associated with poor long term outcomes. Consequently intracoronary or intraperitoneal nicardipine attenuate cardiac ischemic damage induced by toxic or percutaneous coronary intervention [15].

The present study, also demonstrated that hydroxydaunorubicin, leads to a significant elevation in serum LDH levels, due to toxic effects of hydroxydaunorubicin that causes cellular damage and escape of intracellular enzymes, and because of its prevalent allocation throughout the tissues and its release in various disorders such as ischemia, starvation, dehydration, excess heat or cold injury, infection, therefore LDH is considered highly sensitive, but nonspecific cardiac damage biomarker [16]. Pretreatment with calcium channel blockers prevents adrenaline and hydroxydaunorubicin-induced cardio-toxicity and are able to revere the rise in cardiac LDH isoenzyme, like amlodipine has shown to prevent doxorubicin-induced congestive heart failure and reduced infarction size [17]. These findings indicate cardio-protective potential of calcium channel blockers in the prevention of cardio-toxicity. Moreover, hydroxydaunorubicin significantly results in serum MDA level rise, which may be attributed to the free radical generations as a result of hydroxydaunorubicin effects on NADH dependent microsomal lipid peroxidation that initiates, lipid radical

Figure 7: Histopathological changes in rat myocardial architectures (80x photomicrographs) induced by hydroxydaunorubicin and its attenuation by nicardipine as compared to control. (A) Normal control, (B) hydroxydaunorubicin group, (C) nicardipine+hydroxydaunorubicin.
chain reaction, causing oxidative cell membrane injury [18]. Nicardipine has a potent antioxidant property, through direct scavenging activity, preservation of glutathione peroxidase activity, inhibition of lipid peroxidation and modulation of vascular nitric oxide [19]. Additionally, nicardipine and amldipine reduced cardiac oxygen consumption; therefore they ameliorate mitochondrial damage and prevent cardiac ischemic-reperfusion injury. [20]. All these studies pointed out that calcium channel blockers, mainly nicardipine have antioxidant effects which correspond with the findings of the present study in reduction of serum MDA. Furthermore, the present study also demonstrated that hydroxydaunorubicin significantly up-regulated IL-17, which is a pro-inflammatory cytokine secreted from activated T-cells and plays an essential role in different inflammatory conditions [21]. IL-17 produces its inflammatory effects via up-regulated expression of pro-inflammatory genes [22]. Consequently, IL-17 antibody attenuates the histopathological changes in myocarditis [23] accordingly, IL-17 regarded as an important proinflammatory cytokine in induction of hydroxydaunorubicin-induced cardiac damage, further hydroxydaunorubicin has shown to increase IL-17 serum levels which indicates an immunogenic reaction to hydroxydaunorubicin-induced cardio-toxicity [24]. Moreover, T-cell membrane calcium ion channel could be participating in the T-cell stimulation induced by lipopolysaccharide that facilitating the lymphocyte proliferation and differentiation, consequently, nicardipine and verapamil inhibits IL-17 production of stimulated Th17 [25]. In addition nicardipine reduces the circulating adhesion molecules (ICAM) and E-selectin, thus, nicardipine decreases the cellular effects of IL-17, and weaken IL-17 mediated hydroxydaunorubicin-induced cardio-toxicity [26]. Furthermore, verapamil and nicardipine impedes lymphocytic permeation progression, appearance and arrangement of F-actin filaments via reduction in the expression of P-selectin on cardiac and endothelial cells [27]. A number of studied revealed that L-type calcium channel antagonists have beneficial possessions on the ischemic cardiac tissue through slow trans-membrane calcium entry into vascular smooth muscle and cardiac tissue so; pretreatment with nicardipine prevents cardiomyocyte necrosis and damage through reduction in the calcium-induced lipases, ATPase and protease activations [28, 29]. Moreover, nicardipine inhibits ATP production and reduction in the oxygen consumption which leads to inhibition of apoptosis in ATP dependent pathway therefore, nicardipin inhibits hydroxydaunorubicin-induced apoptosis [30].

Regarding histopathological changes in hydroxydaunorubicin-induced cardio-toxicity there are patchy interstitial fibrosis, vacuolated cardiomyocytes and nucleus-chromatin disorganization [31], pretreatment with nicardipine lead to significant amelioration in all cardiomyocyte architectures to a near normal cell which reflect a cytoprotective effect of nicardipine in antagonizing the hydroxydaunorubicin-induced cardio-toxicity [32].

**Conclusion**

In current study, hydroxydaunorubicin has shown to induce cardio toxicity by elevating the cardiotoxicity markers such as Troponin-I which was found to be significantly attenuated by pre-treating the animals with nicardipine. However further studies are needed to elucidate the exact mechanism of action as well as any adverse effects induced by nicardipine.

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**Conflict of interest**

The authors declare that there is no conflict of interest to reveal.
References


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