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Original article

Aliskiren attenuates myocardial apoptosis and oxidative stress in chronic murine model of cardiomyopathy

Azhar Rashikh [a], Shibli Jameel Ahmad [a], Krishna Kolappa Pillai [a], Kanchan Kohli [b], Abul Kalam Najmi [a,*]

[a] Department of Pharmacology, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi-62, India
[b] Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi-62, India

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ABSTRACT

Doxorubicin (DXR) is one of the most effective antineoplastic agents. However, the optimal clinical use of this agent is limited because of marked cardiomyopathy and congestive heart failure. Renin angiotensin system (RAS) plays an important role in the development of cardiac hypertrophy, reperfusion injury and congestive heart failure. Aliskiren (ALK) is a direct inhibitor of renin and does not affect other systems involved in cardiovascular regulation. This study was designed to explore the possible protective effects of ALK (30 and 100 mg/kg, per oral [p.o.] respectively for 42 days) in chronic model of DXR (1.25 mg/kg, intraperitoneally (i.p.) sixteen equal cumulative doses) induced cardiomyopathy in rats. DXR treatment significantly ($P < 0.01$) increased the activities of serum creatine kinase (CK-MB), lactate dehydrogenase (LDH), cardiomyocyte caspase-3 and catalase (CAT). ALK (100 mg/kg) treatment prevented the animals significantly ($P < 0.01$) from rise in the above indices. Furthermore ALK (100 mg/kg) significantly restores the DXR-induced decrease in antioxidant defense, reduced glutathione (GSH) and superoxide dismutase (SOD). Transmission electron microscopic studies showed that DXR caused apoptosis in myocardium, manifested as condensation of chromatin network at the margins and rupture of nuclear membrane which was well protected by ALK (100 mg/kg) treatment. The present study indicates that ALK protected rats from DXR-induced cardiomyopathy.

1. Introduction

Doxorubicin (DXR), an anthracycline antibiotic, is widely used in the treatment of a variety of human malignancies, including breast cancer, small cell carcinoma of the lung and acute leukemia's [1]. However, its therapeutic use is limited because of its acute and chronic side effects. The acute side effects such as myelosuppression, nausea, vomiting and arrhythmias are either reversible or clinically manageable [2,3]. But the chronic side effects represented by the development of cardiomyopathy and congestive heart failure are irreversible [4,5]. The exact causal mechanism of DXR-induced cardiomyopathy is not completely understood. Nevertheless, various mechanisms have been attributed to DXR-induced cardiomyopathy, such as release of vasoactive amines [6], generation of reactive oxygen species (ROS) [3,6,7], induction of apoptosis [8–10], oxidative DNA damage [11], lipid peroxidation [12,13], impairment of enzymatic activity of creatine kinase [14], and induction of renin angiotensin system (RAS) activity [15]. Various agents have been evaluated to treat DXR-induced cardiomyopathy including probucol, amifostine, and dexrazoxane with some protection [16–18]. But, all of these agents have pronounced clinical disadvantages, including a significant decline in HDL levels, an inability to prevent DXR-induced mortality and weight loss, and potentiation of DXR-induced myelosuppression [19]. Numerous studies have suggested that angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) have protective effects against DXR-induced cardiomyopathy [20–23]. There are evidences for the role of RAS in DXR-induced cardiomyopathy. However, ACE inhibitors and ARBs benefited DXR-induced cardiomyopathy to only some extent [24,25]. It may be due to the formation of Ang II by ACE independent pathways (chymase), which is not blocked by ACE inhibitors and ARBs [24,25].

DXR-induced cardiomyopathy involve the over activity of renin that produces angiotensin II [5,26,27]. Thus it was hypothesized that the inhibition of renin at the very first step in RAS cascade may protect the heart from DXR toxicity. Aliskiren (ALK) is an orally active, nonpeptide direct acting renin inhibitor and this novel drug has some advantages over existing RAS blockers such as; it does not have ACE-escape like activity [28], prevents the formation of both angiotensin I and angiotensin II and produces effective blockade of RAS without the compensatory increase in plasma renin activity (PRA) [29].

* Corresponding author.
E-mail addresses: aknajmi@jamiahamdard.ac.in, aknajmi@hotmail.com (A.K. Najmi).

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ALK was found to be cardioprotective against acute DXR-induced toxicity in our previous work [30]. Encouraged from the results of the previous investigation, present study was designed to evaluate the protective potential of ALK in a chronic set up of DXR cardiotoxicity with focus on apoptosis and ultrastructural changes.

2. Materials and methods

2.1. Experimental animals

The study protocol (Protocol No. 574/2010) was approved by the Institutional Animal Ethics Committee (IAEC) of Hamdard University, New Delhi. Albino rats of Wistar strain, with body weight 160–200 g, were procured from Central Animal House Facility, Hamdard university, New Delhi and acclimatized under standard laboratory conditions at 20–25 °C. The animals were kept in propylene cages under controlled conditions of illumination (12-h light and 12-h dark: day: night cycle) and had a free access to commercial pellet diet and water *ad libitum*.

2.2. Drugs and chemicals

DXR (Dabur India Ltd., Sahibabad, Uttra Pradesh, India), ALK (Novartis Pharmaceutical, Basel, Switzerland) and Telmisartan (Glenmark Pharmaceutical Ltd., Kisanpura, Himachal Pradesh) were gratefully received for the study. Caspase-3 inhibitor assay kits from BioVision (USA), LDH and CK-MB assay kits from Reckon Diagnostics Ltd. (India), were purchased. All the other chemicals used were of analytical grade. HPLC grade water was used for all biochemical assays.

2.3. Experimental schedule

After acclimatization, all the animals were randomly allocated into six groups of eight animals each and treated as follows:

- group I received physiological saline (0.5 mL/kg i.p., same schedule as group II) and served as control;
- group II received DXR four times a week in sixteen equal doses over a period of four weeks for a cumulative dose of 1.25 mg/kg, i.p.;
- groups III, IV, and V received 30 mg/kg ALK, 100 mg/kg ALK, 10 mg/kg TEL respectively per day by oral for 42 days along with DXR (1.25 mg/kg, i.p.) as the same schedule as group II;
- group VI received 100 mg/kg ALK alone by gavage for 42 days.

After 24 h of last dose of DXR, the rats were anesthetized with ether for collection of blood samples from the tail vein. The enzymatic parameters (CK-MB and LDH) were estimated in blood. Heart tissue was excised, washed in ice-cold physiological saline. One small piece of heart was cut, weighed and homogenized for biochemical estimations (caspase-3 activity, malondialdehyde [MDA], glutathione [GSH], superoxide dismutase [SOD] and catalase [CAT]). A small piece of 1–2 mm thickness was preserved in phosphate buffer (strength 100 mM, PH 7.4) for ultrastructural studies.

2.4. Hemodynamic measurements

Hemodynamic measurements were carried out using tail cuff method on Biopac Non-Invasive Blood Pressure Recording Instrument (USA). All the rats were initially trained in the restrainer for a period of 15 min every day at least 10–15 days prior to the day of measurement of the hemodynamic parameters (systolic, diastolic, mean blood pressure and heart rate).

2.5. Biochemical estimation in serum

Creatine kinase myocardial band isoenzyme (CK-MB) [31], lactate dehydrogenase (LDH) [32], were estimated in serum by enzymatic kits using an UV-Visible spectrophotometer (Shimadzu, UV-1601, Japan).

2.6. Caspase-3 activity in myocardium

The activity of caspase-3 was determined by the detection of chromophore *p*-nitroanilide after cleavage from the labeled substrate DEVD-*p*-nitroanilide [33]. In brief, 50 μL supernatant from homogenized tissue with cooled lysis buffer was used from each sample and 50 μL of Reaction Buffer was added to each sample. Then, 5 μL of the 4 mM DEVD-pNA substrate (200 μM) was added and incubated at 37 °C for 30 min to permit a dissociation of *p*-nitroanilide (pNA) from the conjugate DEVD-pNA. The activity was read by Elisa at 405 nm using 96 well plate.

2.7. Biochemical assay in cardiac tissues homogenates

Measurement of lipid peroxidation by determination of myocardial MDA content was performed by the method of Ohkawa [34]. Antioxidant enzymes namely GSH, SOD and CAT were determined in cardiac tissue as per standard protocol. GSH was estimated by the Ellman method [35]. The activity of SOD was measured according to the method of Marklund [36]. CAT activity was measured according to Clairbone [37].

2.7.1. Proteins in cardiac tissues

The protein content was determined by the method of Lowry (1951) [38], using bovine serum albumin as a standard.

2.8. Ultrastructural studies in cardiac tissues

Samples were fixed in modified Karnovasky's fluid, buffered with 0.1 M sodium phosphate buffers at pH 7.4. Fixation was done for 8 to 12 h at 4 °C temperature. Subsequent to this the tissues were washed with 0.1 M sodium phosphate buffer. After several washes specimens were desiccated in graded acetone solutions and rooted in CY212 araldite. Ultra thin sections of 60 to 80 nm thickness were incised using an ultracut E ultramicrotome and the sections were stained in alcoholic uranyl acetate (10 min) and lead citrate (10 min) before exploratory the grids in a transmission electron microscope (FEI-Margagni operated 268) Netherlands at 60 to 80 kV [39].

2.9. Data and statistical analysis

All results are expressed as mean ± standard error of mean (S.E.M.). Groups of data are compared with the analysis of variance (Anova) followed by Dunnett's *t* test to identify significance among groups. Values are considered statistically significant at $P < 0.05$.

3. Results

3.1. Hemodynamic parameters

The systolic, diastolic, mean BP and heart rate were significantly increased in DXR alone (group II) as compared to the control (group I). Pretreatment with ALK (100 mg/kg per day) and TEL (10 mg/kg per day) showed significant ($P < 0.01$) reduction in the systolic, diastolic, mean BP and heart rate as compared to the DXR alone group (Table 1).