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Cardioprotective effect of vincristine on isoproterenol-induced myocardial necrosis in rats

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ABSTRACT

This study investigated the protective effect of vincristine (VCR) on isoproterenol (ISO)-induced cardiac necrosis (CN) in rats. Animals ($n=7$ in each group) were pretreated with vincristine (25 μg/kg) intraperitoneal (i.p.) daily in 5-day cycles with 2 days pause between cycles using a 5-day-on, 2-day-off schedule for two weeks and then intoxicated with isoproterenol (100 mg/kg, s.c., for 2 consecutive days). ISO-induced myocardial damage was indicated by changes in electrocardiographic (ECG) patterns, increased activities of marker enzymes such as creatine kinase-MB, serum glutamate pyruvate transaminase and lactate dehydrogenase and the levels of troponin-T in the serum. The levels of lipid peroxide products, (thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (HP)) were increased with a parallel decrease in the activities of antioxidants (superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione) in ISO-induced rats. Furthermore, ISO-induced rats showed increase in the activities of membrane bound enzymes such as Ca^{2+}-ATPase and Mg^{2+}-ATPase with a decreased activity of Na^{+}/K^{+}-ATPase. Triphenyl tetrazolium chloride (TTC) staining of the heart section showed increased area of necrosis in ISO-induced rats. Pretreatment with VCR (25 μg/kg) eliminated all ISO-induced biochemical and histopathological changes, and decreased the myocardial necrosis to a greater extent. Transmission electron microscopic findings on the structure of the heart mitochondria confirmed the protective effects of VCR. Present study provides first scientific report on protective effect of vincristine against ISO-induced cardiac damage in rats.

1. Introduction

Myocardial infarction (MI) or heart attack is the leading cause of death all over the world and incidence of cardiovascular diseases (CVD) is alarmingly increasing throughout the world (Lopez and Murrau, 1998). Obviously search for an effective drug that can ameliorate CVD is imperative. As plant based drugs are considered to be safe and economic, several workers have tried from time to time with phytochemicals to regulate CVD. In this investigation vincristine has been evaluated to explore its potential to ameliorate chemically induced MI.

Vincristine (VCR) is a member of the vinca alkaloid family and is widely used as chemotherapeutic agent for various malignancies (Blasko and Cordel, 1990; Jordon, 2002). As cardiotoxicity is believed to be one of the most significant adverse effects of cancer treatment, and is responsible for considerable morbidity and mortality, in order to prevent adult cardiomyocytes exposed to doxorubicin, supplementation of vincristine is believed to provide substantial protection (Chatterjee et al., 2008). However, no reference is there in the literature to sustain a cause and effect relationship between vincristine and myocardial infarction, despite the fact that the VCR appears to exert cardioprotective effects on cultured adult mouse myocytes exposed to both chemical and hypoxic oxidative stress (Chatterjee et al., 2007). We hypothesize that VCR at moderate dose may protect isoproterenol-induced myocardial injury in *in vivo* through reduction of oxidative stress.

Some previous studies reported signaling responses initiated by vincristine in tumor cell lines (Wang et al., 1998; Stone and Chambers, 2000). An earlier investigation suggested that vincristine activates a pro-survival signaling pathway, resulting in increased phosphorylation of Akt, ERK and GSK-3β and reduced cytochrome C release into the cytosol to trigger cardiac protection (Chatterjee et al., 2007). However, on its mode of action, nothing much had been investigated. An attempt has now been made to reveal the effect of vincristine in oxidative stress-induced injury and to verify the hypothesis that cardio-protection by vincristine, if any, could be the result of inhibition of free radical generated by the MI inducing chemical.

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It is now well recognized that isoproterenol (ISO), a β-adrenergic agonist, causes severe stress in the myocardium, resulting in infarct-like necrosis of the heart muscles (Rona et al., 1959). The pathophysiological and morphologic alterations in the heart of this non-coronary myocardial necrotic rat model are comparable with those taking place in human myocardial alterations (Panda and Naik, 2008; Zhou et al., 2008). However, till to date no scientific report is available on vincristine in ISO-induced myocardial necrosis. Therefore, an effort has been made to study the protective effects of vincristine on ISO induced lipid peroxidation, status of antioxidants and on the extent of necrosis. We also tried to correlate the changes in membrane-bound ATPase enzymes such as Na^+K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase which play a significant role on cellular integrity. The protective effect of VCR on the structure of heart mitochondria was ascertained by transmission electron microscopic (TEM) study.

2. Materials and methods

2.1. Chemicals

Thio-barbituric acid (TBA), sodium dodecyl sulfate (SDS), Ellman's reagent and m-phosphoric acid were obtained from E. Merck Ltd., Mumbai, India. Isoproterenol hydrochloride was purchased from Sigma-Aldrich chemicals (St. Louis, MO, USA) and vincristine sulfate was procured from *Cipla*, India. (1 mg/ml). All other chemicals used were of analytical grade.

2.2. Animals

Albino Wistar rats weighing 190–200 g were considered in this study. Animals were maintained under constant temperature (27 ± 1 °C) and photo-period (14 h light and 10 h dark) with the provision of commercial laboratory feed (Gold Mohur feed, Hindustan Lever Limited, Mumbai, India) and water *ad libitum*. The experimental protocol was approved by the Ethical Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), New Delhi, Ministry of Social Justice and Empowerment, Government of India and approved by the Institutional Animal Ethical Committee of DAVV, Indore.

2.3. Acute toxicity study (data not shown)

Acute toxicity study of VCR was carried out in rats weighing 190–200 g by up and down/staircase method as per OECD guidelines. The drug was administered (i.p) to different groups of rats (comprising 5 animals in each group) at the doses of 2 mg, 1 mg, 500 μg, 100 μg, 50 μg, 25 μg and 12.5 μg/kg. The maximum dose was selected considering the reported LD-50 value of vincristine as 1.9 mg/kg (Pfizer Lmtd, UK). Animals were observed for 48 h to study the general behavior and sign of discomfort. The drug was found to devoid of mortality in animals at 1 mg/kg or less than this dose. However, animals receiving 12.5 μg of the test drug did not show any significant changes as compared to that of controls. Hence from safe and effective point of view 1/10th (100 μg/kg), 1/20th (50 μg/kg) and 1/40th (25 μg/kg) of the dose were selected for preliminary study.

2.4. Experimental design

Two experiments were performed. In a preliminary experiment, to determine the dose dependent effects of VCR, the effects of three different doses of the test drug (25, 50 and 100 μg/kg) were evaluated in ISO-induced animals, considering the cardiac markers CK-MB and LDH as indices. Details of the drug and ISO administration have been described in the next experiment. Since results of this experiment indicated 25 μg/kg as the most effective one, in final experiment this concentration was considered.

In final experiment, total of 28 rats were divided into 4 groups comprising of 7 animals in each. While, animals of group I and II receiving 0.1 ml of vehicle (i.p.) served as control and ISO control; animals of group III and IV were treated with vincristine (25 μg/kg) i.p respectively. The drug was administered daily in 5-day cycles with 2 days pause between cycles, i.e. 5-day-on, 2-day-off schedule comparable to that used previously (Aley et al., 1996). On the day 15th and 16th, ISO (100 mg/kg) dissolved in normal saline was injected subcutaneously (s.c) to group II and III animals at an interval of 24 h for 2 days to induce experimental myocardial injury as described earlier by Kumaran and Prince (2010). After the second dose of ISO, rats of all the groups were anesthetized with ketamine hydrochloride (80 mg/kg, i.p.) and the electrocardiograph patterns were monitored by using a Cardiart 1081 (BPL) ECG machine with 20 mm/mV sensitivity at a paper speed of 50 mm/s. Heart rates were expressed as beats per minute.

All ECG data were scanned, presented and interpreted using a computer system. Alterations in heart rate and ST-segment were measured using Cardio Caliper (Version 3.3, ICONICO software).

After recording the electrocardiogram, all the rats were anesthetized with ketamine and sacrificed by cervical decapitation. Blood was collected and serum separated for various biochemical estimations. The hearts were dissected out immediately and washed with ice-cold saline. 10% (w/v) homogenates in phosphate buffer (50 mM, pH 7.4) were prepared. Homogenates were centrifuged at 10,000*g* in 0 °C for 20 min using Remi C-24 high speed cooling centrifuge. The clear supernatant was used for the assay of lipid peroxidation, GSH, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx).

2.5. Estimation of serum parameters

Activities of CK-MB, LDH, and SGPT in the serum were measured, as done earlier (Panda et al., 2013) using standard commercial kits (Teco Diagnostics, CA, USA). The level of cardiac troponin-T (cTnT) in the serum was estimated, using VITROS immunodiagnostic kit purchased from the Ortho-Clinical Diagnostics, Inc. New York, USA.

2.6. Assessment of biochemical parameters

Clear supernatant was used for the estimation of thiobarbituric acid reactive substances (TBARS), according to the method described by Ohkawa et al. (1979) and lipid hydroperoxides (LOOH) was estimated by Jiang et al. (1992) method. The activity of superoxide dismutase (SOD) was assayed by the method of Marklund and Marklund (1974), catalase by Aebi (1983), glutathione peroxidase (GPx) by Rotruck et al. (1973), while reduced glutathione (GSH) by Ellman (1959). Protein was estimated according to Lowry et al. (1951) using BSA as standard to calculate the protein content of the samples. The membrane-bound enzymes such as Na^+/K^+-ATPase, Ca^{2+}, and Mg^{2+}-ATPase activity were assayed by the method of Bonting (1970), Hjerten and Pan (1983), and Ohnishi et al., (1982), respectively.

2.7. Estimations of myocardial damage by TTC assay method

For the TTC assay, protocol described by Lie et al. (1975) was used. In brief, the heart was frozen immediately after removal. When the tissue was firm, the heart was transversely cut across the left ventricle and sections 1–2 mm thick were incubated in 1% TTC solution prepared in phosphate buffer (pH 7.4) for 30 min at 37 °C. At the end of the incubation period, the heart slices were