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Beneficial role of tamoxifen in experimentally induced cardiac hypertrophy

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ABSTRACT

Background: Protein kinase C (PKC) activation is associated with cardiac hypertrophy (CH), fibrosis, inflammation and cardiac dysfunction. Tamoxifen is a PKC inhibitor. Despite these, reports on effect of tamoxifen on cardiac hypertrophy are not available. Hence, we have investigated effect of tamoxifen (2 mg/kg/day, *po*) on CH.

Methods: In isoproterenol (ISO) induced CH, ISO (5 mg/kg/day, *ip*) was administered for 10 days in Wistar rats. For partial abdominal aortic constriction (PAAC), abdominal aorta was ligated by 4-0 silk thread around 7.0 mm diameter blunt needle. Then the needle was removed to leave the aorta partially constricted for 30 days. Tamoxifen was given for 10 days and 30 days, respectively, in ISO and PAAC models and at end of each studies, animals were sacrificed and biochemical and cardiac parameters were evaluated.

Results: ISO and PAAC produced significant dyslipidemia, hypertension, bradycardia, oxidative stress and increase in serum lactate dehydrogenase and creatine kinase-MB, C-reactive protein. Treatment with tamoxifen significantly controlled dyslipidemia, hypertension, bradycardia, oxidative stress and reduced serum cardiac markers. ISO control and PAAC control rats exhibited significantly increased cardiac and left ventricular (LV) hypertrophic index, LV thickness, cardiomyocyte diameter. Treatment with tamoxifen significantly reduced these hypertrophic indices. There was a significant increase in LV collagen level, decrease in Na^+K^+ATPase activity, and reduction in the rate of pressure development and decay. Tamoxifen significantly reduced LV collagen, increased Na^+K^+ATPase activity and improved hemodynamic function. This was further supported by histopathological studies, in which tamoxifen showed marked decrease in fibrosis and increase in extracellular spaces in the treated animals.

Conclusions: Our data suggest that tamoxifen produces beneficial effects on cardiac hypertrophy and hence may be considered as a preventive measure for cardiac hypertrophy.

Introduction

Cardiac hypertrophy (CH) is an important predictor of cardiovascular morbidity and mortality, associated with diastolic dysfunction [17]. The heart adapts in response to an array of stimuli by increasing myocardial mass through the induction of a hypertrophic response [27]. Studies in humans and animal models have demonstrated that cardiac hypertrophy significantly affects myocardial electronic cell-to-cell coupling, leading to disturbance in action potential duration and sudden cardiac death [29]. Currently, cardiac hypertrophy is associated with several disorders including diabetes mellitus [45] and there is no treatment for reversal of cardiac hypertrophy and hence, preventive measures are strictly required [48].

Protein kinase C (PKC) is a group of closely related serine-threonine protein kinases associated with cardiac hypertrophy, fibrosis, and cardiac dysfunction [40]. Additionally, it has been reported that inhibition of PKC βII prevents cardiac hypertrophy and enhances cardiac contractility [24]. In addition to PKC, estrogen also plays an important role on cardiovascular system. Estrogen effects are mediated by ERα and ERβ, receptors, both of which are expressed in cardiac myocytes, fibroblasts, and vascular cells in human and rodent heart [2]. ERα and ERβ agonist 17β-estradiol attenuates cardiac hypertrophy [46]. It has been reported that pre-menopausal women have a lower prevalence of left ventricular hypertrophy (LVH) than age-matched men [1], and that hormone replacement therapy (HRT) with estradiol reverses left ventricular hypertrophy in postmenopausal women [36]. Similar results have been obtained from animal studies [30,59]. From this

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perspective, it is clear that PKC and estrogen play important role in the prevention of heart disease.

Tamoxifen is a well-known drug from selective estrogen receptor modulator class; basically, used in treatment of breast cancer. In addition to its effect on estrogen receptor, it is also reported to inhibit PKC [23]. Tamoxifen shows inhibitory effect on L-type Ca^{2+} channels in vascular smooth muscle cells and reduced smooth muscle contractility [53]. McDonald et al. [34] have reported that tamoxifen produced a reduction in risk of myocardial infraction. Further, Rutqvist and Mattsson [50] also suggested that tamoxifen decreases cardiac morbidity with long-term treatment. Despite above-mentioned facts, direct reports of effect of tamoxifen on cardiac hypertrophy are not available. Hence, objective of the present study was to evaluate the effect of tamoxifen on experimentally induced cardiac hypertrophy.

Materials and methods

The protocol of the experiment was approved by our institutional animal ethical committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (IPS/PCOL/MPH10-11/009, 17 August 2011 and IPS/PCOL/MPH 10-11/2002, 14 January 2011).

Animals

Adult female Wistar rats of 6- to 8-week of age were chosen for the study and maintained under well-controlled conditions of temperature (22 ± 2 °C), humidity ($55 \pm 5\%$) and 12 h/12 h light–dark cycle. Standard laboratory rat chew and UV-filtered water were provided ad libitum.

Isoproterenol (ISO) induced cardiac hypertrophy

Adult female Wistar rats were used for the procedure. The rats were randomly divided into four groups: CON – control animals, TAM – control animals treated with tamoxifen, DIS – hypertrophic control animals treated with isoproterenol, DIS + TAM – hypertrophic animals treated with treated with isoproterenol and tamoxifen. The rats were injected intraperitoneally (*ip*) with 5 mg/kg isoproterenol in 0.9% sodium chloride solution daily for 10 days. Control rats received equivalent amount of isotonic saline alone.

Partial abdominal aortic constriction (PAAC) induced cardiac hypertrophy

Adult female Wistar rats were used for procedure. The rats were randomly divided into four groups: CON – sham control, TAM – sham control animals treated with tamoxifen, DIS – hypertrophic control animals, DIS + TAM – hypertrophic animals treated with tamoxifen. Treatment of tamoxifen was started from 0th day in sham treated and PAAC treated animals. Surgical procedure was done on 3rd day in PAAC control and PAAC treated animals under anesthesia produced by ketamine (20 mg/kg) and xylazine (10 mg/kg). Incision was made in abdominal wall to expose abdominal aorta. Abdominal aorta was ligated suprarenally with 4.0 silk suture along with 7-0 mm blunt needle. Thereafter needle was removed to leave abdominal aorta partially constricted. Sham control and sham treated animal underwent the same procedure except constriction of abdominal aorta.

Treatment protocol

Tamoxifen was dissolved in saline and was administered orally (*po*) at a dose of 2 mg/kg/day, *po* for 10 days and 4 weeks in ISO and PAAC induced cardiac hypertrophy respectively. The dose of tamoxifen was selected on the basis of the clinical dose converted into rat dose based on body surface area and weights by previously described methods [18,21].

Blood sample collection and serum analysis

At the end of experimental period, blood samples were collected from the retro orbital plexuses under light ether anesthesia, serum was separated and analyzed for total cholesterol, HDL-cholesterol, Triglycerides, LDL-cholesterol. C-reactive protein (CRP), lactate dehydrogenase (LDH), creatinine kinase (CK) spectrophotometrically (Shimadzu UV-1601, Japan) using biochemical diagnostic kits (Labcare Diagnostics Pvt. Ltd., India) [19,43].

Measurement of hemodynamic parameters

At the end of the respective treatment, the carotid artery behind the trachea was exposed and cannulated for the measurement of hemodynamic parameters using a transducer (BP 100) and Labscrib Systems (IWORX, New Hampshire, USA) under anesthetic conditions. The hemodynamic parameters observed were mean arterial blood pressure, heart rate, rate of pressure development (dp/dtmax) and rate of pressure decay (dp/dtmin). All the data were analyzed using Labscrib Software (Version 118) [41].

Measurement of cardiovascular parameters

At the end of the study, animals were sacrificed, hearts were excised, extraneous tissues were separated and wet weight of the entire heart, left ventricle (LV) and right ventricle (RV), femur length and LV wall thickness was noted down using screw gauge micrometer. Cardiac hypertrophic index was calculated as wet heart weight to femur length ratio and left ventricular hypertrophic index was calculated as wet left ventricle weight to wet heart weight ratio [20,42]. Na^+K^+ATPase activity was performed according to method described by Tsimaratos et al. [58]. Quantification of LV myocardial hydroxyproline concentrations [16], malondialdehyde (MDA) [39], reduced glutathione (GSH) [4] and superoxide dismutase (SOD) levels [35] were measured. The LV was subjected for histopathological studies for hematoxylin and eosin (HE) staining. The sections were observed and desired areas were photographed in an Olympus photomicroscope under 40× and 100× magnification and cell diameter measurements were done using Image J analyzer 1.45.

Statistical analysis

Results are presented as mean ± SEM. Statistical differences between the mean of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's test. Data were considered statistically significant at p value < 0.05.

Results

Serum lipid profile

ISO control and PAAC control rats exhibited a significantly ($p < 0.05$) increased level of serum total cholesterol, LDL and triglyceride and significantly ($p < 0.05$) decreased levels of serum HDL as compared to normal control rats. Treatment with tamoxifen (2 mg/kg/day, *po*) showed a significant ($p < 0.05$) reduction in serum cholesterol and serum LDL levels but did not produce any significant ($p < 0.05$) effect on serum triglyceride and HDL levels in both the models of cardiac hypertrophy, i.e. ISO-induced and PAAC-induced hypertrophy (Table 1).