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Isoproterenol-Induced Intramural Cytotoxicity Does Not Correlate with Echocardiographic Functional Abnormalities in Wistar Rat Hearts

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Abstract

Background: Isoproterenol (ISO) is a potent non-selective beta-adrenergic agonist with very low affinity for alpha-adrenergic receptors used for treatment of various cardiac problems including ventricular arrhythmias, asthma and shock. In addition, ISO is also used postoperatively after cardiac surgery. The aim of this study was to analyze the association between histopathological and functional effects of ISO in the rat myocardium.

Methods: 24 Wistar rats (healthy, males) were used in these experiments. The animals were randomly divided two groups. Group I (n=12): all animals received ISO subcutaneous administration (0,3 mg/Kg/day for 8 days). Group II (n=12): all animals received Phosphate Buffered Saline (PBS) subcutaneous administration as a control. One day after drug administration has been completed; echocardiographic analysis of cardiac function was performed. Histopathological analysis of the specimens was made by using H&E, Gomori's Trichrome and picrosirius red staining procedures. The results were analyzed using Student's t-test ($p<0,05$).

Results: Our histopathological results demonstrated: presence of diffuse inflammation, necrosis and fibrosis in the myocardium, and hypertrophy of ventricular septum. Interestingly, we did not find significant alteration in cardiac function ($p>0.05$), however the heart rate differed significantly between both groups ($p=0.0053$).

Conclusion: Short-term ISO administration (8 days) caused notable intramural cytotoxicity of the heart. In contrast, echocardiographic findings did not show any functional abnormalities in accordance with this cytotoxicity.

Keywords: Isoproterenol; Echocardiographic; Atrioventricular block; Ventricular arrhythmia; Histopathological

Introduction

Isoproterenol (ISO) is a potent non-selective beta-adrenergic agonist with very low affinity for alpha-adrenergic receptors. It is widely used in cardiovascular medicine such as in treatment of ventricular arrhythmias and shock. Moreover, ISO is used after cardiac surgery as well as in emergency cases of bradycardia or AV heart block and in pacemaker implant [1].

Administration of ISO is known to cause remarkable stress in the heart via activation of adrenergic system and other neurohumoral systems leading to an upregulation in the L-type Ca^{2+} channel activity, and ultimately disturbances in cardiac contractility, sarcoplasmic reticulum Ca^{2+} load, excitation-contraction coupling and heart failure. Recently, it has been shown that ISO effect on calcium homeostasis in cardiomyocytes can be attenuated by KN-93, an inhibitor of CaMKII phosphorylating activity [2]. ISO is also used to induce cardiomyopathy in experimental models, which is characterized by left ventricular hypertrophy without increased arterial pressure [3-7]. ISO toxicity has been shown to occur in dose and time dependent manner and involves mechanisms of lipid peroxidation and enzyme alterations: catalase, glutatione-S-transferase and superoxide dismutase [8]. Furthermore, ISO administration in high doses could induce heart insufficiency due to cardiac hypertrophy and formation of free radicals such as quinones, superoxides and peroxides of hydrogen. ISO-induced oxidative stress can lead to increased membrane permeability and the calcium influx [9-11]. However, the myocardial hypertrophy induced by ISO might not be an outcome of secondary necrosis because low doses of ISO could induce hypertrophy without necrosis [12,13]. The aim of this study was to analyze the association between histopathological and functional effects of ISO in the rat myocardium. Our results revealed that ISO-induced cardiotoxicity is not accompanied by abnormalities in cardiac function in rats.

Materials and Methods

Ethics: All experiments were carried out according to the principles of treatment of laboratory animals of The National Council for Animal Experiments Control from Brazil (CONCEA) and they were in agreement with the guidelines for the care and use of laboratory animals published by the ARRIVE guidelines and US National Institutes of Health (NIH) [14].

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Experimental model of drug administration

24 Wistar rats were used in this study (healthy male, weigh between 200-300 g). The rats were divided in 2 random groups. Group I (n=12): all animals received Isoproterenol subcutaneous administration (0,3 mg/Kg/day for 8 days) diluted in PBS, once a day. Group II (n=12): all animals received PBS subcutaneous administration as a control.

Analysis of cardiac function

The functional analysis was performed one day after completion of drug administration using a two-dimensional device for transthoracic echocardiography. The echocardiograph apparatus (5500 Sonos, model Hewlet Packard) has S12 (5-12 mHz) sectors conductor and 15L6 (7-15 mHz) which allowed analysis of up to 160 mHz. This apparatus was specifically produced for ultrasonic studies in small animals. The conductor was placed on the left anterior-lateral region of the thorax and the hearts were visualized in two-dimensional form with an axial view of the left ventricle, including the mitral and aortic valves and the apex in the same image. The digital conversion image was performed by delimitation of the ventricular septum and the posterior wall of the left ventricle. The measurements were carried out in a blind fashion by a single observer. The measurements of left ventricular ejection fraction (LVEF), left ventricular-end systolic volume (LVESV), left ventricular-end diastolic volume (LVEDV), Left ventricular diastolic area (LVDA), left ventricular systolic area (LVSA) and heart rate were obtained in 3-5 consecutive cardiac cycles. The correlation coefficient and standardization of the estimated error was calculated in accordance with the Bland and Altman method [4,15].

Histopathological examination

The rats were anesthetized by LD-ketamine- (50 mg/kg) and their hearts were removed. The hearts were then washed quickly in PBS (Gibcco-USA) and cryopreserved in liquid nitrogen. Serial transverse sections (8 μm in thickness) were made by a cryostat (LEICA-model 1850). The sections were stained for histopathological analysis using H&E, modified Gomori's trichrome and picrosirius red.

Statistics

The statistical analysis was performed by Student's t test and p-value of <0.05 was considered significant.

Results and Discussion

In the present study we compared histopathological and functional effects of ISO in the rat myocardium. Our histopathological results indicated presence of diffuse foci of inflammation, necrosis and fibrosis in the myocardium, as well as hypertrophy of the septum and walls of the ventricles (Figures 1-7). In addition, our results did not reveal any significant alteration in cardiac function ($p>0.05$), however the heart rate differed significantly between both groups ($p=0.0053$). One rat of Group I died during anesthesia, before functional analysis (Table 1).

The short-term ISO administration (8 days) caused intramural cytotoxicity of the heart which might not be a sufficient time for causing echocardiographic alterations in accordance with this cytotoxicity. The latter absence of correlation might be explained by probable occurrence of other compensatory mechanisms such as hypertrophy of the ventricles. In addition, it is also possible that ISO affects heart function in dose and time dependent fashions. Earlier investigations have demonstrated increase in oxidative stress upon ISO treatment via formation of free radicals such as quinones, superoxides and peroxides of hydrogen [9]. The increased oxidative stress after ISO

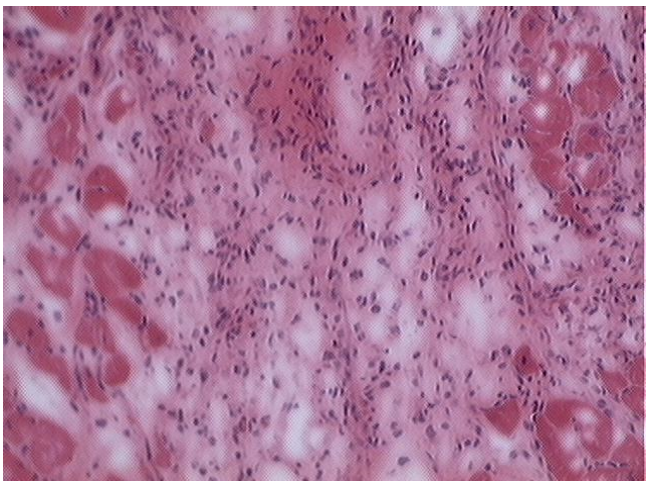

Figure 1: Inflammatory process and necrosis in the myocardium of Isoproterenol treated group, H&E stain, x200.

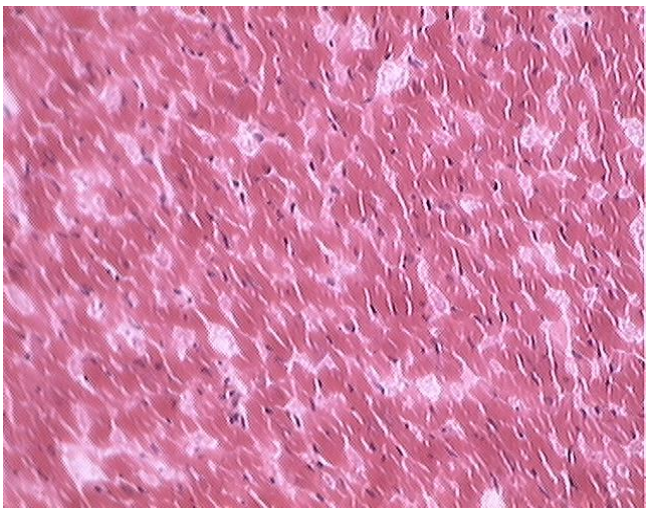

Figure 2: Normal myocardium from group II (control), stained by H&E, x200.

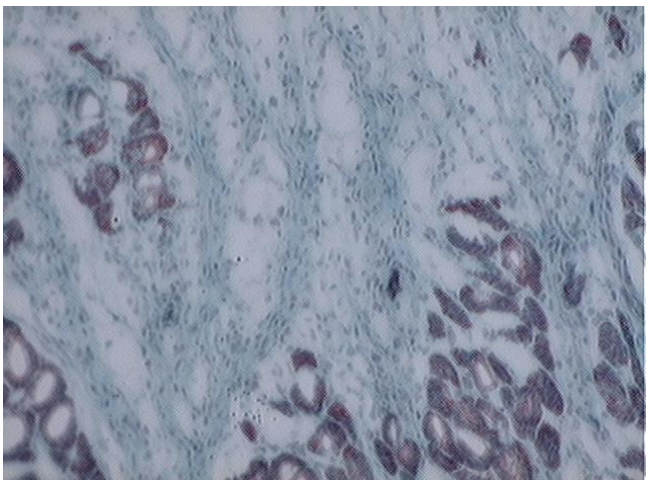

Figure 3: Inflammatory process and necrosis in the myocardium of Isoproterenol treated group, Gomori's Trichrome stain, x200.

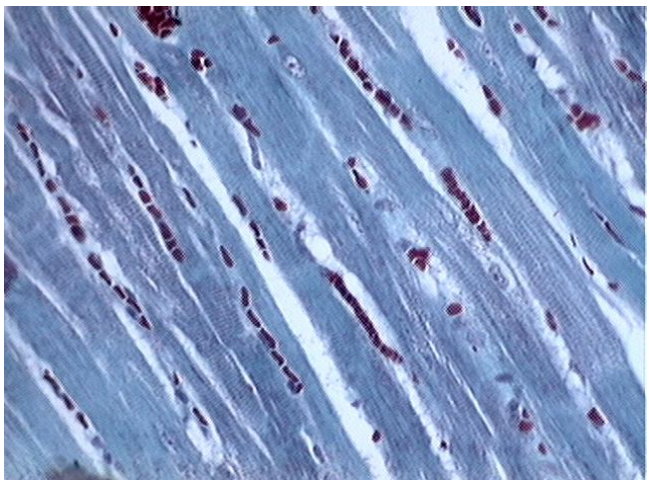

Figure 4: Normal myocardium from control group, Gomori's Trichrome stain, x 400.

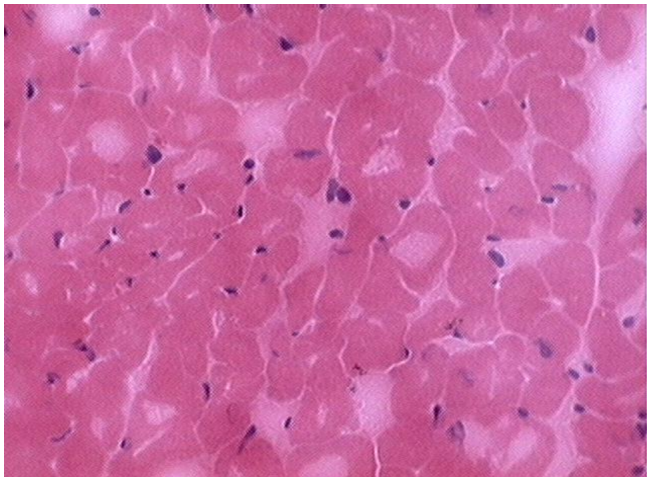

Figure 5: Hypertrophied cardiomyocytes in Isoproterenol treated group, with interstitial fibrosis of ventricular septum, H&E stain, x 400.

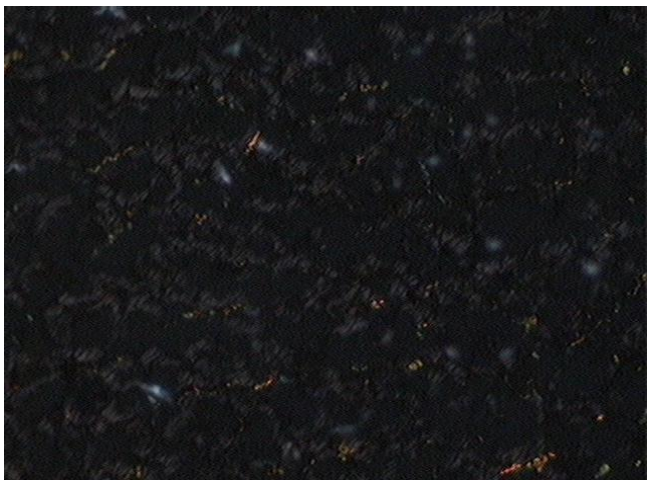

Figure 6: Collagen deposits in the myocardium of control Group, stained with picrosirius red in Polarized Light, x200.

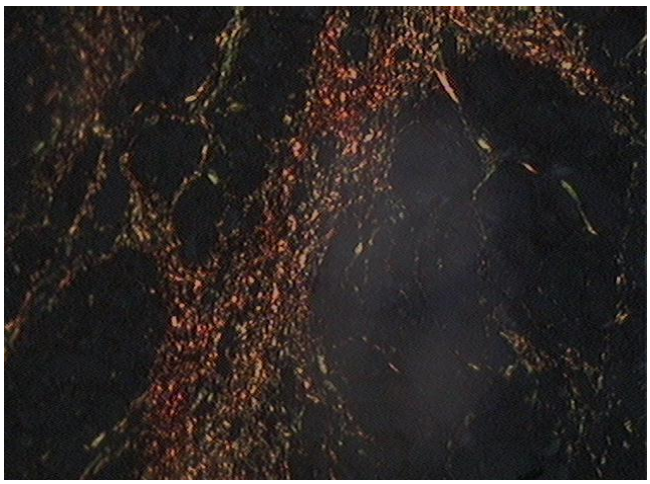

Figure 7: Collagen deposits in the myocardium of Isoproterenol treated group, stained with picro.

Variable	ISO (n=11)		Control II (n=12)		p-value*
	Mean	Standard Deviation	Mean	Standard Deviation	
LVDA	0,881	0,130	0,932	0,073	0,2456
LVEDV	0,439	0,102	0,485	0,071	0,2213
LVSA	0,541	0,115	0,582	0,079	0,3340
LVESV	0,200	0,070	0,225	0,045	0,3087
LVEF%	55,655	5,943	53,575	5,701	0,4015
HR	201,91	25,85	240,75	33,24	0,0053

Left ventricular diastolic area (LVDA) and left ventricular systolic area (LVSA) in millimeters (mm^2); Left ventricular-end diastolic volume (LVEDV) and left ventricular-end systolic volume (LVESV) in milliliters (mL). Left ventricular ejection fraction in percentage (LVEF %). Heart Rate (HR), Sample Number (n). * Student t test, p-value of <0.05 was considered significant.

Table 1: Functional Analysis *p<0.05.

treatment may not be sufficient to perturb cardiac function after 8 days of administration. Long term drug administration is required to clarify the association between ISO cytotoxicity and abnormal heart function.

Previous works on ISO cardiotoxicity were focused on comparing other aspects such as cardiac weight with various histopathological aspects in the heart [9]. Our work is the first to demonstrate absence of correlation between echocardiographic findings and histopathological changes after ISO treatment. Various ISO pro-oxidant actions were described before. These include oxidation of membrane phospholipids, proteins and DNA by superoxide anions ($-O^{2-}$) and hydroxyradicals (-OH). These events are tightly linked with wide range of pathological conditions in the heart such as ischemia-reperfusion injury, aging and drug toxic effects [16-18]. When the production of reactive oxygen species becomes excessive, oxidative stress might have a harmful effect on the functional and structural integrity of biological tissue and may also cause contractile failure and structural myocardial damage. These effects may not always lead to immediate cardiac dysfunction and may thus explain absence of cardiac functional abnormality after 8 days of ISO treatment.

Like echocardiography, non-invasive methods such as magnetic resonance imaging are very valuable in studies of cardiac function, and might thus allow correlation between ISO cardiotoxicity and distinct functional changes in the heart such as abnormal LV geometry and heterogeneous regional myocardial function [19-22]. The histopathological findings of this study are often seen in other

pathological conditions such as myocardial infarction, which is controlled by enzymes dosages [23,24]. The limitations of the study are (a) other parameters such as arterial and ventricular pressures were not measured, (b) although observation of myocyte hypertrophy was made blindly by an expert scientist, the sample size needs to be increased (c) the oxidative stress data discussed in this study were cited from the literature and were not analyzed in our laboratory. Overall, the present results may aid in the understanding of ISO toxicity in the clinical setting and could thus contribute to improving cardiac therapeutic strategies [20,21,25].

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