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PHYSIOLOGICAL AND MOLECULAR ASPECTS OF HEART OF RAT FED WITH CHOLESTEROL REACH DIET - THE IMPACT OF PROCAINE TREATMENT

[1]Cristian Romeo REVNIC, [2]Catalina PENA, [3]Alexandru SONEA, [3]Cosmin SONEA, [4]Gabriel Ovidiu DINU, [2]Flory REVNIC, [5]Bogdan PALTINEANU

[1]Ambroise Pare`Hospital, University of Medicine, Paris VI, France
[2] National Institute of Gerontology and Geriatrics "Ana Aslan", Romania
[3]University of Agronomic Sciences and Veterinary Medicine of Buchares, Romania
[4]University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania
[5]University of Medicine and Pharmacy Tg. Mures, Romania

Abstract

The aim of this study was to investigate the effect of ischemia reperfusion(I/R) upon cardiac physiological parameters(CF) coronary flow,(HR) heart rat as well as LVPD(Left ventricle pressure developed) as well as on apoptosis in adult Wistar rats feed on Cholesterol diet and treated with Procaine. Material and methods:18 male Wistar rats aged 12 months have been used in our experiment divided into 3 groups of 6 rats each: group A Controls, Group B Cholesterol feed rats, group C Cholesterol feed rats treated with Procaine (20mg/kg body weight for 8 weeks). High cholesterol diet (lard mixed with chew) has been used to feed rats for 8 weeks. At the end of treatment the hearts have been excised and mounted in Langendorff reperfusion system. A 45 minutes ischemia has been followed by 120 minutes reperfusion on isolated rat heart in order to measure heart rate, coronary flow and left ventricle developed pressure as well as to assay left ventricle for apoptosis .Our data have pointed out modifications in physiological parameters and the presence of apoptosis in cholesterol treated rats while the Procaine seems to have a benefic effect on these parameters and on DNA integrity.

Keywords*: ischemia reperfusion, rat heart, coronary flow, heart rate, apoptosis*

INTRODUCTION

The heart muscle is largely dependent on uninterrupted blood flow which guarantees delivery of substrates and washout of harmful products of metabolism (Braunwald et al., 1992). The death of cardiac cells during ischemia and reperfusion is partially mediated by apoptosis (Sato et al., 2004; Kaul, 2001).
The aim of this study was to investigate the effect of ischemia reperfusion (I/R) upon cardiac physiological parameters (CF) coronary flow,(HR) heart rat as well as LVPD (left ventricle pressure developed) as well as on apoptosis in adult Wistar rats feed on Cholesterol diet and treated with Procaine (20 mg/kg body weight for 8 weeks.

MATERIALS AND METHODS

The 18 male Wistar rats aged 12 months have been used in our experiment divided into 3 groups of 6 rats each: group A Controls, Group B Cholesterol feed rats, group C Cholesterol feed rats treated with Procaine.
High cholesterol diet (Lard mixed with chew) has been used to feed rats for 8 weeks.
Group C have received also Procaine treatment (20 mg/kg body weight) for 8 weeks.

HEART PREPARATION FOR PERFUSION

The male Wistar rats have been anesthetized with Na Phenobarbital (20 mg/kg body) slowly administrated into the tail vein, together with 250 U heparin. When deep anesthesia has been fully installed, the thoracic cage has been opened and the heart has been quickly removed by excision of big vessels and then passed into cold perfusion, weighted and then mounted on a canula in Langendorff apparatus for retrograde perfusion in 2 minutes maximum.

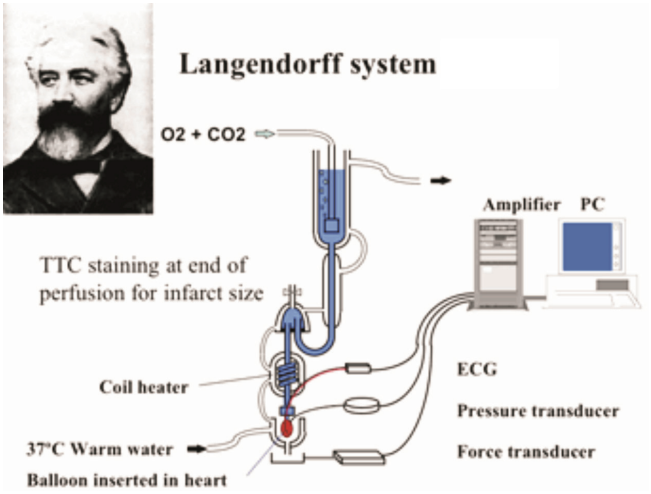

Figure 1

MOUNTING OF HEART IN LANGENDORFF PERFUSION SYSTEM

The hearts have been perfused in a noncirculant system with Krebs Henseleit (KHB) medium aired with 95% O_2 and 5% CO_2 in order to obtain a pH 7.35-7.40. The intracardiac temperature has been permanently monitorised by means of a thermocouple and maintained at 37°C with a thermostat bath.
The pressure developed by the left ventricle (LVPD) has been permanently recorded with a isovolumetric balloon positioned in the left ventricle through an incision of the left atrial apex. The balloon volume has been adjusted at the beginning of the experiment in 8-19 mm Hg. The CF (coronary flow) has been measured during the experiment
We used an ischemia (45 minutes) followed by 120 minutes reperfusion model of isolated rat heart in Langendorff retrograde perfusion.
TACS Apoptotic DNA laddering kit has been used to assay heart cells for apoptosis.
PRINCIPLE OF ASSAY
TACS Apoptotic DNA laddering kit has been used to assay tissues for apoptosis by detecting internucleosomal DNA fragmentation and displaying DNA laddering.

EXPERIMENTAL PROCEDURE
The heart tissue fragments (left ventricle) has been minced into small pieces and frozen in liquid nitrogen, then 0.2-1 g of frozen tissue has been grinded into the powder and then resuspended in 200 µl sample buffer.
20 µl of 10X tissue buffer has been added and incubated at 50°C for 12-14 hours with a gentle shaking.
DNA isolation has been done according with the instruction guide.
Etd.Br. labelling and detection of apoptosis
1 µl DNA has been diluted in 9 µl DNA free water. 2 µl gel loading buffer has been added and the next steps on electrophoresis have been done according with the instructions. Then the gel has been stained for 15 minutes in 0.5 µg/l Etd.Br.
The visualisation of DNA stained with Etd.Br has been done using UV transiluminator. The Photographs have been processed with KodakWratten 22A filter (Yellow).

RESULTS AND DISCUSSIONS

The oxidative stress imposed by 45 minutes ischemia followed by 120 minutes reperfusion intensifies the deleterious effects of pathological state on heart imposed by cholesterol treatment (Ambrosio, 1999; Brar et al., 2000; Braunwald et al., 1992). The effects are reflected upon Coronary flow, heart rate and left ventricular developed pressure.

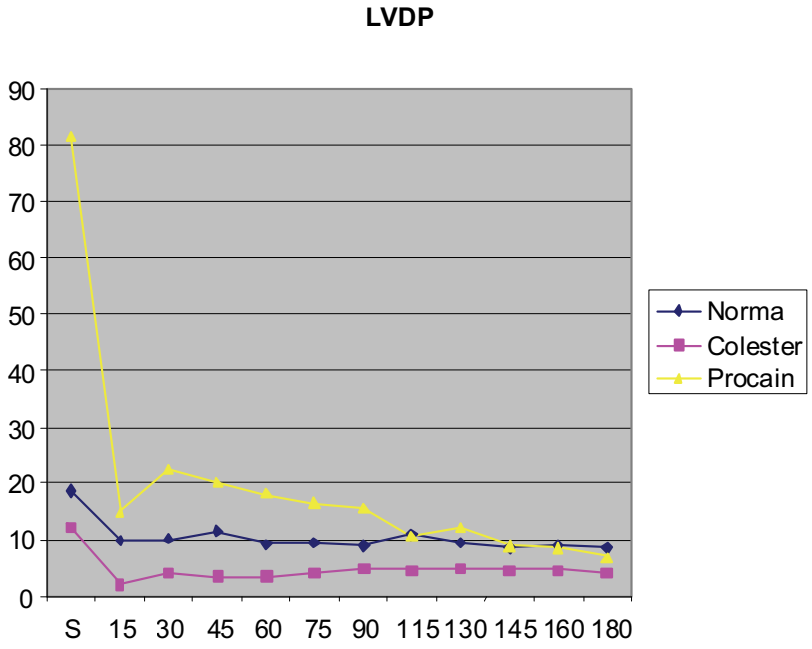

Figure 2

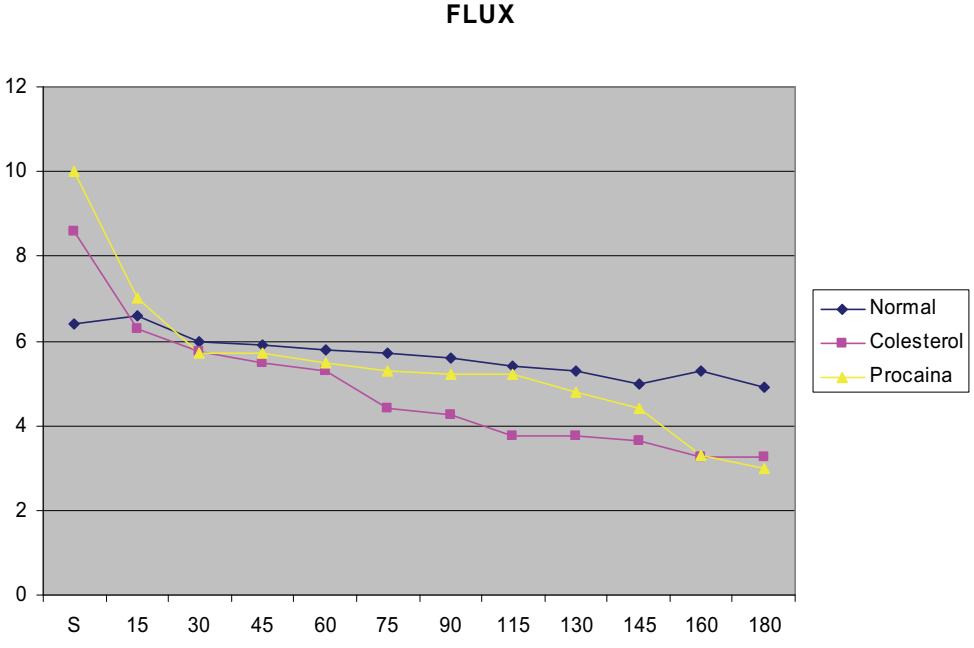

Figure 3

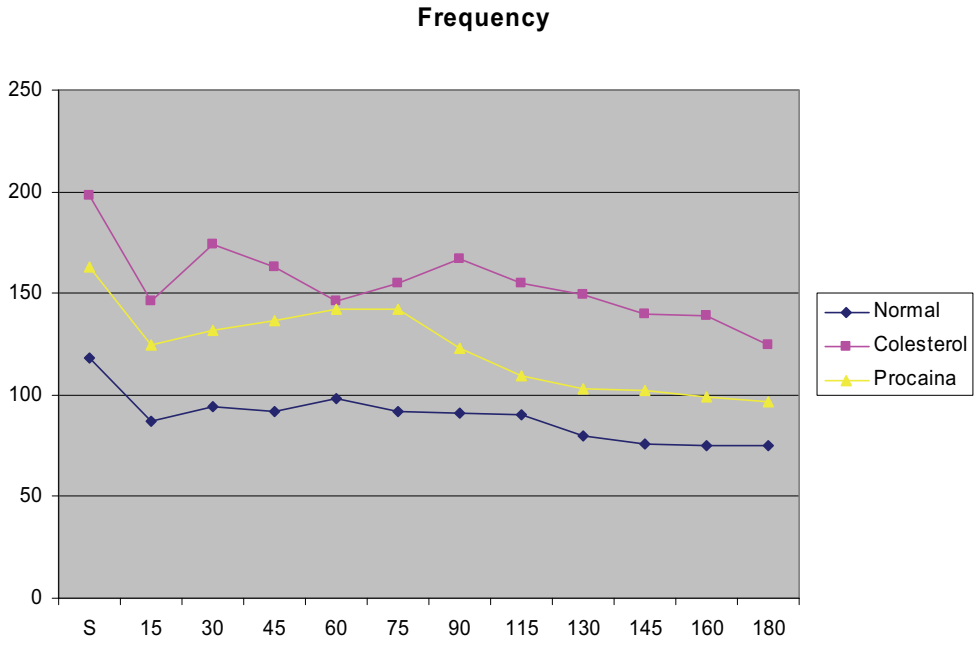

Figure 4

Figure 5. The image of DNA extracted from 12 month old rat ventricle fed on cholesterol reach diet cholesterol and procaine treated

Figure 6. DNA extracted from 12 month old rat ventricle fed on

Cholesterol reach diet induces a pathological state at the level of heart influencing the values of physiological parameters (Braunwald, 1992; Carmeliet, 1999).

The cholesterol diet influences the physiological parameters of heart as we have seen in our results. C.F.(cardiac frequency) which is increased in comparison with Controls. Our results are in accordance with the literature data (Cebbai et al., 1994; Derek et al., 2007).

The effect of treated rats feed with Procaine on Cholesterol diet leads to a decrease in cardiac frequency, approaching the values from the Controls.

The C.F. (coronary flux) is decreased in cholesterol feed rats versus Controls, while in Procaine treated rats the values of C.F. approaching the values from the Controls.

Concerning LVPD (Left ventricle developed pressure), in cholesterol feed rats, the values are decreased versus controls while in Procaine feed rats the values of LVPD are very much increased.

Reperfusion injuries (Kaul, 2001; Opic, 1989; Sato et al., 2004) due to free radicals generated during ischemia associate with 120 minutes reperfusion superimposed on a pathological condition generated by high reach cholesterol diet, generated severe injuries also at the molecular level expressed by internucleosomal fragmentation of DNA.

DNA laddering is present in rat heart feed on cholesterol diet, while in procaine treated rats feed on cholesterol diet, this is absent.

CONCLUSIONS

Our data have pointed out the negative impact of ischemia reperfusion associated with pathological state generated by cholesterol feeding upon heart contractility parameters as well as upon DNA integrity.

Procaine treatment seem to protect cardiovascular system from deleterious effects of cholesterol treatment at the physiological heart level as well as at the molecular level.

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