Effects of the Administration of Beta-Blockers on Ventricular Remodeling Induced by Cigarette Smoking in Rats

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Summary

Background: **The role of the adrenergic system on ventricular remodeling induced by cigarette smoking is unknown.**

Objectives: **To investigate the influence of propranolol on ventricular remodeling induced by exposure to tobacco smoke.**

Methods: **Rats were divided into three groups: 1) C, n=10 – control group; 2) F, n=10 - animals exposed to tobacco smoke; 3) BB, n=10 - animals receiving propranolol and exposed to tobacco smoke (40 mg/kg/day). After 2 months, the animals underwent echocardiographic and morphometric analyses. One-way ANOVA (mean ± standard deviation) or the Kruskal-Wallis test (median and interquartile interval) was used.**

Results: **Group BB showed a lower heart rate than group F (C = 358 ± 74 bpm, F = 374 ± 53 bpm, BB = 297 ± 30; P = 0.02). Group F showed greater end-diastolic diameters (C = 18.6 ± 3.4 mm/kg, F = 22.8 ± 1.8 mm/kg, BB = 21.7 ± 1.8 mm/kg; P = 0.003) and left ventricular (LV) end-systolic diameters (C = 8.6 ± 2.1 mm/kg, F = 11.3 ± 1.3 mm/kg, BB = 9.9 ± 1.2 mm/kg; P = 0.004), adjusted for body weight (BW) and a tendency towards a lower ejection fraction (C = 0.90 ± 0.03, F = 0.87 ± 0.03, BB =0.90 ± 0.02; P = 0.07) than group C. Group BB showed a tendency towards a lower LV/BW ratio than group F (C = 1.94 (1.87 – 1.97), F = 2.03 (1.9-2.1) mg/g, BB = 1.89 (1.86-1.94); P = 0.09).**

Conclusion: **Administration of propranolol attenuated some of the variables of ventricular remodeling induced by the exposure to tobacco smoke in rats. (Arq Bras Cardiol 2009; 92(6) : 443-447)**

Key Words: **Adrenergic beta-antagonists / administration & dosage; ventricular remodeling; smoking; rats.**

Introduction

Cardiac remodeling can be defined as variations in size, geometry, composition, and function of the heart. This process is a response to several stimuli, including myocardial infarction, systemic arterial hypertension, valvular disease, and genetic mutations[1-3]. Ventricular remodeling is initially an adaptive compensatory process, since morphological alterations may restore or preserve cardiac function in response to different aggressions. Chronically, however, the ventricular remodeling process leads to a progressive decline of ventricular function, signs and symptoms of heart failure, and death[1-3].

Recently, several studies have shown that in the rat model, exposure to tobacco smoke results in cardiac remodeling characterized by an increase in the left ventricular cavity, myocyte hypertrophy, and reduced ventricular function[4-9]. However, the mechanisms involved in this phenomenon are not yet determined. Among the mechanisms involved in the modulation of the functional and morphological changes occurring in different models of aggression, the activation of the adrenergic system seems to play a significant role[10,11]. Thus, studies have shown that the use of beta-blockers attenuates cardiac remodeling in different experimental and clinical models[12]. Nevertheless, the effects of the administration of beta-blockers on the remodeling induced by exposure to tobacco smoke are unknown.

Therefore, the aim of this study was to investigate the influence of the administration of propranolol on ventricular remodeling induced by exposure to tobacco smoke in rats.

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Methods

The experimental protocol of this study was approved by the Committee for Ethics in Animal Experimentation at Faculdade de Medicina de Botucatu, and it complies with the Ethical Principles of Animal Experimentation defined by the Brazilian College of Animal Experiments.

Protocol and experimental groups

Wistar male rats weighing 200-230g were allocated to 3 experimental groups: 1) Group C, n=10 – consisting of animals not exposed to tobacco smoke; 2) Group F, n=10 – consisting of animals exposed to tobacco smoke during 2 months; 3) Group BB, n=10 – consisting of animals exposed to tobacco smoke during 2 months and receiving propranolol (40

mg/kg/day) diluted in drinking water. A prior study evidenced that this dose has biological effects in a rat model[13]. As the medication was diluted in water, the daily intake of food and water by the animals was monitored.

Exposure to tobacco smoke

In order to expose the animals to tobacco smoke, we used the method proposed by Wang et al[14] which has already been standardized at our laboratory[4-9]. At the end of the experimental study period, the animals had been exposed to smoke equivalent to 40 cigarettes/day. With this methodology, exposure to tobacco smoke results in cardiac remodeling and functional worsening[4-9].

Tail systolic pressure

Measurement of the systolic arterial pressure (SAP) of the tail was taken two weeks before slaughter. Animals of the smoking group were not exposed to tobacco smoke during a period of 24 hours before the measurements. A tail plethysmograph was used, which consisted of a polygraph (Byo-Sistem PE 300, NARCO) with a sensor placed in the proximal region of the tail, and an electro-sphygmomanometer which enabled graphical recording of arterial pressure. The animals were warmed in a wooden box at 37ºC with heat generated by two incandescent lamps for four minutes, and were then transferred to an iron cylindrical support especially designed to allow total exposure of the animal's tail. A sensor (KSM-microphone) was placed in the proximal region of the tail, and coupled to the plethysmograph.

Echocardiographic evaluation of morphology and function

Twenty-four hours after the end of the observation period, all animals underwent the echocardiographic study performed with a Philips device (TDI 5500 model), with a multi-frequency transducer up to 12 MHz. Cardiac structures were measured as per recommendations of the American Society of Echocardiography[15]. The measurements were taken by the same observer, who did not know which groups the animals belonged to.

Morphometric study

After the echocardiographic study, the animals were euthanized with pentobarbital. The ratio between the weight of the left and the right ventricles adjusted by body weight was used as a parameter of myocardial hypertrophy[16,17]. The measurements were taken by the same observer, who did not know which groups the animals belonged to.

Statistical method

Statistical comparisons were made using one-way ANOVA, followed by the Tukey test when data had a normal distribution. When the distribution was not normal, comparisons between the groups were made using the Kruskal-Wallis test. Data were expressed as means ± standard deviations (for normal distribution), or medians with 25th and 75th percentiles (for non-normal distribution). The significance level was 5%, and the statistical analyses were performed using the SigmaStat for Windows v3.5 software (SPSS Inc, Chicago, IL).

Results

With regard to heart rate, animals in the BB group showed lower values as compared to smoking animals (C = 358 ± 74 bpm, F = 374 ± 53 bpm, BB = 297 ± 30 bpm; P = 0.02). On the other hand, no significant difference was observed in tail SAP among the animals of the three groups (C = 117 (110-120) mmHg, F = 127 (110-140) mmHg, BB = 117 (115-120) mmHg; p = 0.142).

Table 1 shows the echocardiographic study data. Morphological analyses showed that the animals in group F had greater left ventricular (LV) diastolic diameters adjusted for body weight (BW) compared with those of control animals (C = 18.6 ± 3.4 mm/kg, F = 22.8 ± 1.8 mm/kg, BB = 21.7 ± 1.8 mm/kg; p = 0.003). On the other hand, the BB group showed intermediate values for this variable as there was no difference compared to those recorded for both the C and F groups.

Functional analysis showed that the animals in group F had greater left ventricular (LV) systolic diameters adjusted for body weight (BW) (C = 8.6 ± 2.1 mm/kg, F = 11.3 ± 1.3 mm/kg, BB = 9.9 ± 1.2 mm/kg; p = 0.004) than those of the control animals. Once again, Group BB had intermediate values for this variable, as there was no difference compared to the values recorded for both the C and F groups. Likewise, there was a tendency towards a lower ejection fraction (C = 0.90 ± 0.03 %, F = 0.87 ± 0.03 %, BB =0.90 ± 0.02 %; P = 0.07) in group F as compared to group C, and a tendency towards a lower fractional shortening (C = 54 ± 5, F = 50 ± 4, BB = 54 ± 4; P = 0.09) in Group F than in the animals of Groups C and BB. No differences were recorded as to the other variables.

As to the cardiac morphometric variables (Table 2), the animals in group BB showed a tendency towards lower LV

Table 1- Echocardiographic findings

Variables	C (n=10)	F (n=10)	BB (n=10)
LA/BW (mm/g)	10.2 ± 1.6	11.7 ± 1.7	12.8 ± 1.4
LVDd/BW (mm/kg)	18.7 ± 3.4	22.8 ± 1.8*	21.7 ± 1.8
LVSd/BW (mm/kg)	8.6 ± 2.1	11.3 ± 1.4*	9.9 ± 1.9
PWT (mm)	1.50 ± 0.41	1.29 ± 0.2	1.41 ± 0.1
PWT/LVDd	0.20 (0.17-0.21)	0.18 (0.17-0.22)	0.20 (0.17-0.21)
short %	54 ± 5	50 ± 4#	54 ± 4
EF	0.90 ± 0.03	0.87 ± 0.03#	0.90 ± 0.02
E/A	1.63 ± 0.35	1.69 ± 0.31	1.84 ± 0.15

*C - animals not exposed to cigarette smoke; F - animals exposed to tobacco smoke during 2 months; BB - animals exposed to tobacco smoke and treated with propranolol during 2 months; BW - rat's body weight; LA - left atrium diameter; LVDd - left ventricular diastolic diameter; LVSd - left ventricular systolic diameter; PWT - posterior wall thickness; short % - fractional shortening; EF - ejection fraction; E/A - ratio between transmitral flow E and A waves. Data were expressed as means ± standard deviations or medians and interquartile intervals. *p<0.05 vs C; # p<0.1 vs C and BB.*

Table 2 - Morphometric data

Variables	C (n=10)	F (n=10)	BB (n=10)
BW (g)	385 ± 46	343 ± 38	349 ± 34
LV (mg)	0.69 (0.65-0.83)	0.66 (0.64-0.81)	0.66 (0.61-0.73)
LV/BW (mg/g)	1.94 (1.87-1.97)	2.1 (1.9-2.1)	1.89 (1.86-1.94)*
RV (mg)	0.21 ± 0.04	0.19 ± 0.04	0.19 ± 0.03
RV/BW (mg/g)	0.53 ± 0.06	0.57 ± 0.09	0.55 ± 0.06

*C - animals not exposed to tobacco smoke; F - animals exposed to tobacco smoke during 2 months; BB - animals exposed to tobacco smoke and treated with propranolol during 2 months; BW - rat's body weight; LV - left ventricular weight; RV - right ventricular weight. Data were expressed as means ± standard deviations or median and interquartile interval. *p<0.1 vs F.*

weight values adjusted for body weight of the animals (LV/BW) when compared to the smoking animals (C = 1.94 (1.87 – 1.97) mg/g, F = 2.03 (1.9-2.1) mg/g, BB = 1.89 (1.86-1.94) mg/g; P = 0.09). No differences were recorded for the other variables.

Discussion

The aim of this study was to analyze the effect of beta-blocker administration on left ventricular remodeling induced by exposure to tobacco smoke in rats. Results have shown that the blockage of beta-adrenergic receptors was followed by attenuation of some morphological and functional changes caused by tobacco smoking.

Changes in the geometry, volume, mass, and constituents of the heart in response to myocardial aggression or to alterations in loading conditions, have been studied under the name of cardiac remodeling[1-3]. Hypertrophy and increased cavity size have been recognized as important players in the remodeling process. Our findings confirm prior studies[4-9] which showed that smoking was followed by an increase in left ventricular cavity size in the rat model. Additionally, one of the important findings of this study was that administration of propranolol, a non-selective beta-blocker (acting on β1 and β2 receptors), resulted in intermediate values of LV diastolic diameter when compared to smoking and control animals. At first, no differences were found between BB and smoking animals. However, BB values were not different from those of the control group animals. Thus, our data allows us to suggest that propranolol did not prevent, but rather attenuated the increase in cavity size induced by smoking.

Another relevant aspect of cardiac remodeling is that, classically, the process is associated with different degrees of ventricular dysfunction. Initially, the characteristic morphological changes of remodeling may be considered adaptative in face of the new loading conditions to which the affected heart is subjected. Chronically however, genetic, molecular, and cellular changes may occur, resulting in progressive ventricular dysfunction[1-3]. Our data are consistent with this concept, since the increase in LV diastolic diameter induced by smoking was followed by worsening rates in left ventricular function, such as end-systolic diameter, ejection fraction, and LV fractional shortening. Another aspect to be considered is that propranolol resulted in intermediate values between smoking and control animals. Thus, it is possible to conclude that beta-blocker administration did not prevent, but attenuated the functional changes induced by smoking. This effect is even more significant if we consider that the animals treated with propranolol had lower heart rates. Physiologically, a reduced heart rate is associated with a decrease in ventricular ejection rate values, regardless of variations in the contractile status of the myocardium.

The mechanisms responsible for cardiac morphological and functional changes induced by exposure to tobacco smoke have not been fully explained. There is sufficient evidence showing that carbon monoxide is associated with apoptosis, cardiac dysfunction, hypertrophy, and dilation of the left ventricular chamber after experimental myocardial infarction[18,19]. A prior study with a rat model showed that the administration of an antioxidant attenuated remodeling induced by smoking, suggesting that oxidative stress plays a pathophysiological role in the cardiac changes caused by tobacco smoking[6]. Conversely, prior studies, with periods of observation longer than that in this study (4 and 6 months), have not shown changes in PO_2 and in the percentage of hemoglobin saturation with O_2[5,9]. Thus, chronic hypoxemia and changes in blood viscosity secondary to hypoxia, potential explanations for the results in our study, may have not participated in the pathophysiology of the changes induced by smoking. Similarly, no association was found between cardiac remodeling induced by smoking and the activation of **metalloproteinase-2 and -9**[8]. Another potential mechanism is associated with the fact that prior data have shown that the administration of nicotine to alert rats resulted in vasoconstriction. Thus, sympathectomy abolished the vasoactive effects of nicotine[20]. In the same model, tobacco smoke caused a rise in the levels of endothelin-1[21], and the administration of a selective endothelin-A receptor antagonist attenuated the pressure response induced by nicotine[22]. In another study, the administration of nicotine reduced the synthesis of prostacyclin from the vascular endothelium[23]. Additionally, the administration of a vasopressin antagonist attenuated the vasoconstriction induced by nicotine in dermal blood vessels[24]. In our study, however, no differences were observed in the levels of arterial pressure of the animals exposed to tobacco smoke and treated with propranolol versus the animals that did not receive the treatment. Thus, our data suggest that the attenuation of remodeling caused by propranolol did not result from the effects of the drug on arterial pressure.

We must remember that, due to autocrine and paracrine mechanisms, neurohumoral activation could result in changes in intracellular signaling pathways[12,25]. The classical subdivision of β-adrenergic receptors defines 3 subtypes: β1, β2, and β3 which activate adenyl cyclase through the G protein, increasing the levels of cyclic AMP with posterior stimulus by protein kinase A, which in turn causes phosphorylation of several proteins that modulate contractility (for instance: L-type calcium channels, **phospholamban**, troponin I, ryanodine receptors, SERCA2a) and cardiac remodeling (**phosphatidylinositol 3-kinase**, growth factors, ERK,

mitogen-activated protein **kinase,** among others). Thus, blockage of β-adrenergic receptors is associated with less cardiac remodeling secondary to several stimuli, both in experimental and clinical studies[12,25]. Our data are consistent with this evidence since there was attenuation of remodeling induced by exposure to tobacco smoke. Considering that no difference was observed in the pressure levels among the animals of the three groups, our data suggest that attenuation of the remodeling caused by propranolol probably involved intracellular signaling pathways modulated by β-adrenergic receptors, but which most probably did not result from hemodynamic effects.

An important aspect of this study refers to the clinical implications of our results. There is evidence that smoking may cause morphological and functional cardiac changes in humans. For instance, among the participants of the observational study CARDIA, smokers had a greater left ventricular mass than non-smokers as evaluated by echocardiogram[26]. Additionally, acute inhalation of cigarette smoke was accompanied by impairment of diastolic function in patients with documented coronary disease[27,28]. Therefore, it is possible that the administration of beta-blockers may interfere with the changes induced by smoking in humans as well.

Finally, our results should be analyzed considering potential limitations in this study. First of all, the variables analyzed were merely echocardiographic and body weight parameters. Our study did not evaluate other variables involved in the remodeling process, such as interstitial collagen content. Another aspect is that only a few variables were statistically different, fact which was probably due to the small sample in our study. However, despite these limitations, we believe that the variables analyzed were sufficient to characterize the remodeling process induced by smoking and to show biological effects with propranolol.

In conclusion, the administration of propranolol did not prevent, but did attenuate some of the ventricular remodeling variables induced by the exposure to tobacco smoke in rats.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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There were no external funding sources for this study.

Study Association

This study is not associated with any post-graduation program.

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