Original Article

Chronic Cigarette Smoke Exposure Results in Cardiac Remodeling and Impaired Ventricular Function in Rats

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Objective

To determine the cardiac structural and functional alterations caused by cigarette smoke exposure in rats.

Methods

The animals were randomly distributed into the following 2 groups: 1) smokers (S), comprising 10 animals exposed to cigarette smoke at a rate of 40 cigarettes/day; and 2) control (C), comprising 10 animals not exposed to cigarette smoke. After 4 months, the animals underwent morphological and functional study with echocardiography. The variables studied were analyzed by use of the t test or the Mann-Whitney test.

Results

The smoking rats had a greater left atrium (S=4.2±0.7mm; C=3.5±0.6mm; P<0.05), and greater left ventricular diastolic (S=7.9±0.7mm; C=7.2±0.5mm; P<0.05) and systolic (S=4.1±0.5; C=3.4±0.5; P<0.05) diameters. The left ventricular mass index was greater in the smoking animals (S=1.5mg/kg±0.2; C=1.3mg/kg±0.2; P<0.05), and the ejection fraction (S=0.85±0.03; C=0.89±0.03; P<0.05) and the shortening fraction (S=47.8%±3.7; C=52.7%±4.6; P<0.05) were greater in the control group. No differences were observed in the diastolic transmitral flow variables (E wave, A wave, and E/A ratio).

Conclusion

Chronic cigarette smoke exposure results in cardiac remodeling with a decrease in ventricular functional capacity.

Key words

hypertrophy, ventricular function, ventricular dilation

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Active or passive cigarette smoke exposure is an important cause of morbidity and mortality [1,2]. Several studies have shown that chronic smokers have an increased risk of cardiovascular disease, such as atherosclerosis, coronary heart disease, and sudden death. In addition, smoking causes endothelial dysfunction, a decrease in coronary reserve, and vasospasms [3-5].

Although the vascular effects of cigarette smoke exposure are well known, the effects of tobacco smoking on the heart have received less attention. A relevant aspect is that most studies have assessed the effects of the acute administration of nicotine on cardiac variables [6,7]. In a different approach, chronic exposure to carbon monoxide, another constituent of the vapor phase of cigarette smoke, resulted in an increase in gene expression of endothelin-1, inducing cardiac hypertrophy [8].

The effects of acute exposure to specific components, such as nicotine or carbon monoxide, on the heart cannot be extrapolated to chronic exposure to cigarette smoke, because cigarette smoke contains thousands of known substances [9]. Houdi et al [10], in a study to clarify the cardiac effects of cigarette smoke exposure, exposed rats to smoke for 4 days and observed an increase in blood pressure and a decrease in cardiac output. This effect was attenuated by a vasopressin antagonist. Brooks et al [11], using the assessment model of contractility with preparations of isolated papillary muscle, found no differences in cardiac performance between the rats exposed to smoke for 180 days and control animals. In spontaneously hypertensive rats, exposure to smoke for 8 weeks resulted in an increase in blood pressure and a decrease in heart rate compared with that in controls [12]. Yet in the rat model, exposure to cigarette smoke for 6 months resulted in an increase in messenger RNA expression for endothelin 1 in cardiac tissue[13]. On the other hand, in the canine model, both administration of nicotine and exposure to cigarette smoke for 22 months did not modify the ejection fraction and left ventricular end-diastolic pressure compared with that in controls [14].

Therefore, the cardiac effects of chronic exposure to cigarette smoke require better characterization. Thus, this study aimed at assessing the effects of chronic exposure to cigarette smoke on morphological and functional cardiac variables analyzed by use of echocardiography.

Methods

The experimental protocol of this study followed the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation and approved by the Committee on Ethics and Animal Experimentation of our institution.

Male Wistar rats weighing 200-250 g were randomly distributed into the following 2 groups of 10 animals each: 1) smoking group (S), exposed to cigarette smoke at the rate of 40 cigarettes/day for 4 months; and 2) control group (C), not exposed to cigarette smoke.

The animals were exposed to cigarette smoke in a modified incubator according to the method proposed by Wang et al [15] and standardized at our laboratory [16]. The rats were placed in a transparent chamber with a volume of approximately 95x80x65 cm, connected to a smoking device. Puffs of cigarette smoke were collected by use of vacuum in the smoking device, being then thrown into the chamber for 30 minutes. After that period, the smoke was exhausted, and the procedure repeated. During the first week, the smoke was released at a rate of 5 cigarettes, twice a day in the afternoon with 10-minute rest intervals. The number of cigarettes was increased to a rate of 10 cigarettes/30 min, twice in the morning and twice in the afternoon, until the end of the study. Thus, at the end, the animals had been exposed to the smoke of 40 cigarettes/day. The composition of the commercial cigarette used was as follows: 1.1 mg of nicotine, 14 mg of tar, and 15 mg of carbon monoxide.

At the end of the first and third month of observation, blood of the animals in both groups was drawn for blood gas analysis. Samples of the arterial blood of the tail of each animal were obtained with the aid of a "butterfly" connected to a previously heparinized 5-mL syringe. Immediately after blood collection, the analysis was performed in a Technicon automate gas analyzer, RA-XT model, and the following variables were studied: partial pressure of oxygen (PO2); partial pressure of carbon dioxide (PCO2); concentration of carboxyhemoglobin (COHb); and saturation of hemoglobin (SAT O2).

After 3 months of observation, the systolic pressures in the tail of the smoking and control animals were measured by use of a tail plethysmograph with a polygraph (Byo-Sistem PE 300, NARCO), a sensor placed in the proximal region of the tail, and an electrosphygmomanometer, to enable recording tail pressure. The animals were warmed in a wooden box at 37°C with the heat generated by 2 incandescent lamps for 4 minutes and were transferred to an iron support, where the tail was exposed. In the proximal region of the tail, a sensor (KSM-microfone) was placed and coupled to the plethysmograph. Blood pressure was recorded on paper with the polygraph at a 2.5-mm/s velocity.

After 4 months of treatment, the animals of both groups underwent the echocardiographic study according to the previously described method [17]. Briefly, the animals were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (1 mg/kg) through an intramuscular route, and, after epilation of the anterior region of the thorax, they were positioned in the left lateral decubitus position. Echocardiography was performed with Hewlett-Packard equipment, Sonos 2000 model, and an electronic 7.5-MHz transducer. Flows were assessed with the same transducer operating at 5.0 MHz. In the short-axis parasternal view, a transverse left ventricular one-dimensional image was obtained by using the 2-dimensional image as a guide for positioning the ultrasound beam right below the mitral valve plane, between the papillary muscles. Images of the aorta and left atrium were also obtained in the short-axis parasternal view with the M-mode cursor positioned level with the aortic valve. Recording of the one-dimensional image, adjusted for the 100-mm/s velocity, was performed by use of a Sony Co printer, UP-890MD model. Later, the cardiac structures were manually measured with the aid of a precision pachymeter according to the recommendations of the American Society of Echocardiography [18]. The cardiac structures were measured in 3 to 5 consecutive cardiac cycles as follows: left ventricular diastolic diameter (LVDD) and left ventricular posterior wall thickness (LVPWT) were measured at the moment corresponding to the maximum diameter of the cavity; the left ventricular systolic diameter (LVSD) was measured at the moment of the maximum systolic excursion of the posterior wall of the cavity. Left ventricular systolic function was assessed by calculating the percentage of systolic shortening [(LVDD-LVSD)/LVDD x 100] and the left ventricular ejection fraction ($LVDD^3$ - $LVSD^3/LVDD^3$). The transmitral diastolic flow (E and A waves) was obtained with the transducer at the apical 4-chamber view. The peak velocities in the rapid ventricular filling phase (E wave) and during atrial contraction (A wave) were measured directly on the echocardiographic monitor in 5 consecutive cardiac cycles.

After undergoing Doppler echocardiography, the rats were euthanized, the liver and heart were removed and the right and left ventricles (including the ventricular septum) were separated and weighed. Then, the ventricles were put into an incubator, where they remained for 48 hours at the temperature of 80°C, to dehydrate. The relation between the humid and dry weights of the structures could be determined, as well as the tissue water content.

The Student *t* test was used for comparisons between the groups when data had a normal distribution. When their distribution was not normal, the comparisons between the groups were performed by using the U test (or Mann-Whitney test). Data are expressed as mean ± standard deviation (for normal distribution), or median with the quartile 25 and the quartile 75 (for nonnormal distribution). The results were considered statistically significant if $P<0.05$. The analyses were performed with the Sigmastat program.

Results

The body variables of the animals are shown in table I. The smoking rats had a smaller relation between left ventricular humid weight and dry weight, as compared with that of control animals. In regard to the other variables, no differences were found between the groups.

The morphological variables obtained from the echocardiographic study are shown in table II. Smoking rats had greater left atria (S=4.2±0.7 mm; C=3.5±0.6 mm; $P<0.05$) compared with those of control animals. In regard to ventricular diameters, the S group animals had greater left ventricular diastolic (S=7.9±0.7 mm; C=7.2±0.5 mm; $P<0.05$) and systolic (S=4.1 ±0.5; C=3.4±0.5; $P<0.05$) diameters compared with those of control animals. When those variables were adjusted for body weight, the smoking rats had greater atrial and ventricular diameters ($P<0.05$) and left ventricular mass index (S=1.5±0.2; C=1.3±0.2; $P<0.05$).

Considering the functional variables, differences were identified neither in the E and A waves nor in the E/A ratio between the 2 groups (tab. II). The ejection (S=0.85±0.03; C=0.89±0.03; $P<0.05$) and shortening (S=47.8%±3.7; C=52.7%±4.6; $P<0.05$) fractions were greater in the control group.

In regard to blood gas analysis, after one month of exposure to cigarette smoke, the smoking rats had statistically greater concentrations of carboxyhemoglobin than nonsmoking rats did (tab. III). That variable was used to confirm the efficacy of the exposure of smoking animals to cigarette smoke. Regarding the other variables, no differences were found between the groups. After 3 months of evolution, differences in blood gas analysis were observed between the 2 groups only in regard to PCO2, which was smaller in the smoking group.

Regarding blood pressure levels, the group exposed to cigarette smoke showed, after a 3-month exposure, an increase in systolic blood pressure in the tail as compared with that in the control group (S=118±15mm Hg, C=103±16mm Hg; P=0.045) (fig. 1).

Discussion

Our objective was to assess the cardiac effects of prolonged exposure (4 months) to cigarette smoke in the rat model. The results indicate that chronic cigarette exposure leads to cardiac alterations, both morphological and functional, assessed through echocardiography. Thus, the usually neglected cardiac effects may cause additional damage to smokers affected by inhaled smoke. This is an additional effect to the already well-known effects, such as those on the vascular endothelium and lungs.

One of the most relevant pieces of data obtained was that exposure to cigarette smoke resulted in left ventricular morphological alterations. This phenomenon was characterized by an increase in ventricular diameters, both systolic and diastolic. Recently, alterations in cardiac geometry, volume, mass, and constituents in response to myocardial aggression or to alterations in load conditions have been studied under the name of cardiac remodeling [19-21]. Although we have not studied all variables involved in the process, such as cell constitution, tissue structure, and interstitial matrix, our results identified morphological alterations that characterize left ventricular remodeling.

One of the most striking characteristics of cardiac remodeling is that that process invariably results in a progressive decrease in ventricular function. Initially, due to cell growth, remodeling may contribute to maintain or restore cardiac function. Chronically, however, biochemical, genetic, and structural alterations occur, resulting in progressive ventricular dysfunction [19-21]. In agreement with that concept, in rats exposed to cigarette smoke, the remodeling process was accompanied by a significant decrease in the systolic function assessed by use of the ejection and shortening fractions. Our study showed no alteration in diastolic function, at least in the variables assessed through transmitral flow.

Table I - Body variables of the animals

Variables	C Group	S Group	P
BW (g)	439±29	466±43	0.128
LV (mg)	0.98±0.13	0.96±0.09	0.654
RV (mg)	0.29±0.05	0.28±0.04	0.734
LV/BW (mg/g)	2.11±0.17	2.19±0.17	0.331
RV/BW (mg/g)	0.61±0.08	0.64±0.07	0.570
LV H/D (%)	80.97±1.18	79.63±0.51	0.048
RV H/D (%)	79.88 (79.20-80.66)	80.46 (79.88-80.97)	0.273
Liv H/D (%)	69.51±0.66	69.02±0.90	0.206

C - control; S - smoker; BW - body weight; LV - left ventricle weight; RV - right ventricle weight; LV/BW - left ventricle weight adjusted for body weight of the rat; RV/BW - right ventricle weight adjusted for body weight of the rat; LV H/D - ratio between left ventricular humid weight and left ventricular dry weight; RV H/D - ratio between right ventricular humid weight and right ventricular dry weight; Liv H/D - ratio between humid weight and dry weight of the liver.

Table II - Morphological variables assessed on echocardiography

Variables	C Group	S Group	P
LA (mm)	3.5±0.6	4.2±0.7	<0.05
LA/BW (mm/Kg)	7.5±1.5	9.8±1.5	<0.001
LVDD (mm)	7.2±0.5	7.9±0.7	<0.05
LVDD/BW (mm/kg)	15.6±1.1	18.1±0.9	<0.001
LVSD (mm)	3.4±0.5	4.1±0.5	<0.05
LVSD/BW (mm/kg)	7.4±1.1	9.5±1.0	<0.001
PW (mm)	1.3±0.2	1.3±0.1	0.623
LVMI (g/kg)	1.3±0.2	1.5±0.2	<0.05
EF	0.89±0.03	0.85±0.03	<0.05
% SHORTS	52.7±4.6	47.8±3.7	<0.05
E (cm/s)	69.5±7.1	64.5±9.2	0.189
A (cm/s)	42.8±5.5	43.4±9.3	0.862
E/A	1.6±0.2	1.5±0.2	0.294

CC - control; S - smoker; LA - left atrial diameter; LA/BW - left atrial diameter adjusted for the body weight of the rat; LVDD - left ventricular diastolic diameter; LVDD/BW - left ventricular diastolic diameter adjusted for body weight of the rat; LVSD - left ventricular systolic diameter; LVSD/BW - left ventricular systolic diameter adjusted for body weight of the rat; PW - posterior wall diastolic thickness; LVMI - left ventricular mass index; EF - ejection fraction; % SHORT - shortening fraction; E - peak velocity in the phase of rapid filling; A - peak velocity during atrial contraction; E/A - ratio between the E and A waves.

Table III - Blood gas analysis

Variables	C Group	S Group	P
1-month exposure to smoke			
PO2 (mmHg)	55.6±4.7	58.4±6.4	0.305
PCO2 (mmHg)	34.1±2.4	34.8±3.6	0.613
SAT O2 (%)	87.8±2.3	89.9±3.4	0.150
COHb (mg/dl)	0.9±0.7	5.3±2.8	0.008
3-month exposure to smoke			
PO2 (mmHg)	57. 2±6.4	60.7±2.6	0.129
PCO2 (mmHg)	36.9 (33.9-39.7)	32.6 (30.3-34.7)	0.020
SAT O2 (%)	89.90 (87.38-93.07)	92.10 (91.47-92.67)	0.185

C - control; S - smoker; PO2 - partial pressure of oxygen; PCO2 - partial pressure of carbon dioxide; SAT O2 - saturation of hemoglobin; COHb - concentration of carboxyhemoglobin.

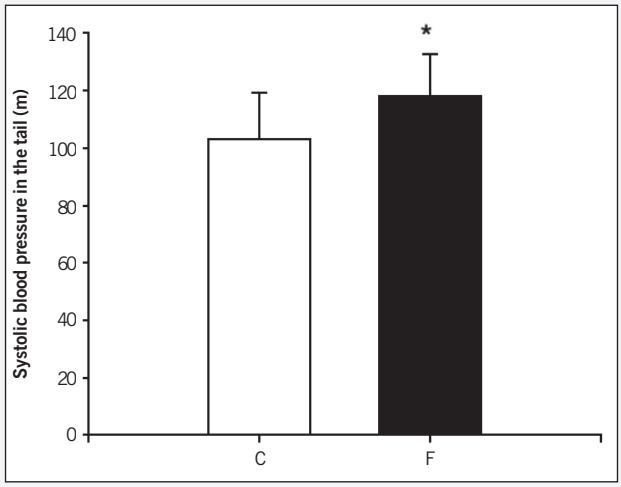

Fig. 1 - Systolic blood pressure values in the tail after 3 months of observation. C - control group; S - group exposed to cigarette smoke; * P < 0.05.

An intriguing fact was that, despite the existence of left ventricular dysfunction, no ventricular or hepatic tissue edema was identified in the animals exposed to cigarette smoke. However, it is worth noting that the remodeling process is evolutionary. Initially, aggression to the heart occurs (stage A). As time goes by, structural alterations, but not functional alterations, are identified (stage B). As the process progresses, asymptomatic ventricular dysfunction appears with signs/symptoms of congestion or of low perfusion pressure [22]. Congestive signs/symptoms appear only in the more advanced phases (stage C) of remodeling. Thus, we believe that the lack of greater water content in the organs of smoking animals suggests the existence of a pathological process, which is not in its most advanced stage.

Other studies have analyzed the effects of cigarette smoke exposure on experimental and clinical models. However, while the vascular effects of smoking have been well characterized, the cardiac effects of cigarette smoke exposure have not yet been completely clarified. In a canine model, the acute administration of nicotine induced an improvement in cardiac muscle contractility[6]. In rats with myocardial infarction, the acute treatment with nicotine resulted in an enlargement of the ventricular cavity, with thinning of the infarcted wall, suggesting that nicotine led to deleterious effects on the remodeling process after infarction [7]. In a previous study [16], we reported that exposure to cigarette smoke for 30 days resulted in a mild decrease in LV function. Some clinical studies have also analyzed the cardiac effects of smoking. Thus, acute inhalation of cigarette smoke was accompanied by disorders in the diastolic function of patients with documented coronary heart disease [23,24]. In the observational CARDIA study, smokers had a greater left ventricular mass when compared with that of nonsmokers on echocardiography [25], suggesting that smoking may induce cardiac alterations.

Considering the mechanisms through which cigarette smoke exposure results in cardiac morphological and functional alterations, our study did not show alterations in PO2 and in the percentage of hemoglobin saturation. Thus, chronic hypoxemia and alterations in blood viscosity secondary to hypoxia, which are potential candidates to explain our results, may not have participated in the pathophysiology of the cigarette-induced alterations. Another possibility could be the participation of hemodynamic factors, particularly arterial vasoconstriction. This phenomenon could result from substances, such as catecholamines, endothelin, and vasopressin, released by the stress or consequent to the inhalation of cigarette smoke. In addition to the hemodynamic alterations, the neurohormonal activation could, through autocrine, paracrine, or endocrine mechanisms result in alterations in the intracellular signaling pathways. Our study found differences in blood pressure between the groups. Although the blood pressure levels were not as high as those in other models, our data suggest that activation of neurohormonal factors may, at least in part, explain the morphological and functional alterations found.

In conclusion, despite the evidence that chronic exposure to cigarette smoke results in cardiac alterations, the exact mechanisms involved in that process remain to be elucidated. Our data indicate that chronic exposure to cigarette smoke for 4 months results in cardiac remodeling with ventricular function impairment in rats.

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