Role of Lipoperoxidation in the Remodeling Intensification Induced by Beta-Carotene after Infarction

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Summary

Background: The mechanisms involved in the biggest remodeling caused by the post-infarct beta-carotene are unknown.

Objective: To analyze the role of lipoperoxidation in the ventricular remodeling after infarct of the myocardium in rats supplemented with beta-carotene.

Methods: Rats were infarcted and divided into two groups: C (control) and BC (500mg/kg/regimen). After six months, echocardiogram and biochemical evaluation were performed. The t test was used, with 5% significance.

Results: The animals from BC group presented highest means of the diastolic (C = 1.57 ± 0.4 mm²/g, BC = 2.09 ± 0.3 mm²/g; p < 0.001) and systolic (C = 1.05 ± 0.3 mm²/g, BC = 1.61 ± 0.3 mm²/g; p < 0.001) areas of LV, which were adapted according to the rat's body weight. The systolic function of LV, evaluated by the area variation fraction, was lower in the animals supplemented with beta-carotene (C = 31.9 ± 9.3%, BC = 23.6 ± 5.1%; p = 0.006). The animals supplemented with beta-carotene presented higher values of the E/A relation (C = 2.7 ± 2.5, BC = 5.1 ± 2.8; p = 0.036). No differences were found between the groups concerning the cardiac levels of the GSH (C = 21 ± 8 nmol/mg of protein, BC = 37 ± 15 nmol/mg of protein; p = 0.086), GSSG (C = 0.4 (0.3-0.5) nmol/g of protein, BC = 0.8 (0.4-1.0; p = 0.19) of protein; p = 0.246) and lipoperoxides (C = 0.4 ± 0.2 nmol/mg of tissue, BC = 0.2 ± 0.1 nmol/mg of tissue; p = 0.086).

Conclusion: The highest remodeling in infarcted rats supplemented with beta-carotene does not depend on the lipoperoxidation. (Arq Bras Cardiol 2009;93(1):31-35)

Key words: Ventricular function; oxidative stress; ventricular dilatation; ventricular dysfunction, left; ventricular remodeling; beta-carotene.

Introduction

After the acute myocardial infarction (AMI), some alterations may happen in the ventricular architecture, assaulting both the infarcted and non-infarcted region. It is accepted that the morphological changes may be reflection of cellular, molecular and interstitial cardiac alterations, which occur as a response to determined aggression. The set of these adaptations - which are clinically detected by changes in composition, mass, volume and cardiac geometry - is called myocardial remodeling[1-4].

The intensity of the ventricular remodeling process is directly associated with a worse prognosis, especially because the remodeling is related to the appearance and progression of ventricular dysfunction. Thus, several strategies have been used to prevent or diffuse the ventricular remodeling process after the AMI[5-7].

One of the main modulators of the remodeling process is the oxidative stress. Among other physiopathological mechanisms, one of the principal consequences of the oxidative stress is the lipoperoxidation. In this manner, it is accepted that the oxidative stress may be an inducer of cellular damages that alter functional and structural cardiac variables, participating in the physiopathology of cardiac insufficiency, which is secondary to a lot of impulses, including AMI[8-11].

Considering that the beta-carotene, for possessing the ability of slowing the reactive species of oxygen, is an antioxidant[12,13], its supplementation could be beneficial after AMI. Thus, in a previous study of our laboratory, the supplementation effects of the beta-carotene in the ventricular remodeling process after AMI were evaluated. However, oppositely to the expected, the supplementation with beta-carotene resulted

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in intensification of the remodeling, followed by worsening of the cardiac function[14]. The mechanisms that are responsible for this phenomenon, however, have not been clarified yet. One of the accepted possibilities is that the beta-carotene, in a situation of great oxidative impulse, would stop practicing its antioxidant activity and start to present a pro-oxidant one[15].

The purpose of this study was to evaluate the participation of lipoperoxidation in the morphological and functional alterations in the cardiac parameters, induced by the supplementation of beta-carotene in infarcted rats.

Material and methods

Experimental groups

The experimental protocol of the present work was approved by the Ethics Committee of Animal Trial of our institution, and it is in accordance with the Ethics Principles in Animal Trial adopted by *Colégio Brasileiro de Experimentação Animal*.

Male Wistar rats were used, weighing between 200 and 250 grams. The acute infarct was produced according to the previously described method[16]. Briefly, the rats (n = 120) were anaesthetized with ketamine hydrochloride (50mg/kg) and xylidine hydrochloride (1mg/kg) and, then, submitted to left lateral thoracotomy. During the experiment, the animals breathed spontaneously, with oxygen supplementation of 100% by catheter. After the exteriorization of the heart, the left atrium was separated and the left coronary artery was connected with a mononylon thread 5,00, between the outlet of the pulmonary artery and the left atrium. Later on, the heart was returned to the thorax, lungs were inflated with positive pressure and the thorax was closed by sutures with cotton 10.

The animals were maintained in cages for recovery; fed with commercial ration and free access to water; with light control, in 12-hour cycles; temperature close to 25ºC and controlled humidity.

After 48 hours of infarct, the animals were randomly divided into two groups: C group (n = 25), composed by infarcted animals which were fed with standard commercial ration; and BC group (n = 27), composed by infarcted animals which received a regimen supplemented with beta-carotene in a 500mg/kg/regimen dose. All the analyses were done by examiners without knowledge of the group of animals.

Morphologic and functional evaluation by echocardiogram

After six months of treatment, the surviving animals were anaesthetized with ketamine hydrochloride (50mg/kg) and xylidine hydrochloride (1mg/kg), for the echocardiographic study. After the tricotomy of the anterior region of the thorax, the animals were positioned in dorsal decubitus in calf chute specially projected, which allows a light left lateral rotation for the realization of the exam, using Philips equipment (model TDI 5500) endowed of a multifrequential electronic transducer up to 12 MHz. The evaluation of the mitral and aortic transvalvar flows was accomplished with the same transducer, functioning in 5.0 MHz. The measures of the cardiac structures were carried out in monodimensional images that were obtained through the ultrasound sheaf guided by the bidimensional image in the parasternal lower axle position. The image of the left ventricular cavity was obtained by positioning the cursor of M-mode between the papilar muscles, right below the mitral valve plan. The images of the aortic and left atrium were obtained from the parasternal lower axle position, with the M-mode cursor, positioned in the level of the aortic valve. The record of the monodimensional image (velocity: 100 mm/s) was carried out by a printer, model UP-890MD of Sony Co. All the measures were made according to recommendations of the American Society of Echocardiography[17], which were already validated in the infarcted rats model[18]. The left ventricular diastolic dimension (LVDD) and the thickness of the left ventricle posterior wall (LVPW) were measured concomitantly to the maximum diameter of the cavity. The LV systolic dimension (LVSD) was measured in the moment of the maximum systolic excursion of the posterior cavity wall. The diastolic (DA) and systolic (SA) areas of LV were measured in the bidimensional mode by planimetry in two parasternal planes: long and minor axle. The LV systolic function was evaluated by calculating the area variation fraction (ACF = DA-SA/DA x 100)[18], obtained by the mean of values of both axles. The diastolic transmitral flow (waves E and A) was attained with the transducer in the apical position of four chambers. The measures of the flows were accomplished directly in the monitor of the echocardiograph.

Morphometric study

After the echocardiographic study, the animals were sacrificed with pentobarbital; their hearts were removed, dissected and the left and right ventricles, including the interventricular septum, were separated and weighted.

Cardiac tissue samples were settled in a 10% solution of formalin within a 48-hour period, according to an already described method[19,20]. The histological cuts were bleached in a flake with solution of hematoxylin-eosin (HE) for the gauging of areas of the oblique section of myocytes, engaging the microscope LEICA DM LS binded to a video camera, which sends digital images to a computer endowed of the image analysis program Image Pro-plus (Media Cybernetics, Silver Spring, Maryland, USA). About 50 to 70 cells were measured per ventricle analyzed. The selected myocytes were sectioned transversally, and presented round shape and visible core in the center of the cell. This care aimed at unifying at most the set of myocytes of different groups. The mean sectioned areas obtained for each group were used as indicators of the cell size.

Flakes with coronary histological cuts of 6 micras - freshed by the Picro Sirius red technique, specific for the collagen visualization - were done for evaluating the interstice of the myocardium of LV. The microscopic reading was made through the microscope LEICA DM LS joined to a video camera, which sends digital images to a computer endowed of the image analysis program named Image Pro-plus (Media Cyberetics, Silver Spring, Maryland, USA).

Lipoperoxidation

For a biochemical evaluation, level dosages of reduced (GSH) and oxidized glutathione (GSSH), relation between GSH/GSSG and lipoperoxides were performed according

to an already standardized technique[21]. The dosages were performed in the liver and heart.

Statistical analysis

As the data presented a normal distribution, the comparisons were made through the Student's t test. Data were expressed as mean ± standard deviation. For the relation GSH/GSSG, they are expressed in median and interquartile interval, and analyzed by means of Mann-Whitney's test. The significance level was 5%. Statistical analysis was accomplished through the program SigmaStat for Windows v2.03 (SPSS Inc, Chicago, IL).

Results

In the end of the study, 13 control animals and 15 animals supplemented with beta-carotene survived to the six-month period ($p > 0.05$).

The results of the echocardiographic study are shown in Table 1. The cardiac frequency was statistically higher in the animals supplemented with beta-carotene (C = 248 ± 31 bpm, BC = 281 ± 40 bpm; p = 0.025). The BC animals presented higher values of the DSLV, adjusted to the rat's body weight (C = 20.5 ± 3.4 mm/g, BC = 23.8 ± 3.8 mm/g; p = 0.025), and lower values of the PWDT (C = 1.4 ± 0.2 mm, BC = 1.2 ± 0.2 mm; p = 0.041), in comparison to the control animals. The animals from BC group presented higher means of the diastolic (C = 1.57 ± 0.4 mm^2/g, BC = 2.09 ± 0.3 mm^2/g; $p < 0.001$) and systolic (C = 1.05 ± 0.3 mm^2/g, BC = 1.61 ± 0.3 mm^2/g; $p < 0.001$) areas of LV, adjusted to the rat's body weight, in comparison to the control animals. The systolic function of LV, evaluated by the area variation fraction, was lower in the animals supplemented with beta-carotene (C = 31.9 ± 9.3%, BC = 23.6 ± 5.1%; p = 0.006). In relation to the diastolic function, the animals supplemented with beta-carotene presented higher values of the E/A relation than the control animals (C = 2.7 ± 2.5, BC = 5.1 ± 2.8; p = 0.036). Considering the other morphometric variables, no differences were observed between groups.

The results of the morphometric study are shown in table 2. The supplementation with beta-carotene resulted in a bigger weight of RV, adjusted to the body weight (C = 0.7 ± 0.4 mg/g, BC = 1.1 ± 0.3 mg/g; p = 0.034). No differences, concerning the other analyzed variables, were observed ($p > 0.05$).

Concerning the biochemical dosages, no differences were found between the groups as the hepatic levels of GSH (C = 17 ± 7 nmol/mg of protein, BC = 27 ± 14 nmol/mg of protein; p = 0.250); GSSG (C = 0.3 ± 0.1 nmol/g of protein, BC = 0.4 ± 0.2 nmol/g of protein; p = 0.246); GSH/GSSG (C = 4377 (2738-8249), BC = 2012 (1116-4488); p = 0.286) and lipoperoxidation (C = 0.3 ± 0.1 nmol/mg of tissue, BC = 0.2 ± 0.1 nmol/mg of tissue; p = 0.159). Likewise, concerning the cardiac levels of GSH, no differences were found between the groups (C = 21 ± 8 nmol/mg of protein, BC = 37 ±15 nmol/mg of protein; p = 0.086); GSSG (C = 0.4 (0.3-0.5) nmol/g of protein, BC = 0.8 (0.4-1.0; p = 0.19) of protein; p = 0.246); GSH/GSSG (C = 56 ±7, BC = 53 ± 14; p = 0.709) and lipoperoxides (C = 0.4 ± 0.2 nmol/mg of tissue, BC = 0.2 ± 0.1 nmol/mg of tissue; p = 0.086).

Discussion

The objective of this study was to analyze the participation of lipoperoxidation in the process of ventricular remodeling after acute myocardial infarction in rats supplemented with beta-carotene. Our study confirmed that the supplementation with beta-carotene intensifies the cardiac remodeling post-AMI. However, in contrast to the expected, worsening of the remodeling does not seem to depend on lipoperoxidation.

The first aspect to be considered refers to the fact that our study has confirmed the previously reported findings related to the supplementation effects' of beta-carotene in the ventricular remodeling process after AMI[14]. In fact, the beta-carotene supplementation resulted in an increase of the ventricular diameters, both systolic and diastolic, indicating worsening of the left ventricular remodeling process. The increase of the ventricular cavity was accompanied by the decrease of the thickness of the LV posterior wall. This fact,

Table 1 - Echocardiographic study

Variables	Control (n = 13)	BC (n=15)	P
CF (bpm)	248 ± 31	281 ± 40	0.025
LA/BW (mm/kg)	12.2 ± 3.6	15.1 ± 4.2	0.076
LVDD/BW (mm/kg)	20.5 ± 3.4	23.8 ± 3.8	0.025
PWDT (mm)	1.4 ± 0.2	1.2 ± 0.2	0.041
E (cm/s)	75.3 ± 24.3	69.4 ± 20.9	0.504
A (cm/s)	44.4 ± 26.8	19.7 ± 11.9	0.004
E/A	2.7 ± 2.5	5.1 ± 2.8	0.036
DA/BW (cm^2/g)	1.57 ± 0.4	2.09 ± 0.3	<0.001
SA/BW (cm^2/g)	1.05 ± 0.3	1.61 ± 0.3	<0.001
AVF (%)	31.9 ± 9.3	23.6 ± 5.1	0.006

Control - infarcted animals; BC - infarcted animals and animals supplemented with beta-carotene; BW - body weight of the rat; LA - left atrial diameter; LVDD - left ventricular end-diastolic diameter; PWDT - posterior wall diastolic thickness; E/A - relation between the evaluated transmitral flow waves E and A; DA - diastolic area; SA - systolic area; AVF - area variation fraction. Data were expressed in mean ± standard deviation.

Table 2 - Morphometric data

Variable	Control (n=13)	BC (n=15)	P
BW (g)	548 ± 68	466 ± 40	<0.001
LV/BW (mg/g)	2.7 ± 0.6	2.6 ± 1.2	0.776
RV/BW (mg/g)	0.7 ± 0.4	1.1± 0.3	0.034
SA (μm^2)	236 ± 7.6	236 ± 8.6	0.812
IC (%)	3.5 ± 12	3.1 ± 1.6	0.482
% AMI	46.3 ± 4.2	48.8 ± 7.8	0.311

Control - infarcted animals; BC - infarcted animals and animals supplemented with beta-carotene; BW - body weight of the rat; LV - weight of the left ventricle; RV - weight of the right ventricle; SA - myocyte sectional area; IC - interstitial collagen fraction; % AMI - size of the infarct. Data were expressed in mean ± standard deviation.

associated with the non-increase of the myocyte's sectional area, may suggest that the beta-carotene supplementation results in cellular growth with eccentric standard.

The second important aspect of our study is associated with the concept that the cardiac remodeling results, unchangeably, in a progressive fall of the systolic ventricular function. Initially, as a consequence of the cellular growth, the remodeling may contribute to the maintenance or repairment of the cardiac function. Chronically, however, there are biochemical, genetic and structural variations that will result in a progressive systolic ventricular dysfunction[2-4]. According to this idea, in the rats supplemented with beta-carotene, this remodeling process was accompanied by the fall of area variation fraction. Other indirect signals of ventricular dysfunction were found in animals supplemented with beta-carotene, such as the increase of cardiac frequency and the right ventricle hypertrophy. It is interesting to notice that alterations in the diastolic function were identified in this study. The increase of the E/A relation was interpreted as a restrictive standard of ventricular fulfillment, characterizing severe diastolic function. The fact that the supplemented animals presented tendency to higher values of the left atrium reinforce this interpretation. Other interesting fact is that no differences concerning the interstitial collagen content were found. Considering that the collagen is an important modulator of the diastolic function[22], the results herein presented suggest that other modulating factors of the diastolic function may have been affected by the treatment with beta-carotene.

The third aspect to be considered is that the responsible mechanisms for intensifying the cardiac remodeling process with the supplementation of beta-carotene are not known. One of the possible mechanisms is related to the lipoperoxidation, since, as already said, the beta-carotene, in situations of great oxidative impulse, can stop exerting its antioxidant activity and could start to present pro-oxidant activity[15]. Another proposed mechanism is that the beta-carotene can induce the cytochrome P450 expression and, thus, increase the catabolism of the retinoic acid, which is an important modulator of the cardiac remodeling[23].

With regard to the oxidative stress, it is known that the reactivated species of oxygen are constantly produced in the organism. The potential damages caused by this production are counterbalanced by the action of the antioxidant defense systems. Among the antioxidant defenses, the enzymatic systems can be emphasized, with the participation, for example, of the GSH and GSSG; and the non-enzymatic systems, with the participation of vitamins, among others. When the balance between the oxidant impulse and the antioxidant defenses is broken, a condition named oxidative stress is established[8-11]. One of the main consequences of the oxidative stress would be the lipoperoxidation. Thus, both the relation GSH/GSSG and the lipoperoxide levels could evaluate the presence or intensity of the oxidative stress[24,25].

Contrarily to our original hypothesis, the supplementation of beta-carotene did not modify the variables that evaluated the lipoperoxidation. In fact, the relation GSH/GSSG, and the levels of GSH, GSSG and lipoperoxides of the group supplemented with beta-carotene were not different from the infarcted animals without supplementation. It should be considered that the concentrations of GSH and GSSG and their association indicate how much of the GSH was used to preserve the antioxidant/oxidant equilibrium. So, the GSH is converted into GSSG to stop the action of the reactive species in the organism. Since there were no differences in the concentrations of GSH, GSSG and in its relation, our results did not confirm the possible pro-oxidant activity of the beta-carotene. On the contrary, as the beta-carotene itself can only present antioxidant effect, there should be no need of consuming GSH, forming GSSG to keep the redox balance. Therefore, if we consider that, despite these factors, there was a consistent intensification of the cardiac remodeling with beta-carotene, it may be suggested that the lipoperoxidation probably did not participate in the enhancement of the ventricular remodeling.

It is important to stand out that our study, by methodological characteristics available at that moment, evaluated only the participation of lipoperoxidation and systems related directly to it. We should consider, however, that the participation of the oxidative stress in the enhancement of the remodeling must not be discarded only by the absence of lipoperoxidation increase, since other producer systems of free radicals of oxygen may be acting, for example, the NADPH-oxidase and thioredoxin systems. Hence, the mechanisms responsible for the deleterious action of beta-carotene, in this model, remain to be determined.

In conclusion, the set of our data suggests that the greatest remodeling in infarcted animals and animals supplemented with beta-carotene does not depend on the lipoperoxidation.

Potential Conflict of Interest

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

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Study Association

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

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