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The effects of supraphysiological supplementation of β-carotene in spontaneously hypertensive rats (SHR and SHR-sp)

Efeitos da suplementação suprafisiológica de β-caroteno em ratos espontaneamente hipertensos (SHR e SHR-sp)

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A B S T R A C T

Objective: To investigate the effect of the administration of supraphysiological â carotene on biological, laboratory and histological parameters of spontaneously hypertensive rats prone to stroke (SHR-sp). **Methods**: We used 36 male rats divided into three groups, each containing 12 rats of the types Wistar SHR, and SHR sp, subdivided into six control animals and six animals treated with supraphysiological doses of â carotene for two ten-week periods, interspersed by a one-week interruption. We carried out daily physical examination and blood pressure assessment. We collected blood for measurement of serum malondialdehyde; the liver and carotid arteries were subsequently harvested for histological examination. **Results**: there was a temporary change in the color of hair, a significant decrease ($p < 0.0001$) in blood pressure (20mg β-carotene supplementation) and of serum levels of malondialdehyde ($p < 0.05$), and increased in the elastic fibers in the carotid artery wall of SHR and SHR-sp rats. **Conclusion**: β-carotene supraphysiological supplementation caused no toxic effects, showed positive response in the modulation of blood pressure and lower serum malondialdehyde. No significant morphological changes were observed in the groups studied, except for an increase in the number of elastic fibers in the carotid muscular layer, suggesting elastosis in SHR and SHR-sp rats.

Key words: Oxidative stress. Malondialdehyde. Betacarotene. Rats.

INTRODUCTION

The cerebrovascular accident (CVA) is one of the most significant public health problems today due to its high incidence and mortality, the disability it causes, the high costs it generates, and the lack of therapeutic strategies [1].

The effects of high blood pressure (HBP) in the structure of the vessels occur both in the larger arteries, where there is remodeling of the vessel wall with increase of its light, which causes increased friction force between blood and the vessel wall, predisposing arteriosclerosis, and in the arteries of small caliber, where there is realignment of the muscle cells and reducing light without alteration in these cells' set, that is, there is restructuring of the smooth muscle around the reduced light, leading to systemic vascular resistance [2].

HBP also exacerbates the atherosclerotic process, possibly by weakening the walls of the arteries at points of higher pressure, leading to injury and invasion of cholesterol and other compounds[3]. Atherosclerotic disease of the carotid arteries can cause symptoms such as *amaurosis fugax*, headache and transient ischemic attack (TIA) and is responsible for 20-30% of stroke cases. The progression of asymptomatic carotid artery stenosis is unpredictable and can be disastrous [4].

Atherosclerosis primarily involves cells of the tunica intima (the innermost layer of the artery wall), and it is believed to originate from a proliferation of fat striae in fibrous-fatty plates[5]. The atherogenesis process is deemed to be triggered when, the arterial wall's subendothelium, macrophages uncontrollably capture oxidized, cholesterol-rich LDL particles, becoming foam cells that accumulate in the arterial wall. The oxidative modification of low density lipoproteins make them preferably capturable by macrophages, starting an inflammatory process that triggers the formation of atheroma plaque, the core element in atherosclerotic vascular lesions [5-7].

Experimental evidence have confirmed the involvement of the macromolecules' oxidative process in endothelial injury of cardiovascular disease, greatly

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increasing interest in research of the probable action of the antioxidant power of vitamins to combat this process. Likewise, recent hypotheses have associated oxidative stress, including inflammation, to stroke [8].

Studies in *Anima Nobile* demonstrated a relationship between markers of oxidative stress, stroke and low serum levels of antioxidant vitamins (alpha tocopherol, ascorbic acid and β-carotene), there being an increasing interest in research on the possible protective action of these nutrients [9].

β-carotene has an important role in combating oxidative stress. Its antioxidant action has been widely debated [10-12]. If this pro-vitamin supplementation is able to control stroke's oxidative stress, it may become a possible adjuvant, or even subsequent, therapy in the management of stroke.

Regarding the experimental study of stroke, the SHR-sp strain (with a tendency to spontaneous hypertensive stroke) described by Maguire *et al.* [13] and Ikeda *et al.* [14], has been chosen as an experimental model for the study of human stroke by developing it spontaneously, resulting in brain damage similar to human stroke, and by presenting with marked hypertension, which can reach 300mmHg, in contrast to the blood pressure of normotensive Wistar Kyoto rats (WKY), which stabilize between 140-150 mmHg and 90-110 mmHg, respectively [15]. Hypertension in this strain of rats occurs around the eighth week of age, and may reach about 250mmHg systolic pressure in adult males, being higher than the SHR's (spontaneously hypertensive) blood pressure [16]. The susceptibility to stroke in this model is also associated with genetic factors independent of systolic pressure, which is why we use this lineage preferentially to SHR in the investigation of brain disease [15,17-20].

The aim of this study was to evaluate the effect of supraphysiological administration of β-carotene on biological, laboratory and histological parameters of spontaneously hypertensive rats prone to stroke (SHR-sp).

METHODS

We used 36 young male rats, weighing between 272 and 356 grams, divided into three distinct groups, each containing 12 rats of the races Wistar, SHR and SHR-sp, respectively, each group consisting of six controls and six treated animals, randomly chosen, obtained from colonies of the vivarium of the School of Nutrition, Federal University of the State of Rio de Janeiro. At the beginning of the experiment the age of the animals was approximately seven weeks in both groups, reaching, respectively, 28 weeks at the end of the survey.

The experimental protocols used in this study were approved by the Ethics Committee for Animal Experiment, Federal University of the State of Rio de Janeiro, and the assays were performed in the Laboratory for Research in Nutrition and Chronic Degenerative Diseases (LINDCD) and at the Laboratory of Anatomy pathology, both from the Federal University of the State of Rio de Janeiro.

The animals were kept in a vivarium with controlled lighting conditions (light-dark cycle / 12h), temperature (21 ± 2° C), humidity (60 ± 10%) and air exhaust cycle (15min / h), in individual metabolic cages.

The animals received food and water *ad libitum* and, after a baseline period of ten days, were subjected to supraphysiological supplementation of β-carotene or placebo by oral gavage via a PE 190 polyethylene catheter.

Initially, we established the dose-effect curve for rats Wistar and SHR rats. The treated animals in Wistar and SHR groups were supplemented with increasing amounts of β-carotene, from 2.5 to 5 mg / day, diluted in 0.3 ml of coconut oil. Each dose was administered for a period of ten weeks. The treatment was divided into two stages, with a break of one week to establish the kinetics of β-carotene.

Simultaneously, the treated SHR-sp supplementation was carried out with administration of β-carotene at a dose of 5 to 20 mg. The initial dose in this group was the same that presented with hypotensive results in the SHR strain, i.e. 5mg. The control group animals were supplemented only with coconut oil.

The animals underwent daily physical examination, following the LINDCD protocol [21] for detecting any signs of toxicity or interaction between nutrients. Concurrently, overall biological parameters (feed intake, water, diuresis, feces and body weight) were evaluated. Systolic pressure was measured twice a week by plethysmography, following the methodology of Magaldi *et al.* modified by Viana *et al.* [22]. The blood pressure measurement is done always of same time to prevent changes from the circadian rhythm. Plethysmography was performed at baseline and throughout the experiment.

For the determination of serum malondialdehyde, obtained from the centrifugation of blood collected in disposable tube without anticoagulant, we used the colorimetric method for the determination of MDA and thiobarbituric acid. The concentration of malondialdehyde was estimated by absorbance at 532nm and the results were expressed in nmol.

Animals were anesthetized by induction of deep coma with inhalation of sulfuric ether and barbiturate (thiopental sodium) intraperitoneally, with doses greater than 25 mg / kg. We held a collection of 5ml of blood by cardiac puncture for MDA dosage.

We then harvested the right and left carotids, the aorta, the heart and liver, which were stored in 10% formalin and sent for histological analysis. The specimens were stained with hematoxylin-eosin or orcein and Masson's trichrome. The morphometric analysis was performed using an optical microscope under magnifications of 10x, 40x, 100x, 160x and 200x.

The determination of liver weight was performed using the Scherle method, which is based on Archimedes' Principle [23].

One Way ANOVA was conducted to evaluate the significance of the results obtained with different doses, considering $p < 0.05$ as significant. We used the Student's t test for comparisons of values between groups (control vs. treatment).

RESULTS

The assessment of physical examinations of animals did not show changes in the general biological parameters: weight, water and food intake, urine output and fecal excretion. We only observed change in coloration of the animals, which returned to normal color during the interruption of treatment. Likewise, motor coordination and behavior of animals remained within the normal range. As for the weight of the liver, we obtained average weight 7.25 ± 3.2 g and the average relationship between liver and body weights equal to 0,0192g, showing that hepatotoxicity did not occur.

Systolic blood pressure in the SHR-sp-treated group showed no reduction in values during treatment with doses of 5 mg and 10 mg of â-carotene. However, when receiving supplementation with 20 mg of β-carotene, it displayed a significant decrease ($p < 0.0001$) in blood pressure, from 233.7 ± 1,39mmHg to 227.5 ± 1,96mmHg, in the first supplementation with this dose.

Discontinuation of treatment for seven days showed an increase in the value of systolic blood pressure, which reached 252.3 ± 0,36mmHg. When supplementation was resumed, this value showed a significant decrease ($p < 0.0001$) to 232.08 ± 1,34mmHg (Figure 1).

The measurement of thiobarbituric acid-reactive substances showed that serum levels of malondialdehyde (MDA) were significantly ($p < 0.05$) lower in the rats treated with β-carotene (0,32nmol ± 1.97) compared with animals in control groups (3.50 ± 1,19nmol – Figure 2).

Histological analysis revealed that the structure of liver was preserved, without steatosis or any other alteration. The morphological evaluation of the carotid arteries showed structural differentiation between control and treated groups.

In both groups we did not observe inflammatory infiltrate in the wall of the arteries, nor the formation of atherosclerotic plaques. However, we observed a slight increase in the amount of elastic fibers that form the wall of the carotid arteries of SHR and SHR-sp control group rats as compared with normotensive Wistar rats (Figures 3 and 4).

DISCUSSION

Hepatotoxicity due to chronic administration of vitamin A in supraphysiological doses causing hepatomegaly (hypertrophy and hyperplasia), portal and periportal fibrosis was reported by Milksad *et al.*, who found portal hypertension in one case, even after five years of interruption of supplemental vitamin A [24]. Still on the overuse of this vitamin, Seiferty *et al.* reported respiratory symptoms caused by hepatic hydrothorax, which suggests the possibility of other clinical manifestations [25].

However, our work has shown that even with the use of supraphysiological doses there were no side effects. The study of the hepatic parenchyma, in order to verify potential development of fatty infiltration or fibrous tissue, rendered no evidence of any change or steatosis in treated rats. Macroscopic analysis of the liver of animals also showed no changes, confirming previous results from our laboratory [26], since the average weight of the organ and the average liver / body weight ratio and body weight were within the normal range, ie, 4g to 100g body weight [27].

A recent study on β-carotene kinetic suggests that its toxicity should be greatly reduced, since high doses are required to maintain the nutritional status of vitamin A, and it has even been attributed to it a hepatoprotective effect in animals with liver fibrosis [25]. In addition, administration of supraphysiological doses of β-carotene did not cause alterations of general biological parameters, thus ruling out the occurrence of possible interaction between fat-soluble vitamins.

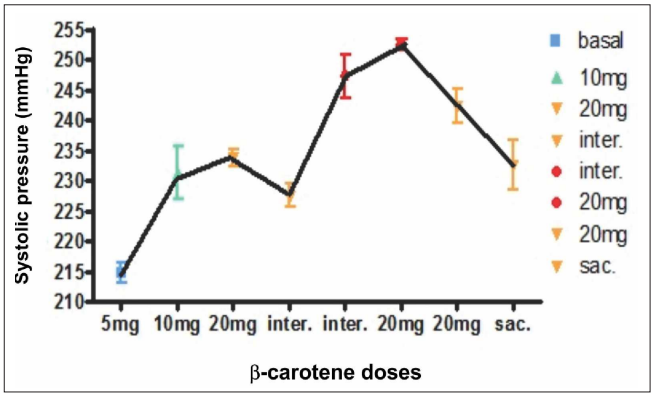

Figure 1 - *Systolic pressure of SHR-sp rats (n = 12). Significance: p <0.05.*

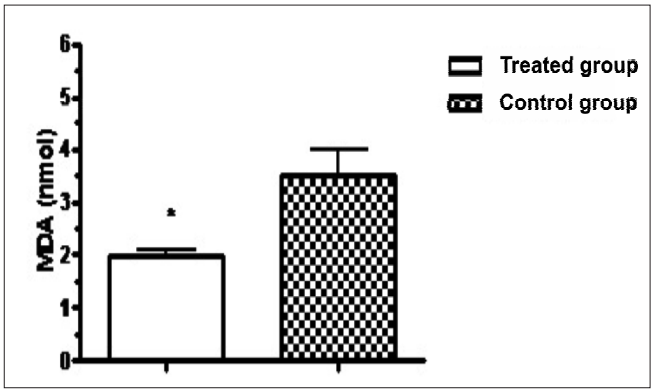

Figure 2 - *Serum levels of Malondialdehyde of SHR-sp rats.*

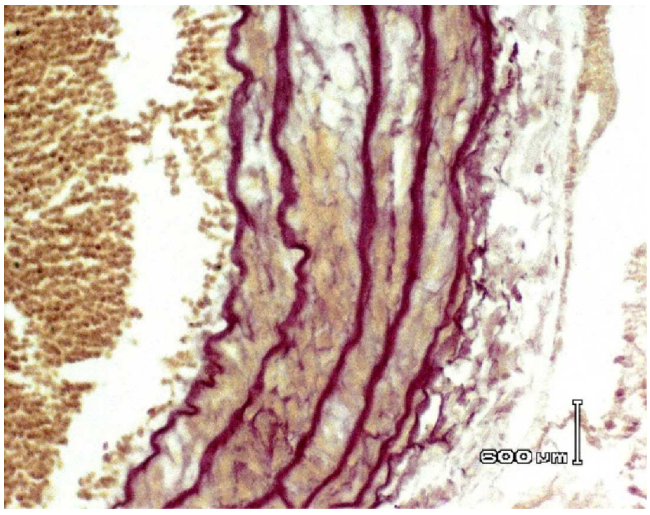

Figure 3 - *Wall of the carotid artery with five layers of elastic fibers, without abnormalities (Wistar); Orcein, 200x.*

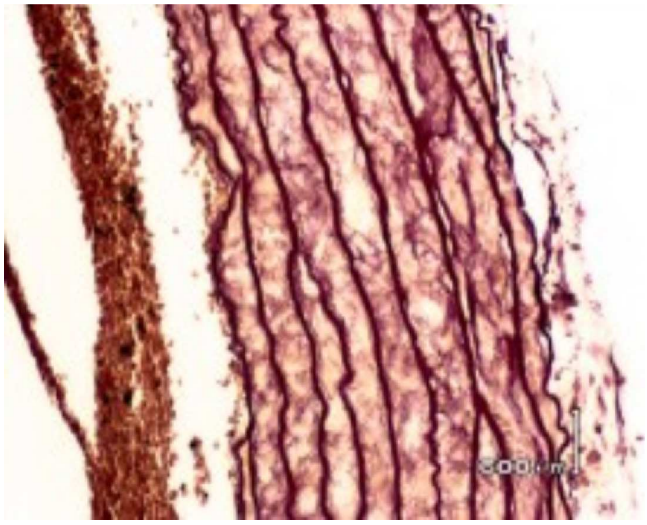

Figure 4 - *Wall of the carotid artery with nine layers of elastic fibers: elastosis (SHR-sp); Orcein, 200x.*

Regarding the modulating effect of β-carotene on blood pressure, the results presented here show a significant hypotensive effect of β-carotene supplementation with doses of 20 mg / day for three weeks in the rats of SHR-sp strain, confirming a previous study described by Oliveira Vianna, in 2004 [26], with SHR rats, which also showed a positive response of β-carotene hypotensive effect, though with doses of 5 mg, which shows greater resistance to treatment by SHR-sp strain. The evaluation of β-carotene kinetics showed a pattern similar to that observed with other fat-soluble vitamins [21,22].

The mechanism of action associated with the hypotensive effect of β-carotene is linked to its antioxidant activity, since there were significantly reduced serum levels of malondialdehyde. Studies in which oxidative stress was experimentally induced indicated increased superoxide radical (O_2^-), hydroxyl radical (OH^-) and HBP. On the other hand, treatment with antioxidants decreased blood pressure and the high level of bioavailable nitric oxide. These results provide strong evidence of the role of reactive oxygen species (ROS) both initially and in maintaining HBP [28] and point to the important role of antioxidant nutrients in controlling blood pressure [29,30].

Histological analysis of carotids in control and treated groups did not reveal formation of atherosclerotic plaque or inflammatory infiltrate, contradicting the proposed idea that the SHR-sp strain, by presenting with hypertension, would had inflammatory morphological changes in the vascular walls. On the other hand, it confirms the absence of atherosclerotic lesions in this lineage, corroborating the findings of Ogata *et al.* [31] and Kritchevskt *et al.* [32].

However, morphometrically, we observed that the number of elastic fibers constituting the wall of the carotid vessels in SHR and SHR-sp strains showed a slight increase, featuring elastosis of the muscular layer as compared with normotensive strain.

Although treatment with β-carotene did result in a change in systolic blood pressure, it was not able to prevent elastosis in the carotid arteries of spontaneously hypertensive rats. We believe that the hypotensive effect of β-carotene is positively associated with its antioxidant action and we do not rule out the possibility that intensified treatment can prevent the morphological changes observed in arteries of hypertensive strains.

RESUMO

Objetivo: *investigar o efeito da administração suprafisiológica de bcaroteno sobre parâmetros biológicos, laboratoriais e histológicos dos ratos espontaneamente hipertensos com tendência ao acidente vascular encefálico (SHR-sp).* ***Métodos:*** *utilizaram-se 36 ratos machos, distribuídos em três grupos, contendo cada um dos 12 ratos das linhagens* Wistar, *SHR e SHR-sp, subdivididos em seis animais controle e seis animais tratados com doses suprafisiológicas de âcaroteno por dois períodos de dez semanas, intercalados por uma semana de interrupção. No experimento foram avaliados diariamente o exame físico e a pressão arterial. Foi coletado sangue para dosagem sérica de malondialdeído; o fígado e as artérias carótidas para exame histológico.* ***Resultados:*** *alteração provisória na coloração dos pelos, diminuição significativa (p<0,0001) da pressão arterial (suplementação de 20mg de bcaroteno) e dos níveis séricos de malondialdeído (p<0,05) e aumento da quantidade de fibras elásticas na parede carotídea dos ratos SHR e SHR-sp.* ***Conclusão:*** *A suplementação suprafisiológica de bcaroteno não causou efeitos tóxicos, apresentou resposta positiva na modulação da pressão arterial e diminuição na concentração sérica de malondialdeído. Não foram encontradas alterações morfológicas significativas nos grupos estudados, exceto um aumento no número de fibras elásticas da camada muscular carotídea sugerindo elastose nos ratos SHR e SHR-sp.*

Descritores*: Estresse oxidativo. Malondialdeído. Betacaroteno. Ratos.*

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