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Alpha-tocopherol supplementation favorable effects on blood pressure, blood viscosity and cardiac remodeling of spontaneously hypertensive rats

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Abstract

Spontaneously hypertensive rats (SHR) were separated into two groups ($n=6$ per group) and, since 5 months old, received alpha-tocopherol (alpha-tocopherol acetate120 IU) or vehicle by daily gavage for 2 weeks. Blood viscosity, blood pressure (BP) and myocardial remodeling were analyzed. The SHRs treated with alpha-tocopherol showed a significant reduction of BP and a major reduction of blood viscosity in comparison with the control SHRs. The cardiac hypertrophy indices showed some differences when the two SHR groups were compared, the LV mass index was not different between the groups; however, the cardiomyocyte size was more than 20% smaller in SHRs treated with alpha-tocopherol than in control SHRs ($P<.05$). The intramyocardial vessels distribution was more than 45% greater in alpha-tocopherol-treated SHRs than in control rats, significantly improving the vessels-to-myocytes ratio in treated SHRs than in control SHRs ($P<.05$). In conclusion, present findings strongly suggest a beneficial effect of alpha-tocopherol supplementation to genetically hypertensive rats. This was observed by a reduction of both blood viscosity and BP, and a consequent cardiomyocyte hypertrophy in treated SHRs; an improvement of vessels-to-myocytes ratio in these rats was also observed.

Keywords: Alpha-tocopherol; Blood pressure; Blood viscosity; Myocardium remodeling; Stereology; Rat

1. Introduction

Primary or idiopathic hypertension is the most common cause of cardiovascular and cerebrovascular complications in humans [1]. Despite the clear benefits of antihypertensive drug therapy, many antihypertensive agents are still expensive and can produce unwanted side effects [2]. Therefore, no pharmacological efforts, including dietary interventions, to lower elevated blood pressure (BP) are welcome. These interventions, if found effective, may be beneficial as a definitive or adjunctive therapy for mild-to-moderate uncomplicated hypertension due to their safety and considerable economic savings [3].

Some epidemiological studies have shown an association between high dietary intake and high serum concentrations of vitamin E and lower rates of ischemic heart disease [4–7]. The Cambridge Heart Antioxidant Study (CHAOS) reported strong protection by high vitamin E doses against the risk of fatal and nonfatal myocardial infarction [8]. A possible alpha-tocopherol action mechanism is to protect low-density lipoproteins from oxidation by peroxy radicals [9,10].

Cell culture studies have shown that alpha-tocopherol causes inhibition of smooth muscle cell proliferation. This event takes place via inhibition of protein kinase C activity. Alpha-tocopherol also inhibits low-density lipoprotein-stimulated smooth muscle cell proliferation and protein kinase C activity [11].

Vitamin E is a powerful antioxidant able to prevent free radical-induced oxidations in biological membranes. Oxidative stress is known to affect cardiovascular function, increase sympathetic nervous activity [12] and enhance atherosclerosis primarily via endothelial cell dysfunction [13]. Altered nicotinamide dinucleotide phosphate activity, for example, after angiotensin II, has been shown to be a crucial initial step in the adaptive remodeling of vascular

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structures [14]. Therefore, the administration of vitamin E seems to be associated with the inhibition of lipid peroxidation, LDL lipoprotein and apoprotein as well. Epidemiological study demonstrated an inverse association between vitamin E intake and coronary disease that became significant with high levels of vitamin E intake reached with supplementation [4]. Furthermore, vitamin E seemed to normalize nitric oxide (NO) bioactivity in most animal studies [15].

The tocopherol-treated platelets presented a significant decrease of platelet aggregation induced by arachidonic acid, phorbol ester or adenosine 5-diphosphate, and such event would be independent of the antioxidant properties of vitamin E [16]. Nevertheless, such findings are very interesting, once platelets hyperactivity, oxidized LDL and lower NO activity are considered risk factors for cardiovascular diseases. In spite of those evidences, vitamin E effect on BP is not clear yet, as there are a number of controversies [17,18], including the alpha and gamma tocopherol differences; gamma-tocopherol shows anti-inflammatory activities in vivo [19,20].

There are some earlier works on the treatment of spontaneously hypertensive rats (SHR) with vitamin E. Those studies showed that antioxidant alpha-tocopherol treatment might increase the NO synthase activity and concomitantly reduce the BP [21] and attenuate adverse renal vascular changes in SHR [22]. Vitamin E has been shown to suppress the elevation in BP and urinary 8-hydroxy-2-deoxyguanosine in stroke-prone SHR, indicating that chronic ingestion of vitamin E produces a significant decrease in BP, lowers the oxidative stress and attenuates the thrombotic tendency [23].

The antihypertensive treatment target is not only BP reduction but also attenuation/prevention of the bad myocardial remodeling that occurs with chronic hypertension characterized by cardiomyocyte hypertrophy, reactive interstitial and perivascular fibrosis, as well as reduction of the intramyocardial artery-to-myocyte ratio [24,25]. Therefore, the present study aim was to investigate the alpha-tocopherol supplementation effect, a well-known antioxidant, on BP, plasma viscosity and myocardial structure of SHR.

2. Material and methods

2.1. Animals

Twelve spontaneously hypertensive male rats obtained from colonies maintained by our laboratory were studied. Rats aged 20 weeks and weighed 200–230 g were maintained in metabolic cages in a temperature (21±2 °C) and humidity-controlled (60±10%) room submitted to a 12-h dark/light cycle (artificial lights, 7:00 a.m.–7:00 p.m.) and air exhaustion cycle (15 min/h). All procedures were carried out in accordance with the conventional guidelines for experimentation with animals (NIH Publication No. 85-23, revised 1996). The experimental protocols used in this study were approved by the Ethics Committee for Animal Experimentation at the Federal University of Rio de Janeiro State.

2.2. Methods

After an adaptation period of 2 weeks, the animals were divided into two groups: control ($n=6$) receiving only the vehicle, and treated ($n=6$) receiving supplementation with a 120-IU dose of alpha-tocopherol acetate (500 UI/kg of body weight) (T-3376 Sigma, St. Louis, MO), dissolved in 0.35 ml of coconut oil. Animals daily received the vehicle or the alpha-tocopherol via a stomach tube (gavage) for a period of 2 weeks. During the study, all rats were fed with standard rat chow (Nuvilab, Nuvital, Brazil) ad libitum in addition to the vehicle or alpha-tocopherol supplement, and had free access to water. Standard rat chow represents 30.0 mg/kg of diet (or 44.7 UI/kg of diet). The average intake of vitamin E in European populations varies between 10 and 30 mg/day [26]. In different trials vitamin E supplementation amounted to 50 [8] and 270–540 mg/day [8,27,28]. It means that the dose used in most supplementation trials was at least 10 times higher than the average intake [29]. The diet and water intake was monitored daily, as well as the diuresis and rat health conditions.

The systolic BP was verified twice a week using the noninvasive method of the tail-cuff plethysmography in conscious rats (Letica LE 5100, Panlab). All animals were weighed weekly throughout the experiment period. Blood collection was done by heart puncture under pentobarbital anesthesia, and blood viscosity was determined by plate viscometer and the results shown as millipascal.

At the end of the experiment, the animals were deeply anaesthetized (intraperitonial Thiopental) and 3 ml of 10% KCl was injected into the left ventricle until diastolic cardiac arrest. The hearts were taken out by excising the vessels at the base, immediately above the aortic and pulmonary valves. The atria were separated from the ventricles and the right ventricle was separated from the left ventricle (including the interventricular septum). The volume of all these cardiac parts was determined according to the submersion method of Scherle [30], in which the water displacement due to the organ volume is recorded by weighing. The left ventricle mass (LV)/body mass (BM) ratio was determined as LV mass index=LV (mg)/BM (g).

2.3. Stereology

Fragments of the left ventricular myocardium were obtained through the orientator method [31] then fixed for

Table 1
Biological parameters in control and alpha-tocopherol-treated SHRs (mean±SD)

Groups	Daily			Final body mass (g)
	Water intake (ml)	Food intake (g)	Diuresis (ml)	
Control	30.7±2.5	40.5±2.8	6.2±0.8	208.5±4.6
α-Tocopherol	32.5±2.3	41.8±2.18	6.3±0.9	210.4±1.63

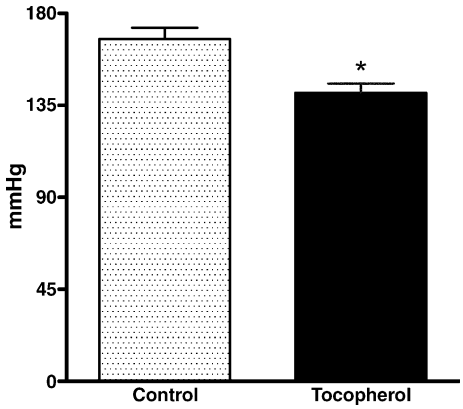

Fig. 1. Systolic blood pressure (mean±SD) after 2 weeks of experiment (*P<.05).

48 h in phosphate buffered 4% formaldehyde pH 7.2 [32], embedded in paraplast plus (Sigma) and sectioned 3 μm in thickness. The sections were stained with Masson and picro Sirius red trichrome methods.

The left ventricle myocardium was analyzed considering the cardiomyocyte and the cardiac interstitium (composed of blood vessels and connective tissue with nerves). Several myocardial fragments per animal (up to 10 fragments) were embedded together in a block. From each block, several nonserial slices were cut and five microscopic fields were randomly analyzed blindly moving the stage of the microscope. The stereological parameters were analyzed only considering well-preserved structures and not crossing the forbidden line [33–35].

The analysis used video microscopy (Leica model DMRBE microscope, Kappa CF 15/5 video camera, Sony trinitron monitor), and a test system with 42 test points. The reference volume was estimated by point counting using the test points that hit the global myocardium (P_T). The number of points hitting the cardiomyocytes and the cardiac interstitium including vessels (Pp) was counted to estimate the volume densities of such structures ($Vv:=Pp/P_T$) (:= is used to indicate it is an estimate) [36]. A test area was constructed upon the monitor and calibrated with a Leitz micrometer 1 mm/100. The number of structures counted in a two-dimensional 2800 μm^2 test frame area (Q_A) was performed with cardiomyocytes (cm) and intramyocardial arteries (art) to determine its mean cross-sectional area: $A[structure]:=Vv[structure]/2Q_A[structure]$ μm^2. The vessels-to-myocytes ratio was determined as Vv[art]/Vv[cm] and the intramyocardial arteries length density ($Lv[art]:=2Q_A[art]$) was also estimated [33].

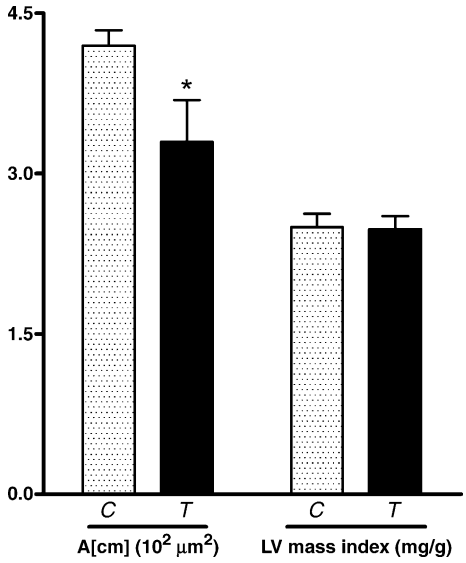

Fig. 3. Heart hypertrophic indices measured after 2 weeks of treatment (mean±SD) (*P<.05). A (cm) is the cardiomyocyte mean cross-sectional area, C is control group, LV is left ventricle, T is alpha-tocopherol-treated group.

2.4. Data analysis

The differences in biometric parameters were tested with the unpaired t-test with the Welch correction. Differences in stereology were tested with the nonparametric

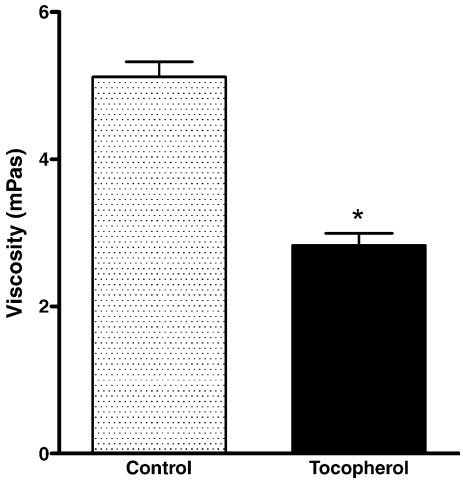

Fig. 2. Blood viscosity measured after 2 weeks of treatment (mean±SD) (*P<.05).

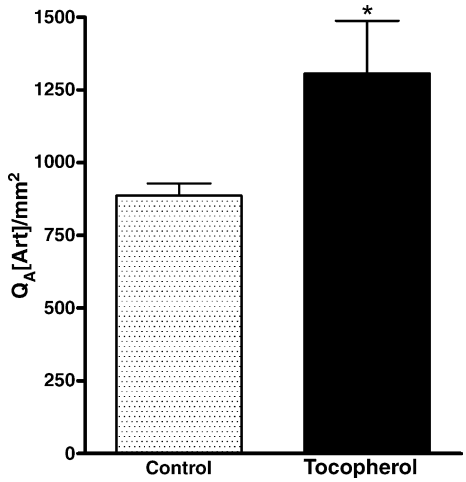

Fig. 4. Intramyocardial arteries density per area (mean±SD) after 2 weeks of treatment (*P<.05).

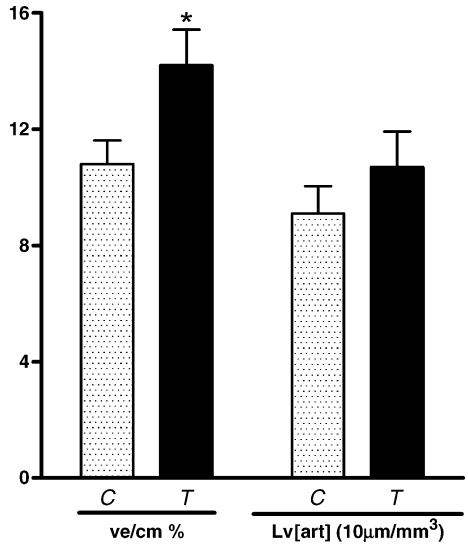

Fig. 5. Myocardial vascularization indices measured after 2 weeks of treatment (mean±SD) (*P<.05). C is control group, Lv[art] is myocardial arteries length density, T is alpha-tocopherol-treated group, ve/cm is the vessels-to-myocytes ratio.

Mann–Whitney U test. In all cases P<.05 was considered significant [37]. All analyses were performed using GraphPad Prism version 4.01 for Windows (GraphPad Software, San Diego, CA).

3. Results

At the end of the 2-week experiment, body mass, water and food intake, and diuresis values were compared in the two groups, and there were no alterations of other biological parameters investigated (Table 1).

The SHRs treated with alpha-tocopherol showed a significant reduction of BP (Fig. 1; around a 15% reduction) and a major reduction of blood viscosity (Fig. 2; more than 45% reduction) in comparison with the control SHR group.

The cardiac hypertrophy indices, the LV mass index and the cardiomyocyte size (A[cm]) showed some differences between the groups (Fig. 3) also. While A[cm] was significantly smaller in SHRs treated with alpha-tocopherol (reduction of more than 20% in the cardiomyocyte size), LV mass index was not different between control SHRs and SHRs treated with alpha-tocopherol.

The intramyocardial arteries distribution was more than 45% greater in alpha-tocopherol-treated SHRs than in control SHRs (Fig. 4), which improved the vessels-to-myocytes ratio by more than 30% in treated SHRs, but did not alter these vessels length density (Fig. 5).

4. Discussion

The SHR are by far the most widely used rat model for hypertension, although they only reflect a rare subtype of human hypertension, i.e., primary hypertension that is inherited in a Mendelian fashion. The BP rises around 5–6 weeks of age and steadily increases to reach a systolic BP of 180–200 mm Hg, and these animals develop many hypertensive end-organ damage characteristics, as cardiac hypertrophy, cardiac failure and renal dysfunction [38]. Therefore, the SHR presented chronic hypertension progressing to heart failure during the last 6 months of their life span of about 2 years [39]. As hypertrophy in humans is usually associated with chronic hypertension, the SHR are a realistic model of human hypertrophy [40].

In this study, the genetic hypertensive lesions normally observed in SHRs were altered after 2 weeks of treatment with alpha-tocopherol. Besides the BP and the blood viscosity reduction due to the treatment, the myocardial structure showed better indices in the treated animals than in the control ones. The major beneficial effects were observed in the cardiomyocyte hypertrophy and in the myocardial vascularization.

The responsiveness to alpha-tocopherol treatment focuses on two main points: one is related to its hypotensive action that is not always observed. Such controversy has been attributed either to the methodological differences regarding the vitamin E form used, via the administration, dosage, and even time of supplementation, or due to the heterogeneity among the animal models [17,18].

In the present assay, the alpha-tocopherol treatment provoked a hypotensive effect in genetically hypertensive rats. The normotensive rat strains seem to present more efficient compensatory mechanisms which might explain the absence of a response to the dietetic maneuvers usually reported [41,42]. The other point of concern refers to the possibility of side effects resulting from the administration of supra physiological doses. An interaction between vitamin E and retinal has been reported [43], and a pro-oxidant action as well [44]. However, the present assay demonstrated that the hypotensive effect was markedly accentuated with a 120-IU dose.

Additionally, findings showed a significant decrease of SHR blood viscosity after treatment. Increased resistance to blood flow is a haemodynamic characteristic of hypertension. This resistance is a function of the vessels geometric aspect and its rheological blood properties. So, an occurrence of high blood viscosity in cardiovascular diseases has been previously demonstrated [45,46]; and a positive correlation between viscosity and BP has been observed in hypertensive patients [47]. Additionally, increased red blood cell aggregation and blood viscosity have also been well documented in early stages (≅3 weeks age) of hypertension of SHRs [48]. In fact, SHR presents abnormalities on calcium binding and a compromised cellular membrane of erythrocytes fluidity which might result on modifications of cellular membrane physical state and its mechanical properties provoking a decrease of the membrane elasticity and an increase of the resistance to blood flow [49].

The endothelium plays an important role in the maintenance of vascular homeostasis. Central to this role is the

endothelial NO production, synthesized by the constitutively expressed endothelial isoform of NO synthase. Vascular diseases, including hypertension, diabetes, and atherosclerosis, are characterized by impaired endothelium-derived NO bioactivity that may contribute to clinical cardiovascular events. Growing evidence indicates that impaired endothelium-derived NO bioactivity is partly due to an excess vascular oxidative stress [50]. Concerning the hypotensive effect, vitamin E improved the activity of the endothelium-derived NO, and more specifically, the alpha-tocopherol appears to enhance platelet NO release both in vitro and in vivo through antioxidant and protein kinase C-dependent mechanisms [50,51], and this effect was not dependent upon the antioxidant protection of LDL. In fact, vitamin E improved endothelial function in part due to the inhibition of protein kinase C stimulation. This vitamin E activity was examined in platelets, and vitamin E partly inhibited platelet aggregation through a mechanism that involves protein kinase C [52–54].

This study did not detect any myocardial alteration in the interstitial or perivascular connective tissue content in alpha-tocopherol-treated SHRs. Cardioreparation implies the regression of myocyte hypertrophy and myocardial fibrosis. Myocyte hypertrophy is primarily a response to chronic pressure or volume overload of the ventricles, whereas myocardial fibrosis depends on the activation of circulating and tissue renin–angiotensin–aldosterone systems (RAAS) [55].

SHRs develop cardiac and renal lesions during their lives. Antihypertensive treatment only attenuates end-organ damage in case it decreases BP. Moreover, an effective antihypertensive sometimes even attenuates end-organ damage in nonhypotensive doses. On the other hand, some agents do decrease BP, but do not prevent end-organ damage (e.g., hydralazine in SHR) [38]. Our group previous studies demonstrated the RAAS enhancement in SHRs [25,56].

Present findings demonstrated the cardiomyocyte response to the BP attenuation in SHRs supplemented with alpha-tocopherol during 2 weeks (reduction of the cardiomyocyte hypertrophy); however, it did not apparently alter RAAS in SHRs in a significant way and, consequently, did not alter the myocardial connective tissue in treated SHRs in comparison with the control ones.

The intramyocardial arteries stereological indices were higher in the alpha-tocopherol group, which supports the findings about the maintenance of the myocardial blood vessels of SHRs fed with alpha-tocopherol. Treatment with vitamin E prevented cardiomyocyte/capillary mismatch in the left ventricle of rats with chronic renal failure [24]. In an experimental study on ischemia-reperfusion injury in rats using the Langendorf preparation, functional recovery of heart rate, left ventricular diastolic pressure and coronary flow were significantly improved by alpha-tocopherol [57]. Those characteristics altogether might explain the benefit of vitamin E supplementation on systolic BP, blood rheology and the density per area of the vessels reported here.

In conclusion, present findings strongly suggest a beneficial effect of alpha-tocopherol supplementation to genetically hypertensive rats. This was observed by the reduction of both blood viscosity and BP, and the consequent cardiomyocyte hypertrophy in treated SHRs, as well as by the microvessels-to-myocytes ratio improvement in these rats.

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