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Short communication

Enalapril does not prevent the myocardial ischemia caused by the chronic inhibition of nitric oxide synthesis

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Abstract

In rats, chronic administration of the nitric oxide (NO) inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME) causes arterial hypertension, cardiac hypertrophy and myocardial ischemic alterations such as necrosis and fibrosis. In this study, we evaluated the effect of 8 weeks of treatment with enalapril maleate on cardiac weight and on the development of the histological alterations induced by L-NAME. Enalapril significantly inhibited the development of both arterial hypertension (117.2 ± 5.8, 161.8 ± 8.8 and 122.0 ± 10.6 mm Hg, for control, L-NAME- and L-NAME + enalapril-treated animals, respectively) and left ventricular hypertrophy (1.36 ± 0.13, 1.60 ± 0.04 and 1.48 ± 0.05 mg/g, for control, L-NAME- and L-NAME + enalapril-treated animals, respectively), but had no effect on the myocardial lesions. These findings demonstrate that although the renin-angiotensin system plays a major role in the development of arterial hypertension and cardiac hypertrophy, it does not modulate the ischemia-induced myocardial alterations observed in this model.

Keywords: Nitric oxide (NO); Hypertension; Enalapril; Renin-angiotensin system; Myocardial ischemia

1. Introduction

The chronic administration of the nitric oxide (NO) inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME), Moore et al., 1989) to rats causes arterial hypertension accompanied by functional alterations and structural lesions in the kidneys (Baylis et al., 1992; Ribeiro et al., 1992) and heart (Moreno et al., 1994). Since inhibition of the renin-angiotensin system prevents the alterations induced by L-NAME in the kidney (Ribeiro et al., 1992), we have evaluated wheter the renin angiotensin system is also involved in the L-NAME-induced cardiac alterations in rats.

2. Material and methods

2.1. Experimental design

Male Wistar rats (150–200 g at the start of the study) provided by CEMIB-UNICAMP were divided into the following groups: (1) control ($n = 10$), rats that received tap water alone; (2) L-NAME ($n = 8$), rats that received L-NAME (20 mg/kg/day; Ribeiro et al., 1992; Moreno et al., 1994); (3) enalapril ($n = 9$), rats that received enalapril maleate (25 mg/kg/day; Childs et al., 1990); (4) L-NAME + enalapril ($n = 9$), rats that received both L-NAME and enalapril maleate (20 and 25 mg/kg/day, respectively). Both L-NAME and enalapril maleate were dissolved in the drinking water. The animals were killed after 8 weeks of treatment.

2.2. Blood pressure measurements

Mean arterial pressure was measured twice a week by a tail-cuff method (Zatz, 1990) and the mean of

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these two determinations was considered to be the mean for that week. The same procedure was applied to the weight gain.

2.3. Cardiac weight indexes and histological analysis

The rats were killed with ether and the heart was dissected out and washed with saline. The atria were then removed and the ventricles weighed in order to obtain the heart weight. The left ventricular weight was determined by excising the right ventricle and weighing the remaining tissue. The heart weight index and the left ventricular weight index were calcuted by dividing the heart weight and the left ventricular weight by the body weight. After fixation, in 10% formalin for 24 h, the left ventricle and the septum were cut into five equidistant rings perpendicular to the long axis of the ventricle. The rings were then embedded in paraffin, and 5 μm sections were stained with hematoxylin-eosin. From each rat, one section of each of the five ventricular rings was studied by light microscopy. The histological analysis had been done as a blind study. For semiquantitative analysis, we adopted the following classification (Factor et al., 1981: (+) grade one: one or two foci of clear-cut previous cell loss replaced by granulation tissue or a scar less than 500 μm in its greatest diameter; (++) grade two: more than two foci of clear-cut previous myocardial cell loss replaced by granulation tissue or scars, all less than 500 μm in their greatest diameter. There must be a broad rim of viable myocardium between individual foci; (+++) grade three: confluent foci without a continuous rim of myocardium separating them, thus creating larger areas of previous cell (> 500 μm in their largest diameter) replaced by granulation or scar tissue with only rare islands of very few surviving cardiomyocytes in it. We did not include myocardial fibrosis, which is defined as an increase of interstitial collagen, without apparent loss of cardiomycytes, since we were only interested in lesions caused by cell loss.

2.4. Drugs

N^{ω}-Nitro-L-arginine methyl ester (L-NAME) was purchased from Sigma (USA). Enalapril maleate was provided by Biosintética (Brazil).

2.5. Statistical analysis

Results are expressed as the mean ± S.E.M. Analysis of variance (ANOVA) was applied to repeated measurements in order to assess the differences in body weight and tail-cuff pressure. When the ANOVA results were significant, Duncan's test was applied to determine the level of significance and a P value < 0.05 was considered to be significant. For comparison of the myocardial damage in the different study groups, the Kruskall-Wallis and Dunn tests (Primer of Statistics, from software Stanton Glanz) were used.

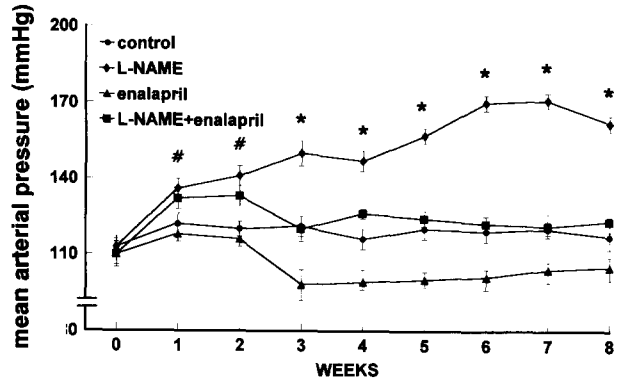

Fig. 1. Mean arterial pressure (mm Hg) during the 8 weeks of study. (●) control ($n = 15$), (♦) L-NAME ($n = 8$), (▲) enalapril ($n = 9$), (■) L-NAME + enalapril ($n = 9$). Results are expressed as mean ± S.E.M. $^{*}P < 0.05$ (ANOVA); Duncan test: L-NAME > control = enalapril = L-NAME + enalapril; $^{\#}P < 0.05$ (ANOVA); Duncan test: L-NAME = L-NAME + enalapril > control = enalapril.

3. Results

3.1. Body weight and tail-cuff pressure

The body weight of control, L-NAME, enalapril and L-NAME + enalapril rats did not differ significantly from each other. L-NAME treatment induced a time-dependent increase in the blood pressure up to the sixth week of treatment after which the levels stabilized (Fig. 1). Enalapril prevented the hypertension in the L-NAME + enalapril rats after the second week of treatment (Fig. 1). Enalapril alone significantly reduced the blood pressure after the second week of treatment ($P < 0.05$; Fig. 1).

3.2. Cardiac weights

Chronic treatment with L-NAME significantly elevated the heart weight index (1.90 ± 0.18 and 2.12 ± 0.05 mg/g for control and L-NAME-treated animals, respectively; $P < 0.05$). This increase was abolished by enalapril (1.84 ± 0.06 mg/g; $P < 0.05$). Similarly, L-NAME caused a significant increase in the left ventricular weight index (1.36 ± 0.13 and 1.60 ± 0.04 mg/g, for control and L-NAME-treated animals, respectively; $P < 0.05$) which was also prevented by enalapril (1.48 ± 0.05 mg/g; $P < 0.05$). In the animals receiving enalapril alone, there were no significant alterations in the heart weight index (1.89 ± 0.07 mg/g) and left ventricular weight index (1.50 ± 0.08 mg/g) when compared with the control group.

Table 1
Myocardial alterations at the end of the study (week 8)

Groups	Myocardial alterations			
	(−)	(+)	(++)	(+++)
Control ($n = 15$)	15	0	0	0
L-NAME ($n = 8$)	4	2	0	2
Enalapril ($n = 9$)	9	0	0	0
L-NAME + enalapril ($n = 9$)	5	2	0	2

Alterations: (−) none; (+) grade one; (++) grade two; (+++) grade three. Kruskall-Wallis: $P < 0.05$; $H = 8.920$. Dunn test: control = enalapril ≠ L-NAME = L-NAME + enalapril.

3.3. Histological alterations

In both the control and enalapril groups, no heart was classified as positive. L-NAME-treated animals developed myocardial alterations which were not prevented by enalapril (Table 1). The lesions observed consisted mainly of areas of dense interstitial and replacement fibrosis consistent with organized myocytolytic necrosis. Fresh myocardial necrosis was also encountered. Subendocardial infarcts were common, sometimes occupying up to 80% of the circumference of the left ventricle. In the L-NAME-treated rats, thickened arterial walls with perivascular fibrosis could be found (Moreno et al., 1994).

4. Discussion

Our results clearly demonstrate that, while enalapril presented the development of both arterial hypertension and left ventricular hypertrophy, it had no effect on the myocardial alterations resulting from ischemia.

Angiotensin-converting enzyme inhibitors reduce cardiac mass when used as short-term therapy for clinical and experimental hypertension (Dunn et al., 1984). Our results on cardiac hypertrophy support previous observations showing that both enalapril (Nemoto et al., 1994; Ueki et al., 1994) and the angiotensin II receptor blocker losartan (Jover et al., 1993) prevent the left ventricular hypertrophy induced by the chronic inhibition of NO synthesis.

Previous studies (Lund and Tomanek, 1978; Buttrick et al., 1986) have demonstrated that left ventricular hypertrophy due to arterial hypertension is associated with a limited coronary vasodilator reserve and that this situation is a predisposing factor for the development of myocardial ischemia. Interestingly, the activation of the renin angiotensin system leads to the development of left ventricular hypertrophy (Weber et al., 1991) and angiotensin II causes myocyte acute injury in rats (Tan et al., 1991). Although the above findings indicate a close relationship between renin angiotensin system activation and myocardial ischemia, the failure of enalapril to prevent the development of ischemic lesions indicates a clear dissociation between left ventricular hypertrophy and the ischemic process in this model of chronic NO synthesis blockade. Indeed, renovascular hypertensive rats are known to develop left ventricular hypertrophy, yet no ischemic lesions are observed in the hearts of these animals (Moreno et al., 1994), thus supporting the concept that the ischemic process may be unrelated to the development of cardiac hypertrophy (Brush et al., 1988).

Since as noted above arterial hypertension and left ventricular hypertrophy are not related to the development of myocardial ischemia, we propose that NO plays an essential role in the autoregulation of coronary blood flow by acting as vasodilator and inhibitor of platelet function. This model of chronic NO synthesis inhibition may therefore reflect clinical syndromes in which patients present with arterial hypertension and associated myocardial ischemia, but have no concomitant left ventricular hypertrophy.

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