

Structural Alterations in the Heart after Long-Term L-NAME and D-NAME Treatment

O PECHÁŇOVÁ¹, I BERNÁTOVÁ¹ AND P BABÁL²

¹ *Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic*

² *Department of Pathology, University of South Alabama, Mobile, USA*

Abstract. N^G-nitro-D-arginine methyl ester (D-NAME), considered as an inactive enantiomer of NAME, is generally used as a negative control for NO synthase inhibition with L-NAME. The aim of this work was to compare the effect of L-NAME (20 and 40 mg/kg/day), and D-NAME (40 mg/kg/day) on hemodynamic and structural parameters in the rat cardiovascular system. After 4 weeks of treatment, blood pressure and left ventricle weight/body weight ratio increased significantly in all studied groups versus control. Myocardial fibrosis (in %) represented 0.94 ± 0.04 in control, 4.70 ± 0.39 in L-NAME (20 mg/kg/day), 10.54 ± 0.91 in L-NAME (40 mg/kg/day) and 5.25 ± 0.46 in D-NAME (40 mg/kg/day) group. We conclude that in a long-term experiment D-NAME provokes similar changes in cardiovascular system like L-NAME.

Key words: D-NAME — L-NAME — Hypertension — Hypertrophy — Myocardial fibrosis

Introduction

The best known inhibitors of NO synthase are L-arginine analogues, while D-arginine analogues are considered as their inactive enantiomers. However, Wang *et al* (1991) showed that the D-enantiomer of N^G-hydroxy-arginine can inhibit NO synthesis when given in high doses. It is likely that N^G-nitro-D-arginine methyl ester (D-NAME) has similar properties, since D-NAME treatment increased arterial blood pressure during a long-term experiment (Turner *et al* 1997). Recently, development of hypertension associated with the elevation of left ventricle weight/body weight ratio (LVW/BW) and myocardial fibrosis enlargement have been shown after long-term N^G-nitro-L-arginine methyl ester (L-NAME) treatment (Moreno *et al* 1996, Babál *et al* 1997). The aim of this study was to compare the effect of 4 week-lasting L-NAME and D-NAME treatment on hemodynamic and structural parameters in the rat cardiovascular system.

Correspondence address: Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Sienkiewiczova 1, 813 71 Bratislava, Slovakia
E-mail: pechan@unpf.savba.sk

Materials and Methods

Male Wistar rats, 12 weeks old, were randomly divided into four groups. The first group served as control ($n = 8$). In the second group ($n = 8$), L-NAME (Sigma Chemical Co, Germany) was given in the dose of 20 mg/kg/day (L-NAME₂₀), in the third group ($n = 8$), L-NAME was given in the dose of 40 mg/kg/day (L-NAME₄₀), and the fourth group ($n = 12$) received D-NAME (Sigma Chemical Co, Germany and Biomol GmbH, USA) in the dose of 40 mg/kg/day. The substances were given in drinking water for four weeks. Systolic blood pressure (SBP) was measured by the non-invasive method of tail-cuff plethysmography every day. After 4 weeks rats were sacrificed, the body weight, heart weight and left ventricle weight were determined and the LVW/BW ratio was calculated. DNA concentration was analysed by spectrophotometry according to Sambrook et al (1989).

For histology, representative samples of myocardium were taken from the middle between the apex and sulcus coronarius. After a 24 hour fixation in 10% phosphate buffered formalin, specimens were routinely processed in paraffin. Serial 5 μ m thick sections were stained with hematoxylin and eosin and by Van Gieson's staining.

Morphometric evaluation was performed under an Olympus light microscope equipped with a two-dimensional image analyzer Alphamager 2000. Van Gieson's histochemical staining was applied to enhance the red color contrast of collagen. The fibrous tissue was expressed as % of the total measured area of the heart slice.

Statistical analysis. Results were expressed as mean \pm S.E.M. For the analysis, one-way ANOVA and Bonferroni test were used.

Results

After the fourth week of the experiment, SBP was 124 ± 3 mmHg in the control group. In the L-NAME₂₀ and L-NAME₄₀ group SBP increased significantly by 33% and 37%, respectively, vs control group. In the D-NAME group, SBP increased significantly by 26%. The LVW/BW ratio was 1.28 ± 0.03 in the control group. In the L-NAME₂₀ and L-NAME₄₀ group the ratio increased significantly by 14% and 27%, respectively, vs the control group. In the D-NAME group, LVW/BW ratio increased significantly by 13%.

Table 1. Effect of 4 weeks lasting L-NAME (20 and 40 mg/kg/day) and D-NAME (40 mg/kg/day) treatment on the systolic blood pressure (SBP), left ventricle weight/body weight ratio (LVW/BW), DNA concentration, and myocardial fibrosis. * $p < 0.05$ as compared to control, + $p < 0.05$ as compared to L-NAME (40 mg/kg/day) group.

	Control	L-NAME ₂₀		L-NAME ₄₀		D-NAME	
SBP (mm Hg)	124 ± 3	165 ± 5	*	170 ± 6	*	157 ± 6	*
LVW/BW (mg/g)	1.28 ± 0.03	1.46 ± 0.03	*	1.60 ± 0.02	*	1.44 ± 0.03	*
DNA concentrations (mg/g)	0.62 ± 0.03	0.76 ± 0.03	*	0.83 ± 0.04	*	0.74 ± 0.02	*
Myocardial fibrosis (%)	0.94 ± 0.04	4.70 ± 0.39	*+	10.54 ± 0.91	*	5.25 ± 0.46	*+

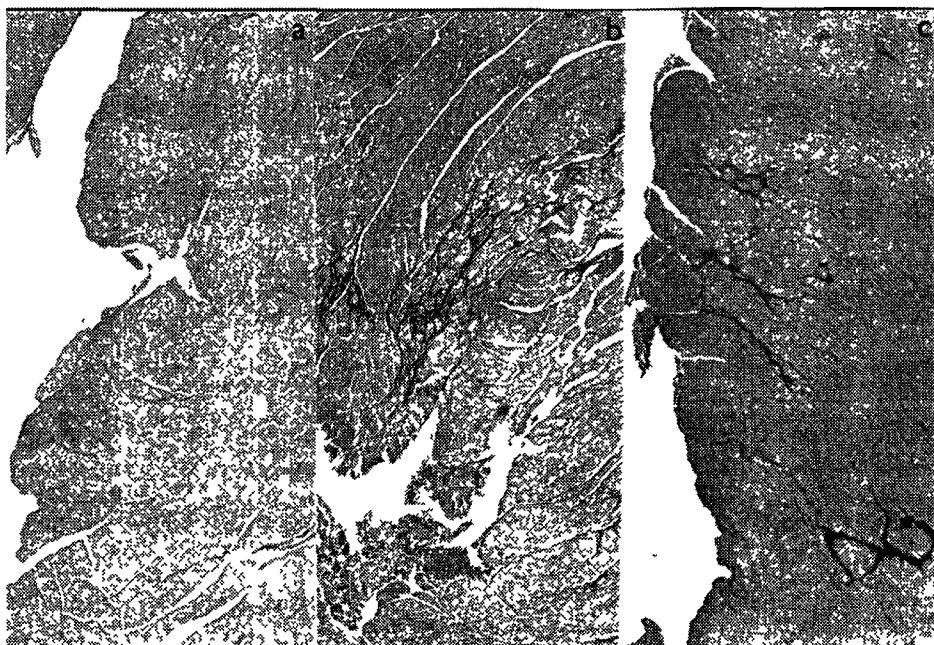


Figure 1. Subendocardial area of the left ventricle in the control heart (a) and focal myocardial fibrosis in L-NAME (40 mg/kg/day) (b) and D-NAME (40 mg/kg/day) (c) treated rats Van Gieson, 200 \times

There were no significant changes in SBP and LVW/BW ratio among the groups treated with 20 and 40 mg/kg/day of L-NAME or D-NAME (Tab 1)

DNA concentration was 0.62 ± 0.03 in the control group. In the L-NAME₂₀ and L-NAME₄₀ group the concentration increased significantly by 23% and 34%, respectively, vs control group. In the D-NAME group the concentration increased significantly by 20%. There were no significant changes in DNA concentration among the groups treated with 20 and 40 mg/kg/day of L-NAME or D-NAME (Tab 1)

Histological investigation revealed large areas of fibrosis in the myocardium of rats treated with L-NAME as well as D-NAME (Fig 1). Maximal changes were in the subendocardial location of the left ventricle, papillary muscles, and in the interventricular septum. Areas of acute necrosis with inflammatory cells accumulation in close vicinity immediately next to foci of fibrosis documented the ischemic changes in the myocardium of L-NAME and D-NAME treated animals.

Morphometry. Fibrous tissue represented $0.94 \pm 0.04\%$ of the myocardium area on slices from the control group. In the L-NAME₂₀ group, fibrous tissue area was elevated five times and in the L-NAME₄₀ group it was elevated eleven times vs control group. Fibrous tissue in D-NAME group was elevated approximately six times.

Discussion

The present study showed that in long-term experiment, besides the raise in blood pres-

sure, both L-NAME and D-NAME administration resulted in the development of LV hypertrophy, increase in DNA concentration and myocardial fibrosis enlargement. As L-NAME is easily dissolved, it became the most frequently used orally active NO synthase inhibitor, for the first time applied in Brattleboro rats by Gardiner *et al* (1990). In their long-term experiment, orally applied L-NAME (0.1 mg/ml) caused significant increase in blood pressure. D-NAME treatment (1 mg/ml) was reported to produce similar effect increasing arterial blood pressure in Sprague-Dawley rats when delivered in drinking water for a duration of 18 days (Turner *et al* 1997). Our experiment with 4 week-lasting D-NAME treatment confirms this result. Moreover, D-NAME-induced blood pressure increase was accompanied by LV hypertrophy and increase in DNA concentration and myocardial fibrosis. Our previous findings of increased RNA concentration and [^{14}C]leucine incorporation into proteins of the left ventricle in the long-term L-NAME treated rats provided direct biochemical evidence of increased proteosynthesis, typical for the period of developing hypertrophy (Babál *et al* 1997). As fibrocytes represent a substantial number of myocardial cells, increase of DNA concentration after L-NAME and D-NAME treatment may be ascribed to fibrotic tissue enlargement. Indeed, our morphometric investigation revealed significant enhancement of fibrotic tissue in the left ventricle. Moreover, the comparable elevation of cardiac fibrosis in L-NAME₂₀ and D-NAME group was shown. Structural changes consisted of extensive areas of cardiac fibrosis and necrosis after long-term L-NAME treatment was observed also by other authors (Moreno *et al* 1996). This is the first evidence that long-term D-NAME treatment, although weaker than L-NAME treatment, may provoke similar alterations in the cardiovascular system. Thus, the consideration of D-NAME as an inactive enantiomer of L-NAME is questionable.

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