Hydralazine reduces myocardial tissue damage in rats submitted to chronic inhibition of systemic nitric oxide synthesis during 4, 14 and 28 days

Recebido em 28/08/02
Aceito para publicação em 15/10/02

Hidralazina reduz lesões teciduais miocárdicas em ratos submetidos à inibição crônica da síntese sistêmica do óxido nítrico durante 4, 14 e 28 dias

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key words

Nitric oxide
L-Name
Arterial hypertension
Cardiac hypertrophy
Heart morphology
Cardiomyopathy
Arteriosclerosis

abstract

The aim of the present work was to develop a qualitative chronopathological study concerning abnormalities in myocardium, due to nitric oxide (NO) blockage. We used 60 Wistar normotensive young male rats from several breeds. Groups of rats were submitted to L-Name (L) via oral administration dissolved in water (750mg/l) during days 4, 14 and 28. Other groups were submitted concomitantly to L-Name and hydralazine hydrocloride (L + H) (120mg/l). On days 4 and 14 (L group) we have found myocardial abnormalities and lesions while in L + H we could not identify abnormalities. Considering L group on day 28, the myocardium presented characteristic fibrosis (reactive and reparative), vascular damage with increasing wall thickness due mainly to proliferation of the arterial smooth muscle cell. Total obliteration of vessels was noted only in this period. We also observed reactive fibrosis between muscle cells of the vascular wall and proliferation of cells in the intimal layer. In L + H (day 28), similar vascular abnormalities described for L group (less frequent and less apparent) were also observed. In L + H we did not identify total vascular obstructions. In L + H, infarct areas were not observed. Control groups did not present any abnormalities. Our results support the idea that, at least in some cases, hypertrophy vascular abnormalities and myocardial lesions in arterial hypertension can occur because of the reduction in organic nitric oxide production. Our results also suggested that these morbid processes can be postponed by the use of hydralazine which, however, does not avoid abnormalities after long-term experimental blockage of NO.

resumo

O alvo do presente trabalho foi desenvolver um estudo cronopatológico experimental referente a anormalidades miocárdicas com origem no bloqueio sistêmico da síntese do óxido nítrico. Utilizamos 60 ratos wistar machos jovens, oriundos de várias ninhadas. Grupos de ratos foram submetidos ao L-Name (L), por administração via oral, dissolvido em água (750mg/l), durante quatro, 14 e 28 dias. Outros grupos foram submetidos concomitantemente ao L-Name e ao hidrocloreto de hidralazina (L + H) (120mg/l). Aos quatro e 14 dias (grupo L) encontramos anormalidades miocárdicas e lesões. Contudo, nos grupos L + H, aos quatro e 14 dias, não pudemos encontrar quaisquer alterações. Considerando o grupo L aos 28 dias, o miocárdio apresentou fibrose característica (reativa e reparativa) e lesões vasculares com aumento da espessura da parede vascular, principalmente por razão da proliferação das células musculares lisas arteriais. Obliterações totais dos vasos sangüíneos foram notadas apenas neste período. Observamos ainda fibrose reativa entre as células musculares da parede vascular e proliferação das células da túnica íntima. No grupo L + H (28 dias), anormalidades vasculares similares àquelas descritas para o grupo L (menos freqüentes e menos aparentes) foram também observadas. Os grupos controle não apresentaram quaisquer anormalidades. Nossos resultados sustentam a idéia de que, ao menos em alguns casos, a hipertrofia, as anormalidades vasculares e as lesões miocárdicas na hipertensão arterial podem ocorrer por razão da redução orgânica da síntese do óxido nítrico. Nossos resultados também sugerem que estes processos mórbidos podem ser postergados pelo uso da hidralazina, a qual, contudo, não evita anormalidades miocárdicas após longa exposição experimental de bloqueio sistêmico da síntese do óxido nítrico.

unitermos

Óxido nítrico
L-Name
Hipertensão arterial
Hipertrofia cardíaca
Morfologia cardíaca
Cardiomiopatia
Arteriosclerose

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This work is part of the Ph.D. thesis of the main author (PCM/ICB/UFRJ) and also received honor mention in the XXIII Brazilian Congress of Pathology – 2000.

Introduction

The nitric oxide (NO) is an endothelium vasorelaxing factor and its deficit is involved in some human morbid processes. The L-Name – N^{ω}-nitro-arginine-methyl-ester – is an analog and antagonist of L-Arginine (substract of NO) and via oral or parenteral administration interrupts the NO synthesis which is necessary for maintenance of normal arterial pressure. That causes arterial hypertension, cardiac hypertrophy, vascular abnormalities and lesions in the myocardium (1-6). Nowadays the degree of direct influence of NO and the influence of its hypertension in the origin of lesions found in heart and vessels is disputed. Since the reduction of arterial hypertension in this model does not reduce significantly the cardiac and vascular abnormalities, these morbid processes are probably related mainly to NO deficit and not to hypertensive processes (1, 3-6). Moreno Jr. *et al.* (3, 5), using L-Name + enalapril, identified that enalapril avoided arterial hypertension and ventricular hypertrophy; however, did not avoid the development of myocardial lesions. Numaguchi *et al.* (1) showed that on day 56 of concomitant submission to L-Name and hydralazine, necrotic areas and reparative fibrosis in rats' myocardium occurred. Felix *et al.* (7), utilizing infused dosis of aldosterone and angiotensin II in rats, found similar reactive and reparative fibrosis which were also similar to the changes noted in our previous (6) work. However, the beginning of this process is not well-known. In other words, does addition of hydralazine delay the beginning of arterial and myocardian lesions or not?

The aim of this work is to develop a qualitative chronopathological study in rats' myocardium submitted to L-Name or L-Name + hydralazine during days 4, 14 and 28.

Material and methods

A total of 60 normotensive young adults Wistar rats from several breeds were used. Tail arterial pressures were obtained using hydraulic pletismography (6). Initially, the pressure of all animals was not above 119mmHg. After checking arterial pressure and weight, each animal was placed in individual plastic boxes. To perform nitric oxide blockage we utilized L-Name (hydrochloride of N^{ω}-nitro-L-arginine methyl ester, Sigma Chemical) via oral administration, concentration of 750mg/l in drinking water (about 60-80mg/Kg of rat's body weight a day (6, 8)). To prevent hypertension we utilized hydralazine hydrocloride (Sigma Chemical), concentration of 120mg/l (1) in drinking water solution (same bottle of L-Name). On days 4, 14 and 28, the rats' arterial pressure and weight were measured, they were anesthetized with ethylic ether and necropsies were performed. The hearts were weighed and fixed in formaldehyde 4% buffered pH 7.2-7.4, processed using routine methods, embedded in paraffin, sectioned in 4, 5 or 8μm and stained using: hematoxilin-eosin, Masson's trichrome, picro-sirius red polarization, Weigert's resorcinol fuchsin solution (with and without oxon). The treatment with Weigert's resorcinol fuchsin solution using oxon (10% in water solution during 45 min) is important because the oxon produces oxidation which reveals the oxytalan fibers in violet (9, 10). Large amounts of oxytalan fibers suggest increasing stiffness of tissue because these fibers are the less stretching of the elastic fibers. The picro-sirius red technique was performed following Dolber and Spach (11, 12).

Group 1 (4 days): 21 rats were used. Eight rats were submitted to L-Name (group 1L). Seven rats were submitted concomitantly to L-Name plus hydralazine hydrocloride (group 1L + H). Six rats were used as control group (group 1C). Group 2 (14 days): 19 rats were utilized. Seven rats were submitted during 14 days to L-Name (group 2L). Six rats were submitted concomitantly to L-Name plus hydralazine hydrocloride (group 2L + H). Six rats were used as control group (group 2C). Group 3 (28 days): 21 rats were used. Eight rats were submitted to L-Name (group 3L). Seven rats were submitted to L-Name plus hydralazine hydrocloride (group 3L + H). Six rats were used as control group (group 3C) (**Tables 1** and **2**). Anova one way, following Duncan Test of multiple comparisons, was utilized to statistical evaluations using software Statistica version 5.5, copyright 1984-2000 by Statsoft, Inc.

Results

Water ingestion, arterial blood pressure, body weight and cardiac weight

Our results (**Table 3**) show a similar ingestion of water for control (C) (33.24ml) and L-Name (L) (33.52ml) groups ($p > 0.05$). Nevertheless, comparing C (33.24ml) with L-Name + hydralazine hydrocloride (L + H) (28.01ml) we found significant reduction in water ingestion from C to L + H groups ($p < 0.001$). Concerning arterial pressure (Table 1), group 1 had no significant difference comparing 1C with 1L and 1C with 1L + H ($p > 0.05$). In group 2, comparing 2C with 2L, we found significant difference on day 6 ($p < 0.05$) and also on day 13 ($p < 0.001$) of submission.

Table 1 Tail-cuff arterial pressure (mmHg) measured using a hydraulic pletismography (mean/standard error)

	Control	L	*p*	L + H	*p*
Group 1: Control (*n* = 6); L (*n* = 7); L + H (*n* = 7)					
Initial AP	106.2/3.6	106.6/2.3	> 0.05	106/3.1	> 0.05
Final AP	108.5/2.7	110.1/2.7	> 0.05	106.9/2.3	> 0.05
Group 2: Control (*n* = 6); L (*n* = 7); L + H (*n* = 6)					
Initial AP	104.2/3.9	111.4/1.2	> 0.05	103/3.9	> 0.05
AP day 6	111.5/1.8	121.4/1.9	< 0.05	106.2/2.9	> 0.05
AP day 13	106.3/2.6	145.9/3.5	< 0.001	111.7/4.4	> 0.05
Group 3: Control (*n* = 6); L (n = 8); L + H (*n* = 7)					
Initial AP	111.2/2	103.6/2.4	< 0.05**	104.4/1.8	< 0.05**
AP day 6	110.5/3.1	115.1/2.4	> 0.05	98.6/3.7	< 0.05**
AP day 13	114.2/1	141.6/5	< 0.001	113.4/2.7	> 0.05
AP day 20	105.3/1.8	165/4.9	< 0.001	111.1/3	> 0.05
AP day 27	106.5/1.9	200.5/3.1*	< 0.001	112.3/3	> 0.05

AP: arterial pressure; L: submitted to L-Name; L + H: submitted to L-Name + hydralazine; *p* = test of similarity evaluating the probability of arterial pressure of control group compared with groups submitted to L-Name and L-Name + hydralazine being equal to zero; **n* = 6, two animals presented intense paralysis of the limbs and because of terminal stage of that were killed, one on day 24 and the other on day 27; **note that this arterial pressure had a significant inferior value comparing with control group.

Table 2 Body weight during the experiments (mean/standard error of mean)

	Control	L	*p*	L + H	*p*	*p* (L x LH)
Group 1: Control (*n* = 6); L (*n* = 7); L + H (*n* = 7)						
Initial	240.8/8	237/5.1		215.7/13.6		
Final	288.7/6.6	278.1/5.9		260.9/12.7		
Final/initial	47.8/4.3	41.1/6.2	> 0.05	45.14/9.5	> 0.05	> 0.05
Variation (%)	16.6	14.9		17.3		
Group 2: Control (*n* = 6); L (*n* = 7); L + H (*n* = 6)						
Initial	152.1/6.7	194.3/3.5		195/6.96		
Day 7	188.9/7.2	211.7/9.6		228.4/11.3		
Day 14	215.4/10.2	226/14.2 (*n* = 6)*		244.5/12.7		
Final/initial	63.3/5	31/6.9	> 0.05	48.7/18.7	> 0.05	> 0.05
Variation (%)	29.4	14		20.3		
Group 3: Control (*n* = 6); L (*n* = 8); L + H (*n* = 7)						
Initial	211.7/4.4	213.5/4.9		205/15.7		
Day 7	255/9.1	239.9/7.4		230.4/7		
Day 14	278.7/7.8	258/10.1		241.6/4.9		
Day 21	294.8/9.1	269.9/12.2		257.1/6.4		
Day 27	312.7/10.6	271/12.1 (*n* = 7)**		260.7/6.2		
Final/initial	101/7.2	56.14/14	< 0.05	55.71/16.3	< 0.05	> 0.05
Variation (%)	32.3	21.2		21.4		

L: submitted to L-Name; L + H: submitted to L-Name + hydralazine; final/initial: difference between final and initial body weight. In these procedures were utilized: *n* = 6 in group 2 and *n* = 7 in group 3; *it was not possible to evaluate the weight of one of the animals; **one animal presented great paralysis of all limbs and, in terminal stage, was killed on day 24.

Table 3 Mean of daily water ingestion (ml) during the experiments (mean/standard error of mean)

Control	L	*p*	L + H	*p*
33.24/0.66 (*n* = 42 days*)	33.52/0.68 (*n* = 43 days**)	> 0.05	28.01/0.64 (*n* = 43 days***)	< 0.001
min = 23/max = 42.9	min = 22.4/max = 43.2		min = 15/max = 37.3	

L: submitted to L-Name; L + H: submitted to L-Name + hydralazine; min: minimum value; max: maximum value; *p* = test of similarity evaluating probability of arterial pressure of control group compared with groups submitted to L-Name and L-Name + hydralazine being equal to zero; *mean of 3 days (1C) + 13 days (2C) + 26 days (3C). Sum equal to 42 days. A total of 20 control rats; **mean of 3 days (1L) + 13 days (2L) + 27 days (3L). Sum equal to 43 days. A total of 19 L-Name rats; ***mean of 3 days (1L + H) + 13 days (2L + H) + 27 days (3L + H). Sum equal to 43 days. A total of 21 L-Name rats.

Nevertheless, comparing 2C with 2L + H groups significant difference concerning arterial pressure was not found ($p > 0.05$). In group 3, significant difference in arterial pressure comparing 3C and 3L was only noted beginning on day 13 of experiment, continuing on day 20 and also on day 27 ($p < 0.001$). Nevertheless, comparing 3C and 3L + H we had no significant difference on days 13, 20 or 28 of experiment ($p > 0.05$). Variations of body weight were considered in Table 2. For day 4 we found variations of 16.6%, 14.9% and 17.3% respectively to C, L and L + H. For day 14 we found variations of 29.4%, 14% and 20.3% respectively to C, L and L + H. For day 27 we found variations of 32.3%, 21.2% and 21.4% respectively to C, L and L + H. Then, on day 27, both L and L + H groups had similar deficit of about 1/3 in gain of weight when compared to control groups ($p < 0.05$). Estimating cardiac weight (**Table 4**), we also utilized a relation of cardiac weight (mg)/body weight (g) to eliminate weight interference of each animal in our evaluations. Our results did not show significant difference on days 4 and 14 of experiment ($p > 0.05$). Nevertheless, on day 28, the L + H group presented a near significant cardiac hypertrophy compared with the control group ($p = 0.052$). In the L group, cardiac hypertrophy was not significant probably because of the wide estimated standard error of arithmetic mean.

Pathohistological results

Concerning histological myocardial alterations, in 1L (day 4) we found myocardial abnormalities and lesions mainly in right ventricles while in 1L + H we could not identify myocardial abnormalities or lesions in myocardial tissue. In all control groups, perivascular connective tissue was visualized as more delicate and granular than in L-Name groups and also in group 3L + H (day 28) (**Figure**). The fibroses (reactive and reparative) in 1L were

Table 4 Cardiac weight during the experiments (mean/standard error of mean)

	Control	L	*p*	L + H	*p*
Group 1: Control (*n* = 6); L (*n* = 7); L + H (*n* = 7)					
CW	1.21/0.03	1.28/0.07	> 0.05	1.19/0.07	> 0.05
CW/BW	4.19/0.12	4.61/0.3	> 0.05	4.59/0.31	> 0.05
	(max = 4.51/min = 3.8)	(max = 6.04/min = 3.59)		(max = 5.71/min = 3.73)	
Group 2: Control (*n* = 4, two animals could not be considered); L (*n* = 7); L + H (*n* = 6)					
CW	1.14/0.07	1.16/0.05	> 0.05	1.42/0.11	> 0.05
CW/BW	3.05/0.62	2.53/0.11	> 0.05	2.88/0.07	> 0.05
	(max = 5.18/min = 2.13)	(max = 3.03/min = 2.14)		(max = 3.18/min = 2.68)	
Group 3: Control (*n* = 6); L (*n* = 8); L + H (*n* = 7)					
CW	1.38/0.1	1.4/0.06	> 0.05	1.44/0.09	> 0.05
CW/BW	4.41/0.23	5.51/0.45	> 0.05	5.5/0.29	= 0.052
	(max = 5.15/min = 3.6)	(max = 7.29/min = 3.99)		(max = 6.53/min = 4.39)	

L: submitted to L-Name; L + H: submitted to L-Name + hydralazine; CW: cardiac weight; CW/BW: relation of cardiac weight/body weight; min: minimum value; max: maximum value; *p* = test of similarity evaluating the probability of arterial pressure in control group compared with groups submitted to L-Name and L-Name + hydralazine being equal to zero.

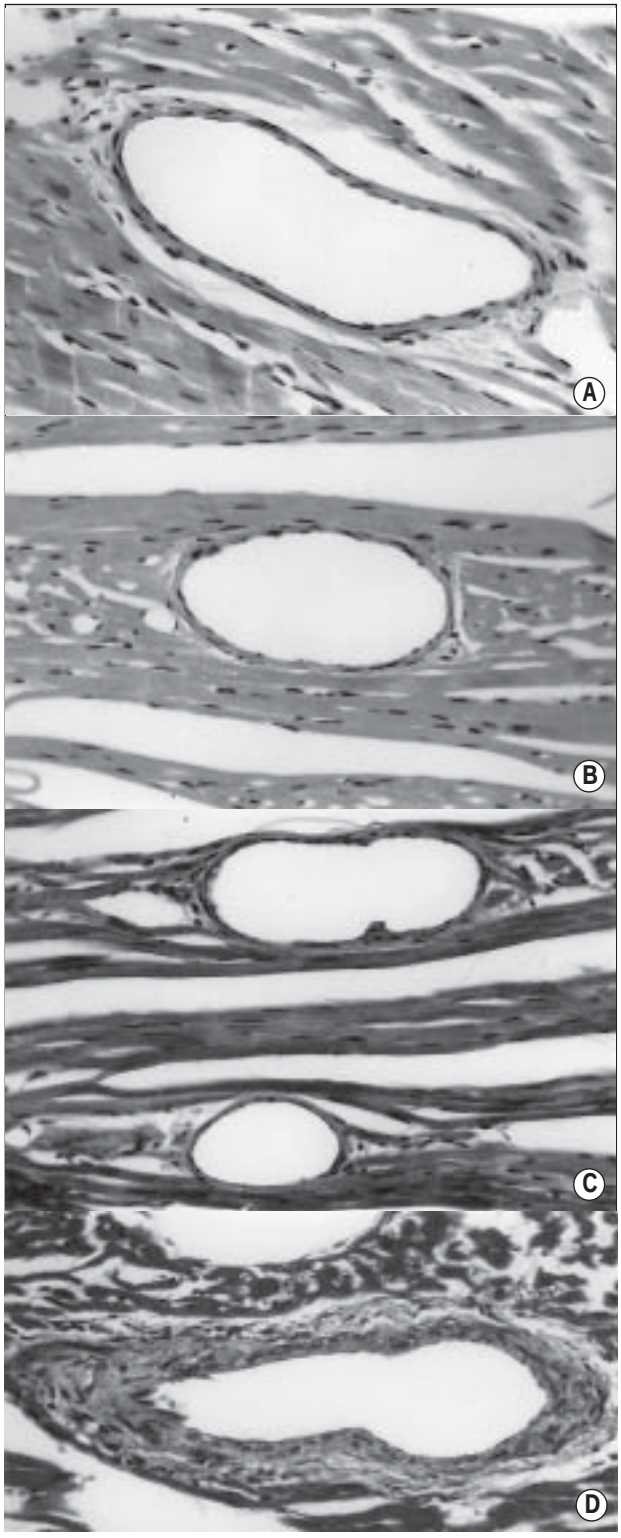

Figure – *A – Normal vessels in control rat's myocardium (hematoxilin-eosin, 400x); B – Normal vessels in myocardium of rats submitted concomitantly to L-Name and hydralazine during 4 days (hematoxilin-eosin, 400x); C – Normal vessels in myocardium of rats submitted concomitantly to L-NAME and hydralazine during 14 days (Masson's trichrome, 400x); D – Abnormal arterial vessels in rats submitted concomitantly to L-Name and hydralazine during 28 days. In D, note proliferation of smooth muscle cells, proliferation of cells in the tunicae intima and also thickness of arterial wall with degenerative fibrotic process (Masson's trichrome, 400x)*

less frequent than in 2L and in 2L less frequent and less apparent than in 3L. In 1L vascular abnormalities were generally in a more initial state and not so common as in 2L, and so on. However, in some cases they were very intense. In 1L, 2L and 3L the right ventricular walls were more affected than the left ventricular walls. In 1L – in some cases – we had similar lesions (reparative fibrosis) of those here described in 2L. Nevertheless, in other animals, we found myocardium in a normal or near normal state. In 1L, 2L, 3L and 1L + H, 2L + H, 3L + H reactions to Weigert's resorcinol fuchsin at repair areas was near null. Considering 3L (day 28) myocardium presented characteristic lesions (reactive and reparative fibrosis), vascular damage with increasing wall thickness related mainly to proliferation of the arterial smooth muscle cells. The total obliteration of small vessels was noted only in this period. We also observed occurrence of reactive fibrosis between muscle cells of vascular wall. The hypertrophic process of arterial smooth muscle cells and smooth muscle cell's nuclei was observed, which probably contributes to the thickness of arterial wall. Proliferation of cells in the intimal layer and its thickening were also observed. In 3L + H (day 28) we also observed similar vascular abnormalities described for 3L subgroup. Nevertheless, it was observed less frequently and less apparently. In subgroup 3L + H, we did not identify total vascular obstructions. Reparative fibrosis (infarct areas) was not noted in 3L + H and reactive fibrosis occurred mainly in perivascular region (Figure).

Discussion

In the present work, using NO systemic blockage (via L-Name) we have identified thickening in vascular wall, perivascular and myocardial fibrosis. At least part of our results are similar to those demonstrated by Hocher *et al.* (13) in renovascular hypertension and also by Felix *et al.* (7) using angiotensin II. Hocher *et al.* (13) suggested that in renovascular hypertension stimulation of endothelin receptor-A (ETA) was related to abnormalities in media/lumen ratio of intramyocardial vessels. Treatment of these renovascular hypertensive rats with ETA antagonist prevented this alteration. In the same work, stimulation of endothelin receptor-B (ETB) was related to increase of fibrotic tissue in left ventricle, however, had no influence in perivascular fibrosis and treatment of these renovascular hipertensive rats with ETB antagonist avoids this alteration. ET-1 promotes growth of cardiomyocytes and induces colagen synthesis (13).

NO blockage also promotes vasoconstriction, myocardial fibrosis, cellular proliferation and hypertrophy (6, 14). Angiotensin II stimulates the synthesis of endothelin-1 (ET-1) in many cellular types including cardiomyocytes. ET-1 causes contraction and mitogenesis at least in mesanglial cells. NO is known to inhibit endothelial cell ET-1 production and this occurs probably via cGMP dependent mechanism. Most endothelial cells express only ET-B receptors. ET-A receptors are widely distributed in vascular smooth muscle cells. Stimulation of ET-A receptor mediates most of the vasoconstrictor response to endothelins. NO counteracts with the vasoconstrictor effect of ET-1. Human and animal studies have suggested that there is a feedback mechanism between ET-1 and NO synthesis that acts reciprocally to regulate vascular tone (15). Almost all biological actions of angiotensin II were considered mediated by angiotensin receptor-I. However, nitric oxide releases because high level of angiotensin II has been recently attributed to angiotensin receptor-II. In angiotensin dependent hypertension the hypotensive effect occurred at least in part because of angiotensin receptor type 2. Then we have a contra-regulatory protector angiotensin receptor type 2 effect mediated by nitric oxide (16).

Considering all these results together, it is probable, in L-Name model, that the involvement of angiotensin II in myocardial remodeling is mediated – at least partially – by the paracrine cardiac endothelin system promoting tissue fibrosis in the heart. Our data suggests that lesions in cardiac tissue occurred in L-Name model not mainly because of the hypertensive process, since in nitric oxide blockade/not hypertensive rats the same lesions occurred.

Sander *et al.* (17) showed that L-NMMA and L-Name significantly increase the arterial pressure of human beings. Nevertheless, blood pressure returns to baseline 24 hours after stopping infusion or after administration of L-arginine (200mg/kg). They also showed that L-Name (4mg/kg, 60 minutes of infusion time) was much more effective than L-NMMA (50mg/Kg, 120 minutes of infusion time). Using these doses the greatest increase of mean arterial pressure was 23 ± 3mmHg (L-Name) and 15 ± 2mmHg (L-NMMA). The largest effect of L-Name was on diastolic blood pressure with a maximum value of 109mmHg. D-Name and D-arginine had no effect in blood pressure. Also α-adrenergic blockage reversed in 40% the peak increase of blood pressure after L-Name infusion meaning that this increase is in part sympathetically mediated. These authors conclude that, in human beings – although inhibition of endothelium-dependent vasodilatation is the primary mechanism underlying the initiation of the hypertensive response to L-Name – the sympathetic nervous system plays an important role in the full expression and maintenance of this large blood pressure-raising effect.

Mice submitted to endotelial nitric oxide sinthases (eNOS) knockout present an arterial pressure level raising in about 15mmHg (18) or 47mmHg (19) compared with control animals. Also the basal arterial pressure is reduced in about 20mmHg in mice with eNOS overexpression (18).

In this work, concerning L-Name group on day 28, fibrosis and lesions were greater than those we found in a previous work on day 21 and inferior to that found on day 35 (6). Lesions on day 35 (6) were more similar to that found on day 43 and were very wide (20). Our previous (6, 20) and present data suggest that after day 28 of submission to L-Name (concentration 750mg/l in drinking water) the cardiac tissue becomes more susceptible reducing its stability. So, considering the data here presented and other results (6, 20), we can say that the L-Name model presents progressive lesions directly proportional to the time of submission. In this model the right ventricular wall is more affected.

In present work, on all days of submission to L-Name the reaction to Weigert's resorcinol fuchsin (with or without oxon) in myocardial or perivascular fibrotic areas is nearly null. In a previous work, however, on days 35 and 43 the lesions were greatly reactive and using oxon, a more intense reaction was seen (6, 20). This indicates that initial repair in L-Name model is made more by collagen than by elastic fibers and in extended periods (days 35 and 43) a substantial amount mainly of oxitalanic fibers in reparative region was observed, which may originate diastolic deficiency (6, 20).

Concerning day 28, the group L + H presented a near significant cardiac hypertrophy compared with control group (p = 0.052). This may suggest that blockage of hypertension using hydralazine does not avoid heart hypertrophy. Then, these results may contribute to the knowledge that the hypertrophic process is mainly due to nitric oxide deficit. In human species, the increase of load in arterial pressure induces hypertrophy at left ventricular wall. In some patients the heart may double its weight; nevertheless, in others – with the same arterial overloaded pressure – even after several years the heart may suffer just a slight hypertrophy (21). In the present

work, a non-significant difference in heart weight comparing 3C with 3L groups probably occurred because L group presented a wide standard error of mean (SEM = 0.45), because not all animals developed significant cardiac hypertrophy.

Arnal *et al*. (22) found that both hypertension and myocardial abnormalities were avoided using concomitant submission of L-Name and angiotensin convert enzyme inhibitor trandolapril or calcium blocker channel verapamil. These results were not confirmed by our present results and neither by those of Numaguchi *et al*. (1) or Moreno Jr. *et al*. (3-5). Moreno Jr. *et al*. (3-5), utilizing L-Name + enalapril, found that angiotensin convert enzime inhibitor avoided arterial hypertension and left ventricular hypertrophy, nevertheless, did not prevent myocardial lesions. In the present work, on day 28 of submission in 3L + H group (L-Name 750mg/l + hydralazine 120mg/l), similar vascular abnormalities were described for 3L. Nevertheless, the abnormalities in 3L + H were less frequent and less apparent. Group 3L presented severe vascular obstructions, infarct and repair areas, however, in group 3L + H these lesions were not seen. In 3L + H we could only see vascular abnormalities. Results on day 28 are also similar to abnormalities and lesions occurring in human arteriosclerosis (23). NO was also suggested to be an antiatherogenic, antiproliferative and antithrombotic factor (15). Numaguchi *et al*. (1), using concomitant submission to L-Name and hydralazine in rats (56 days), found necrotic areas, reparative fibrosis and vascular obstructions in myocardium. However, in doses of L-Name 100mg/l the lesions were greater than those found in L-Name 1g/l + hydralazine 120mg/l. In doses of L-Name 1g/l the lesions were greater than those found in doses of L-name 100mg/l. In the present work on day 28 of concomitant submission to L-Name 750mg/l + hydralazine 120mg/l we did not find necrotic areas, reparative fibrosis and vascular obstructions found by Numaguchi *et al*. (1) on day 56 of submission. Our results suggest that the morbid process in the present model can be postponed by using hydralazine which, however, does not avoid these events after moderate or long-term experimental submission to L-Name.

Acknowledgements

This article was supported by Capes, CNPq, IPPMG/UFRJ, CIC-HUCFF/UFRJ and AXV. During this work, the main author had a Ph.D. fellowship from Capes, Brasília/Brazil. The main author thanks his Lord and God Jesus Christ and the 1st Baptist Church of Niterói, RJ.

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