Effect of oral arginine administration over blood pressure and cardiac parameters in rats submitted to chronic inhibition of nitric oxide synthesis

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ABSTRACT

It has been clearly established that chronic inhibition of nitric oxide synthesis results in a sustained increase in blood pressure, cardiac remodeling and fibrosis. It was also demonstrated by our group that arginine supplementation was able to increase the skeletal muscle resistance to fatigue, but its mechanism remains uncertain. The experimental treatment of rats with L-NAME is one of the most common models employed to induce hypertension. The expected compensatory response against increases in systemic vascular resistance would be ventricular hypertrophy. However, the presence of cardiac hypertrophy still controversial. The aim of the present study was to verify the effects of nitric oxide inhibition through oral L-NAME administration on the cardiac tissue of rats, and the possible reversion by L-arginine. Thirty male Wistar rats (250-350 g) were kept in controlled conditions of temperature, light, humidity, with water and food “ad libitum”. At the end of 4 weeks or treatments the animals were sacrificed by CO2 inhalation and the hearts were removed. Soon after, the hearts were dissected, to separate atria and ventricles, obtaining the total heart weight. After the retrieval of the right ventricle, the remaining part was weighed, to obtain the left ventricular weight (LVW, mg); the difference between the total heart weight and the LVW was considered the right ventricular weight (RVW, mg). These values were corrected in function of the corporal weight obtained in the last week of treatment. L-NAME was able to induced hypertension and increases in double product but without any heart hypertrophy. The increase arterial pressure and double product were reversed by L-arginine administration in a dose-dependent way. Preliminary findings demonstrated a reversion of heart fibroses induced by L-NAME, after arginine treatment. We concluded that arginine may constitute a valuable tool in preventing hypertension and cardiac remodeling mainly related to vascular dysfunctions and maybe also in athletic activities.

INTRODUCTION

The arterial hypertension (AH) is considered a primary risk factor for cardio and cerebrovascular diseases and can occur in all ages and in both genders[1-2]. It has also been known as a silent hazard due to its lack of early signs. Many cardio and cerebrovascular diseases have straight relation with the altered pressor levels. The Framingham model used for risk foreseeing of the AH mentions the PAS as an important related risk factor in significant coronary diseases[3]. The arterial pressure (AP) increase may cause, among other things, vascular endothelial dysfunctions and lesions with migration of atherogenic elements, including LDL, monocytes and macrophages[4,5].

The vascular endothelium, a monolayer of cells that covers the blood vessels and separates the circulatory flow from the vascular smooth muscle, is not simply a dialysis membrane, but it has intense metabolic activity. It is involved in many endogenous mediators, namely the nitric oxide, the prostaglandins and the endothelins. Many substances derived from the endothelium seem to be involved in the physiological modeling of the local control of the tonus and the vascular flow: a) vasodilators substances – the nitric oxide (NO) and the prostacyclin (PGI2); b) vasoconstrictor substances – endothelin and tromboxane A2, for instance. Those substances that are in some cases continuously produced by the endothelial cells in small amounts, can be liberated in much bigger amounts through mechanical and humor stimuli[6].

Hundreds of researchers worldwide have being studying the role of the vascular endothelium in the relaxing of the blood vessel process, due to the interest related to the NO biological functions. The interest for such issue began in a research conducted by Furchgott; Zawadzki[7], who demonstrated that the vascular relaxation induced by acetylcholine depended on the endothelium and showed evidence that the demonstrated effect was mediated by a humor factor, later known as endothelium derived relaxing factor (EDRF). Raoport and Murad[8], suggested that the EDRF mechanism, which caused vascular relaxing, was mediated by the cyclic guanosine monophosphate (GMP). Seven years after the EDRF discovery, Palmer[9] and Ignarro[10], almost simultaneously, demonstrated that this relaxing factor derived from the endothelium was a free radical, the NO. It was suggested that the EDRF and the NO were indistinguishable in the biological activity, chemical stability and susceptibility to inhibitors or potentializers. Moreover, both had their action inhibited by hemoglobin and potentIALIZED by the superoxide dismutase.

Rats treated with n-nitro-l-arginine-methyl-ester (L-NAME), a potent inhibitor of the nitric oxide (NO) synthesis, are a model of systemic arterial hypertension widely used[11,12]. The compensatory response to the post-load increase is the left ventricular hypertrophy. However, the left ventricular hypertrophy in the L-NAME model is controversial. Previous studies have reposted all kinds of

Keywords: L-NAME, Arginine, Hypertension, Rats.
cardiac response in animals treated with L-NAME, from no hyper-
trophy to mild and moderate hypertrophy[13-17].

On the other hand, it has been reported in animals and humans as well, that the infusion of L-arginine (amino acid that acts as sub-
strate for the nitric oxide synthesis) induces a significant improve-
ment in the vasodilatation dependent on endothelium in hypercoles-
theromelia conditions. This situation also suggests that the
decrease of substrate availability can be responsible for the de-
creased vascular responsiveness which is observed in these con-
ditions[18-19]. Recent tests verified the possibility of the L-arginine
oral supplementation through diet partly reestablish the endothe-
lial function. Moreover, the L-arginine oral administration is able to
improve hemodynamic factors and the ability to do physical activ-
ities[20-22].

The literature data indicate that the inhibition or the deficient
production of nitric oxide in the body can be responsible for a se-
ries of changes that act in synergy with other cardiovascular risk
factors for events such as AVEs, infarcts and vasospasms. On the
other hand, the L-arginine amino acid oral administration can be a
simple and safe tool for the reversion of the deleterial effects of
the dysfunction in the endogenous nitric oxide production.

METHODS

30 Wistar male young adult rats, weighing between 250 and
300 g were used. The animals were obtained in the Research and
Development Institute of the Universidade do Vale do Paraíba
(UNIVAP) biotherium, and kept in controlled conditions of temper-
ature, light and humidity. They were given water and food (Purina
Brazil) “ad libitum”.

Experimental groups

30 animals were randomly divided in 5 groups with 06 individu-
als each, as described in the following protocols:
Protocol # 1: Control group received standard food and fresh
water.
Protocol # 2: Group treated with daily 60 mg/Kg of L-NAME in a
0,5 ml volume for 28 days.
Protocol # 3: Group treated with daily 60 mg/Kg of L-NAME and
daily 10 mg/Kg of L-Arginine for 28 days.
Protocol # 4: Group treated with daily 60 mg/Kg of L-NAME and
daily 30 mg/Kg of L-Arginine for 28 days.
Protocol # 5: Group treated with daily 60 mg/Kg of L-NAME and
daily 100 mg/Kg of L-Arginine for 28 days.

Surgical procedure and hemodynamic parameters

In order to verify the pressor values and heart rate, the rats were
anesthetized with Tiletamine and Zolazepam (40 mg/Kg), intraper-
toneally administrated (i.a); the anesthesia was complemented
by another intraperitoneal injection of 15 mg/kg of Tiletamine +
Zolazepam before the control period and whenever necessary. The
rats were tracheostomized, intubated with a poliethilenum tube
and immobilized with pancuronio bromet (1 mg/kg i.v), with sup-
plementary doses of 1 mg/kg whenever necessary. The animals
were artificially ventilated through a mechanical breather (UGO
BASILE 7052, continuous volume of 2 ml/kg, and respiratory rate
of 75 cycles/min). The right jugular vein was catheterized to re-
ceive the intravenous injections of pancuronio bromet. The arte-
reral pressure was continuously monitored through a catheter placed
in the left carotid artery and connected to a transducer of arterial
pressure (UGO BASILE), connected to GEMINI 7070 physiogra-
phers (UGO BASILE). The pulse arterial pressure (systolic and di-
astolic) was obtained directly from the pressor register; and the
average arterial pressure calculated by the PAM formula = (systol-
ic pressure - diastolic pressure)/3 + diastolic pressure. The heart
rate was evaluated every 5 minutes by the heart beats, directly
from the register, increasing the register velocity.

RESULTS

Evaluation of the cardiac weight

After the animal’s sacrifice, their hearts were removed for later
analysis. They were washed with sodium chloride solution (0,9%,
p/v) for clots removal. Afterwards, the hearts were dissected, the
atriums removed and the ventricules weighted, thereby obtaining
the total cardiac weight. (TCW, mg). After left ventricle removal,
the remaining tissue was weighted, obtaining the left ventricular
weight (LWV, mg). The difference between the total cardiac weight
and the left ventricular weight is the right ventricular weight (RWW,
mg). Such values were corrected in relation to the body weight
obtained in the last week of treatment. Thus, they were finally
expressed as relative cardiac weight (RCW = TCW/body weight,
mg/g), index of the left ventricular weight (ILVW = LWV/body weight,
mg/g) and the index of the right ventricular weight (IRVW = RWW/
body weight mg/g).

Statistical analysis

The results were expressed as average ± average standard erro-
. The analysis of variance (ANOVA) for repeated measures was
applied to evaluate differences in the body weight and in the arte-
rerial pressure. One-way ANOVA will be used to compare the car-
diac weight.

Double product

The Double Product index was used as an indirect indicator of
the cardiac work, calculated through by the formula: DP = systolic
pressure X heart rate.

Euthanasia protocol

Four weeks later, all animals’ arterial pressure and heart rate
were checked and they were submitted to the euthanasia protocol
that consists of 0,1 ml of xylazine chlorhydrate + 0,1 ml of Keta-
mine chlorhydrate intraperoneally injected and after the expected
anesthesia effect, the animals were placed in a mortuary chamber
and sacrificed by CO2 inhaling.

Evaluation of the L-Arginine effect in the prevention of the
pressor levels increase induced by L-NAME

Graph 1 represents the values of the systolic arterial pressure in
rats in the different groups. The increase of the systolic arterial
pressure in the animals treated with L-NAME can be observed.
Such increase was statistically significant when compared to the
control group (Control (82 ± 4) versus L-NAME (134 ± 5), L-NAME
+ L-Arginine (10 mg) (119 ± 8) and L-NAME + L-Arginine (30 mg)
(119,1 ± 6) L-NAME + L-Arginine (100 mg) (100 ± 2).

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Graph 1 - Systolic arterial pressure in rats after 28 days of treatment with
L-NAME and L-Arginine. The data represent the average ± E.P.M, n = 6,* =
P < 0,05 when compared with the Control group, # = P < 0,05 when com-
pared with the L-NAME group.

Graph 2 represents the diastolic arterial pressure in rats in the
different groups. The diastolic arterial pressure presented a statisti-
cally significant increase when compared to the Control group
(63 ± 5 mm Hg); L-NM E (118 ± 5 mm Hg), L-NM E + L-Arginine (10 mg) (110 ± 8), L-NM E + L-Arginine (30 mg) (97 ± 6 mm Hg); L-NM E + L-Arginine (100 mg) (81 ± 3).

When comparing the L-NM E group (36 ± 2) in relation to the L-NM E group + L-Arginine (10 mg) (23 ± 3), a significant reduction of the double product in the groups that received the L-Arginine amino acid was observed, the same occurring to the L-NM E group + L-Arginine (100 mg) (21 ± 2).

**Evaluation of the effect of the preventive L-Arginine on the cardiac weight - Total and Partial - after the treatments with L-NM E and L-Arginine**

In graph 5 the total cardiac weight in rats presented a significant decrease in all treated groups, when compared to the control group.

Evaluation of the L-Arginine preventive effect in the cardiac work increase process (Double Product) induced by L-NM E

In graph 4 the double product (indicator of cardiac work and oxygen consumption by the myocardium) in rats in the different groups. The treatment with L-NM E was able to induce a statistically significant increase of the Double Product when compared to the control group. Control (19 ± 1) versus the L-NM E group (36 ± 2).

In graph 6 the weight of the left ventricle did not present statistically significant alterations in all treated groups, when compared to the Control group.

In graph 7 it can also be observed that the relative cardiac weight did not present significant alterations after the treatments, when compared to the Control group.

Graph 2 - Diastolic arterial pressure in rats after 28 days of treatment with L-NM E and L-Arginine The data represent the average ± E.P.M, n = 6, * = P < 0.05 when compared with the Control group, # = P < 0.05 when compared with the L-NM E group.

Graph 3 - Average arterial pressure in rats after 28 days of treatment with L-NM E and L-Arginine The data represent the average ± E.P.M, n = 6, * = P < 0.05 when compared with the Control group, # = P < 0.05 when compared with the L-NM E group.

Graph 4 - Double Product in rats after 28 days of treatment with L-NM E and L-Arginine The data represent the average ± E.P.M, n = 6, * = P < 0.05 when compared with the Control group, # = P < 0.05 when compared with the L-NM E group.

Graph 5 - Total cardiac weight in rats after 28 days of treatment with L-NM E and L-Arginine The data represent the average ± E.P.M, n = 6, * = P < 0.05 when compared with the Control group, # = P < 0.05 when compared with the L-NM E group.

Graph 6 - Left ventricle weight in rats after 28 days of treatment with L-NM E and L-Arginine The data represent the average ± E.P.M, n = 6, * = P < 0.05 when compared with the Control group, # = P < 0.05 when compared with the L-NM E group.

Graph 7 - Index of the weight of the left ventricle in rats after 28 days of treatment with L-NM E and L-Arginine The data represent the average ± E.P.M, n = 6, * = P < 0.05 when compared to the control group Control, # = P < 0.05 when compared to the L-NM E group.
The discovery that the NO synthase inhibitors increase the vasoconstrictor activity in vitro, enabled researchers to postulate the hypothesis in which such inhibition could induce to hypertension in vivo. Actually, with the chronic administration of NO synthase inhibitors, the induction of a long term pressor effect that seems to be dependent on the dose is possible.\textsuperscript{28-34}

According to a work conducted by Ribeiro et al.,\textsuperscript{13}, the oral administration of an L-arginine analogous, the L-NAME in Wistar rats for four to six weeks, induces severe and progressive hypertension, vasoconstriction and renal dysfunction. According to the same authors, with a week inhibition, the hypertension can be partially reverted by supplementation of high doses of L-arginine.

Another work showing the hypotensor effect of the L-arginine was done by Wong et al.,\textsuperscript{28}, where the authors investigated the effect of the oral administration of L-arginine in the arterial pressure, in some metabolic parameters and of coagulation in six healthy individuals for one week. The results indicated that a moderate increase of the L-arginine plasmatic concentration significantly reduces the arterial pressure.

A study conducted by Hambrecht et al.\textsuperscript{29} associated the daily physical activity with oral supplementation of 8 g daily of L-arginine in patients with chronic cardiac diseases and reached to the conclusion that both the regular physical activity and use of L-arginine improve the vasodilator properties of the endothelium and that the association of the two interventions can improve the vasodilatation-endothelium dependent.

Clarkson et al.,\textsuperscript{30}, in a study using L-arginine oral supplementation, demonstrated that the L-arginine plasmatic levels increased after its ingestion, as well as the vasodilatation-endothelium dependent. Moreover, our group's studies with healthy volunteers demonstrated that the L-arginine oral supplementation was able to increase the muscular resistance to fatigue, evaluated through isokinetic dynamometry. Such effect was supposedly attributed to the improvement of the local circulation in the limbs involved in the conducted physical effort.\textsuperscript{21} These results demonstrated the efficiency of the oral supplementation with the L-arginine amino acid in humans, probably through an improvement mechanism of the vasodilatation in the skeletal muscles induced during the effort. Consequently, a better adaptation of the blood demand and the local muscular fatigue delay are observed.

The systemic, or even local vascular resistance increase, is able to induce a compensatory increase of the local NO liberation, opposite to the vasoconstriction. Such fact reveals an important physiological mechanism of vasomotor tone regulation, and consequently of the vascular resistance and arterial pressure. When a failure in the basal or even stimulated liberation of NO occurs, the increase of the vascular resistance and consequently of the arterial pressure, may occur. The administration of the NO-synthase inhibitor used in the present study determined the increase of the systemic arterial pressure and the cardiac work as well. The cardiac work was indirectly evaluated by the Double Product. Since it would be normal for a muscle that works against an increased resistance, show more mass (demonstrated by the increase observed of the Double Product), it would be also expected to find increase in cardiac muscular mass, even in a short period of four week-treatment. The double product, also called MTTS (Modified Tension Time Index), is considered an important metabolic parameter that helps in the estimated calculation of the maximum consumption of myocardial oxygen. The double product is a parameter that allows a linear correlation establishment between the product of the cardiac frequency and the maximum systolic arterial pressure (SAPmax) with the myocardial oxygen consumption.\textsuperscript{21}

However, this increase of arterial pressure was not accompanied by an increase of the total cardiac mass, not even the left ventricle, in the analyzed period. On the other hand, preliminary analyses (not demonstrated in this work) indicate significant increase of diffuse interstitial fibrosis, which was reversed by the L-arginine administration. These results agree with those found by Rossi et al.,\textsuperscript{32}, who did not observe cardiac or left ventricular hypertrophy, with interstitial fibrosis increase, though.

The experimental model of H.A. with L-NAME causes a fibrosis (peri-vascular and repairing interstitial) and a disorganization of the cardiac muscle apparently more intense than the ones observed in the renovascular model.\textsuperscript{31}

Some results found in the literature suggest that the myocardial lesions in animals that were submitted to the L-NAME would not be exclusively due to the H.A., but would be mainly associated with the chronic inhibition of the nitric oxide synthesis and the vascular endothelium lesion.\textsuperscript{32-34} In the H.A. derived from the L-NAME administration, the increase of the resulting myocardial metabolic demand occurs at the same time of the narrowing of the micro vases, hypertrophy and myocytes necrosis. Besides that, the local production of angiotensine II, endothelins and/or catecholamines related to the H.A., represent important roles in the myocardial necrosis and fibrosis.\textsuperscript{34}

As a whole, the data presented suggest a real efficiency of the oral administration of the L-arginine amino acid in the reversion of the cardiovascular effects induced by the inhibition of the NO-synthase enzyme. Moreover, it can be suggested that L-arginine can be used in the future as a cardiovascular risk prevention agent, as well as in the athletic performance improvement. Finally, the amino acid could also be used in cardiac rehabilitation protocols or in cardiovascular risk prevention in post-infant patients or hypertensives. However, long term studies are still necessary to confirm such hypothesis.

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