Original Research

The effect of L-arginine and L-NAME on myocardial capillary density in normal rats

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Abstract

Background: This study evaluated the effect of L-arginine (Nitric Oxide (NO) precursor) and L-NG-Nitroarginine Methyl Ester (L-NAME) (NO synthase inhibitor) on myocardial capillary density in normal rats.

Materials and Methods: Eighteen male rats were divided into three groups: Group 1: Received L-NAME (10 mg/kg/day; ip), Group 2: Received L-arginine (50 mg/kg/day; ip), and Group 3 (control) received normal saline. After 3 weeks, blood samples were taken and myocardial capillary density was evaluated using immunohistochemistry method.

Results: Serum NO concentration in control group was 6.45 ± 0.44 μmol/lit. Treatment of animals with L-arginine increased serum NO concentration (7.90 ± 0.75 vs. 6.45 ± 0.44 μmol/lit, respectively) and L-NAME decreased (4.86 ± 0.40 vs. 6.45 ± 0.44 μmol/lit, respectively) compare to control group. L-arginine significantly increased serum vascular endothelial growth factor (VEGF) concentration (353.01 ± 7.03 vs. 100.5 ± 6.61 pg/ml; $P < 0.05$), however, did not change myocardial capillary density.

Conclusion: Although L-arginine alters some serum angiogenic factors, either L-arginine or L-NAME could not improve myocardial capillary density in normal rats.

Key Words: Angiogenesis, L-arginine, L-NG-Nitroarginine Methyl Ester, myocardium

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INTRODUCTION

Diabetes is the 5th global leading cause of death.[1] Among diabetes complications, cardiovascular diseases cause most deaths due to coronary artery complications.[2]

Angiogenesis, the process of formation of new vessels from existing ones in tissues, is predominantly seen during in uterus, and on occasions, after birth and even in adults. Obvious examples of angiogenesis after birth are in physiological cases like healing of the wounds and menstrual cycle, and in pathological conditions such as diabetic retinopathy, endometriosis, and blood vessel growth in tumors.

There are several factors involve in creation of new vessels including nitric oxide (NO) and vascular endothelial growth factor (VEGF).[3,4] Angiogenesis plays an important role in physiological adaptation with ischemia, but in pathological conditions such as obesity, hypertension, and diabetes mellitus, it may be sluggish or insufficient. Today, angiogenesis

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therapy is used for wound healing, inflammatory diseases, ischemic heart disease, peripheral artery diseases, myocardial infarction (MI), cancer, and diabetic retinopathy.[5] Previous studies have shown that NO regulates VEGF, and causes migration and differentiation of endothelial cells that turn into capillary.

This study aims to investigate the effects of NO agonists (L-arginine) and antagonists (L-NAME) on coronary angiogenesis in heart and serum NO and VEGF levels in normal rats.

MATERIALS AND METHODS

Animals and experimental groups

A total of 18 male rats, purchased from the Pasteur Institute, spent a week at the Animal Physiology room to adjust to new condition. They were then randomly divided into three equal number groups. The first and the second groups received intraperitoneal injection of L-NG-Nitroarginine Methyl Ester (L-NAME) (10 mg/kg),[6] and L-arginine (50 mg/kg),[7] respectively, and the third group received normal saline. The animals were kept under 12 h light/dark cycle and normal room temperature conditions with unlimited access to food and water. After 3 weeks, the rats were anesthetized with a 75 mg/kg dose of ketamine and blood samples were taken for assessment of serum concentrations of NO and VEGF. To investigate the effect of L-NAME and L-arginine on coronary angiogenesis, the rats were sacrificed with a high dose of anesthetic drug, their hearts were removed and the tips of hearts were placed in 10% formalin.

Measuring serum VEGF level

ELISA (Enzyme-Linked Immuno Sorbent Assay) method was used to measure serum VEGF concentration (R and D systems, Minneapolis, USA) with Recombinant Standard and Reagents.[8]

Evaluation of capillary density in the hearts

The immunohistochemical (IHC) technique was used to assess capillary density and angiogenesis. IHC was performed with the use of anti-CD31 antibody (rat-monoclonal antibody against murine CD_{31}) as endothelial cell indicator. Ten microscopic fields were randomly selected from each tissue preparation and capillaries with positive CD31 cells were counted. Capillary density was expressed as the number of capillaries per square millimeter.

Measuring serum NO level

Measurement of serum NO level was done by Promega kit with Griess reagent method in which NO metabolites are measured by existing reagents in the kit (sulfanilamide and N-1-naphthylethylenediamine dihydrochloride (NED) solutions) using spectrophotometer and compared with the standard curve.

Analysis of the data

The Statistical Product and Service Solutions (SPSS) 16 software was used to analyze the data. Changes in serum factors before and after experiment were assessed by paired *t*-test, and changes between groups were compared using one-way analysis of variance (ANOVA). The level of significance was considered as $P < 0.05$.

RESULTS

Serum NO level

Before the experiment, the differences in the NO serum levels in the three groups were not significant ($P > 0.05$). By the end of the experiment, serum NO level in normal rats was 6.45 ± 0.44 μmol/lit. L-arginine caused an increase and L-NAME caused a decrease in the NO serum level, however, the difference was not significant ($P > 0.05$) [Figure 1].

Serum VEGF level

At the beginning of the experiment, serum VEGF level in the three groups was not significantly different between groups ($P > 0.05$), but at the end of study, serum level of VEGF in the control group was 78.22 ± 5.14 pg/ml. L-arginine significantly increased this level to 353.01 ± 7.03 pg/ml ($P < 0.05$), while L-NAME could not alter significant differences in serum VEGF level [Figure 2].

The effect of L-arginine and L-NAME on angiogenesis of the heart

Neither L-arginine nor L-NAME made a significant difference in capillary density and coronary angiogenesis [Figure 3]. Example of IHC slides

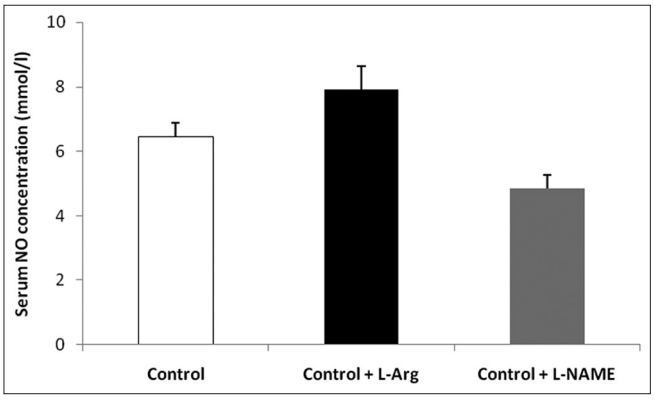

Figure 1: Serum NO level (μmol/lit) in experimental groups at the end of study

prepared from the hearts of animals are presented in Figure 4.

DISCUSSION

The results of this study showed that administration of L-arginine increased NO serum level, but L-NAME decreased it. Also, L-arginine increased serum VEGF level, however, L-arginine and L-NAME could not significantly change angiogenesis of the heart.

Endothelial cells use L-arginine and oxygen molecules to produce NO by NO synthase enzyme. This enzyme converts L-arginine to NO and L-Citrulline.[9] In this experiment, L-arginine caused an increase in NO serum level. The beneficial effects of L-arginine on cardiovascular system are indicated in various studies. Previous studies have shown that L-arginine causes an increase in vasodilatory function of endothelial cells in coronary arteries and aorta in diabetic rats.[10,11] NO has important and beneficial effects on cardiovascular system including improvement of endothelial function, reduced proliferation of smooth muscle cells, atherosclerosis, plaque collection, and, most importantly, increasing angiogenesis. Therefore, agents that can increase or decrease NO are expected to have stimulatory or inhibitory effects on angiogenesis.

Another important and well-known factor in proliferation of endothelial cells is VEGF.[12] Although most studies have indicated the role of angiogenesis in the retina of diabetic patients,[13,14] fewer studies have addressed the potential role of VEGF in vascular disorders of other tissues. In this study, serum VEGF level after using L-arginine significantly increased in normal rats, but L-NAME failed to make any significant differences.

Angiogenesis is the proliferation and migration of endothelial cells and formation of new capillaries from preexisting vessels. Several factors have an impact on the process of angiogenesis. Various studies have indicated the important role of NO in an angiogenesis process (see John *P* Cooke review).[15] NO has an important role in the inhibition of apoptosis, proliferation, and migration of endothelial cells,[16,17] it can also increases the synthesis and releases of VEGF from vascular cells.[18] There are interactions between NO and VEGF. VEGF expression causes an increase in NO synthesis and NO biosynthesis from cultured endothelial cells.[19] Pre-incubation of endothelial cells with L-arginine increases basal NO and VEGF-stimulated NO.[15] Use of L-NAME as NO antagonist prevents the formation of new vessels[20] and causes inhibition of angiogenic effects of the *P* matter or β-TGF.[17] In rabbit cornea, the use of L-NAME blocked VEGF-induced angiogenesis.[21]

This study showed that coronary angiogenesis in the rats, who were receiving L-arginine and L-NAME,

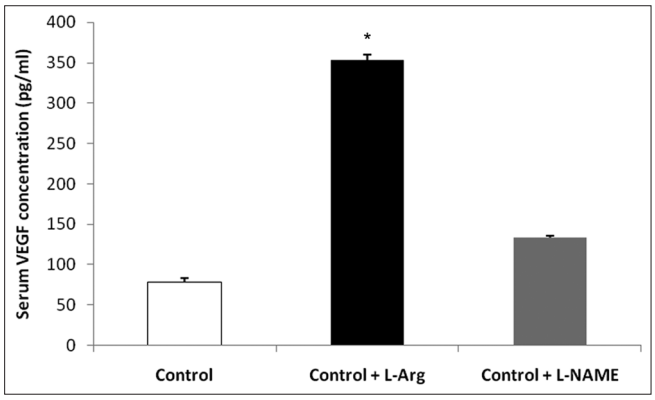

Figure 2: Serum VEGF level (pg/ml) at the end of study (*P < 0.05 compared with other groups)

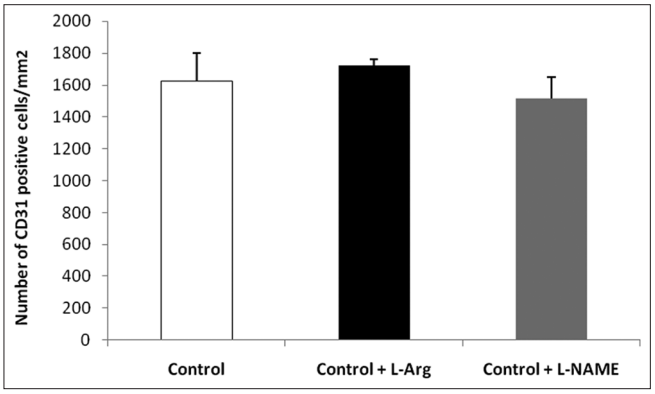

Figure 3: Capillary density in the heart muscles (expressed as CD31 positive cells/mm^2)

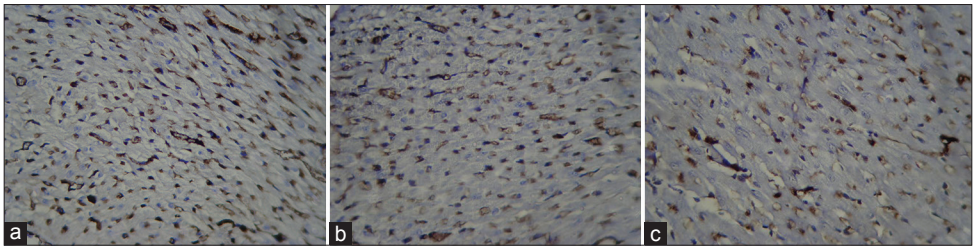

Figure 4: Microscopic view of the heart muscle stained with anti-CD31 antibody using immunohistochemistry method in (a) Control, (b) recipients of L-arginine, (c) recipients of L-NAME. Images are with magnification of ×400

did not have a significant difference compared with saline Group E, which means that despite an increase in serum NO and VEGF concentrations, L-arginine was unable to affect angiogenesis of the heart. Although, doses of medications used in this experiment were selected according to those used in previous studies, perhaps higher doses of L-arginine are required for its angiogenic effects on the heart muscle. A major limitation in this study was the investigation of L-arginine and L-NAME effects on angiogenesis in rat hearts without ischemia, and since hypoxia-induced ischemia is the most important stimulus in angiogenesis, it may be better to investigate the effects of L-arginine and L-NAME in cardiac ischemia models in future studies.

CONCLUSION

Although L-arginine causes changes in the serum levels of some of the angiogenic factors in normal rats, its use as NO precursor and use of L-NAME as NO syntheses enzyme inhibitor cannot affect a significant changes in coronary angiogenesis. Further studies are needed in different models in hypoxia settings to better assess the effects of these medications on angiogenesis.

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