DO L-ARGININE AND L-NAME ALTER CORONARY VASCULAR AND AORTIC ENDOTHELIAL PERMEABILITY IN NORMAL- AND HIGH-CHOLESTEROL-FED RATS?

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Abstract

INTRODUCTION: The objective of this study was to evaluate the effect of L-arginine (L-Arg) and L-NAME on coronary vascular and aortic endothelial permeability in normocholesterolemic (NC) and hypercholesterolemic (HC) rats.

METHODS: Forty-eight male rats were divided into NC and HC groups and each group was divided into L-Arginine-treated, L-NAME-treated and control subgroups. L-Arg (2.25%) and L-NAME (0.75 mg/ml) were dissolved in drinking water and control groups received tap water. After 8 weeks, endothelial permeability was assessed by using the Evans Blue (EB) dye method.

RESULTS: Aortic endothelial permeability was significantly higher in HC group compared to NC group (15.1±0.7 vs. 7.7±0.8 μgEB/g tissue, respectively; $P<0.05$). L-Arg and L-NAME treatment decreased aortic endothelial permeability in HC animals (L-Arg: 8.4±0.4 & L-NAME: 10.8±0.6 vs. 15.1±0.7 μgEB/g tissue, respectively; $P<0.05$). There was no significant difference in endothelial permeability in coronary circulation between HC and NC groups and L-Arg and L-NAME did not alter endothelial permeability.

CONCLUSION: It seems that L-Arg and L-NAME have different effects on endothelial permeability based on physiological and pathological conditions and type of vessel.

Keywords: L-Arginine, L-NAME, nitric oxide, endothelium, permeability.

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Introduction

Endothelium is a single layer of vascular surface which consists of approximately 1×10^{13} cells with an estimated mass of 1.5 kg. Endothelial cells act as the source of numerous vasoactive factors which regulate the control of endothelial permeability, vascular tone, leukocyte adhesion, vascular smooth muscle cell growth and platelet aggregation.

Endothelial dysfunction is a marker of vascular disease and plays an important role in the initiation and progression of disease. Much interest in the functioning of endothelial cells has originated from the concept that endothelial cell injury is involved in many disease processes, including atherosclerosis, hypertension, diabetes mellitus, hypertrophic cardiomyopathy or viral myocarditis. The earliest changes that precede formation of atherosclerotic lesions take place in the endothelium.[1] Injury to endothelial cells or endothelial dysfunction is considered as one of the critical events in the development of fatty streaks and plaque formation.[2] These damages include increased monocyte adherence, permeability to monocytes/ macrophages and lipoprotein particles and accumulation in the subintimal space, smooth muscle cell migration and proliferation, and platelet activity and aggregation.[1]

Nitric oxide (NO) is a potent vasodilator and performs a pivotal role in the normally functioning cardiovascular system. NO is synthesized by endothelial cells from L-arginine and molecular oxygen. NO synthase catalyzes the reaction which converts L-arginine

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to citrulline and NO. Vasodilatation is the best documented activity of NO in the cardiovascular system. This action led to the discovery of endothelium-derived relaxing factor (EDRF) 20 years ago.[3] NO also prevents the proliferation of vascular smooth muscle cells[4] playing a key role in the narrowing the lumen of blood vessels in coronary artery disease;[5,6] it inhibits platelet activation and adhesion,[4] reduces leukocyte adhesion,[7] and inhibits oxidation of free fatty acids and low-density lipoprotein (LDL) particles.[8] In this study, we investigated the possible role of NO in endothelial permeability in aorta and coronary circulation in normocholesterolemic (NC) and hypercholesterolemic (HC) rats. For this purpose, we used L-Arginine as NO donor and L-NAME as NO synthase inhibitor.

Materials and Methods

Forty-eight male rats were purchased from the Razi Institute of Iran. The animals were housed in 2 cages, under standard animal room conditions of 12-hour light/dark cycle, room temperature (26 °C) and were given free access to food and water. After one week of habituation, blood samples were collected from the retroorbital sinus under anesthesia and stored at -70 ºC for further analysis. Then, the animals were randomly divided into NC and HC groups and each group was divided into three subgroups: L-Arg-treated, L-NAME-treated and control groups as follows:

Group 1: normal diet + usual drinking water
Group 2: normal diet + L-Arg (2.25%)
Group 3: normal diet + L-NAME (0.75mg/ml)
Group 4: high-cholesterol diet (4%) + usual drinking water
Group 5: high-cholesterol diet (4%) + L-Arg (2.25%)
Group 6: high-cholesterol diet (4%) + L-NAME (0.75 mg/ml)

High-cholesterol diet was consisted of cholesterol (4%) as previously described.[9] In groups with L-Arg supplementation (groups 2 and 5), L-Arg (Sigma) was added to drinking water (2.25%).[10] L-NAME-treated animals (groups 3 and 6) were received L-NAME (0.75 mg/kg) dissolved in drinking water.

After 8 weeks, the animals were anaesthetized by intraperitoneal injection of ketamine (75mg/kg). Right common carotid arteries were cannulated. Blood samples were taken and the Evans Blue (EB) dye technique was used to measure coronary vascular and aortic endothelial permeability. This technique is based on the principle that EB dye binds to the intravascular albumin. This technique has been used in previous studies.[11,12] Briefly, the EB (Merck, Germany) diluted in normal saline (20 mg/ml) was administered through the right common carotid artery catheter. After 20 minutes allowing the circulation, the rats were sacrificed. Heart and aorta (from base of heart to renal arteries) were isolated and cleaned from the surrounding connective tissues and were weighed immediately. Then the tissues were placed in formamide solution (heart: 4ml; aorta: 2ml) for 24 hours at room temperature for EB dye extraction. The extracted amount of EB was determined by spectrophotometer at 620 nm wavelength. The results were plotted on a standard curve of EB in 0.2 to 10 μg/ml formamide using the regression analysis to find the relationship between EB concentration and optical density. Concentration of EB in these tissues was expressed in μg/g (μg/g) tissue.

SPSS 11 was used for data analysis. Data are reported as mean value ± SEM (standard error of mean). Comparison of data between the groups was performed using the ANOVA. P values less than 0.05 were considered statistically significant.

Results

Results showed that plasma cholesterol, triglyceride, LDL and the ratio of LDL to HDL (high-density lipoprotein) were significantly higher in HC animals compared to normal-diet groups (data not shown). L-Arg supplementation, not L-NAME, decreased plasma cholesterol level in HC rats. Figure 1 illustrates that aortic endothelial permeability was significantly higher in HC animals compared to NC animals (15.1±0.7 vs. 7.7±0.8 μg EB/g tissue, respectively; $P<0.05$). L-Arg and L-NAME did not alter aortic endothelial permeability in NC rats. However, in HC rats, both L-Arg and L-NAME significantly decreased aortic endothelial permeability ($P<0.05$) (Figure 1). Endothelial permeability in coronary circulation was not significantly different between NC and HC rats (2.8±0.3 vs. 2.5±0.6 μg EB/g tissue, respectively; $P>0.05$) and remained unaltered by L-Arg and L-NAME (L-Arg: 2.5±0.3; L-NAME: 2.3±1.4 μg EB/g tissue) (Figure 2).

Discussion

The aim of this study was to evaluate the effect of NO (using L-Arg: NO donor; L-NAME: NO antagonist) on endothelial permeability in aorta and coronary circulation. Our results showed that L-Arg and L-NAME decreased aortic endothelial permeability in HC rats, however, coronary vascular permeability was unaffected by these drugs.

It has been proposed that atherosclerosis results from arterial response to chronic injury to endothelium.[1]

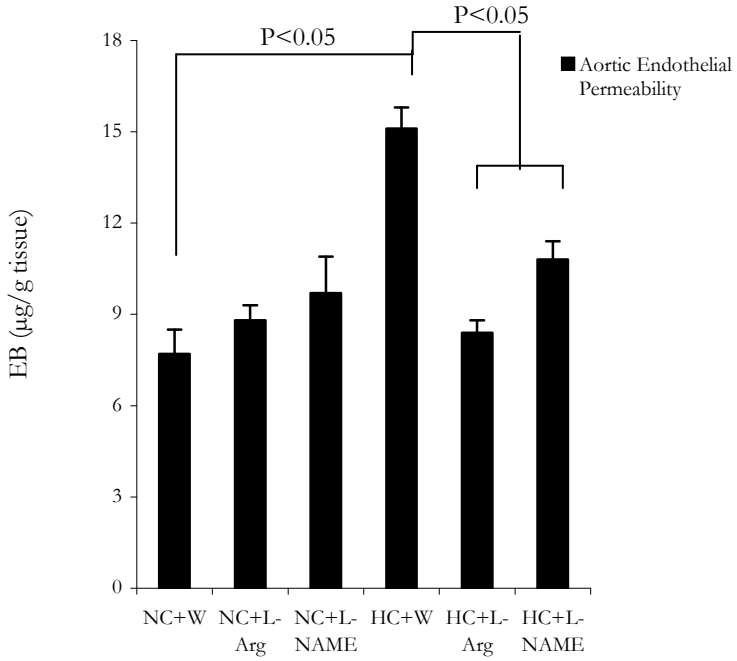

FIGURE 1. Changes of aortic endothelial permeability during L-Arg and L-NAME treatment.
(NC: normocholesterolemic; HC: hypercholesterolemic; W: water; L-Arg: L-arginine; mean±SEM).

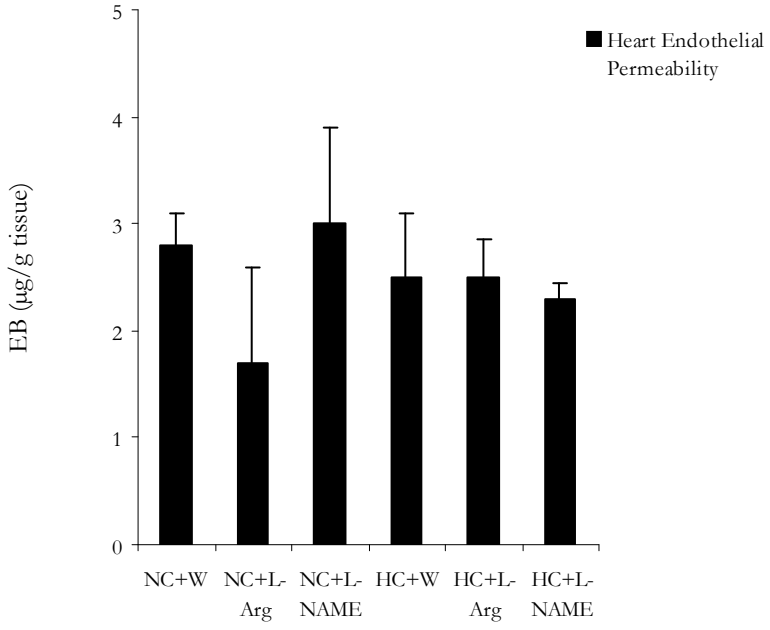

FIGURE 2. The effect of L-Arg and L-NAME on coronary vascular permeability.
(NC: normocholesterolemic; HC: hypercholesterolemic; W: water; L-Arg: L-arginine; mean±SEM; P>0.05).

Changes in injured endothelium lead to disruption of its permeability characteristics, thus permitting the interaction between elements of the blood and the arterial wall, especially atherogenic lipoproteins.[13] We

demonstrated that aortic endothelial permeability in HC rats was higher than in NC animals. In a recent study which supports our results, Lamack et al.[14] found that endothelial permeability to albumin was greater in hypercholesterolemic pigs than in those on a normal diet. Our results also showed that L-Arg supplementation did not alter aortic endothelial permeability in NC animals; however, it reduced endothelial permeability in HC animals. It has been demonstrated that acute or chronic L-Arg supplementation in HC animals has beneficial effects on vascular function.[15,16] Since there is a link between endothelial permeability and progression of atherosclerosis,[17] in this study, the reduced aortic endothelial permeability after L-Arg supplementation in HC animals may have contributed to the protective effect of L-Arg. However, there are conflicting reports regarding the effect of NO inhibition on endothelial permeability.[7,18,19] Some studies have reported that NOS inhibitor L-NAME increased microvascular leakage to protein in various tissues including stomach, intestine, liver, spleen, pancreas, kidney, and lung;[7,18] whereas in other studies, NO inhibitor decreased microvascular permeability of venules.[19] We found that aortic endothelial permeability in L-NAME-treated HC animals was significantly lower than in the control group, however, L-NAME did not affect coronary vascular permeability. These different results may be related to in-vitro or in-vivo study protocols, type of tissues or vessels and/or endothelial or epithelial studies.

L-Arg and L-NAME did not alter coronary vascular and aortic endothelial permeability in normal animals but decreased aortic endothelial permeability in HC animals; there is apparently a difference in the effect of NO on endothelial permeability in physiological and pathological conditions and the physiological level of NO is essential for endothelial permeability in hypercholesterolemic conditions. The exact mechanism of this response and evaluation of the role of NO in other pathological conditions such as hypertension and diabetes should be addressed in future studies.

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