PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 7 (1): 126-129, 2008
ISSN 1680-5194

Low Dose of L-Arginine Does Not Change Endothelial Permeability of Aorta and Coronary Arteries in Rat

Mehdi Nematbakhsh, Zahra Heydarzadeh, Lila Borjian and Shaghayegh Haghjooyejavanmard
Department of Physiology and Applied Physiology Research Center,
Isfahan University of Medical Sciences, Isfahan, Iran

Abstract: Nitric Oxide (NO) donors such as L-arginine are a candidate to restores endothelial function. NO itself may change endothelial permeability which is an important factor to control vascular homeostasis. So, the effect of L-arginine on aorta and coronary arteries permeability was the objective of this study. 20 male rats were randomly divided into two groups of oral administrated L-arginine (4 g/l) and control. After five weeks of experiment, the serum level of nitrite by Griess reagent method and direct blood pressure were measured. The aorta and coronary arteries permeability were determined by extravasation of injected Evan Blue dye. The results indicate that after the 5 weeks of experiment, L-arginine increases mean arterial pressure (group I: 157±6, group II: 108.2±10.1 mmHg; $p<0.05$) and the serum level of nitrite(group I: 35.69±6.88, group II: 23.21±2.72 µmol/l; $p<0.05$) significantly. No statistical differences were observed in endothelial permeability of aorta (group I: 66.6±5.4, group II: 85.23±10.64 µg/g.tissue) and coronary arteries (group I: 28.2±3.17, group II: 22.83±1.39 µg/g. tissue) between the groups. Low dose L-arginine may increases the level of nitrite; last metabolite of NO in plasma, but its effect on aorta and coronary arteries permeability is not certain.

Key words: L-arginine, Nitric Oxide, Endothelial permeability, aorta, Coronary artery, blood pressure

Introduction

The endothelium is an important and interesting monolayer that covers the blood vessels surfaces. In an adult human, the surface of Endothelial Cell (EC) is composed of approximately 1 to 6×10^{13} cells, weighs approximately 1kg and covers a surface area of approximately 1 to $7m^2$. The EC monolayer, separating the blood from all other tissues, controls the movement of cells, liquid and small and large solutes (e.g., plasma proteins) from the blood to interstitial space (Cines *et al*., 1998; Galley and Webster, 2004), so it not an inactive barrier, but it has an important gate keeping role in the body with important metabolic and synthetic activities. The low permeability of the endothelium under basal conditions is crucial for maintaining vascular homeostasis. The EC generates Nitric Oxide (NO). NO maintains basal tone by relaxing vascular smooth muscle cells through the binding of NO to the heme prosthetic group of guanylyl cyclase and it inhibits platelet adhesion, activation, secretion and aggregation. In addition, NO inhibits leukocyte adhesion to the endothelium and inhibits smooth muscle cell migration and proliferation (Cines *et al*., 1998; Galley and Webster, 2004; Luscher and Barton, 1997). However, the role of NO in regulating vascular permeability was subject of controversial researches in the literature (Hinder *et al*., 1997; van Nieuw Amerongen and van Hinsbergh, 2002; Rumbaut and Huxley, 2002; Filep *et al*., 1993; Baldwin *et al*., 1998; Addicks *et al*., 1995; Nematbakhsh *et al*., 2002). Inhibition of NO synthesis by NG-nitro-L-arginine methyl ester (L-NAME) increases venular permeability (Baldwin *et al*., 1998), while the NO-donors did not enhance microvascular permeability (Addicks *et al*., 1995). In general, the endothelial permeability disorder occurred in condition of endothelial dysfunction which is characterized by reduced bioavailability of NO (Napoli *et al*., 2006). One straightforward approach to increase NO bioavailability is providing additional substrate for NO synthase (Megson and Webb, 2002). L-arginine is the substrate of endothelial NO synthase (eNOS) and the main precursor of NO in the vascular endothelium. Data from numerous studies imply that L-arginine supplemen-tation restores endothelial function in several disease states associated with endothelial dysfunction (Boger *et al*., 1997; Cooke and Tsao, 1997; Gornik and Creager, 2004; Hayashi *et al*., 2005; Loscalzo, 2003). Now, due to importance of cardiovascular diseases prevention and the role of L-arginine in the treatment process, understanding the role of L-arginine as a NO donor in permeability of aorta and coronary arteries is important and in this study, we addressed the possible role of NO constitutively released by eNOS, using L-arginine in regulating basal endothelial permeability in aorta and coronary arteries.

Materials and Methods

Animals: 20 male rats weighing 268±6.5g were used in this experiment. All animals were housed four per cage at room temperature with 12 h light/dark cycle. The animals were fed with standard chow diet with free access to drinking water.

Table 1: Systolic, Diastolic and Mean Arterial Pressures (SP,DP, MAP) in two groups of experimental animals. Data are expressed as mean±SE

Groups	n	SP (mmHg)	DP (mmHg)	MAP (mmHg)
I	11	175.1±7.9*	148±5.5*	157±6*
II	9	127.3±11.2	98.7±9.6	108.2±10.1

*Significant difference from other group ($p<0.05$); Significant difference from other group ($p<0.05$)

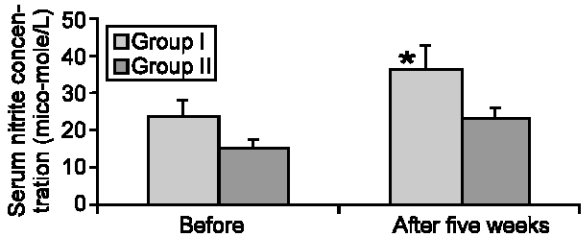

Fig. 1: Serum nitrite concentrations (µmole/l) in two groups of study before and after the five weeks of experiment. There is significant difference between the groups after the five weeks ($p<0.05$)

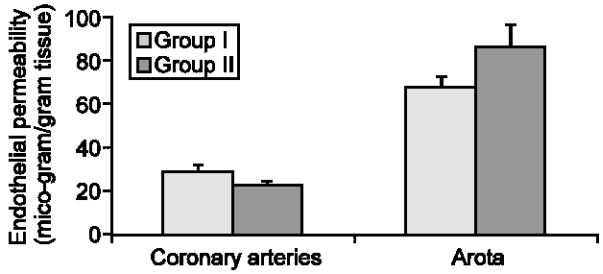

Fig. 2: The endothelial permeability (express as quantitative extravasation of EB) of aorta and coronary arteries in two groups of experiments after the five weeks. There are no significant differences between the groups either in aorta nor coronary arteries

Experimental design: The animals were randomly divided into two groups. The blood samples were taken and centrifuged in 3000 C/S for 12 min. The supernatant was poured in Eppendorff tubes and kept in-70°C until analysis. Then, the first group was subject to receive 4 g/l of L-arginine (Sigma) in dinning water. The group II was received only dinning water as control. After five weeks, the animals were anesthetized with an intraperitoneal injection of ketamine. A polyethylene catheter was inserted into the right common carotid artery and direct Systolic Pressure (SP) and Diastolic Pressure (DP) were measured by physiograph (Bioscience, England). Mean Arterial Pressure (MAP) was calculated by; MAP = (SP+2DP)/3. Then, blood samples were taken, centrifuged and kept in separate Eppendorff tubes at-70°C for measurement of serum nitrite concentrations.

Serum Nitrite measurement: Serum nitrite concentration (the last metabolite of NO) was determined by Griess reagent method (Promega Corp, Madison, USA). Briefly, serums were added into wells (96-well enzymatic assay plate). A sulfanilamide solution was added to all experimental samples and after incubation, N-1-naphtylethylenediamine dihydrochloride solution was added. Then, absorbance was measured by a micro reader (Hyperion, USA) in 520 nm wavelength. The samples nitrite concentration were determined by comparison to nitrite standard reference curve. The limit detection was 2.5 µM nitrite.

Measurement of endothelial permeability: Coronary and aortic endothelial permeability were measured in two groups of animals. Endothelial permeability was determined by extravasation of injected Evan Blue dye (EB) as previously described (Hulthen *et al.*, 1996; St-Pierre *et al.*, 2004). Briefly, EB diluted in normal saline was administrated through the catheter (20 mg/kg weight). After 20 minutes, the animals were sacrificed. Heart and aorta were isolated and cleaned from surrounding connective tissues. In order to obtain the EB dye extraction from the heart and the aorta, they were then weighted immediately and put into form amide solution (aorta: 2 mL, heart: 5 mL) for 24 hours at 80°C. The extracted amount of EB diluted in form amide was determined by spectrophotometer (Secomam, France) at 623 nm wavelength. The EB concentration in these tissues was express in µg/g weight of tissue.

Statistical analysis: Data are reported as mean±SEM. The Student t-test was used for comparison of data between the groups. p-value less than 0.05 was considered statistically significant.

Results

Blood pressure: The data for the blood pressures are shown in Table 1. Comparison of blood pressure showed that there was significant difference between two groups ($p<0.05$).

Serum nitrite concentration: Serum nitrite concentrations are shown in Fig. 1. Results showed that the level of nitrite was not significantly different between the groups at the beginning of study (23.45±4.79 vs 14.98±2.35 µmol/l), but in L-arginine group, serum nitrite concentration was increased significantly after five weeks (35.69±6.88 vs. 23.21±2.72 µmol/l; $p<0.05$).

Endothelial permeability: In two groups of study, there were no significant difference in endothelial permeability (express as quantitative extravasation of EB) of aorta (66.6±5.4 vs 85.23±10.64 µg/g.tissue) and coronary

arteries (28.2±3.17 vs 22.83±1.39 µg/g.tissue). These data are shown in Fig. 2.

Discussion

The main purpose of this study was to investigate the role of L arginine in aorta and coronary arteries permeability. L-arginine supplementation has been frequently appreciated as a restoring factor for endothelial integrity and function because of its positive effect on NO production. The results indicate that L-arginine supplementation (4 mg/ml) in drinking water increases systemic blood pressure, but it has no effect on endothelial permeability of aorta and coronary arteries. This result is much different and controversial from other studies. The correction of hypertension by L-arginine was mentioned in the literature (DE Fatima Cavanal *et al*., 2007; Palloshi *et al*., 2004; Gouvea *et al*., 2003; Penttinen *et al*., 1998; Gokce, 2004). It is also reported that L-arginine might provide additional blood flow (Ohta, 2007) and it increases contraction of the rat portal vein (Shimamura *et al*., 2003). It seems that L-arginine's effects not only are depended on dose and duration used, but also are related to the level of NO formation from endothelial barrier. NO has positive effect on myocardial contraction which is mediated by cGMP (Kojda, 1997). In one side, there is many evidence that endogenous NO increases micro vascular permeability and molecular inhibition of eNOS reduces eNOS-regulated permeability and on the other side reduction of endothelial permeability by lower NO also are reported (Hinder *et al*., 1997; van Nieuw Amerongen and van Hinsbergh, 2002; Rumbaut and Huxley, 2002; Filep *et al*., 1993; Baldwin *et al*., 1998; Addicks *et al*., 1995; Nematbakhsh *et al*., 2002; Baldwin *et al*., 1998; Kubes, 1993). Other reports indicate that, L-arginine may increase vascular permeability (Lundblad and Bentzer, 2007; Motlekar *et al*., 2006) and NO has no effect on myocardial capillary permeability (Hansen and Haunso, 1995). Therefore, it seems that the effect of L-arginine on vessel permeability followed by formation of NO is different from one situation to another condition and from one organ to another organ (Anderson, 1999; Mayhan, 1993; Kubes, 1993; Jeremy *et al*., 1996; Radomski and Salas, 1995; Chen *et al*., 2003; Susic *et al*., 2001; Artigues *et al*., 2000). Our result for endothelial permeability is consistent with the results of other studies in which intravenous or oral L-arginine had no effect on endothelial function in healthy patients.

It is concluded that oral administration of low dose L-arginine may increases the level of nitrite; last metabolite of NO in plasma, but its effect on aorta and coronary arteries permeability is not certain.

Acknowledgment

This study (Grant # 83165) was supported by Isfahan University of Medical Sciences, Isfahan, Iran.

References

Addicks, K., S. Arnhold, P. Fasching, C. Hoffmann and W. Bloch, 1995. Nitric oxide mediates microvascular permeability in the isolated perfused rat mesentery? Agents Actions Suppl., 45: 139-44.

Anderson, T.J., 1999. Assessment and treatment of endothelial dysfunction in humans. J. Am. Coll. Cardiol., 34: 631-8.

Artigues, C., V. Richard, C. Roussel, F. Lallemand, J.P. Henry and C. Thuillez, 2000. Increased endothelium--monocyte interactions in salt-sensitive hypertension: effect of L-arginine. J. Cardiovasc Pharmacol., 35: 468-73.

Baldwin, A.L., G. Thurston and H. al Naemi, 1998. Inhibition of nitric oxide synthesis increases venular permeability and alters endothelial actin cytoskeleton. Am. J. Physiol., 274: H1776-84.

Boger, R.H., S.M. Bode-Boger, R.P. Brandes, L. Phivthong-ngam, M. Bohme and R. Nafe, 1997. Dietary L-arginine reduces the progression of atherosclerosis in cholesterol-fed rabbits: comparison with lovastatin. Circulation, 96: 1282-1290.

Cines, D., E. Pollak and Buckc, 1998. Endothelial in physiology and in phathophysiology of vascular disorder. Blood, 91: 3527-3561.

Chen, J., P. Kuhlencordt, F. Urano, H. Ichinose, J. Astern and P.L. Huang, 2003. Effects of chronic treatment with L-arginine on atherosclerosis in apoE knockout and apoE/inducible NO synthase double-knockout mice.Arterioscler Thromb Vasc Biol., 23: 97-103.

Cooke, J.P. and P.S. Tsao, 1997. Arginine: a new therapy for atherosclerosis? Circulation, 95: 311-312.

DE Fatima Cavanal, M., G.N. Gomes, A.L. Forti, S.O. Rocha, M.D. Franco, Z.B. Fortes and F.Z. Gil, 2007. The Influence of l-Arginine on Blood Pressure, Vascular Nitric Oxide and Renal Morphometry in the Offspring from Diabetic Mothers. Pediatr. Res., 62: 145-150.

Filep, J.G., E. Földes-Filep and P. Sirois, 1993. Nitric oxide modulates vascular permeability in the rat coronary circulation. Br. J. Pharmacol., 108: 323-6.

Galley, H.F. and N.R. Webster, 2004. Physiology of the endothelium. BRJA., 93: 105-13.

Gokce, N., 2004. L-arginine and hypertension. J. Nutr., 134: 2807S-2811S; discussion 2818S-2819S.

Gornik, H.L. and M.A. Creager, 2004. Arginine and endothelial and vascular health. J. Nutr., 134: 2880S-2887S.

Gouvea, S.A., M.R. Moyses, N.S. Bissoli, J.G. Pires, A.M. Cabral and G.R. Abreu, 2003. Oral administration of L-arginine decreases blood pressure and increases renal excretion of sodium and water in renovascular hypertensive rats. Braz. J. Med. Biol. Res., 36: 943-9.

Hansen, P.R. and S. Haunso, 1995. Effects of nitric oxide synthase inhibition on myocardial capillary permeability and reactive hyperaemic response. Cardiovasc Res., 29: 862-6.

Hayashi, T., P.A. Juliet, H. Matsui-Hirai, A. Miyazaki, A. Fukatsu and J. Funami, 2005. l-Citrulline and l-arginine supplementation retards the progression of high-cholesterol-diet-induced atherosclerosis in rabbits. Proc. Natl. Acad. Sci. USA, 102: 13681-13686.

Hinder, F., M. Booke, L.D. Traber and D.L. Traber, 1997. Nitric oxide and endothelial permeability. J. Appl. Physiol., 83: 1941-6.

Hulthen, U.L., Z. Cao, J.R. Rumble, M.E. Cooper and C.I. Johnston, 1996. Vascular hypertrophy and albumin permeability in a rat model combining hypertension and diabetes mellitus. Effects of calcium antagonism, angiotensin converting enzyme inhibition and angiotensin II-AT1-receptor blockade. Am. J. Hypertens., 9: 895-901.

Jeremy, R.W., H. McCarron and D. Sullivan, 1996. Effects of dietary L-arginine on atherosclerosis and endothelium-dependent vasodilatation in the hypercholesterolemic rabbit. Response according to treatment duration, anatomic site and sex. Circulation., 94: 498-506.

Kojda, G., K. Kottenberg and E. Noack, 1997. E. Inhibition of nitric oxide synthase and soluble guanylate cyclase induces cardiodepressive effects in normal rat hearts. Eu. J. Pharmacol., 334: 181-90.

Kubes, P., 1993. Nitric oxide-induced microvascular permeability alterations: a regulatory role for cGMP. Am. J. Physiol., 265: H1909-15.

Loscalzo, J., 2003. Adverse effects of supplemental L-arginine in atherosclerosis: consequences of methylation stress in a complex catabolism? Arterioscler Thromb. Vasc. Biol., 23: 3-5.

Lundblad, C. and P. Bentzer, 2007. Effects of L-arginine on cerebral blood flow, microvascular permeability, number of perfused capillaries and brain water content in the traumatized mouse brain Microvasc Res., 74: 1-8.

Luscher, T. and M. Barton, 1997. Biology of the endothelium. Clin. Cardiol., 20: s3-s10.

Mayhan, W.G., 1993. Role of nitric oxide in leukotriene C4-induced increases in microvascular transport. Am. J. Physiol., 265: H409-14.

Megson, I.L. and D.J. Webb, 2002. Nitric oxide donor drugs: current status and future trends. Expert Opin. Investig. Drugs, 11: 587-601.

Motlekar, N.A., K.S. Srivenugopal, M.S. Wachtel and B.B. Youan, 2006. Modulation of gastrointestinal permeability of low-molecular-weight heparin by L-arginine: *in-vivo* and *in-vitro* evaluation. J. Pharm. Pharmacol., 58: 591-8.

Napoli, C., F. de Nigris, S. Williams-Ignarro, O. Pignalosa, V. Sica and L.J. Ignarro, 2006. Nitric oxide and atherosclerosis: an update. Nitric Oxide, 15: 265-279.

Nematbakhsh, M., P. Hayat-Davoodi, P. Rajabi and S.H. Samarian, 2002. The effect of estrogen on endothelial permeability of aorta and the level of serum nitrite concentration in cholesterol-fed ovariectomized rabbit. Iran Biomedical. J., 6: 77-82.

Ohta, F., T. Takagi, H. Sato and L.J. Ignarro, 2007. Low-dose L-arginine administration increases microperfusion of hindlimb muscle without affecting blood pressure in rats. Proc. Natl. Acad Sci. USA, 23: 1407-11.

Palloshi, A., G. Fragasso, P. Piatti, L.D. Monti, E. Setola, G. Valsecchi, E. Galluccio, S.L. Chierchia and A. Margonato, 2004. Effect of oral L-arginine on blood pressure and symptoms and endothelial function in patients with systemic hypertension, positive exercise tests and normal coronary arteries. Am. J. Cardiol., 93: 933-5.

Penttinen, J., J. Toyry, S. Eerikainen, H. Litmanen, T. Rinkinen and E. Lansimies, 1998. Does plasma L-arginine level play a role in regulation of blood pressure during exercise and autonomic nervous function? Clin. Physiol., 18: 539-43.

Radomski, M.W. and E. Salas, 1995. Nitric oxide-biological mediator, modulator and factor of injury: its role in the pathogenesis of atherosclerosis. Atherosclerosis, 118 Suppl: S69-80. Review.

Rumbaut, R.E. and V.H. Huxley, 2002. Similar permeability responses to nitric oxide synthase inhibitors of venules from three animal species. Microvasc Res., 64: 21-31.

Shimamura, K., M. Zhou, M. Toba, S. Kimura, T. Higuchi, H. Kawaguchi, F. Sekiguchi and S. Sunano, 2003. Effects of L-arginine on spontaneous contraction of the rat portal vein. Pflugers Arch., 446: 30-5.

St-Pierre, P., L. Bouffard and P. Maheux, 2004. Rosiglitazone increases extravasation of macromolecules and endothelial nitric oxide synthase in skeletal muscles of the fructose-fed rat model. Biochem. Pharmacol., 67: 1997-2004.

Susic, D., J. Varagic and E.D. Frohlich, 2001. Isolated systolic hypertension in elderly WKY is reversed with L-arginine and ACE inhibition. Hypertension., 38: 1422-6.

van Nieuw Amerongen, G.P. and V.W. van Hinsbergh, 2002. Targets for pharmacological intervention of endothelial hyperpermeability and barrier function. Vascul Pharmacol., 39: 257-72.