JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1999, 50, 4, 653—660

K. HERBACZYŃSKA-CEDRO

POSITIVE AND NEGATIVE OUTCOMES OF L-ARGININE THERAPY IN CARDIOVASCULAR DISEASES

Cardiovascular Laboratory, Research Centre of Experimental & Clinical Medicine, Polish Academy of Sciences, Warsaw

The author reviews controlled clinical investigation on the effectiveness of L-argine in cardiovascular diseases. Positive results were observed in hyperlipidemic subjects and in patients with a critical stage of the peripheral arterial occlusive disease. Patients with stable ischemic heart disease responded to L-argine to some extent, while results of L-arginine therapy in congestive heart failure are inconsistent.Null effects if L-arginine has been documented in essential hypertension.

Key words: *L-arginine therapy, cardiovascular diseases, nitric oxide.*

Dysfunction of the vascular endothelium, characterized by vasoconstriction to the endothelium-dependent vasodilators, is present in most vascular diseases indicating that the production and /or release or availability of endothelium-derived nitric oxide (NO) is impaired. Arterial vasodilator responses to acetylcholine, the „prototype" of endothelium-dependent vasodilators (1) are attenuated, abolished or changed into paradoxic vasoconstiction in the coronary (2, 3) or peripheral (4—7) vascular bed in patients with atherosclerosis (2—4) hyperlipidemia (5), diabetes (6), essential hypertension (7) and heart failure (8).

Impaired endothelial function resulting from NO deficiency is associated with a number of adverse effects in the vascular system, including hypercontractility of the arterial vessels , enhanced platelet adhesiveness, aggregability and proliferation of vascular smooth muscles. All these disturbances are directly related to the atherosclerotic process. Therefore, the correction of endothelial dysfunction might prevent or attenuate the occurence of cardiovascular consequences of atherosclerosis. Therapy widely tested to improve vascular NO activity is the supplementation of L-arginine, the

substrate for NO synthase (NOS), given with the aim to facilitate production of NO and augment endothelium-dependent dilation.

In fact, in asymptomatic young subjects with **hypercholesterolemia**, administration of L-arginine, acute intravenous (5) or chronic dietary (9), improved endothelial function in the peripheral vessels. These studies confirmed earlier experimental observations (10)and extended them to humans. Intracoronary L-arginine has also augmented coronary blood flow response to acetylcholine in hyperlipidemic patients diagnosed for evaluation of chest pain (11). The mechanism whereby L-arginine improves endothelial function in this clinical setting is uncertain, but some possibilities have emerged from subsequent studies. First, exogenous L-arginine may overcome the effects of an endogenous competitive inhibitor of NOS, asymetric dimethylarginine (ADMA), elevated in serum of hypercholesterolemic animals (12) and humans (13) and thereby restore or increase NO synthesis. Second, deficiency of an essential NOS cofactor, tetrahydrobiopterin (BH4) found in hypercholesterolemic subjects (14) may contribute to decreased NO formation and increased degradation.The latter may result from an enhanced generation of oxygen free radicals associated with hyperlipidemia and with functional changes of NOS itself (15). Whether the supplementation of L-arginine increases availability of BH4 and BH4-dependent NO formation remains to be established.

In patients with angiographicaly documented **ischemic heart disease**, acetylcholine evoked paradoxical vasoconstriction in the coronary circulation (2). Subsequently, the occurence of abnormal endothelium-dependent vascular responses was documented not only in the coronary vessels with atherosclerotic changes (16, 17) but also in angiographically normal coronary arteries of patients with only risk factors for coronary atherosclerosis (18). Short-term administration on of L-arginine appeared effective in attenuating or reversing endothelial dysfunction associated with aging (19) or smoking (20). In patients with ischemic heart disease without substantial coronary artery stenosis, long-term (6 months) oral L-arginine supplementation increased coronary blood flow response to acetylcholine and improved symptoms (21).

The question whether L-arginine therapy alleviates exercise-induced myocardial ischemia, seemed of particular clinical importance and was raised by two randomized investigations. In our study (22), 6g L-arginine/day for 3 days improved exercise capacity in patients with stable angina as shown by decreased maximum ST segment depression and delayed appearance of ST depression. In line with these results, Kobayashi *et al.* (23) have documented that acute administration of L-arginine to the patients with stable angina improved recovery from myocardial ischemia reducing the time for ST segment normalization although the threshold of exercise — induced ischemia

was not altered. Beneficial effects were also noted in the patients with intractable angina pectoris (24) to whom no further invasive or pharmacological intervention could be offered. As shown by this pilot study, clinical effects of 3 month oral L-arginine therapy were favourable in these problematic patients. The mechanism by which L-arginine supplementation helped patients with ischemic heart disease might be multifactorial and relate to NO-mediated increase in the coronary and /or peripheral perfusion, attenuation of vasoconstrictor effects of sympathetic stimulation (25), particularly pronounced in patients with endothelial dysfunction (26) and antagonism of endogenous vasoconstrictors. Indeed, the decrease in plasma endothelin concentration was reported after prolonged L-arginine supplementation (21, 27) and might result from an inhibition of endothelin production by NO (28).

Favourable clinical effects of L-arginine were also obtained in patients with **peripheral arterial occlusive disease** in whom 3 week oral treatment prolonged the pain-free and absolute walking distances and improved endothelium--dependent vasodilation of the lower limb (29). Similarly, the patients with advanced peripheral atherosclerosis and critical limb ischemia showed an increase in femoral blood flow paralleled by increased urinary NO_3^- and cGMP excretion (30).

Endothelial dysfunction in **chronic heart failure**, documented by diminished vasodilation to acetylcholine and attenuated hyperemic responses to exercise and ischemia (31) may contribute to the impaired exercise capacity of these patients. Therefore, it was tempting to test the possibility that facilitation of NO production by NO substrate supply may improve vasodilator capacity and produce a clinical benefit. Early results of L-arginine administration to the patients with heart failure were promising: both acetylcholine- and exercise-induced vasodilation improved after intraarterial L-arginine (32). Howewer, subsequent studies on the efficacy of dietary supplementation of L-arginine on endothelium — dependent vasodilation to acetylcholine (33), forearm exercise (27) and on the functional status of the patients with heart failure (27, 33) brought conflicting results showing improvement (27) or no effect (33) with high dosage, long-term, well controlled treatment. The results of our controlled study, still in progress, have also been disappointing : oral L-arginine (9g/day for 7 days) remains ineffective in improving exercise capacity in the patients with congestive heart failure. In the same study, L-arginine therapy does not affect the markers of free radical activity (serum lipid peroxides and thiols, free radical production by leukocytes) known to be increased in heart failure (34). There are several possible reasons for conflicting results of L-arginine in the patients with chronic congestive heart failure. Enhanced peripheral vasoconstriction and altered vascular

structure are pathophysiological characteristics of this syndrome which may lead to endothelium-independent dysfunction. This may be a partial cause for ineffectiveness of vasodilation induced by NO itself and by NO-dependent vasodilator stimuli. The medication may also influence vascular responses and the metabolic fate of high oral doses of L-arginine in the patients with severe congestive heart failure is uncertain (31). Furthermore, if L-arginine administration augments NO biosynthesis and/or NO activity, it may not improve exercise capacity. In fact, it has recently been shown that acute administration of L-arginine to the patients with chronic heart failure increased lower limb reactive hyperaemia but failed to influence objective indices of exercise such as peak oxygen consumption and anaerobic threshold (35).

Patients with **essential hypertension** also present a defective response of the peripheral (36) and coronary (37) arteries to acetylcholine whereas the vasodilator capacity of the vascular system to endothelium-independent relaxing factors is maintained. L-arginine does not alter endothelium-dependent vascular responses in patients with essential hypertension (38) indicating that the defect in the NO system in hypertensive vessels does not seem to be due to decreased availability of NO precursor but to yet undefined abnormality within the endothelial cell. Observations from animal models of hypertension suggested that enhanced generation of superoxide anions may be responsible for increased NO inactivation (39) and this possibility seemed attractive also in humans. In fact, L-arginine increases vasodilation to acetylcholine in patients with essential hypertension pretreated with indomethacin (40) indicating that cyclooxygenase inhibition restores NO-mediated vasodilation. These observations suggest that substances produced by cyclooxygenase pathway(superoxide? endoperoxide ?) inactivate NO. Moreover, endothelium-dependent vasodilation in hypertensive patients was improved by vitamin C (42), supporting the view that superoxide may account for endothelial dysfunction in these patients. However, the possibility of NO inactivation by oxygen free radicals still remains speculative as neither superoxide dismutase, protecting against extracellular NO inactivation (42) nor oxypurinol, protecting from NO inactivation within the endothelial cell (43) improve acetylcholine-induced vasodilation in hypertensive patients. It is important to note that the defect in the NO pathway was also documented in normotensive subjects with genetic predisposition to hypertension. In young healthy individuals with at least one hypertensive parent, vasodilating response to acetylcholine was blunted and increased after L-arginine (44). This effect is at variance with null responses to L-arginine in older patients with established hypertensive disease (38). Whether these differences are due to yet undefined age-related alterations in the L-arginine — NO pathway is speculative and further studies are needed to evaluate the nature of this defect.

In summary, controlled clinical investigations on the effectiveness of L-arginine in cardiovascular diseases provided positive results in hyperlipidemic subjects (5, 9) and in the patients with a critical stage of the peripheral arterial occlusive disease (29). The patients with stable ischemic heart disease responded to L-arginine with improvement of some parameters of exercise test (22, 23) and alleviation of symptoms (24). So far, the results of L-arginine therapy in congestive heart failure are inconsistent (27, 33) and null effect of L-arginine has been documented in patients with essential hypertension (38).

The future directions in which L-arginine treatment seems worth exploring include prevention of atherosclerotic vascular changes in heart transplant recipients and prevention of restenosis after coronary bypass graft surgery and coronary angioplasty. Preliminary data related to these topics are promising (45, 46).

The list of potential mechanisms through which L-arginine therapy exerts beneficial effects in the vascular system increased considerably in recent years. Besides NO-dependent vasodilation, it also includes ADMA antagonism (13), endogenous vasoconstrictor antagonism (21, 28), antiplatelet action (47, 48), stimulation of insulin-dependent NO production (49, 50) antioxidant effect (51) and a non-enzymatic NO generation (52). Whether and to what extent these potential mechanisms contribute to beneficial effects of L-arginine in the particular pathological conditions remains to be investigated.

REFERENCES

1. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; 288: 373—376.
2. Ludmer PL, Selwyn AP, Shook TL *et al.* Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med* 1986; 315: 1046—1051.
3. Otsui S, Nakajima O, Waku S *et al.* Attenuation of acetylcholine — induced vasoconstriction by L-arginine is related to the progression of atherosclerosis. *Am Heart J* 1995; 129: 1094—1100.
4. Anderson TJ, Vehata A., Gerhard M *et al.* Close relation of endothelial function in the human coronary and peripheral circulation. *J Am Coll Cardiol* 1995; 26: 1235—1241.
5. Creager MA, Gallagher SJ, Girerd XJ *et al.* L-arginine improves endothelium — dependent vasodilation in hypercholesterolemic humans. *J Clin Invest* 1992; 90: 1248—1253.
6. Johnstone MT, Creager SJ, Scales KM *et al.* Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation* 1993; 88: 2510—2516.
7. Panza JA, Casino PR, Badar DM, Quyyumi AA. Effect of increased availability of endothelium-derved nitric oxide precursor on endothelium-dependent vascular relaxation in normal subjects and in patients with essential hypertension. *Circulation* 1993; 87: 1475—1481.
8. Kubo SH, Rector TS, Bank AJ, Williams RE, Heifetz SM. Endothelium dependent vasodilation is attenuated in patients with heart failure. *Circulation* 1991; 84: 1589—1596.

9. Clarkson P, Adams MR, Powe AJ *et al.* Oral L-arginine improves endothelium-dependent dilation in hypercholesterolemic young adults. *J Clin Invest* 1996; 97: 1989—1994.
10. Girerd XJT, Hirsch AT, Cooke JP, Creager MA. L-arginine augments endothelium-dependent vasodilation in cholesterol-fed rabbits. *Circ Res* 1990; 67: 1301—1308.
11. Drexler H, Zeiher AM, Meinzer K, Just H. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolaemic patients by L-arginine. *Lancet* 1991; 338: 1546—1550.
12. Yu X, Li Y, Xiong Y. Increase of an endogenous inhibitor of nitric oxide synthesis in serum of high cholesterol fed rabbits. *Life Sci* 1994; 54: 753—758.
13. Boger RH, Bode-Boger SM, Szuba A *et al.* Asymetric dimethylarginine (ADMA): A novel risk factor for endothelial dysfunction. Its role in hypercholesterolemia. *Circulation* 1998; 98: 1842—1847.
14. Stroes E, Kastelein J, Cosentino F *et al.* Tetrahydrobiopterin restores endothelial function in hypercholesterolemia. *J Clin Invest* 1997; 99: 41—46.
15. Pritchard KA, Groszek L, Smalley DM *et al.* Native low-density lipoprotein increases endothelial cell nitric oxide synthase generation of superoxide anion. *Circ Res* 1995; 77: 510—518.
16. Nabel E, Selwyn AP, Ganz P. Large coronary arteries in humans are responsive to changing blood flow: an endothelium-dependent mechanism that fails in patients with atherosclerosis. *J Am Coll Cardiol* 1990; 16: 349—356.
17. Forstermann U, Mugge A, Alheid U, Haverich A, Frolich JC. Selective attenuation of endothelium-mediated vasodilation in atherosclerotic human coronary arteries. *Circ Res* 1988; 62: 185—190.
18. Vita JA, Treasure CB, Nabel EG *et al.* Coronary vasomotor response to acetylcholine relates to risk factors for coronary artery disease. *Circulation* 1990; 81: 491—497.
19. Chauhan A, More RS, Mullins PA, Taylor G, Petch MC, Schofield PM. Aging-associated endothelial dysfunction in humans is reversed by L-arginine. *J Am Coll Cardiol* 1996; 28: 1796—1804.
20. Campisi R, Czernin J, Schoder H, Sayre JW, Schelbert HR. L-arginine normalizes coronary vasomotion in long-term smokers. *Circulation* 1999; 99: 491—497.
21. Lerman A, Burnett JC, Higano ST, McKinley LJ, Holmes DR. Long-term L-arginine supplementation improves small-vessel coronary endothelial function in humans. *Circulation* 1998; 97: 2123—2128.
22. Ceremużyński L, Chamiec T, Herbaczyńska-Cedro K. Effect of supplemental oral L-arginine on exercise capacity in patients with stable angina pectoris. *Am J Cardiol* 1997; 80: 331—333.
23. Kobayashi N, Nakamura M, Hiramori K. Effects of infusion of L-arginine on exercise-induced myocardial ischemic ST-segment changes and capacity to exercise of patients with stable angina pectoris. *Cor Art Dis* 1999; 10: 321—326
24. Blum A, Porat R, Rosenschein U *et al.* Clinical and inflammatory effects of dietary L-arginine in patients with intractable angina pectoris. *Am J Cardiol* 1999; 83: 1488—1490.
25. Zanzinger J, Czachurski J, Seller H. Inhibition of sympathetic vasoconstriction is a major principle of vasodilation by nitric oxide *in vivo*. *Circ Res* 1994; 75: 1073—1077.
26. Vita JA, Treasure CB, Yeung AC *et al.* Patients with evidence of coronary endothelial dysfunction as assessed by acetylcholine infusion demonstrate marked increase in sensitivity to constrictor effects of catecholamines. Circulation 1992; 85: 1390—1397.
27. Rector T, Bank AJ, Mullen KA *et al.* Randomized, double-blind, placebo-controlled study of supplemental oral L-arginine in patients with heart failure. *Circulation* 1996; 93: 2135—2141.
28. Boulanger C, Luscher TF. Release of endothelin from the porcine aorta: inhibition by endothelium-derived nitric oxide. *J Clin Invest* 1990; 85: 587—590.

29. Boger RH, Bode-Boger SM, Thiele W, Creutzig A, Alexander K, Frolich JC. Restoring vascular nitric oxide formation by L-arginine improves the symptoms of intermittent claudication in patients with peripheral arterial occlusive disease. *J Am Coll Cardiol* 1998; 32: 1336—1344.
30. Bode-Boger SM, Boger RH, Alfke H *et al.* L-arginine induces nitric oxide-dependent vasodilation in patients with critical limb ischemia. A randomized, controlled study. *Circulation* 1996; 93: 85—90.
31. Hirooka Y, Imaizumi T, Tagawa T *et al.* Effects of L-arginine on impaired acetylcholine-induced and ischemic vasodilation of the forearm in patients with heart failure. *Circulation* 1994; 90: 658—668.
32. Kubota T, Imaizumi T, Oyama J, Ando S, Takeshita A. L-arginine increases exercise-induced vasodilation of the forearm in patients with heart failure. *Jap Circ J* 1997; 62: 471—480.
33. Chin-Dusting JPF, Kaye DM, Lefkovits J, Wong J, Bergin P, Jennings GL. Dietary supplementation with L-arginine fails to restore endothelial function in forearm resistance arteries of patients with severe heart failure. *J Am Coll Cardiol* 1996; 27: 1207—1213.
34. Belch JJF, Bridges AB, Scott N, Chopra M. Oxygen free radicals and congestive heart failure. *Br Heart J* 1991; 65: 245—248.
35. Kanaya Y, Nakamura M, Kabayashi N, Hiramori K. Effects of L-arginine on lower limb vasodilator reserve and exercise capacity in patients with chronic heart failure. *Heart* 1999; 81: 512—517.
36. Panza JA, Quyyumi AA, Brush JE, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 1990; 323: 22—27.
37. Quyyumi AA, Mulcahy D, Andrews NO, Husain S, Panza JA, Cannon RO. Coronary vascular nitric oxide activity in hypertension and hypercholesterolemia. Comparison of acetylcholine and substance P. *Circulation* 1997; 95: 104—110.
38. Panza JA, Casino PR, Badar DM, Quyyumi AA. Effect of increased availability of endothelium-derived nitric oxide precursor on endothelium-dependent vascular relaxation in normal subjects and in patients with essential hypertension. *Circulation* 1993; 87: 1475—1481.
39. Nakazano K, Watanabe N, Matsuno K, Sasak J, Sato T. Does superoxide underlie the pathogenesis of hypertension. *Proc Natl Acad Sci* 1991; 88: 1045—1048.
40. Taddei S, Virdis A, Ghiadoni L, Magagna A, Salvetti. Cyclooxygenase Inhibition restores nitric oxide activity in essential hypertension. *Hypertension* 1997; 29: 74—279.
41. Taddei S, Virdis A, Ghiadoni L, Magagna A, Salvetti A. Vitamin C improves endothelium-dependent vasodilation by restoring nitric oxide activity in essential hypertension. *Circulation* 1998; 97: 2222—2229.
42. Garcia CE, Kilcoyne CM, Cardillo C, Cannon RO, III, Quyyumi AA, Panza JA. Effect of copper-zinc superoxide dismutase on endothelium-dependent vasodilation in patients with essential hypertension. *Hypertension* 1995; 26: 863—868.
43. Cardillo C, Kilcoyne CM, Cannon RO, III, Quyyumi AA, Panza JA. Xanthine oxidase inhibition improves endothelium-dependent vasodilation in hypercholesterolemic but not in hypertensive patients. *Hypertension* 1997; 30: 57—63.
44. Taddei S, Virdis A, Mattei P, Ghiadoni L, Sudano I, Salvetti A. Defective L-arginine-nitric oxide pathway in offspring of essential hypertensive patients. *Circulation* 1996; 94: 1298—1303.
45. Drexler H, Fischell TA, Pinto FJ *et al.* Effect of L-arginine on coronary vessel wall morphology. *Circulation* 1994; 89: 1615—1623.
46. Schwarzbacher SP, Lim TT, Wang BY *et al.* Local intramural delivery of L-arginine enhances nitric oxide generation and inhibits lesion formation after ballon angioplasty. *Circulation* 1997; 95: 1863—1869.

47. Adams MR, Forsyth CJ, Jessup W, Robinson J, Celermajer D. Oral L-arginine inhibits platelet aggregation but does not enhance endothelium-dependent dilation in healthy young men. *J Am Coll Cardiol* 1995; 26: 1054—61.
48. Wolf A, Zalpour C, Theilmeir G *et al.* Dietary L-arginine supplementation normalizes platelet aggregation in hypercholesterolemic humans. *J Am Coll Cardiol* 1997; 29: 479—485.
49. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. *J Clin Invest* 1994; 94: 1172—1179
50. Giugliano D, Marfella R, Verrazzo G *et al.* The vascular effects of L-arginine in humans. The role of endogenous insulin. *J Clin Invest* 1997; 99: 433—438.
51. Wascher TC, Posch K, Wallner S, Hermetter A. Vascular effects of L-arginine: anything beyond a substrate for the NO-synthase. *Biochem Biophys Res Commun* 1997; 234: 35—38.
52. Nagase S, Takemura K, Ueda A *et al.* A novel nonenzymatic pathway for the generation of nitric oxide by the reaction of hydrogen peroxide and D- or L-arginine. *Biochem Biophys Res Commun* 1997; 233: 150—153.

R e c e i v e d: June 21, 1999
A c c e p t e d: September 21, 1999

Author's address: K. Herbaczyńska-Cedro, Cardiovascular Laboratory Research Centre of Experimental & Clinical Medicine Polish Academy of Sciences 02 106 Warsaw 5, Pawińskiego str.