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Review

L-arginine and cardiovascular system

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Abstract:
L-arginine is a basic endogenous amino acid. Its significant metabolic role as the product of ammonia detoxification, the urea cycle metabolite, the precursor of proteins, ornithine, urea and creatinine, and the amino acid involved in the formation of active enzyme centers was very well established. The current interest in this amino acid refers mainly to its close relation with an important signal molecule nitric oxide (NO). Literature review demonstrates that L-arginine, the only substrate of the NO production, affects cardiovascular system (blood vessels and heart). The majority of experimental and clinical studies clearly show a beneficial effect of L-arginine on endothelium in conditions associated with its hypofunction and thus with reduced NO synthesis. Some clinical studies involving healthy volunteers or patients suffering from hypertension and diabetes indicate that it may also regulate vascular hemostasis. Moreover, experiments performed on animals and *in vitro* data also suggest that L-arginine may have a complex antiaggregatory, anticoagulatory and profibrinolytic effect. Therefore, a novel therapeutic potential of L-arginine should be taken into consideration.

Key words:
L-arginine, cardiovascular system, hemostasis

Abbreviations: ACE – angiotensin converting enzyme, ADP – adenosine 5'-diphosphate, ECLT – euglobulin clot lysis time, FAD – flavin adenine dinucleotide, FMN – flavin mononucleotide, GMP – guanosine monophosphate, GTP – guanosine triphosphate, ip – intraperitoneal, iv – intravenous, 2K1C – two kidney, one clip renovascular hypertension model, L-NAME – N^G-nitro-L-arginine-methyl ester, NADPH – reduced nicotinamide adenine dinucleotide phosphate, NO – nitric oxide, NOHA – N-hydroksy-L-arginine, PAI-1 – plasminogen activator inhibitor-1

L-arginine-metabolism

L-arginine is a basic endogenous amino acid discovered by Schultze in 1886. Its significant metabolic role as the ammonia detoxification product, the urea cycle metabolite, the precursor of proteins, ornithine, urea and creatinine, and the amino acid involved in the formation of active enzyme centers was very well established.

However, current interest in this amino acid refers mainly to its close relation to an important signal molecule nitric oxide (NO). L-arginine is the only substrate in the production of NO. Two basic forms of NO synthase can be distinguished, namely inducible and constitutive, which are dioxygenases and contain heme molecule. In a complex reaction of oxidation, in the presence of N-hydroxy-L-arginine (NOHA), reduced nicotinamide adenine dinucleotide phosphate (NADPH), and with the involvement of such cofactors as flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme and tetrahydrobiopterin catalyzed *via* NO synthase, L-arginine gives rise to L-citrulline molecule and NO [57]. NO as a free radical easily reacts with other free radicals and is

rapidly inactivated. One of these free radicals is the superoxide anion (OO^{-}), reactive oxygen species produced by cellular oxidases in the vascular wall. NO reacts rapidly with superoxide, producing peroxynitrite. The balance between these radicals has implications for pathophysiology of cardiovascular system. The relations between NO, superoxide and peroxynitrite formation in human vessels remain unclear. The interaction between NO and superoxide depletes NO bioactivity and is functionally important since NO is a pivotal mediator of the vascular system. The loss of NO bioactivity associated with increased vascular superoxide plays an important role in pathogenesis of hypertension and atherosclerosis. On the other hand, the formation of peroxynitrite may also generate nitrosylated thiols that function as endogenous NO donors capable of inducing vasorelaxation and inhibiting platelet aggregation. This mechanism seems to operate in the deficiency of L-arginine metabolism when production of superoxide is augmented [37]. Hence, NO exerts its effect in the vicinity of the release site and is regarded to be a local transmitter. The target of NO is guanylate cyclase. When dissolved in water, NO gains an electron and as a lipophilic molecule easily diffuses through the cellular membrane, binds to iron of heme group of the cytosol guanylate cyclase and activates it. The activation of guanylate cyclase leads to conversion of guanosine triphosphate (GTP) into cyclic guanosine monophosphate (GMP) [57].

Due to the presence of the L-arginine/NO pathway in many cells and organs, the significance of L-arginine may be associated with the biological effect of NO and consists in e.g. maintenance of normal peripheral vascular resistance, neuronal transmission, modulation of the immune response and vascular wall thromboresistance [53].

L-arginine – pharmacological effects

A. Effect of L-arginine on blood vessels and heart

Experimental studies

The experimental studies were performed both *in vitro* on isolated fragments of rat blood vessels and *in vivo* mostly on rabbits. In 1991 Schini and Vanhoutte [63], using isolated fragments of rat blood vessels, showed that a dilating effect of L-arginine occurred both in the presence and absence of endothelial cells and they suggested the existence of the L-arginine/NO metabolic pathway also in the vascular smooth muscle cells. Briones et al. [18] recently found that *in vitro* L-arginine more dilates brain vessels of hypertensive than normotensive rats.

Boegehold [13] administered L-arginine by ionophoresis and he examined arteriolar network in the spinotrapezius muscle by intravival microscopy in experiments performed on Dahl salt sensitive rats on high and low salt diets. He noted lack of vessel dilation in hypertensive rats after L-arginine application, while in normotensive rats the dilation was visible.

Oral administration of L-arginine for 10 days to rabbits on high-cholesterol diet according to Cooke et al. [24] markedly improved the vasodilating, endothelium-dependent effect compared to control and reduced the formation of atheromas. Hamon et al. [39] and Tarry and Makhoul [71] examined the normocholesterolemic rabbits on L-arginine-rich diet. Hamon et al. observed that L-arginine reduced neointimal thickening after balloon denudation and improved neoendothelial-dependent relaxation [39]. Tarry and Makhoul [71] found that this amino acid enhanced NO production at sites of vascular healing and reduced intimal hyperplasia. Wang at al. [75, 76] reported that oral administration of L-arginine prevented intimal thickening in hypercholesterolemic rabbits. However, in a similar study, Singer et al. [65] noted a dissonance between a satisfactory antiatherogenic effect and practically unchangeable vessel reactivity after oral application of the drug. Böger et al. [15] examined the effect of L-arginine administered *po* on blood vessels of rabbits receiving high-cholesterol diet. In hypercholesterolemia, NO synthesis was impaired, production of peroxide anions was increased, but L-arginine restored normal endothelial function. The same authors [14, 16] using a similar experimental animal species, confirmed again that L-arginine applied in a diet improved NO-dependent vasodilating function, inhibited enlargement of atheromas by normalization of substrate availability for NO synthase and reduced vascular oxidative stress in animals with hypercholesterolemia. Davies et al. [26–28] observed also that L-arginine-rich diet ameliorated hypercholesterolemia-induced functional abnormalities in endothelial cells. Jeremy et al. [45] reported that long-term L-arginine *po* administration limited atherosclerosis only in descend-

ing aorta and preserved endothelium-dependent vasodilation in resistance arteries, but this treatment effect is not sustained. Aji et al. [4] in experiments performed on the model of familial hypercholesterolemia in mice showed that L-arginine had a beneficial effect on vascular endothelium and inhibited atheroma growth, but the effect was reversed by L-arginine analog that blocked NO production.

In three consecutive experiments performed by Hutchison et al. [42–44], rabbits with normal cholesterol level and hypercholesterolemia were exposed to tobacco smoke. A 10-week *po* administration of L-arginine caused alleviation of endothelial dysfunction induced by tobacco smoke in both groups. Le Tourneau et al. [48] reported that L-arginine applied in a diet inhibited while L-NAME stimulated neointimal hyperplasia after balloon angioplasty in the hypercholesterolemic rabbits. In contrast, studies performed on the mouse model of atherosclerosis demonstrated that L-arginine supplementation did not reduce lesion formation [20].

Sun et al. [69] observed that L-arginine administrated *ip* increased cerebral blood perfusion and improved vasomotions of microvessels by enhancing NO levels and decreasing endothelin-1 levels in blood in subarachnoid hemorrhage rat model.

The other authors reported that L-arginine administered *po* in hypertensive rat model decreased blood pressure and reduced left ventricular wall thickness [70]. Gouvea et al. [35] in experiments performed also in rat model of hypertension (2K1C) found after oral L-arginine therapy, a significant reduction of mean blood pressure and an increase in renal excretion of sodium and water. Hiraoka et al. [40] observed that oral administration L-arginine prevented congestive heart failure in murine viral myocarditis.

Alexander et al. [5] reported that L-arginine applied *po* in pregnant rats decreased blood pressure; thus, it may be beneficial to attenuating the hypertension in preeclampsia.

The majority of studies cited above clearly show a beneficial effect of L-arginine on endothelium in conditions associated with its hypofunction and, thus, with reduced NO synthesis.

Clinical studies

Clinical observations performed in groups of healthy volunteers and patients suffering from various ailments seem to confirm the results of experiments on animals.

Bode-Böger et al. [11] investigated the effects of intravenous (*iv*) infusion of L-arginine (a dose of 30 g for 30 min) on blood pressure in healthy volunteers, compared to placebo. L-arginine markedly reduced this parameter, affecting diastolic pressure more than systolic pressure. Marietta et al. [49], who conducted their study in a group of young healthy women receiving 30 g of L-arginine *iv*, observed a marked decrease in both systolic and diastolic pressure at 30 min of the infusion followed by return to the initial values. Another study of Siani et al. [64] showed that the intake of L-arginine-enriched diet by healthy volunteers caused a reduction in arterial blood pressure. In contrast to that, Adams et al. [2] administered L-arginine *po* to young healthy men (21g daily) for 3 days and observed no correlation of this amino acid with blood pressure and brachial artery dilation in this group. In the study by Chin-Dusting et al. [22], healthy men received L-arginine *po* (20 g daily) for 28 days. However, these authors showed no effect of L-arginine on arterial blood pressure. Lerman et al. [47] administered L-arginine *po* to healthy subjects (9 g daily) for 6 months and found no significant effect on arterial pressure. However, they noted that long-term administration of this amino acid had a favorable effect on endothelium, improving its function and reducing concentration of endothelin.

Hishikawa et al. [41] administered L-arginine *iv* at a dose of 500 mg/kg in patients with primary and secondary hypertension and observed a considerable reduction both in systolic and diastolic pressure in all the cases. Panza et al. [58] reported that in patients with arterial hypertension, acetylcholine caused a smaller increase in blood flow and a smaller drop in peripheral resistance than in normotensive subjects. These findings indicate the impairment of endothelial NO production in hypertension at the level of reduced NO synthesis and release. This phenomenon was not caused by a decrease in the substrate availability, as *iv* infusion of L-arginine did not increase blood flow. Mimran et al. [52] performed their study in 4 groups of patients: normotensive, with essential never treated hypertension, treated with angiotensin converting enzyme (ACE) inhibitors (more than 2 years), treated with other drugs (excluding ACE inhibitors). L-arginine was administered *iv* at a dose of 30 g for 60 min. The mean blood pressure decreased most in untreated hypertension, then in ACE-treated condition,

slightly in normotension and was almost unchanged in patients treated with drugs other than ACE inhibitors.

According to Rector et al. [62], oral 6-week application of L-arginine (5.6 or 12.6 g/day) in patients with heart failure, causes a drop in arterial blood pressure. Adams et al. [3], who administered L-arginine *po* (21 g for 3 days) to young men with coronary artery disease observed no changes in arterial pressure, despite the fact that the brachial artery was dilated, and the dilatation could be endothelium-dependent.

Giugliano et al. [33] reported that the induction of acute hyperglycemia in healthy subjects and the subsequent administration of L-arginine (30 g) *via* infusion (1 g/min) caused a rapid drop in arterial pressure, both systolic and diastolic, already at 10 min of drug administration, which lasted till the end of the experiment. The same research group [34] administered L-arginine in an *iv* bolus at a dose of 3 g to healthy subjects and patients with insulin-independent diabetes, hypercholesterolemia and primary hypertension. In healthy subjects, these authors observed a decrease in blood pressure; they noted the greatest reduction (by 8 ± 1 mmHg) in blood pressure in the youngest age group (< 30 years) and the smallest (by 2.8 ± 0.4 mm Hg) in the group of over 60 years of age, which may indicate that endothelial dysfunction progresses with age. In patients with hypercholesterolemia and diabetes, the drop in blood pressure was not so pronounced (2.8 ± 1.8 mm Hg and 2.2 ± 1.8 mm Hg, respectively). In hypertensive patients, the decrease in blood pressure was comparable to that noted in healthy subjects (mean 4.1 mm Hg). The authors emphasized the fact that the hypertensive group included only patients with freshly recognized and never treated disease and thus the L-arginine/NO pathway dysfunction could not be regarded as the primary cause of hypertension but rather secondary one, complicating its course.

Creager et al. [25] found that *iv* acute administration of L-arginine improved endothelium-dependent dilating function of large blood vessels in patients with hypercholesterolemia. Clarkson et al. [23], who examined young subjects with this ailment after oral application of L-arginine (21 g daily for 4 weeks), confirmed also marked endothelium-dependent dilatation of the brachial artery. In contrast, 14-day oral application of L-arginine-enriched medical food had no effect on endothelial function [1].

It has been demonstrated that L-arginine administered *iv* is an effective drug in patients with obliterative atheromatosis of the lower limbs. Clinical improvement was manifested in the extension of painless intermittent claudication distance, shortening of pain regression after walking the maximum distance, improvement of lower limb blood supply and increase in ankle-arm pressure ratio [17, 36]. Bode-Böger et al. [10] observed a marked reduction in diastolic and systolic pressure and an increased blood flow in the femoral artery in patients with critical limb ischemia, even after a single *iv* infusion of L-arginine at a dose of 30 g for 60 min.

Long-term oral administration of L-arginine in patients: with congestive heart failure [21], with stable angina pectoris and healed myocardial infarction [19], with coronary artery disease [8] and postmenopausal women [7] was ineffective in influencing endothelial function. In contrast, others reported that dietary L-arginine might have clinical beneficial effects in patients with stable [50] and intractable angina pectoris [9], and with chronic heart failure [38]. *Iv* administration of L-arginine in patients with angina pectoris increased basal forearm blood flow and decreased endothelin-1 level [59]. Zimmermann et al. [79] observed that *iv* administration of L-arginine in patients with cardiovascular risk of stroke was a potential marker for endothelial dysfunction and independent indicator for an increased risk of stroke.

Other authors performed intra-coronary infusion of L-arginine in patients with: hyperlipidemia [6, 32], coronary artery disease and stable angina pectoris [73] and cardiac transplant recipients [6]. Berkenboom et al. [6] after infusion of L-arginine observed attenuation of serotonin-induced constriction in hyperlipidemic group but not in heart transplant recipients (one year after transplantation). Infusion of the amino acid did not change the arterial pressure in the two groups. Tousoulis et al. [73] reported that L-arginine dilated coronary segments and stenoses but did not increase the magnitude of response to atrial pacing in proximal and distal segments and in coronary and their reference segments. Gellman et al. [32] showed that the amino acid not only improved abnormal microvascular responses to sympathetic activation, but also restored the coupling that normally exists between coronary blood flow and cardiac work.

Oral administration of L-arginine in women with preeclampsia did not reduce mean diastolic blood pressure [67]. Piatti et al. [60] observed that oral L-arginine treatment in diabetic patients (type 2) significantly improved but did not completely normalize

peripheral and hepatic insulin sensitivity. *Iv* administration of this drug in hypertensive patients with type 2 diabetes induced vasodilation of the renal vasculature, which was not different in comparison to young, healthy reference group [29].

Oomen et al. [56] performed the observational study of L-arginine intake based on dietary history, but did not prove the hypothesis that dietary arginine lowers the risk of mortality due to coronary heart disease.

Thus, numerous studies of *iv* L-arginine in humans have shown that endothelial function was improved in subject, e.g. with hypertension, hypercholesterolemia and diabetes.

Recently, Preli et al. [61] summarized the results of studies concerning the effect of oral L-arginine supplementation on cardiovascular system in humans. In contrast to animal studies, the results from human studies have varied. Five of the 17 human studies showed no vascular health benefit from oral L-arginine supplementation. The remaining 12 studies demonstrated beneficial effect of oral L-arginine supplementation as evidenced by decreased platelet aggregation and adhesion, decreased monocyte adhesion or improved endothelium-dependent vasodilation.

B. Effect of L-arginine on blood platelets

Experimental studies

Wang et al. [77] found marked inhibition of platelet aggregation induced by adenosine 5'-diphosphate (ADP), collagen or thrombin, both in oral and *iv* administration of L-arginine to rabbits. Tsao et al. [74] and Bode-Böger [12] using hypercholesterolemic rabbits also demonstrated that long-term administration of this amino acid considerably inhibited ADP-induced platelet aggregation. Mendez and Zarzoza. [51] tested platelet aggregation potential in rats with alloxan diabetes and found the inhibition of thrombin-activated aggregation. Dewanjee et al. [30], during cardiopulmonary bypass in pig model, confirmed decreased incidence of thrombi and emboli after L-arginine infusion. Only Thomas et al. [72] in experiments on rats observed no significant changes in collagen-induced aggregation under the influence of L-arginine.

Thus, the results of studies on animal models clearly show an inhibitory role of L-arginine in the platelets activity.

Clinical studies

Like experiments on animals, also clinical studies provide well-documented and defined results on the effect of L-arginine on platelet aggregation. Marked inhibition of platelet aggregation after *iv* [11, 49] and oral [2] administration of this amino acid has been confirmed in healthy subjects. Gryglewski et al. [36], using L-arginine *iv* for 7 consecutive days to treat peripheral arterial obstructive disease, observed inhibition of spontaneous, collagen- and ADP-induced aggregation. In the study by Giugliano et al. [34], *iv* therapy with this amino acid decreased platelet capability of ADP-induced aggregation in patients with hypercholesterolemia, insulin-independent diabetes and arterial hypertension. Wolf et al. [78] also noted normalization of collagen-induced aggregation in patients with elevated cholesterol level after oral application of L-arginine. Its positive effect was also observed in acute coronary failure. The drug improved endothelial function and thus exercise tolerance, and inhibited ADP-induced platelet aggregation [66]. Facchinetti et al. [31] showed a decrease in platelet aggregation following L-arginine administration in pregnant women with normal blood pressure, but not in the preeclamptic state. Similarly, Neri et al. [54, 55] found a correlation between L-arginine administration and inhibition of platelet aggregation in pregnant women with normal blood pressure and with chronic hypertension, but not in the preeclamptic state. Abdelhamed et al. [1] reported that L-arginine-enriched medical food had no effect on platelet function.

C. Effect of L-arginine on coagulation and fibrinolysis

There are not many reports on the effect of L-arginine on the plasma coagulation components in literature. Stief et al. [68] exposed human plasma to the action of L-arginine at various concentrations *in vitro*. They found that L-arginine in a concentration-dependent manner prolonged the prothrombin time and activated partial thromboplastin time.

The effect of L-arginine on the fibrinolytic system was investigated by Gryglewski et al. [36], who administered this amino acid *iv* to patients with obliterative atheromatosis of the lower limbs. They found shortening of the euglobulin clot lysis time (ECLT) and reduced activity of plasminogen activator inhibitor-1 (PAI-1). Similar conclusions were reported by Kawa-

bata and Hata [46], who revealed that simultaneous administration of L-arginine and L-NAME reversed action of the latter, i.e. ECLT shortening, lowering of PAI-1 activity and reduction of fibrinogen concentration.

Thus, the above-described results suggest that L-arginine to some extent can modify hemostasis by inhibition of coagulation and/or activation of fibrinolysis.

Conclusion

Literature review demonstrates that L-arginine, the only substrate of the NO production, affects cardiovascular system (blood vessels and heart). Some clinical studies involving healthy volunteers or patients suffering from hypertension and diabetes indicate that it may also regulate vascular hemostasis. Moreover, experiments performed on animals and *in vitro* data also suggest that L-arginine may have a complex effect on platelets, coagulation and fibrinolytic systems. Therefore, a novel therapeutic potential of L-arginine should be taken into consideration.

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