

L-Arginine and vascular diseases: lights and pitfalls!

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Summary. L-Arginine, a semi-essential cationic amino acid involved in multiple areas of human physiology and metabolism, is a precursor of nitric oxide, and, recently, it has been found to crucially influence endothelial function. Arginine appears to be safe and effective therapy for many health conditions, particularly vascular diseases responsive to modulation of endothelial-derived relaxing factor including intermittent claudication, angina pectoris and erectile dysfunction. However, L-arginine metabolism is still not fully understood, highlighting a complex implication for its pharmacological use in clinical practice. In this review, we want to point out the lights and pitfalls of L-arginine as potential therapeutic option in vascular disorders.

Key words: L-arginine; Vascular diseases; NO

Introduction

L-Arginine (L-arg) is a basic, semiessential amino acid that has a key role in the detoxification of ammonia from human body via urea cycle. Its metabolism is complex and not yet fully understood. L-arg serves as an amino acid in protein synthesis as well as a substrate of different metabolic pathways, such as nitric oxide (NO), creatine and agmatine. Since the first ever animal experiment proving the beneficial effect of Arg coronary injection in subjects with acute coronary disease, the indication for the pharmacological use of Arg to prevent vascular disorders has broadened (1,2).

To this end, aim of this review was to better characterize the biochemistry and pharmacological effects of L-arg in various vascular diseases.

Biochemistry and Pharmacology

L-arg is the common name of the 2-amino-5-guanidino-pentanoic acid, a semi-essential amino acid with a central role into the nitric metabolic path-

way (fig 1). It plays also a role in several metabolic pathways, such as creatine and agmatine synthesis, as well as in vascular health due to NO metabolism (2).

In healthy adult individuals, serum L-arg concentration is primarily determined by protein turnover (about 80% of L-arg); the remaining 20% is kept from dietary protein, with an average intake of 4-5g/die, and from de novo synthesis (1). Altered nutritional status, such as dietary program or during infection, older age and increased L-arg consumption during

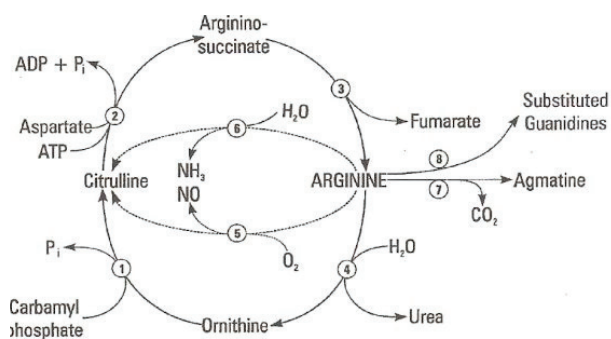


Figure 1. Shows the complex L-arg metabolism.

prolonged stress period may lead to a decreased serum L-arg concentration with a subsequent increased risk in endothelial dysfunction.

De novo synthesis of L-arg is supported from citrulline by the small intestine during perinatal phase; after the weaning phase, there is a gradual transition to an "intestine-renal axis", where the citrulline produced by intestine is kept by proximal tubule of kidney and then converted to L-arg. This axis is limited in elderly by the activity of arginosuccinate synthase (ASS) and arginosuccinate lyase (ASL) enzymes, which gene expression is slowly decreased with age (1,4).

L-arg could be administered via intravenous or oral route; its half-life is about 1.5-2 hours after an oral dose of 6 gr. Blood concentration peak are achieved within 20-30 minutes after the intravenous

administration and after 60 minutes when L-arg is taken per os. Plasma level of oral L-arg is limited by the intestinal γ -transporter system for cationic aminoacid, which could led to a L-arg bioavailability of $21 \pm 4\%$ (5). Intravenous administration of L-arg is characterized by a bioavailability of $68 \pm 9\%$ instead. Thus, intravenous route are preferred when L-arg has to be administered in acute and short therapy, such as after myocardial infarction, but oral L-arg supplementation is the best way in cases of prolonged treatment (i.e. senescence) (3).

L-arg plays a central role in several metabolic pathways, mostly in ammonia detoxification and NO synthesis. L-arg is the substrate of four different enzymes, namely Arginase (ARG), nitric oxide synthase (NOS), arginine decarboxylate (ADC) and arginine:glycine aminotransferase (AGAT).

The first study on L-arg showed its centrality in ammonia detoxification in healthy individuals, as the normal urea cycle takes place from L-arg through ARG activity; ARG catalyses the conversion of L-arg into L-Ornithine, an important byproduct that is also a precursor for polyamines synthesis (4,6). ADC mediates the catabolism of L-arg into Agmatine, a polyamine which role has not been fully elucidated (7). AGAT converts L-arg into creatine, an important storage of energy for muscle contraction, and an important ammonia scavenger for central nervous system during embryonic development (1,2). However, clinical use of L-arg growing in importance after the 1988,

when NO was evidenced as the endothelium-derived relaxing factor (8,9). NO is formed from endothelium NOS (eNOS) extracting the Nitric group from L-arg.

Interestingly, in animal models (10-12) acute or chronic L-arg has been showed to improve endothelium function in cases of hypercholesterolemia and atherosclerosis (3). Then, using L-arg supplementation in preventing or treating vascular diseases (such as in coronary disease or cerebrovascular disorders) increase its chance.

The L-Arginine paradox

Oral supplementation of high dose of L-arg has proven to be effective in many vascular disorders such as coronary artery disease (13-17), peripheral artery disease (18-20) and in vascular senescence (21,22).

All the aforementioned studies have agreed that a periodic oral administration of L-arg, at a mean dosage of 9 g/die (i.e. 3 g tid) for 4 weeks, seems to improve endothelium function in selected groups of patients, such as hypercholesterolemic patients with coronary artery diseases.

However, the absence of specific guidelines in dose regimen and protocol administration for L-arg treatment makes a wide variability in the results of these clinical trials. Nevertheless, a minimum dosage of 6.6 for a week (23) as the smallest dosage with clinical effect, and the maximum dose of 24 gr for 8 week (16) before of presenting clinical adverse effects were recommended (3).

Although these studies are of some interest, the use of oral implementation of L-arg in clinical practice delays to begin of standard use due to the growing conflicting results. In a recent study on individuals with peripheral artery diseases (25), the recent hypothesis of a reduced effect of L-arg on endothelium function when chronically administered for long periods (24) has been confirmed. Indeed, the authors concluded that there is no benefit in long-term administration of L-arg, which, furthermore, impairs vascular functional capacity (25). To this end, the VINTAGE study (26), performed on 2006, was closed only after 6 month-follow-up because of an high percentage of deceases in patients assuming oral L-arg.

This particular clinical paradox, i.e. if a chronic implementation of L-arg is ineffective or even detrimental in vascular health although the aminoacid is effective in animal models, reflects the complex L-arg metabolism in a puzzling findings called “arginine paradox”. According this theory, the plasma concentration of L-arg is up 15-30 fold higher of that needed for the Km of NOS.

Then, plasma L-arg concentration is largely saturating the NOS, but each L-arg dietary implementation produce an increasing in NO synthesis, with a subsequent NO overdose possibly leading to death in specific patient’s categories.

Moreover, the kinetics of ARG in the healthy individuals remains hard to understand, as the lowered availability of L-arg does not seem to be rate limiting (3). The cytoplasm of endothelium cells contains a concentration of L-arg largely saturating the NOS activity, and thus, any acute introduction of exogenous arginine elicits an increase in NO production (24). This cytosolic compartmentalization of L-arg could explain the arginine paradox, as eNOS utilizes only the extracellular L-arg, which is lower than expected in whole cell homogenates (3,27).

Recently, Bode-Boger S.M. et al. (3) pointed out the importance of asymmetrical dimethylarginine (ADMA) in understanding the clinical arginine paradox. ADMA is a byproduct of L-arg after post-transcriptionally methylation of proteins and subsequent hydrolysis. In particular, the L-arg/ADMA ratio seems to modulate the NOS activity and subsequent NO production after L-arg supplementation (28). Indeed, elevated ADMA levels were found in several pathological conditions, such as hypercholesterolemia, hypertension and hyperhomocysteinemia, as well as in altered health status such as inflammations and infections or in the elderly (3). Moreover, in a recent review, Dioguardi (24) highlighted the genetic implications of arginine paradox concluding that only a balanced formulations of essential aminoacids are the key to promote the physiological vascular health.

Clinical Implications

L-arg treatment in clinical trials is increasing in the last years with a wide spectrum of potential and interesting clinical applications. Herein, we describe

the most important and recent studies focusing on the link between L-arg and vascular diseases.

Cardiovascular Disease (CVD)

The clinical applications of L-arg in patients with CVD have been largely showed since 1997, with conflicting results (29-31). Interestingly, all these clinical trials concluded about the effectiveness of L-arg in selected groups of individuals affected by different CVD, including hyperhomocysteinemia (16), diabetes (15), peripheral arterial disease (19) and tabagism (17).

In 2009, Tao Sun et al. (32) in a meta-analysis on the use of L-arg after myocardial infarction, found that both the two eligible controlled trials, - i.e. the l-arginine in acute myocardial infarction (ARAMI) study (33) and the vascular interaction with age in myocardial infarction (VINTAGE M1) study (26), - demonstrated a reduction of 7% in mortality of patients treated with L-arg compared with placebo, but without a statistical significance. Thus, they concluded that L-arg treatment is unsuccessful in changing the risk of total events, even if encouraging data from ARAMI study showed the L-arg effectiveness in improving the clinical course in patients affected by hypercholesterolemia (3,33). Unfortunately, the safety monitoring committee early closed the enrollment in VINTAGE study as a mortality rate of 8.6% in treated patients group was reached (26). Nevertheless, the authors, after considering the positive results of in vitro study on endothelial dysfunction, and the small number of trials with a fair heterogeneity on methods included in their meta-analysis, concluded about the need of more efforts on higher quality of standardized study about use of L-arg on myocardial infarction (33).

Mitochondrial myopathy, encephalopathies, lactic acidosis and stroke-like (MELAS)

In the last 10 years several studies have analyzed the role of L-arg in the acute treatment and during interictal phase of stroke-like episode in MELAS patients (34-36).

The cause of vascular stroke-like episodes in these patients is still under debate. The most validated hypothesis is a microcirculation impairment due to el-

evated intracellular concentration of reactive oxygen species (such as NO). Administration of L-arg in acute phase seems to improve the clinical outcome in MELAS patients, as well as oral L-arg supplementation in interictal phase prolongs the wellness period (36). L-arg effects are evidenced in both MRS findings (35) and clinical outcome (37), with the endothelium function in MELAS patients that harmonized with plasma level of L-arg (38).

L-arg supplementation seems to increase NO production in MELAS patients with a subsequent enhanced vasodilatation leading to a reduced ischemic damage, energy failure and lactate accumulation. However, more studies are needed to unveil the exact beneficial effects of L-arg on MELAS abnormalities (1).

Erectile dysfunction (ED)

The NO pathway seems to be a critical issue in physiology of bladder function and penile erection. Several evidence indicates that NO contributes to the physiological erectile function via non adrenergic non-cholinergic nerves leading to relaxation of corpus cavernosus (39,40). Interestingly, ED in the young-adults has been often considered as a clock alarm for CAD, especially for angina, and it highlights how this two vascular disorders share common risk factors (such as hypertension, diabetes, insulin resistance, smoking) involving the NO pathway (41). Dietary implementation of high dose of L-arg in ED patients is largely used as a good therapeutically option (42-44), especially in young individuals with arteriogenic ED. In particular, recent studies have showed a possible link between ADMA level and ED, as demonstrated by the alterations at the penile dynamic doppler. To this end, Ioakeimidis N et al. have showed how ADMA levels are independently associated with poor penile outflow in arteriogenic ED (45). This results have been recently confirmed by Paroni et al., who evidenced a lower L-arg/ADMA ratio in patients with arteriogenic ED (46).

However, a clinical opposite effect could be evidenced when L-arg plasma level is too high, demonstrating how only a formulation of different aminoacids could support the physiological penile erection (43).

Vascular senescence

Aging is worldwide considered as an emerging risk factor for CVD leading to a progressive endothelial dysfunction (47) in many vascular districts, with regard to forearms and coronary arteries (48-51). NO is considered a potent vasoactive hormone playing a key role in maintaining vascular wall health. NO acts via inhibition of inflammation, cellular proliferation and thrombosis induced by shear stress (52,53). Aging seems to reduce the NO production/bioavailability leading to endothelial dysfunction. As reported by Hefferman and coworkers (54), available data indicates that endothelial dysfunction is highly prevalent if not ubiquitous in elderly individual; moreover, many authors consider the endothelial dysfunction as the primarily phenotypic expression of normal human aging (55,56). Moreover, NO acts with pleotropic effect on hormone secretion; in particular growth hormone and insulin are the main NO-dependent hormones associated with endothelial dysfunction (57-59). Thus, a chronic implementation of oral L-arg at low dosage seems to slow this physiological process in the elderly without significant effect on young subjects with normal vascular health (54).

Indeed, it has been demonstrated that 6 month of oral L-arg supplementation at 1.2 gr/L reduces the clinical effect of NO impairment, such as peripheral resistance and coronary hemodynamics (60,61). However, more studies have to be conducted to better understand the efficacy and safety of L-arg in the elderly. Interestingly, recent studies have demonstrated how L-arg, and in particular ADMA/L-arg ratio, does not affect the flow mediated dilation, that is the typical feature of endothelium dysfunction in elderly (62). Thus, a balanced combination of different aminoacids seems to reduce clinical effect of endothelial dysfunction also in elderly subjects (3).

Conclusive remarks

L-arg is an aminoacid linked to vascular physiology. As the main NO donor, it serves for the maintaining of normal vascular health. Actually, NO is responsible of the endothelium-dependent vasodilatation

subsequent to several molecules, such as acetylcholine, serotonin, thrombin and bradykinin (63,64). All these local stimuli activate eNOS on endothelial cells; therefore, the produced NO diffuse into the smooth muscle cells causing the vasodilation (65). On the strength of these process, all the conditions that alter physiological endothelial cells could explain the so-called endothelium dysfunction, whereas reduced NO synthesis plays the key role in this impairment.

For this reason, in the last decades several studies has been conducted to test L-arg effects on selected groups of subjects; then, patients affected by hypercholesterolemia, hyperhomocystinemia, diabetes and vascular atheromatic disorders, as well as shear stress in cell-culture, have confirmed a positive role of L-arg in reducing endothelial dysfunction.

This is the reason why our study group has recently postulated a possible positive effect of L-arg in treatment of cerebral small vessels disease, such as leukoaraiosis (66, 67).

Unfortunately, the extreme variability and heterogeneity of the published studies lead to inconclusive results when applied to a large numbers of patients. Then, despite the proved beneficial effects of L-arg in small and large vascular diseases, the clinical pharmacological use of L-arg delays to begin. To date, the clinical use of L-arg is considered effective only as secondary prevention in individuals with endothelium dysfunction, since the highest efficacy of L-arg is evidenced not when administered alone but in combination with other aminoacids (i.e. citrulline, glutamate, etc.) to prevent and reduced the arginine paradox.

In conclusion, we want highlight the following L-arg clinical implications:

- Oral L-arg implementation is effective as prevention treatment in subjects with chronic detrimental of endothelial function, such as senescence, aging and MELAS;
- The useful implementation with other aminoacids in a balanced formulation to better enhanced the vascular effect of L-arg and reduced the arginine paradox;
- The need of more standardized study to better define the efficacy and safety of L-arg in patients with vascular disorders.

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