The Pharmacodynamics of L-Arginine

Rainer H. Böger*

Clinical Pharmacology Unit, Institute of Experimental and Clinical Pharmacology, University Hospital Hamburg-Eppendorf, Germany

Abstract

L-Arginine is a precursor for nitric oxide (NO) synthesis. NO is a ubiquitous mediator that is formed by a family of enzymes named NO synthases. In the brain, NO acts as a neurotransmitter; in the immune system, NO acts as a mediator of host defense; and in the cardiovascular system, NO mediates the protective effects of the intact endothelium, acting as a vasodilator and endogenous antithromogenic molecule. About 5 g of L-arginine is ingested each day in a normal Western diet. L-Arginine plasma levels are not significantly reduced in most disease conditions, except end-stage renal failure. L-Arginine plasma levels are not significantly reduced in most disease conditions, except end-stage renal failure. L-Arginine is involved in various metabolic pathways, such as synthesis of creatine, L-ornithine, L-glutamate, and polyamines. Besides its role in protein metabolism, L-arginine is a conditionally essential, proteinogenic amino acid that is a natural constituent of dietary proteins. Its role in protein metabolism, L-arginine is involved in various metabolic pathways, such as synthesis of creatine, L-ornithine, L-glutamate, and polyamines. Decarboxylation of L-arginine can produce agmatine, a biogenic amine metabolite. L-Arginine is also involved in protein degradation by the ubiquitin-proteasome pathway. A biologically important pathway involves L-arginine as the substrate of a family of enzymes named nitric oxide synthases (NO synthases, NOS)3 (3). Three different isoforms of NOS have been characterized that are named according to the cell type from which they were first isolated: neuronal NOS (nNOS, NOS I), inducible NOS (iNOS, NOS II), and endothelial NOS (eNOS, NOS III) (4). nNOS and eNOS are expressed constitutively, their activity is regulated by calcium/calmodulin, and they produce NO at low rates. iNOS is induced in inflammatory cell types on cytokine stimulation; its activity is independent of calcium because of tight binding of calmodulin to the enzyme, and it produces NO at high rates. Recently, expression regulation of eNOS has been observed (5), so that the simple discrimination between constitutively and inducibly expressed enzymes is no longer correct; however, this nomenclature is still broadly used.

The reaction mechanism of NO synthases involves a 2-electron transfer from molecular oxygen via a number of cofactors to L-arginine, resulting in the release of NO and L-citrulline. N°-Hydroxy-L-arginine is formed as a relatively stable intermediate product of this reaction (6). NO exerts a range of critical roles in the regulation of the function of diverse organs throughout the body, depending on the cell type and tissue and the NOS isoform responsible. NO plays an important role as a mediator in nonadrenergic, non-cholinergic neurotransmission, in learning and memory, synaptic...
plasticity, and neuroprotection (7,8). In the cardiovascular system, NO produced by eNOS in response to stimulation of mechanoreceptors by the shear stress of the flowing blood is critically important for the homeostasis of vascular tone, interactions between the vascular wall and circulating blood cells (mainly thrombocytes and leukocytes), and for vascular structure. These functions exceed the scope of the present article; they have been reviewed extensively in recent years (9–13). Impaired formation or function of NO in the vasculature is an important pathogenic factor in the development of vascular diseases such as atherosclerosis, hypertension, and diabetic angiopathy (9).

L-Arginine has been studied extensively as a precursor for NO production, as evidenced by animal and human studies that have been reviewed extensively in recent years (9–13). Impaired formation or function of NO in the vasculature is an important pathogenic factor in the development of vascular diseases such as atherosclerosis, hypertension, and diabetic angiopathy (9).

The relative amounts of L-arginine in various proteins range from 3% to 15% (15). Soy protein, peanuts, walnuts, and fish are relatively rich in L-arginine, with 7–15% of their amino acid content being L-arginine (16). In contrast, cereals are relatively poor in L-arginine, with only 3–4% of their low protein content being L-arginine. Therefore, differing dietary habits between populations may account for differences in L-arginine plasma levels in various parts of the world. The usual range of L-arginine plasma levels has been determined as 6.7 ± 7.3 mmol/L in young men and 7.3 ± 7.8 mmol/L in young women (17) and 113.7 ± 19.8 μmol/L in elderly men and women, as compared with 72.4 ± 6.7 μmol/L in young men and 88.0 ± 7.8 μmol/L in elderly women (18).

Although intracellular L-arginine levels have been demonstrated to be considerably higher than L-arginine levels in the extracellular fluid or in plasma (19,20), evidence has been provided that extracellular L-arginine can be rapidly taken up by endothelial cells and contribute to NO production (21). Furthermore, dietary L-arginine is absorbed in the small intestine and transported to the liver, where the majority is taken up and used in the hepatic urea cycle; however, a small part of dietary L-arginine passes through the liver and is utilized as a substrate for NO production, as evidenced by animal and human studies that used L-arginine as a precursor (22,23).

How supplemental L-arginine might work: mechanisms of action

L-Arginine has been studied extensively as a precursor for NO synthesis in human subjects. One peculiar aspect in these studies was that the early studies were performed with high intravenous doses, and low doses have only recently been adopted in oral supplementation studies. The early, high doses stem from reports that L-arginine stimulated pituitary growth hormone secretion (24). A single dose of 30 g of L-arginine administered intravenously during a 30-min period was shown to induce vasodilation in human subjects (25–27). This vasodilation appeared rapidly after the initiation of the infusion in healthy human subjects (25), and it was reproducible in patients with arterial disease (26) and in patients with coronary artery disease but not in patients with primary pulmonary hypertension (28). L-Arginine-induced vasodilation was associated with increased release of NO metabolites, nitrite and nitrate, into urine. These data suggested that the reaction was NO-dependent; however, subsequent studies demonstrated that hormone release induced by such high doses of L-arginine also contributed to the vasodilator effect. In 1 study intravenous L-arginine resulted in a significant increase in the plasma concentration of growth hormone and insulin, and this endocrine effect of L-arginine was blocked by somatostatin coinfusion, and the vasodilator effect was partly abolished (29). Another study in healthy subjects also showed release of growth hormone after intravenous L-arginine (30), and this effect was antagonized by octreotide pretreatment and restored by coadministration of recombinant growth hormone with L-arginine.

Other mechanisms have been shown to contribute their parts to vasodilation induced by extremely high doses of parenterally administered L-arginine: Calver and co-workers (31) infused L-arginine locally into dorsal hand veins of human subjects, either using L-arginine or D-arginine (the latter is not a substrate for NO synthase), and both given as their free base form or hydrochloride salts, respectively (31). They found that both the L- and the D-forms of arginine induced vasodilation at local plasma concentrations estimated to be in the range of 4 to 13 mmol/L, suggesting that this vasodilator effect was nonspecific, possibly related to osmolality or pH effects and certainly unrelated to enhanced endothelial NO formation. All of these effects have been observed only at plasma concentrations of L-arginine that were in the very high micromolar to millimolar range. None of these mechanisms has been demonstrated to play a role at the lower plasma L-arginine concentrations likely to be achieved by oral supplementation with relatively low doses. In contrast, 1 study reported that plasma growth hormone and insulin-like growth factor-1 levels were unchanged by oral supplementation with 8 g of L-arginine twice daily in elderly humans (32).

From the different doses and routes of administration that have been used in these studies it can be concluded that the effects of L-arginine and the underlying mechanisms vary according to the plasma concentration range that is reached (Fig. 1). There is no indication to date of acute, pharmacologic effects of oral L-arginine in the dose range below 15 g of L-arginine per day. An acute vasodilator effect has been shown only in studies in which L-arginine was administered via a parenteral route, i.e., either intravenously or intraarterially. Acute hemodynamic effects of L-arginine at higher intravenous or intraarterial doses...
can be related to endocrine secretagogue and unspecific vaso-
dilator actions, which have been shown to be absent in the low
dose range. These data do not explain how t-arginine modulates
NO-dependent biological effects in a plasma concentration range
that closely resembles its physiological concentration range or
provide an explanation for the variable effects of oral supple-
mentation with t-arginine in different patient populations.

Although plasma levels of t-arginine have been reported to be
unchanged in vascular disease in all studies except 1 (34), it is
possible that the local availability of t-arginine as a substrate for
NO synthase may nonetheless be reduced by the activity of
arginase. Arginase utilizes t-arginine for the production of urea
and ornithine and thus competes with NO synthase for substrate
availability (35). Several studies have demonstrated that induct-
ion or activation of arginase I or arginase II can lead to impaired
NO production and endothelial dysfunction (36–40).

Recently evidence has emerged that accumulation of an endo-
genous inhibitor of nitric oxide synthase, asymmetric dimethyl-
arginine (ADMA), impairs nitric oxide formation in certain
pathophysiological conditions (41). The relation of elevated
ADMA levels with cardiovascular disease has been reviewed
recently (42). ADMA competes with t-arginine for binding to
NOS and thus competitively antagonizes the enzyme’s catalytic
activity, giving rise to the hypothesis that t-arginine may be
beneficial in patients with elevated ADMA but have no effects on
NO-dependent mechanisms in subjects with low or normal
ADMA levels (Fig. 2) (43).

**FIGURE 2** The “L-arginine paradox.” L-Arginine is the substrate of
NO synthase. The enzyme kinetics of endothelial NO synthase have
been determined biochemically in vitro. Data show that physiological
plasma L-arginine concentrations are in a range that enables full
activity of the enzyme in the presence of physiological, low ADMA
levels (A). However, in the presence of elevated levels of ADMA, a
competitive inhibitor of NO synthase, the conversion of L-arginine to
NO is impaired, resulting in decreased biological actions of NO (B).
Under such circumstances, even small changes in L-arginine concen-
tration secondary to dietary supplementation with L-arginine may result
in restoring NO production to near-normal levels (C). Adapted from
Böger (42).

**Clinical trials with L-arginine in cardiovascular disease**

Based on observations from experimental clinical studies like
those cited above, which showed vasodilation and enhanced NO
production after administration of L-arginine, a series of clin-
ical trials have been performed to investigate the potential of
this amino acid to improve the symptoms of cardiovascular
disease.

The first clinical application of L-arginine in an attempt to
improve vascular function in patients with cardiovascular dis-
ease was published in 1991 by Drexler and co-workers (44).
They infused L-arginine into the coronary arteries of patients
with coronary artery disease during a cardiac catheterization
and measured the coronary flow response to acetylcholine be-
fore and after L-arginine. These investigators showed that
L-arginine enhanced the blood flow response to acetylcholine
in coronary artery disease but not in controls. Since then, there
have been many studies with L-arginine in healthy human
subjects or in patients with various cardiovascular conditions.

Although it is beyond the scope of this article to give a com-
plete overview of all published clinical studies with L-arginine, it
becomes clear even from studying recent studies that L-arginine
has led to discrepant findings. As an example, Ceremuzynski
et al. (45) reported a significant improvement of exercise capac-
ity in 22 patients with coronary artery disease who received oral
L-arginine, 6 g/d, for 3 d in a double-blind, placebo-controlled
design (45). Bednarz et al. (46) later confirmed these findings in a
virtually identical study design in which 25 patients with stable
coronary artery disease underwent exercise testing before and
after 3 d of oral L-arginine (6 g/d) or placebo. L-Arginine signifi-
cantly improved exercise duration but did not affect QT
segment depression in exercise electrocardiogram. Rector and
co-workers (47) performed a study in 15 patients with moderate
to severe heart failure who received, in random sequence,
L-arginine 5.6 to 12.6 g/d or matching placebo for 6 wk.
Compared with placebo, supplemental oral L-arginine signifi-
cantly increased forearm blood flow during forearm exercise,
6-min walking distance, and arterial compliance as well as
subjective well-being as assessed by the Living with Heart
Failure Questionnaire. In another study (48), 21 patients with
stable heart failure were given sequential exercise tests before
and after L-arginine or placebo in a double-blind crossover study
comparing 9 g/d of L-arginine or placebo for 7 d. This study
confirmed a significant improvement in exercise duration time
by oral L-arginine as compared with placebo.

In contrast, there are several relatively small, clinical studies
with experimental endpoints that failed to show beneficial ef-
ects of L-arginine on vascular function. In a study including 30
patients with stable coronary heart disease receiving optimized
medical treatment according to current guidelines, Blum et al.
(49) found no significant improvement of endothelium-dependent
vasodilation, blood flow, or inflammatory marker serum levels
by dietary L-arginine at a dose of 9 g/d as compared with pla-
cebo, given for a period of 1 mo. In another study, 40 patients
with coronary heart disease and angiographically proven steno-
sis of >50% received L-arginine 15 g/d or placebo for 2 wk (50).
L-Arginine supplementation had no significant effect on endo-
thelial function, blood flow, markers of oxidative stress, or exer-
cise performance. Finally, supplementation with 6 g/d of L-arginine
vs. placebo for 2 wk after coronary stent implantation resulted in
no significant change of coronary neointima formation or in-
tent restenosis in 60 patients with stable angina pectoris and
angiographically proven stenosis of >50% (51).

Taken together, these clinical studies with experimental de-
signs suggest that there may be subgroups of patients whose
vascular function is improved by L-arginine supplementation, although there are other patients or subgroups of patients who do not profit from such dietary intervention. Diagnostic markers are needed that allow prospective identification of patients who have a high probability of showing a response to dietary intervention with L-arginine. To this end, patient characteristics of different studies need to be analyzed carefully to define differences between studies that may account for such apparently conflicting results. In addition to the dose of L-arginine (daily doses below 2 to 3 g/d appear to be without beneficial effect), patient selection appears to be a major factor affecting study outcome. Patients on "optimized medical treatment" may be less responsive, and patients with advanced coronary stenoses also showed less effect. By contrast, L-arginine was more effective when early, functional changes of vascular function were chosen as endpoints, and vascular disease may have been less advanced.

In addition to the relatively small experimental trials, 2 recent comparatively large clinical trials investigated the effects of oral supplementation with L-arginine in patients with coronary heart disease. In 1 study (52), 792 patients with coronary artery disease were included within 24 h after the onset of acute myocardial infarction. More than 85% of the patients received thrombolytic therapy for the acute myocardial infarction. Patients were randomized to receive oral L-arginine (3 g 3 times daily) or matching placebo for 1 mo. The composite clinical endpoint (cardiovascular death, reinfarction, recurrent myocardial ischemia, successful resuscitation, or shock/pulmonary edema) was not significantly different between the 2 groups, but there was a strong trend in favor of L-arginine (OR 0.63, 95% CI 0.39–1.02, P = 0.06). The endpoint was significantly reduced by L-arginine in a predefined subgroup of hypercholesterolemic patients (19 vs. 31 events, P < 0.05), and a reduced incidence of events was observed in each of the components of the composite clinical endpoint. Adverse events were rare and not significantly different between the L-arginine and placebo groups, with gastrointestinal disorders (mostly loose stools) being the most frequently observed side effect.

The second study included 153 patients with stable coronary artery disease at 3–21 d after their first ST-segment elevation infarction (53). Patients were randomized to 3 g of L-arginine or placebo 3 times daily for a period of 6 mo. The primary endpoint was left ventricular ejection fraction, with several measures of vascular stiffness and clinical events being secondary endpoints. Close to 90% of the patients in this trial had received acute percutaneous coronary intervention for the acute myocardial infarction. In this study, the ejection fractions were not significantly different between the 2 groups, nor were differences between the 2 groups reported for any of the secondary endpoints. However, a strong trend can be seen in the data reported for L-arginine to decrease pulse wave velocity, a measure of arterial elasticity and endothelial function (54), as compared with placebo. Concern was raised because this study was stopped prematurely after 6 deaths had occurred in the L-arginine group vs. none in the placebo group. A close analysis of the deaths reveals that 4 of the deaths were most probably unrelated to treatment (1 myocardial rupture at reinfarction, 2 presumed sepsis, and 1 sudden death at wk after study treatment had ended), and a causal relation could neither be confirmed nor excluded for 2 patients who were found dead at their homes during the course of the study. The study has been criticized because the authors failed to show elevation of plasma L-arginine levels during supplementation with this amino acid, and a causal relation between the dietary intervention and any of the deaths could not be ascertained (55,56). Both aspects make it hard to determine the risk-benefit relation of dietary L-arginine in this trial.

Conclusion: Disease prevention with L-arginine supplementation?
Currently available data point to the fact that oral supplementation with L-arginine can affect endothelium-mediated vascular functions such as enhanced vasodilation, decreased platelet aggregation, and reduced endothelial monocyte adhesion. These effects occur when L-arginine plasma concentrations are elevated minimally above the physiological concentration range. At higher L-arginine plasma concentrations, like those reached during intravenous or intraarterial infusion, other effects that are not directly linked to NO production can be observed, such as hormone release and nonspecific vasodilation.

Beneficial (endothelium-dependent, NO-mediated) vascular effects of dietary L-arginine are more likely to be reached when the following conditions are fulfilled: 1) Dietary supplementation with L-arginine can be effective when the endothelial L-arginine–NO metabolism is impaired in a fashion that is reversible by L-arginine. Among possible causes for such impairment are increased arginine losses (e.g., during hemodialysis treatment), increased metabolic use of L-arginine by NO-independent pathways [e.g., induction of arginases (57,58)], or the presence of elevated levels of ADMA, the endogenous inhibitor of NO synthase that displaces L-arginine from the substrate binding site of this enzyme (42,43) and is a common cause of relative arginine deficiency in vascular pathologies. 2) L-Arginine supplementation is more efficient in patients who are not maximally treated with pharmacologic agents. Such optimized medical management probably does not allow any room for improvement of vascular function by L-arginine. In addition, several pharmacologic agents used in secondary prevention of cardiovascular disease have been shown not only to improve vascular function but also to reduce ADMA levels (59); they may thereby diminish the ability of L-arginine to improve vascular function. 3) L-Arginine appears to affect pathophysiological mechanisms that contribute to the progression of atherosclerosis. Such pathological mechanisms may be more strongly affected by dietary L-arginine in relatively early stages of the disease, when functional changes are still reversible, whereas structural atherosclerotic changes of the vascular wall may be less responsive to L-arginine. Therefore, L-arginine has a place as a nutraceutical agent in the modification of functional impairment and in the prevention of vascular disease but not as a therapy to reverse manifest atherosclerosis.

Future studies should be planned after carefully considering these influencing factors, and patients should be preselected by a marker that allows prediction of a higher-than-average probability of responding to L-arginine supplementation, such as arginase induction or elevated ADMA concentration. Diagnostic tools to determine ADMA levels easily and rapidly have been made available recently (60–62) and should therefore diminish the obstacles for such studies. Long-term studies are needed to determine whether there is a difference in the availability of dietary L-arginine when it is given during short- or long-term periods.

Literature Cited


