Reduced Nitric Oxide Production by L-Arginine Deficiency in Lysinuric Protein Intolerance Exacerbates Intravascular Coagulation

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Lysinuric protein intolerance (LPI) results in low serum L-arginine, hyperammonemia, mental retardation, thrombocytopenia, and an increased frequency of bowel movements. Our objective was to evaluate the effects of low serum L-arginine, the essential substrate for reactions catalyzed by nitric oxide synthetase (NOS), on the serum nitric oxide (NO) level and coagulation activity in a patient with LPI. A 37-year-old Japanese man who presented with abdominal pain and subnormal fasting levels of serum L-arginine and L-lysine was found to have LPI. The result of oral administration of diamino acids was an increased in urine and a decrease in serum, thus confirming the diagnosis. A decrease in the platelet count and an increase in the plasma levels of thrombin–antithrombin III complex (TAT) and fibrin degradation products (FDPs) indicated the presence of subclinical intravascular coagulation. Serum levels of NO derivatives and L-arginine were determined after intravenous administration of L-arginine. The effects of intravenous L-arginine or transdermal nitroglycerin on the plasma level of TAT were also investigated. Serum levels of NO derivatives were significantly reduced in the LPI patient versus the healthy control group (n = 5). Intravenous administration of L-arginine increased the serum level of NO derivatives and the platelet count and reduced plasma TAT and FDP levels. The plasma level of TAT was also reduced by transdermal nitroglycerin. A decrease in the serum level of L-arginine in patients with LPI appears to result in a decrease in NO production. The improvement in plasma TAT levels produced by administration of intravenous L-arginine or transdermal nitroglycerin suggests that intravascular coagulation is exacerbated by the decrease of NO production in patients with LPI.

IN PATIENTS WITH lysinuric protein intolerance (LPI), an autosomal recessive disorder of amino acid transport, impaired intestinal absorption and renal wastage of L-arginine, L-ornithine, and L-lysine lead to a deficiency of diamino acids. The prevalence of this autosomal recessive disease is one in 60,000 in Finland, whereas it is much lower in Japan. The main symptoms are due to ornithinopenic failure of the urea cycle, with postprandial hyperammonemia.[1] Other important symptoms include abdominal pain, vomiting, and diarrhea.[2] The platelet count is decreased in patients with LPI, and the cause of this is unknown.

Nitric oxide (NO) is synthesized in a tightly regulated manner by three types of NO synthetases (NOSs) in various tissues and a variety of cell types.[3] The regulation of the timing and amount of NO production, as well as its sites of synthesis, is crucial for the proper function of NO. NO exerts a variety of physiological effects such as vasodilation,[4] a decrease of gastrointestinal motility,[5] macrophage-induced cytotoxicity,[6] and inhibition of platelet aggregation.[7] Because L-arginine is the only physiological nitrogen donor for the NOS-catalyzed reaction, the availability of this essential substrate is expected to determine the cellular rate of NO synthesis. L-Arginine can be obtained in the body by uptake from the extracellular fluid, intracellular protein degradation, or endogenous synthesis.[8] It was recently suggested that a deficiency of L-arginine may lead to a decrease in NO production in vivo.[9]

It is postulated that a decrease in serum L-arginine in patients with LPI results in a decrease of NO production. Our objective was to examine the relationship between serum L-arginine and NO levels in a patient with LPI. Moreover, since NO inhibition of platelet aggregation and thrombosis is one of the complications of LPI, the effects of L-arginine and NO levels on coagulation activity were also investigated in this patient.

SUBJECTS AND METHODS

Case Report

A 37-year-old Japanese man (weight, 50 kg; height, 150 cm; body mass index, 22.2 kg/m^2) with LPI was admitted to our hospital because of abdominal pain. He has no family history of LPI. Additional findings included mental retardation, subaverage height, vomiting, and diarrhea. Elevated serum levels of ammonia (51 μg/dL; normal range, 5 to 43), lactate dehydrogenase (640 U/L; normal range, 130 to 290), ferritin (688 ng/mL; normal range, 23 to 263), and thyroxine-binding globulin (31 μg/mL; normal range, 14 to 28) were observed in the patient. These findings also confirmed the presence of LPI according to a previous report.[2] He had decreased serum levels of L-arginine, L-lysine, and L-ornithine and increased urinary excretion of the diamino acids (Table 1). A test involving oral administration of L-arginine, L-lysine, L-ornithine, and L-citrulline indicated impaired intestinal absorption of L-arginine, L-lysine, and L-ornithine but not L-citrulline (the only substrate tested that was not a diamino acid). Fasting serum levels of L-arginine and L-lysine were subnormal after their oral administration, and diamino acid attained only the lower limit of the fasting normal range (Fig 1). These data confirmed that the patient has LPI. The patient and the control subjects (n = 5) provided informed consent to participate in the study.

Determination of Serum NO Derivatives

Fasting serum concentrations of NO derivatives were measured in the patient with LPI and five healthy control subjects using the chemiluminescence method.[10] Serum samples were collected from the patient at five different times. The NO derivatives nitrite and nitrate were assayed in serum using a Sievers NO analyzer (Taiyo Toyo Koso, Osaka, Japan) after reduction by vanadium trichloride (Wako Pure Chemical, Osaka, Japan).

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Table 1. Serum Level and Urinary Excretion of L-Arginine, L-Ornithine, and L-Lysine in a Patient With LPI

Amino Acid	Serum (nmol/mL)	Urine (mmol/d)
L-Arginine	13 (54-130)	615 (10-60)
L-Ornithine	21 (30-100)	435 (7-50)
L-Lysine	68 (110-240)	12,254 (50-2,000)

NOTE. The normal range for each amino acid is shown in parentheses.

Measurement of Serum Amino Acid Levels After Oral Loading

An oral bolus dose (20 g) of L-arginine, L-lysine, L-ornithine, or L-citrulline was administered to the patient, blood was collected at defined intervals, and the serum was separated by centrifugation. The amino acid content was measured by an amino acid autoanalyzer (model L-8500 with column 2622SC; Hitachi, Tokyo, Japan).

Determination of Serum L-Arginine and NO Derivatives After Intravenous Administration of L-Arginine

A mixture of L-arginine (20 g) and L-glutamate (20 g) was infused intravenously over 60 minutes into the patient. Serum levels of L-arginine and NO derivatives were measured at defined intervals.

Measurement of Thrombin–Antithrombin III Complex Levels After Administration of Intravenous L-Arginine and Transdermal Nitroglycerin

The effect of intravenous daily administration of a mixture of L-arginine (20 g) and L-glutamate (20 g) and transdermal delivery of a NO donor (nitroglycerin 25 mg/d) on the plasma level of thrombin–antithrombin III complex (TAT) in the patient was evaluated for several months. Blood was taken from a vein of the patient in the fasted state and immediately mixed with disodium EDTA as an anticoagulant. Plasma levels of TAT were measured by enzyme immunoassay (Enzygnost TAT Micro kit; Behring Hoechst Japan, Tokyo).

Measurement of Plasma Fibrin Degradation Products and Cyclic Guanosine Monophosphate

Plasma levels of fibrin degradation products (FDPs) were measured by a latex agglutination test (LPIA-200; Daiayatoron, Tokyo, Japan), and plasma levels of cyclic guanosine monophosphate (cGMP) were measured by radioimmunoassay.[11]

Statistical Methods

Results are presented as the mean ± SD. Statistical significance in comparisons between two measurements and among groups was determined by the two-tailed Student's *t* test.

RESULTS

Serum Concentration of NO

The fasting serum concentration of NO derivatives was markedly decreased in the patient with LPI compared with the five healthy controls (Fig 2). Serum NO levels were elevated after intravenous administration of L-arginine following the elevation in serum L-arginine levels. Similar results were obtained for L-arginine administration in three healthy controls (Fig 3A). To ensure that the serum concentration of NO derivatives was correctly measured, we measured the plasma concentration of cGMP, the second messenger of NO. Figure 3B shows that plasma cGMP levels were concomitantly ele-

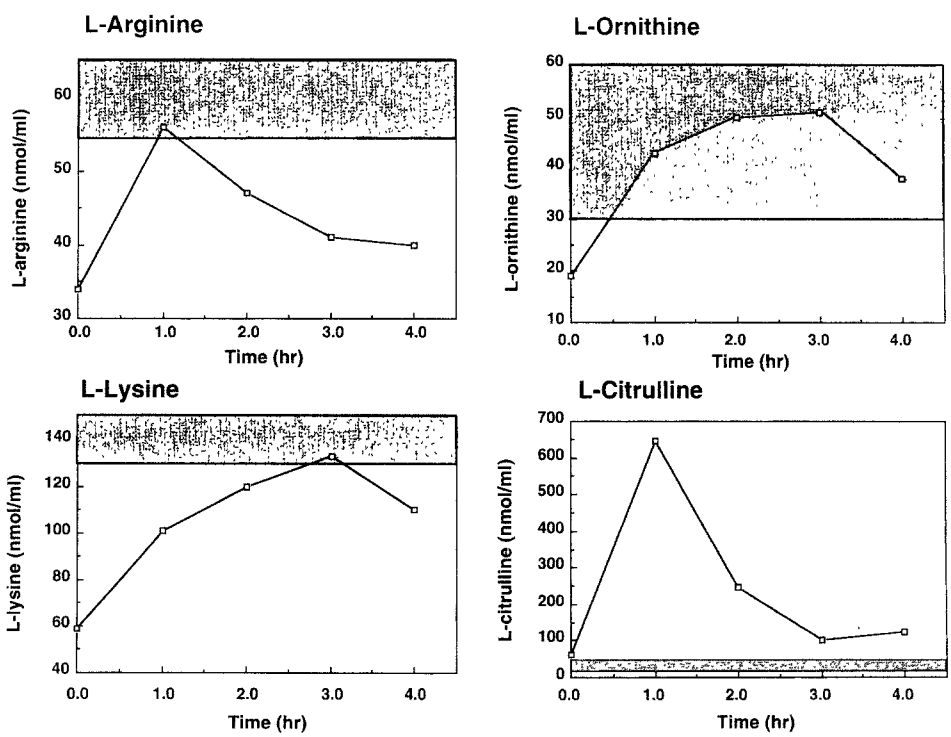

Fig 1. Serum amino acid concentrations after loading. Twenty grams of each indicated amino acid was administered orally to the patient at time 0. (▭) Fasting normal range.

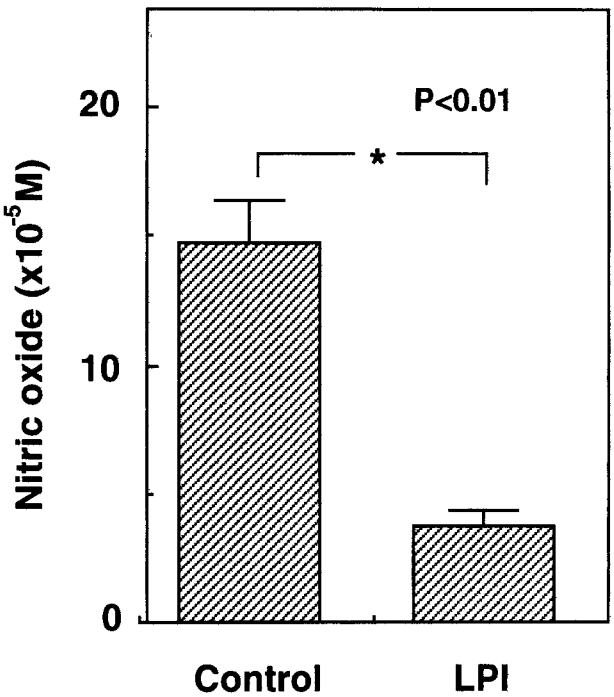

Fig 2. Effect of LPI on serum NO derivatives. Fasting serum levels are shown for NO derivatives in 5 healthy controls and the patient with LPI (mean ± SD). Five samples from the patients were measured.

vated after intravenous administration of L-arginine (before, 2.43 ± 0.22 pmol/mL; after. 7.55 ± 2.23; normal range, 1.8 to 4.8).

Intravascular Coagulation Activity

In the patient with LPI, the platelet count was decreased (12.4×10^4/μL; normal range, 13.0×10^4 to 32.0×10^4), and the plasma levels of TAT (126 μg/L; normal range, <3) and FDP (47.1 μg/mL; normal range, <5.0) increased spontaneously. These findings suggest that this patient has subclinical intravascular coagulation. The increased coagulation activity of this patient improved during intravenous administration of L-arginine 20 g daily. The platelet count increased (15.0×10^4 μL) and the plasma level of TAT decreased (40.9 μg/L), as did plasma FDP (22.9 μg/mL), 2 weeks after intravenous administration was started.

Effect of Intravenous L-Arginine or Transdermal Nitroglycerin on Serum TAT

TAT levels in plasma were dramatically decreased during treatment with L-arginine or the nitroglycerin patch, ie, the NO donor (Figs 4 and 5). Figure 5 shows that the NO donor also decreased TAT levels significantly. TAT levels began to increase during the interval between treatments. Abdominal pain was diminished during either treatment, while administration of either scopolamine butylbromide, a muscarinic receptor antagonist, or diclofenac sodium, a nonsteroidal antiinflammatory, was less effective for abdominal pain.

DISCUSSION

The abnormality of the diamino acid transporter leads to an impairment of intestinal absorption and a renal loss of L-arginine, L-ornithine, and L-lysine, resulting in a deficiency of these diamino acids. The reduced serum concentration and increased urinary excretion of L-arginine in the patient confirmed that he has a chronic deficiency of L-arginine. Since L-arginine is the only substrate that regulates NO production,[12] the patient with LPI would be expected to have a reduced NO production due to the low serum concentration of L-arginine.[2] We found that the serum concentration of NO derivatives was markedly decreased in this patient with LPI. A decrease in the supply of L-arginine from the extracellular fluid seemed to be responsible for the impairment of intracellular NO production by NOS. Intravenous administration of L-arginine resulted in an increased concentration of serum NO derivatives. These data suggest that L-arginine, when administered intravenously, is transported into NO-producing cells and is converted to NO by NOS.

The present study suggests that an impairment of NO production by a deficiency of L-arginine caused this patient's

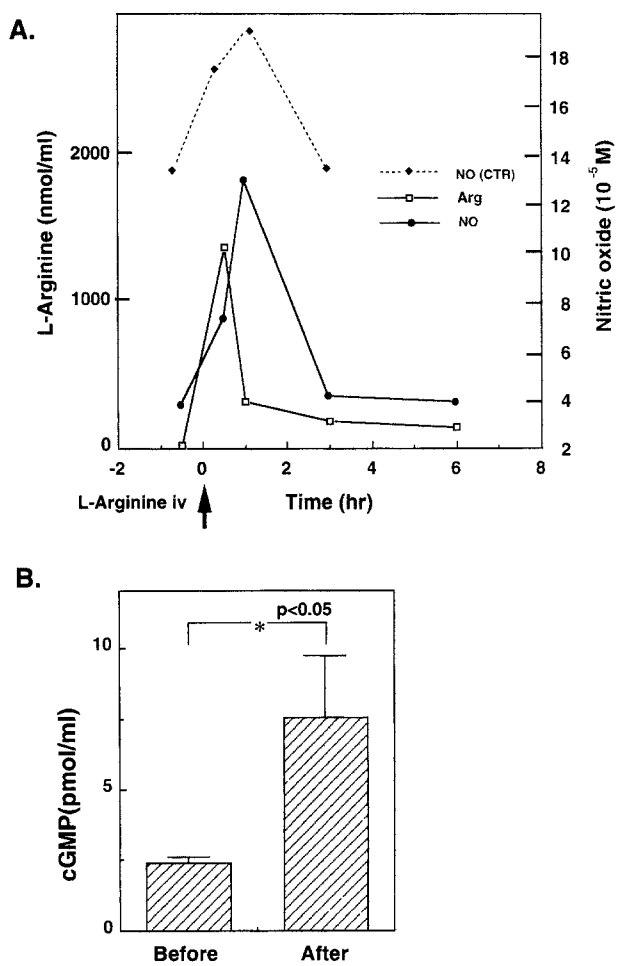

Fig 3. Effect of intravenous infusion of L-arginine on serum L-arginine and NO derivatives in a patient with LPI. (A) Twenty grams of L-arginine and L-glutamate mixture was administered intravenously to the patient with LPI. Serum (□) L-arginine and (●) NO derivatives were measured at the indicated times; (◆) serum NO derivatives in 3 controls were measured at the indicated times. (B) Plasma cGMP levels were measured before treatment and 1 hour after intravenous administration. Three samples in each group were measured. *$P < .05$ by Student's t test.

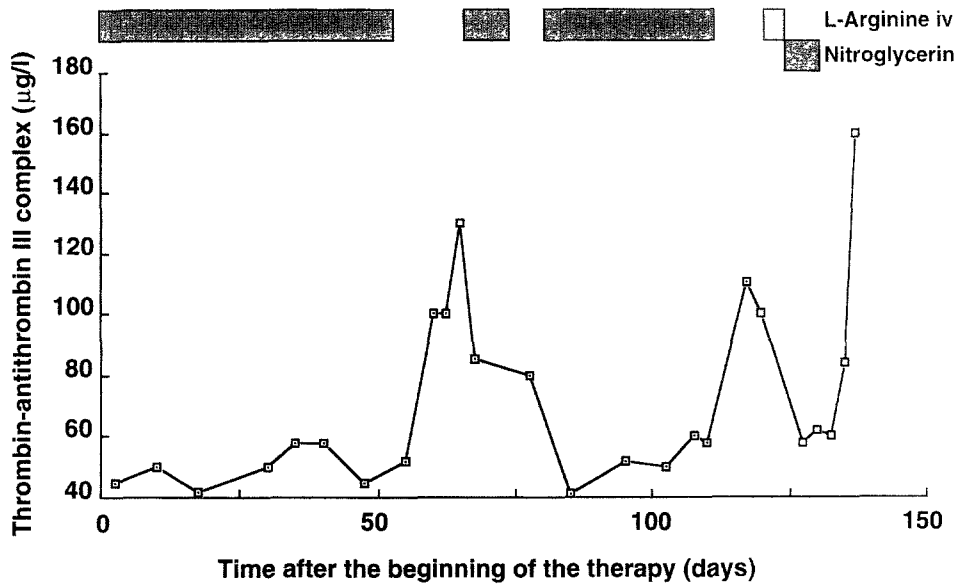

Fig 4. Effect of intravenous L-arginine and transdermal nitroglycerin (a NO donor) on plasma TAT levels in a patient with LPI. Intravenous L-arginine (20 g) and L-glutamate (20 g) (□) or transdermal nitroglycerin 25 mg/d (■) were administered daily.

symptoms. NO is important in vasodilation, intestinal relaxation, and inhibition of platelet aggregation, and in increasing the cytotoxicity of the macrophage. In this patient, the elevated plasma levels of TAT and FDP and decreased platelet count suggested the presence of subclinical intravascular coagulation due to an impairment of NO production, probably in endothelial cells. Abnormal pulmonary macrophages containing elevated amounts of iron are present in LPI.[13] The intracellular accumulation of iron suggests the occurrence of subclinical hemorrhages in the lung tissue[13] consistent with the easy bleeding found in patients with LPI. Pregnancy in a patient with LPI carries the risk of severe hemorrhage during labor.[2] Plasma TAT levels were significantly decreased in our patient during each therapy session with L-arginine or nitroglycerin, whereas the levels quickly increased in the absence of therapy (Fig 4). Such treatments improved the clinical symptoms in this case, such as abdominal pain, diarrhea, and vomiting, as well as the biochemical abnormalities.

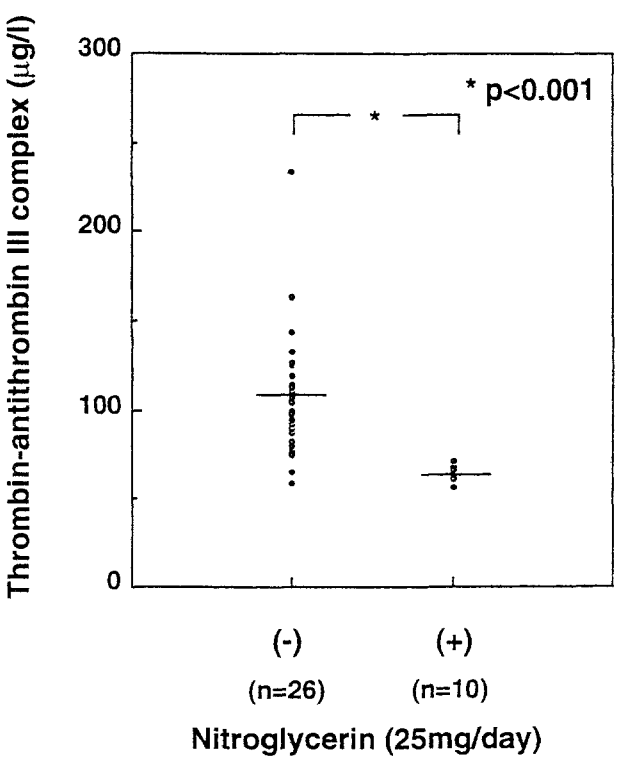

Fig 5. Influence of nitroglycerin on plasma TAT in a patient with LPI. Plasma TAT levels were measured daily after administration of transdermal nitroglycerin 25 mg. Horizontal bars represent the mean ± SD; n indicates the number of samples in each group. *$P < .001$ by Student's t test.

The results are consistent with the association of NO with the junctional potential in nonadrenergic, noncholinergic nerves and with the relaxation of the gastrointestinal tract.[7] Recently, the cytokine-dependent NO production by rat cardiac myocytes was found to be a consequence of increased expression of the inducible isoform of NO synthase and of the cationic amino acid transporter.[14] The evidence indicates that the availability of L-arginine is required for the production of appropriate amounts of NO. Therefore, NO production in humans seems to depend on L-arginine uptake and its concentration in serum.

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