

## Effect of Short-Term Administration of High Dose L-Arginine on Restenosis After Percutaneous Transluminal Coronary Angioplasty

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### Abstract

**Background.** A single and local administration of L-arginine after balloon angioplasty enhances nitric oxide (NO) generation and inhibits lesion formation in animals.

**Objectives.** The present study assessed the effect of increasing NO to inhibit restenosis after percutaneous transluminal coronary angioplasty (PTCA) in humans by local and systemic administration of L-arginine, a precursor of NO in humans.

**Methods.** L-arginine was administered to 34 consecutive patients with angina pectoris or old myocardial infarction via a cardiac catheter (500 mg/4 min) before PTCA, and via a peripheral vein (30 g/4 hr, for 5 days) after PTCA. Patients were treated between December 1998 and December 2000. Plasma concentrations of L-arginine, NO (as nitrite + nitrate) and cyclic guanosine monophosphate (cGMP) were measured before and after L-arginine administration. The control group consisted of 90 patients who underwent PTCA successfully without L-arginine administration in the period between July 1996 and November 1998. Baseline clinical and angiographic characteristics were compared between the two groups. All patients were followed by coronary angiography for 3 months after PTCA. Quantitative coronary angiography and restenosis rate were studied.

**Results.** Baseline clinical and angiographic characteristics were not different between the two study groups. Despite a significant elevation in plasma L-arginine concentration after L-arginine administration, NO and cGMP did not increase significantly. After PTCA, the difference in restenosis rates between L-arginine and control subjects (34% vs 44%) was not significantly different.

**Conclusions.** Short-term administration of high dose L-arginine did not significantly change the restenosis rate after PTCA.

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### Key Words

■ Myocardial infarction, treatment    ■ Angioplasty (PTCA)    ■ Restenosis  
■ Nitric oxide (L-arginine administration)

### INTRODUCTION

Percutaneous transluminal coronary angioplasty (PTCA) is commonly used as a nonsurgical treatment for occlusive or stenotic atherosclerotic coronary artery disease. Despite an initial success rate

greater than 90%, patency does not continue in the long-term because of restenosis. Indeed, restenosis affects as many as 30 - 40% of successfully dilated lesions<sup>1)</sup>. Experimental and necrotic tissue studies suggest that restenosis is secondary to balloon-induced injury to proliferating vascular smooth

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muscle cells<sup>2</sup>). To address this problem, various techniques, including anticoagulants and angiotensin converting enzyme inhibitors and new devices have been tried to decrease restenosis, but most have failed<sup>1-3</sup>). Only stents have decreased restenosis<sup>4,5</sup>). The rate of in-stent restenosis remains about 20%, judging by neointimal formation consisting of migration and proliferation of smooth muscle cells with deposition of extracellular matrix<sup>6</sup>). Despite this significant reduction in the restenosis rate in primary studies of intracoronary brachytherapy<sup>7</sup>), edge restenosis and late coronary occlusion remain unsolved<sup>8,9</sup>). Drug coated stents decrease the restenosis rate even further<sup>10,11</sup>). However, the long-term results and side effects remain unknown.

Recently, it was established that nitric oxide (NO) has many biological effects. For example, NO can interfere with monocyte adhesion and chemotaxis, platelet adherence and aggregation, and vascular smooth muscle cell proliferation<sup>12-18</sup>). Thus, NO may reduce restenosis after PTCA. A single intramural delivery of L-arginine, the precursor of NO, improved vasomotion and attenuated neointimal lesion formation after balloon angioplasty in previous reports<sup>19,20</sup>). Therefore, the present study examined the potential of L-arginine administration to reduce restenosis after PTCA in humans.

## SUBJECTS AND METHODS

### Study population

This study included 35 consecutive patients admitted to our hospital with angina pectoris or old myocardial infarction in the period between December 1998 and December 2000. All patients had angiographical coronary artery stenosis greater than 50% and exercise thallium myocardial scintigraphy indicated that they had myocardial ischemia. This study excluded patients with recent myocardial infarction (< 3 weeks), recent unstable angina, restenosis, left main trunk lesion, chronic total occlusion, severe left ventricular dysfunction (ejection fraction < 40%), uncontrolled diabetes mellitus (hemoglobin A<sub>1c</sub> > 8.0%), bronchial asthma, renal failure (serum creatinine level > 2.0 mg/dl), acidosis, amino acid metabolic dysfunction, or other disease using steroid hormone. The protocol for administration of L-arginine was approved by the hospital ethics committee and written informed consent according to the Helsinki Declaration was

obtained from all patients. The control subjects consisted of 90 patients successfully treated with PTCA without L-arginine administration at our hospital in the period between July 1996 and November 1998. They underwent follow-up coronary angiography on average 3 months after PTCA. All patients enrolled in this study received isosorbide dinitrate (60 mg/day), an angiotensin converting enzyme inhibitor (enalapril 5 mg/day or temocapril 2 mg/day), a calcium antagonist (long acting nifedipine 30 mg/day or long acting diltiazem 300 mg/day), acetylsalicylic acid (81 mg/day) and ticlopidine (100 mg/day). Treatment was started at least 2 weeks before PTCA and was continued for 3 months.

### Percutaneous transluminal coronary angioplasty procedure

PTCA was performed by the femoral approach with a bolus dose of 10,000 U of heparin. A guiding catheter (8F Brite Tip; Cordis), guide wire (0.014 High torque floppy; ACS) and semi-compliant balloon catheters (Bandit; Boston Scientific) were used in all patients. Balloon inflation for 1 min was repeated until the residual stenosis became less than 25%. Patients who underwent emergent coronary artery bypass grafting or stent implantation due to acute occlusion after PTCA with flow-limiting dissection were excluded from this study.

### Quantitative analysis of coronary angiography

Angiography was recorded from multiple projections for all patients before and after PTCA. Quantitative analysis of the lesions was carefully performed based on standard criteria for preprocedural lesion morphology, and the lesions were also categorized according to the modified American College of Cardiology/American Heart Association (ACC/AHA) classification system. Quantitative coronary angiography (edge detection method; MAC HEART DATABASE SYSTEM, Baxter) was used to evaluate the initial and long-term results of PTCA. Cinefilm was used as a medium. One experienced cardiologist not involved in this study measured the diameter of the coronary artery three times and the mean value was used for analysis. The minimal lumen diameter of the stenotic lesion, and the proximal and distal sites of the lesion were measured five times: immediately before L-arginine administration, after L-arginine administration via guiding catheter, after isosorbide dinitrate

administration, immediately after PTCA, and 3 months after PTCA. A guiding catheter filled with contrast medium was used as the scaling device.

Initial success was defined as percentage diameter stenosis (%DS) of < 25% after PTCA without major complications (death, emergent coronary artery bypass grafting, stent implantation, Q-wave infarction). Restenosis was defined as %DS > 50% at follow-up angiography.

#### L-arginine administration

i) L-arginine (500 mg/4 min; Hoechst Marion Roussel) was initially administered into the coronary artery via guiding catheter before PTCA.

ii) PTCA was performed after intracoronary infusion of 5 mg of isosorbide dinitrate.

iii) Systemic administration of L-arginine via peripheral vein was started from the beginning of PTCA and was repeated once a day for the following 4 days at the same rate (30 g/4 hr).

#### Measurement of plasma L-arginine concentration

A 5-French NIH catheter was inserted into the coronary sinus through the internal jugular vein during PTCA in nine patients. Plasma L-arginine concentrations in the coronary sinus and peripheral veins were measured before and after intracoronary L-arginine administration. Plasma L-arginine concentrations in the peripheral vein were measured before, immediately after, 6 hr after, and 12 hr after the systemic administration of L-arginine in 10 patients. All samples were measured with an amino acid analyzer at a commercial laboratory (SRL).

#### Measurement of nitric oxide and cyclic guanosine monophosphate

The production of NO in blood was evaluated by the measurement of nitrite ion ( $\text{NO}_2^-$ ) + nitrate ion ( $\text{NO}_3^-$ ) by the Griess method using a chemiluminescence NO analyzer (SPD-10A, Shimadzu Industries) at a commercial laboratory (SRL). Cyclic guanosine monophosphate (cGMP) was measured by radioimmunoassay with a gamma counter (ARC-950, AROKA) at a commercial laboratory (SRL) according to the manufacturer's protocol. The same blood sample collected for the measurement of L-arginine was also used for the measurements of NO and cGMP.

**Table 1** Baseline patient characteristics

	Arginine( + ) ( n = 34 )	Arginine( - ) ( n = 90 )	p value
Age( yr )	67 ± 10	63 ± 10	NS
Male( % )	71( 24/34 )	71( 64/90 )	NS
Risk factors( % )			
Hypertension	26( 9/34 )	29( 26/90 )	NS
Diabetes mellitus	18( 9/34 )	16( 14/90 )	NS
Hypercholesterolemia	12( 4/34 )	14( 13/90 )	NS
Smoking	24( 8/34 )	22( 20/90 )	NS
Previous myocardial infarction	44( 15/34 )	48( 43/90 )	NS
Total cholesterol( mg/dl )	199 ± 44	193 ± 39	NS
HDL-cholesterol( mg/dl )	52 ± 10	44 ± 14	NS
Triglyceride( mg/dl )	169 ± 136	159 ± 119	NS
Uric acid( mg/dl )	5.6 ± 1.7	5.9 ± 1.6	NS
Blood sugar( mg/dl )	120 ± 31	119 ± 32	NS

Continuous values are mean ± SD.

HDL = high-density lipoprotein.

#### Statistical analysis

Data was expressed as mean ± standard deviation. The chi-square test was used to assess differences in categorical variables. The paired Student's *t*-test was used to assess differences in continuous variables between the two groups. *p* values of less than 0.05 were considered significant.

## RESULTS

**Table 1** shows the clinical characteristics of patients in both groups. There were no statistically significant differences between the two groups with respect to age, sex, total cholesterol, triglyceride, high-density lipoprotein-cholesterol, uric acid, fasting blood sugar, coronary risk factors and prior myocardial infarction. **Table 2** shows the angiographic characteristics of the two groups. Lesion vessels and ACC/AHA classification types showed no difference. PTCA was successfully performed in 34 patients (success rate of 99%). One patient was defined as failed PTCA because the residual stenosis was 50% after PTCA and he was excluded from the study.

L-arginine was administered to all 34 patients via the coronary artery and peripheral vein. One patient had severe headache and the infusion speed of L-arginine was decreased (30 g/4 hr → 30 g/8 hr). Plasma L-arginine concentration after intracoronary and systemic administration of L-arginine signifi-

cantly increased (Tables 3 and 4). The diameter of the coronary artery did not change significantly after intracoronary administration of L-arginine. NO and cGMP did not increase significantly in the coronary sinus or the peripheral vein after intracoronary administration of L-arginine ( $n = 9$ ; Table 3). After systemic administration of L-arginine, NO did not increase significantly ( $n = 10$ ; Table 4). Follow-up angiography was performed in 32 patients (94%). The parameters of quantitative

coronary angiography and the restenosis rate in the L-arginine group after PTCA were not significantly different from those of the control group (Tables 5 and 6).

## DISCUSSION

The restenosis rate after PTCA is 30 - 40% and is still the biggest limitation of PTCA. The mechanism of restenosis consists of platelet aggregation within 48 hr after PTCA, followed by intimal hyperplasia, proliferation and migration of smooth muscle cells for 3 or 6 months, and vascular remodeling by proliferating extracellular matrix<sup>1-3</sup>). Intimal hyperplasia and vascular remodeling are the most important processes. Many experimental studies have shown that NO produced from L-arginine by constitutive NO synthetase in endothelial cells regulates intimal hyperplasia<sup>16,21,22</sup>). Although endothelial cells are injured after PTCA and regenerate quickly, NO production remains disturbed, due to the decrease in constitutive NO synthetase in the regenerated endothelium<sup>23,24</sup>). However, NO synthetase is induced in the vascular smooth muscle cells of injured arteries in response to the cytokines produced at the injured site. Then, NO is produced by the smooth muscle cells<sup>25</sup>). The improvement in endothelial function due to the administration of L-arginine to levels that exceed the Km is called the arginine paradox, and may be explained by a relative intracellular deficiency in L-arginine caused by the competition of asymmetric

**Table 2 Baseline lesion angiographic characteristics**

	Arginine( + ) ( $n = 34$ )	Arginine( - ) ( $n = 90$ )	<i>p</i> value
Vessel( % )			NS
LAD	5( 17/34 )	6( 60/90 )	
LCX	1( 5/34 )	7( 6/90 )	
RCA	3( 12/34 )	2( 24/90 )	
ACC/AHA lesion type( % )			NS
A	2( 8/34 )	1( 16/90 )	
B	6( 21/34 )	7( 71/90 )	
C	1( 5/34 )	3( 3/90 )	
Maximal inflation pressure( atm )	9.7 ± 3.1	8.3 ± 2.2	NS
Balloon-artery ratio	1.04 ± 0.14	1.05 ± 0.15	NS
Total inflation time( sec )	360 ± 180	300 ± 144	NS

Continuous values are mean ± SD.

LAD = left anterior descending artery; LCX = left circumflex coronary artery; RCA = right coronary artery; ACC/AHA = American College of Cardiology/American Heart Association.

**Table 3 Changes in plasma concentration of L-arginine, nitric oxide and cyclic guanosine monophosphate during intracoronary administration of L-arginine**

	L-arginine( nmol/ml )		NO( $\mu$ mol/l )		cGMP( pmol/ml )	
	Before	After	Before	After	Before	After
Coronary sinus	72 ± 19	1,670 ± 1,801*	31 ± 11	32 ± 12	8.2 ± 4.3	8.3 ± 4.2
Peripheral vein	64 ± 16	326 ± 57*	32.7 ± 11.1	30.8 ± 10.8	7.9 ± 4.0	8.2 ± 4.3

Values are mean ± SD ( $n = 9$ ). \* $p < 0.05$  vs control before L-arginine administration.

NO = nitric oxide; cGMP = cyclic guanosine monophosphate.

**Table 4 Serial changes of L-arginine, nitric oxide, and cyclic guanosine monophosphate during systemic administration of L-arginine**

	Before	Just after	6 hr later	14 hr later
L-arginine( nmol/ml )	108 ± 97	2,417 ± 1,733*	340 ± 381	151 ± 36
NO( $\mu$ mol/l )	33 ± 4	28 ± 1	39 ± 7	36 ± 7

Values are mean ± SD ( $n = 10$ ). \* $p < 0.05$  vs control before L-arginine administration.

Abbreviation as in Table 3.

**Table 5 Results of quantitative coronary angiography analysis**

	Arginine( + ) ( n = 32 )	Arginine( - ) ( n = 90 )	p value
Reference diameter( mm )			
Before PTCA	2.80 ± 0.54	2.78 ± 0.52	NS
After PTCA	2.81 ± 0.55	2.79 ± 0.52	NS
Follow up	2.81 ± 0.54	2.78 ± 0.53	NS
MLD( mm )			
Before PTCA	0.77 ± 0.34	0.76 ± 0.32	NS
After PTCA	2.15 ± 0.47	2.13 ± 0.48	NS
Follow up	1.64 ± 0.58	1.55 ± 0.57	NS
Changes in MLD( mm )			
Acute gain	1.39 ± 0.50	1.38 ± 0.49	NS
Late loss	0.52 ± 0.58	0.58 ± 0.57	NS
Net gain	0.87 ± 0.60	0.80 ± 0.59	NS
Loss index	0.38 ± 0.76	0.42 ± 0.74	NS
Percentage of stenosis( % )			
Before PTCA	72.5 ± 10.3	72.7 ± 10.6	NS
After PTCA	23.5 ± 7.3	23.7 ± 7.6	NS
Follow up	41.6 ± 18.6	44.2 ± 19.2	NS

Values are mean ± SD.

MLD = minimal lumen diameter; PTCA = percutaneous transluminal coronary angioplasty.

dimethyl L-arginine with L-arginine and the dysfunction of the cationic acid transporter of L-arginine<sup>26-29</sup>). Therefore, the administration of a high dose of L-arginine may increase production of NO and reduce restenosis after PTCA.

The rate and the dose of intracoronary administration of L-arginine in our study were similar to those in experimental studies in animal or humans<sup>30,31</sup>). The rate of systemic administration of L-arginine was also designed to be similar to that of intracoronary administration to maintain the same L-arginine concentration in the coronary artery. The daily dose of systemic infusion was also similar to that of oral administration in animals<sup>22</sup>). Ethical limitations in Japan did not permit us to use L-arginine for oral administration at that time. The plasma concentration of L-arginine at the end of the period of systemic administration was equal to that at the end of the period of intracoronary administration. The use of a high dose of L-arginine was safe in our patients with intracoronary or systemic administration. Only one patient (3%) experienced headache during systemic administration and deceleration of the rate of administration improved the symptom.

**Table 6 Restenosis rate at follow up**

	L-arginine( + ) ( n = 32 )	L-arginine( - ) ( n = 90 )	p value
Restenosis rate( % )	34( 11/32 )	44( 40/90 )	NS
Follow up rate( % )	94( 32/34 )	100( 90/90 )	NS

We used plasma concentration of nitrate and nitrite as an indicator of NO production. After 12 hr of fasting, as much as 90% of the circulating nitrite is derived directly from the L-arginine: NO pathway<sup>32</sup>). However, the circulating nitrate concentration is usually influenced by dietary intake, especially by nitrate-rich foods like lettuce<sup>33</sup>). All the patients enrolled in this study received their usual diet except for lunch just before PTCA. Therefore, the fact that the plasma concentration of nitrate and nitrite did not change after L-arginine administration in both the coronary sinus and the peripheral vein may have been due to dietary influences. The plasma cGMP concentration is also an indicator of NO production. However, local cGMP elevations in the endothelial cells or in the fibroblasts in small vessels like the coronary artery may not have affected plasma cGMP levels.

Follow-up angiography showed that the minimal lumen diameter after PTCA was slightly bigger and the restenosis rate was slightly lower in patients with L-arginine administration than in control subjects. However, the difference was not statistically significant. Recently, it has been reported that vascular remodeling is the main determinant of lumen size after arterial injury, rather than intimal hyperplasia, in atherosclerotic rabbits. However, L-arginine supplementation did not decrease the in-stent reocclusion rate, which suggests that positive or negative vascular remodeling can be excluded<sup>34</sup>). Furthermore, neither L-arginine nor N<sup>G</sup>-nitro-L-arginine methyl ester( L-NAME ) administration after balloon angioplasty in hypercholesterolemic rabbits significantly changed lumen size, as L-arginine inhibited whereas L-NAME stimulated intimal hyperplasia and vascular enlargement<sup>35</sup>). In contrast, oral L-arginine administration from 2 days prior to 4 weeks following catheter-induced injury to the rabbit thoracic aorta and iliac artery attenuated the development of intimal hyperplasia in experimental studies<sup>21,22</sup>). Furthermore, a single and small dose of L-arginine administration through a Dispatch catheter decreased intimal hyperplasia.

The catheter was shown to maintain a high concentration of drug in the coronary artery for a long time after a single administration<sup>19,20</sup>. These results may depend on the bifunctional regulation of apoptosis according to the NO concentration. Physiologically relevant levels of NO seem to suppress apoptosis of endothelial cells. However, higher levels of NO induction may overwhelm cellular protective mechanisms and exert proapoptotic and cytotoxic effects on endothelial and smooth muscle cells<sup>36</sup>.

Additionally, the administration of L-arginine improved endothelial dysfunction in the microcirculation but not in the macrocirculation in animal and human experiments<sup>30,31</sup>. The minimal and reference vessel diameter after a single intracoronary injection of L-arginine did not change in our study. Thus, the endothelial dysfunction of the coronary artery in the epicardium might not be improved enough to inhibit the growth of smooth muscle cells that result from the administration of L-arginine in humans.

### Study limitations

The present study included a small number of patients. A larger population study may be required to clarify whether NO can reduce restenosis after PTCA or not. Although the baseline clinical and angiographic characteristics of both groups were similar and without significant differences, our study was not a randomized study. As stated above, we could not use purified L-arginine for oral supplementation because of ethical limitations in Japan. Furthermore, we could not use an infusion catheter because of the small stock available in Japan at that time. The long-lasting elevation of plasma L-arginine levels after low dose but not high dose administration of L-arginine using these products may affect the restenosis rate after PTCA.

### CONCLUSIONS

This study showed that the short-term administration of high dose L-arginine did not significantly change the restenosis rate after PTCA.

### 要 約

#### 冠動脈形成術後再狭窄に及ぼすL-アルギニン高用量短期投与の効果

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背景・目的: 動物実験においてアルギニンの局所単回投与により, 一酸化窒素(NO)の産生が増強され, 冠動脈形成術後再狭窄病変の予防効果が証明されている。本研究ではヒトにおいて, NOの前駆物質であるアルギニンの局所および全身投与により, 冠動脈形成術後の再狭窄が抑制されるか否かを検討した。

方 法: 対象は1998年12月-2000年12月の間に当院に入院した, 狭心症ないし陳旧性心筋梗塞患者連続34例である。カテーテルを通して, 4分間で500mgのL-アルギニンを投与したのち, 冠動脈形成術を行い, 終了後30gのL-アルギニンを経静脈的に1日1回4時間かけて投与し, これを5日間行った。冠静脈洞および末梢血血漿中のL-アルギニン, NO(亜硝酸および硝酸イオン), サイクリックGMPを, L-アルギニンの投与前後で測定した。対照には1996年7月-1998年11月にL-アルギニン非投与下に冠動脈形成術を行い, 成功した90症例とした。対照群, L-アルギニン投与群の臨床指標および病変形態を比較した。両群ともに3ヵ月後に冠動脈造影を行い, 術前後の冠動脈径の定量的評価を行い, 再狭窄率を求めた。

結 果: 両群間で臨床指標および病変形態には差はみられなかった。L-アルギニン投与後の血漿アルギニン濃度は有意に上昇したが, NOおよびサイクリックGMP濃度は, 冠静脈洞ならびに末梢血中ともに増加しなかった。3ヵ月後の再狭窄率は, アルギニン投与群, 非投与群の間で有意差がなかった(再狭窄率: 投与群34%, 非投与群44%)。

結 論: NOの前駆物質であるL-アルギニンの短期投与は, 冠動脈形成術後の再狭窄率を有意には変化させなかった。

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