Vol. 193, No. 1, 1993
May 28, 1993

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 291-296

# L-ARGININE INHIBITS BALLOON CATHETER-INDUCED INTIMAL HYPERPLASIA

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Received April 5, 1993

Intimal hyperplasia that results from therapeutic revascularization is an important etiologic factor in the failure of these procedures (i.e., restenosis). Drugs which donate nitric oxide have been shown to inhibit the proliferation of vascular smooth muscle cells *in vitro*. We tested the hypothesis that administration of L-arginine (0.5 g/kg/day), the precursor of nitric oxide, would inhibit development of intimal hyperplasia following balloon catheter-induced injury. L-arginine administration from 2 days prior to and 2 weeks following catheter-induced injury to the rabbit thoracic aorta attenuated the development of intimal hyperplasia by 39% as compared with untreated controls. This effect was due to decreased intimal area. The effect of L-arginine was inhibited by co-administration of an inhibitor of nitric oxide synthase, $N^G$-nitro-L-arginine methyl ester (0.5 g/kg/day). These data demonstrate that L-arginine attenuates intimal hyperplasia and suggest that the mechanism for this effect is the conversion of L-arginine to nitric oxide.


Vascular injury produced during attempted revascularization (e.g., balloon catheters, bypass surgery) damages the endothelial cell layer lining the arterial wall. This injury is followed by the growth of a neoendothelium and by proliferation and/or migration of medial smooth muscle cells through fenestrae of the internal elastic lamina resulting in the formation of intimal hyperplasia (1,2). Intimal hyperplasia has been implicated as a main etiologic factor in restenosis within 1 to 12 months after therapeutic revascularization procedures. It has been noted that regression of intimal hyperplasia is associated with neoendothelial formation (1). Endarterectomized carotid arteries seeded with autogenous endothelial cells showed a marked reduction in the development of intimal hyperplasia at 6 weeks (3).

Prostacyclin and NO are formed by the endothelium and have been reported to inhibit vascular smooth muscle proliferation (4-6). We have previously shown that neoendothelial

Abbreviations: NO, nitric oxide; L-NAME, $N^G$-nitro-L-arginine methyl ester; I/M, intimal area/medial area x 100.

0006-291X/93 $4.00

prostacyclin formation by rabbit thoracic aorta returns to control levels 3-4 weeks after catheter injury; however, NO formation as determined by acetylcholine-induced relaxation of vascular rings, was attenuated by 75% and development of intimal hyperplasia was progressive over the 8-week study (7). These data suggested that neoendothelial NO may play a more important role than prostacyclin in inhibiting intimal hyperplasia.

Endothelium-derived NO is synthesized from L-arginine by a membrane-bound, constitutive isoform of NO (8). We, therefore, tested the hypothesis that administration of L-arginine, which would increase NO formation, inhibits catheter-induced intimal hyperplasia.

## METHODS

### Operative Preparation and Harvesting of Aortae

Male New Zealand White rabbits weighing between 2 and 4 kg were randomly divided into 6 groups. One group was sham operated and received treatment with L-arginine (0.5 g/kg/day); another group underwent balloon catheter injury and received no treatment; another group underwent balloon catheter injury and received L-arginine (0.5 g/kg/day); two groups underwent balloon catheter injury and received L-arginine plus L-NAME (0.5 g/kg/day or 0.1 g/kg/day); another group underwent balloon catheter injury and received L-NAME (0.5 g/kg/day). Treatment with L-arginine was initiated 2 days prior to induction of catheter injury whereas treatment with L-NAME was initiated the day of injury. All animals were sacrificed 2 weeks after induction of catheter injury. L-arginine and L-NAME were administered in the drinking water or by gavage.

The animals were anesthetized with ketamine HCl (20 mg/kg i.m.). Through an arteriotomy in the superficial femoral artery, a 4 French embolectomy catheter was passed until the tip was positioned in the ascending aorta. The balloon was inflated with water and withdrawn to the level of the abdominal aorta. This procedure then was repeated 3 additional times. In order to provide a consistent degree of injury and complete denudation of the vascular endothelium, the catheter was withdrawn attached to a force gauge and a calculated shear force of 140-175 cm was transmitted to the vessel wall. The femoral artery was then ligated. Sham-operated animals underwent simple ligation of the superficial femoral artery. These procedures were conducted under sterile conditions in a vivarial operating room. Following operation, the rabbits were housed in the vivarium and given water and standard rabbit chow ad libitum until sacrifice. Blood pressure was measured in anesthetized animals with a Gould IM 1000 computer monitor immediately prior to sham operation/cather injury and to sacrifice. These procedures are as previously published (7).

At the time of sacrifice, the animals were euthanized with a lethal dose of pentobarbital (150 mg i.v. push), and the thoracic aortae were removed. After removal, the aortae were fixed in gluteraldehyde.

### Histologic Studies

Arterial specimens were embedded in paraffin, sectioned at 5 micron intervals, and stained with the Verhoeff-Van Gieson stain for elastic tissue.

### Measurement of Intimal Hyperplasia

In order to obtain a semiquantitative measurement of intimal hyperplasia, specimens prepared for light microscopy as described above were examined morphometrically using

videomicroscopy and computerized digital image analysis system (Optimas, BioScan, Inc., Edmonds, WA). The absolute area of the intima, as defined by the tissue beween the lumen of the vessel and the internal elastic membrane, was divided by the absolute area of the media between the internal and external elastic membranes to obtain the intima to media (I/M) ratio for each specimen. Multiple sections (n=2-4) were examined for each specimen; torn or distorted sections were excluded; and the average I/M ratio for each specimen obtained. Findings were averaged for each group and reported as mean ± SE. The segments were fixed under atmospheric rather than physiological pressure. It is felt that qualitative assessment of this data is justified, as all segments were prepared under similar conditions. These procedures were as previously published (7,10).

Data Analysis

Data obtained from within a group were averaged and reported as mean ± SE. The data were analyzed using analysis of variance and the Scheffe F-test to determine if differences existed between groups. A $P < 0.05$ with 95% confidence intervals was considered significant. The n refers to the number of animals in a group.

## RESULTS

The data in Fig. 1 demonstrate that following balloon catheter injury there was a significant increase in the I/M ratio; however, following administration of L-arginine (0.5 g/kg/day) from 2 days prior to and 2 weeks after injury, there was a significant reduction in I/M as compared with the no treatment group (P=0.0015). Moreover, this attenuation produced by L-arginine was significantly inhibited by co-administration of L-NAME (0.5 g/kg/day) for 2 weeks after injury (P=0.0109). The I/M ratio in animals receiving the combination of L-arginine and L-NAME was not significantly different from that in animals receiving no treatment (P=0.9641). However, co-administration of a lower dose of L-NAME (0.1 g/kg/day) over the 2-week interval did not significantly attenuate the response to L-arginine (12.4 ± 0.9, n=5, P=0.3967 as compared with L-arginine treatment). Administration of L-NAME (0.5 g/kg/day) alone for 2 weeks after injury did not significantly change the I/M ratio (18.5 ± 2.6, n=3) as compared to the no treatment group (P=0.5410).

Administration of L-arginine, L-NAME alone or in combination did not affect blood pressure, which at the time of sacrifice was: 118/92 ± 6/5, 98/71 ± 6/5, 87/62 ± 6/1, 103/77 ± 10/2, and 105/78 ± 6/4 for L-arginine sham, NoTx ballooned, L-arginine ballooned, L-arginine + L-NAME (0.5 g/kg/day) ballooned, and L-NAME ballooned, respectively. These blood pressures were similar to those measured prior to catheter injury.

The effect of balloon catheter injury and of L-arginine treatment on the intimal and medial areas is shown in Fig. 2A and B, respectively. The injury produced a significant increase in the intimal area as compared with sham-operated controls. L-arginine significantly attenuated this response (P=0.0148). Co-administration of L-NAME with L-arginine significantly reversed this effect of L-arginine (P=0.0007). The intimal area of animals

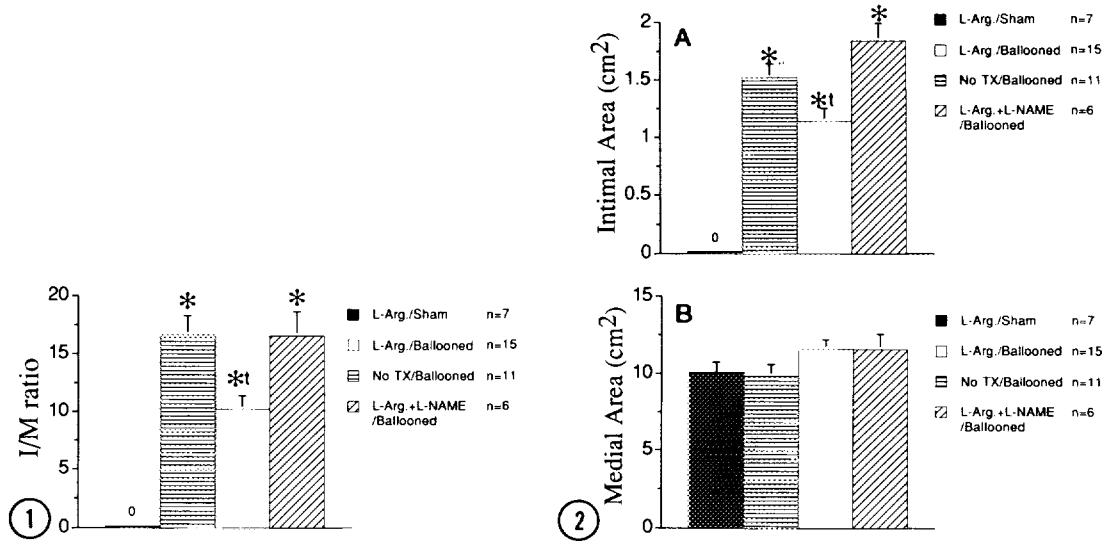


Fig. 1. Development of intimal hyperplasia following balloon catheter-induced arotic injury as determined by the ratio of the area of the intima/area of the media (I/M); L-arginine or L-NAME was given in a dose of 0.5 g/kg/day; L-arginine was given from 2 days prior to 2 weeks following catheter injury; L-NAME was given for 2 weeks from day of injury. The data are presented as mean ± SE; n = number of animals; NoTx = no treatment; * = significant difference vs. L-arginine/sham; t = significant difference vs. NoTx/ballooned or L-arginine + L-NAME/ballooned.

Fig. 2. Changes in the intimal (A) and medial (B) areas of rabbit aorta following balloon catheter-induced injury; L-arginine or L-NAME was given in a dose of 0.5 g/kg/day, as in the legend to Fig. 1. The data are presented as mean ± SE; n = number of animals; NoTx = no treatment; * = significant difference vs. L-arginine/sham; t = significant difference vs. NoTx/ballooned or L-arginine + L-NAME/ballooned.

receiving a combination of L-arginine and L-NAME was not significantly different from that of animals receiving no treatment (P=0.0844). Co-administration of a lower dose of L-NAME (0.1 g/kg/day) did not significantly attenuate the response to L-arginine (1.39 ± 0.12 $cm^2$, P=0.2237). Administration of L-NAME (0.5 g/kg/day) for 2 weeks following injury to a group of 3 animals significantly increased the intimal area as compared to the no treatment group (2.13 ± 0.26 $cm^2$ vs. 1.52 ± 0.12 $cm^2$, P=0.0129); however, when these data were expressed as an I/M ratio, this effect of L-NAME was not evident. Neither balloon catheter injury nor any of the treatments significantly affected the medial area, as compared with the L-arginine sham group (Fig. 2B).

## DISCUSSION

Administration of the precursor of NO, L-arginine (0.5 g/kg/day) attenuated the development of intimal hyperplasia as characterized by the I/M ratio. This effect of L-arginine

was reversed by co-administration of an inhibitor of NO synthase, L-NAME. The reversal by L-NAME was dose-dependent as co-administration of a lower dose of L-NAME was without effect. It has been previously reported that NO-releasing vasodilators (e.g., nitroglycerin, sodium nitroprusside) inhibit mitogenesis and proliferation of cultured vascular smooth muscle cells (6). Therefore, these data suggest that the conversion of L-arginine to NO by NO synthase is the mechanism by which L-arginine inhibited intimal hyperplasia. No synthase is constitutively present in the endothelial cell and has been reported to be induced in the blood vessel wall 24 hr after balloon catheter injury (10). The constitutive form is agonist-stimulated whereas the inducible isozyme is not. It is interesting to speculate that catheter injury results in induction of NO synthase which converts the exogenous L-arginine to NO within medial smooth muscle thereby attenuating intimal hyperplasia. However, it is uncertain whether the induced forms remain present and/or active for longer than 24 hr.

The changes in the I/M ratio reflect changes in the area of the intima not the media as neither catheter injury nor any treatment altered the medial area as compared to sham controls whereas injury increased and L-arginine treatment attenuated this increase in the intimal area. However, it is not possible to ascertain from the present data whether the inhibitory effect of NO was at the level of smooth muscle cell proliferation, migration, or both. The observation that administration of L-NAME (0.5 g/kg/day) alone for 2 weeks following injury significantly increases the intimal area suggests that NO produced from endogenous L-arginine may play a role in attenuating intimal hyperplasia.

In conclusion, the data suggest that endogenous biosynthesis of NO from L-arginine plays a role in preventing intimal hyperplasia. Interference with the synthesis of endothelium-derived NO, which occurs in endothelium-damaged arteries, could lead to the develop of intimal hyperplasia. Moreover, it was recently reported that administration of L-arginine improves endothelium-dependent vasorelaxation and reduces atherogenesis in thoracic aortae from hypercholesterolemic rabbits (11). Therefore, administration of L-arginine may be therapeutically beneficial in inhibiting intimal hyperplasia/restenosis following revascularization attempts employing balloon catheters.

## ACKNOWLEDGMENTS

The authors thank Ms. Janice Ignarro for editorial assistance. This work was supported in part by NIH grant HL15580 and by grants-in-aid from the American Heart Association and the American Heart Association-Louisiana, Inc.

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