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N^G-NITRO-L-ARGININE METHYL ESTER DOES NOT AFFECT BALLOON CATHETER-INDUCED INTIMAL HYPERPLASIA IN RATS

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The L-arginine derived NO-cGMP pathway's role in the response of the arterial wall to balloon catheter injury was examined. Rats were given the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (10 mg/kg po twice daily) or vehicle for 6 days before and 2 weeks after balloon catheter injury. N^G-nitro-L-arginine methyl ester treatment increased blood pressure and inhibited acetylcholine responses in aortic rings but did not alter the lesions produced by balloon injury. Our results suggest that the L-arginine derived NO-cGMP pathway does not play a significant role in the response of the artery wall to balloon injury in the rat.

As a response to injury and endothelial damage, vascular smooth muscle proliferation leads to intimal thickening (1). This process contributes to the common problem of restenosis following angioplasty or coronary artery bypass procedures (2).

The endothelium via the L-arginine derived nitric oxide (NO)-cGMP pathway (3) plays an important role in mediating vascular smooth muscle function. Agents acting via this pathway have been shown to inhibit vascular smooth muscle cell proliferation in vitro (4). Two recent studies in the rabbit have indicated that stimulation

Abbreviations:

NO, nitric oxide; cGMP, cyclic guanosine 3'-5' monophosphate; L-NAME, N^G-nitro-L-arginine methyl ester; ACE, angiotensin converting enzyme.

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of the L-arginine derived NO-cGMP pathway will inhibit intimal hyperplasia following cholesterol feeding or balloon catheter injury (5,6).

In the present study, we examined the role of the L-arginine derived NO-cGMP pathway in the intimal hyperplasia following balloon catheter-induced injury in the rat carotid artery model. To interrupt the L-arginine -NO pathway, an inhibitor of NO synthase, N^G-nitro-L-arginine methyl ester (L-NAME), was used (7).

MATERIALS AND METHODS

Balloon Injury Model

The left carotid artery of male Sprague-Dawley rats (410-440 g, Charles River Breeding Laboratories, Charles River, MA) was subjected to balloon catheter injury using the method of Baumgartner (8) as modified by Clowes et al. (9). Using aseptic technique and ether anesthesia, a 2F Fogarty embolectomy catheter was inserted via the left external carotid artery and advanced 5 cm. The balloon was inflated and the catheter was retracted. This was repeated three times turning the catheter 90° each time it was retracted. Rats were dosed orally with L-NAME (10 mg/kg twice daily) or received an equivalent volume of 0.4% aqueous methylcellulose vehicle. This regimen was followed for 6 days prior to balloon injury and continued for 14 days post-balloon injury.

All rats were anesthetized with ether the day prior to sacrifice. The caudal artery was cannulated with PE_{50} tubing for direct determination of arterial blood pressure as previously described (10). Mean blood pressure and heart rate were assessed in fully conscious rats after a 90 min recovery period. Cannulas were sealed and taped and the rats returned to their cages after blood pressure readings were obtained.

At sacrifice, rats were anesthetized with a mixture of ketamine and xylazine (75 mg/kg and 15 mg/kg, im respectively) and exsanguinated via aortic transection. Rings were cut from the thoracic aorta and mounted for isometric force measurements in organ baths containing a modified Krebs-Henseleit bicarbonate solution at 37°C. Phenylephrine (0.3 μM) was used to contract the rings and acetylcholine was added to the organ bath in cumulative fashion.

Three 5 mm segments of the left carotid artery (LC-1,LC-2 and LC-3) beginning 5 mm above the aortic origin were taken for histological and morphometric analysis. Tissues were fixed in 4% paraformaldehyde containing 10% sucrose (w/v) and paraffin-embedded sections were stained with Gomori-trichrome-aldehyde fuschin. Computer-based morphormetric analysis was conducted using a Bioquant System IV image analysis system. Animals were treated and cared for as recommended by the NIH Guide for the Care

and Use of Laboratory Animals and the Animal Welfare Act in a vivarium certified by the American Association for the Accreditation of Laboratory Animal Care. The experimental protocol used was reviewed and approved by the Schering Plough Research Institute's Animal Care and Use Committee.

Statistics

Data are expressed as means±SEM. Analysis of variance and Scheffe's F-statistic were used to examine statistical differences between treatment groups. P values < .05 were considered significant.

Materials

L-NAME was obtained from Sigma (St. Louis, MO) and 2F Fogarty embolectomy catheters from Baxter Healthcare Corp., (McGraw, Il). Other materials were of reagent grade.

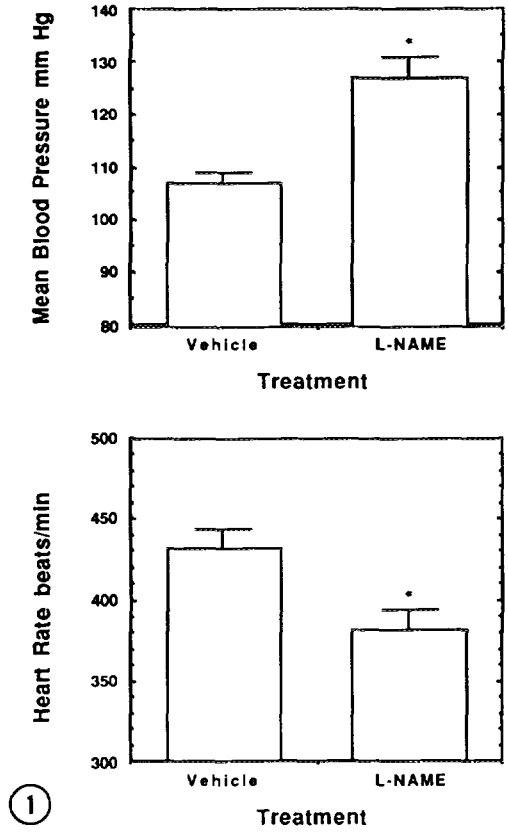
RESULTS

Figure 1 shows that mean blood pressure in rats treated with L-NAME was significantly higher than in rats treated with 0.4% aqueous methylcellulose vehicle. Heart rate was also significantly lower in L-NAME treated rats. The response of aortic rings taken from L-NAME treated rats to the endothelium-dependent vasodilator acetylcholine was significantly depressed compared to those rings harvested from control rats (Figure 2).

The results of morphometric analysis of the balloon catheter-induced lesions are summarized in Table 1. There was no significant difference in intimal lesion area, medial lesion area or of the ratio of intimal to medial areas between treatment groups. That is to say, L-NAME treatment did not cause the anticipated exacerbation of the intimal proliferative response in any of the individual carotid artery segments or on an overall basis.

DISCUSSION

Our findings lead us to conclude that the endogenous L-arginine -NO pathway may not be an important control mechanism in the long term response of the artery wall to local (i.e., balloon catheter) injury *in the rat*. We do not believe that the inability to observe an effect upon intimal hyperplasia reflects the fact that the manipulation we utilized, L-NAME treatment to inhibit NO synthase, was inadequate to perturb the endogenous L-arginine -NO pathway. The dose of L-NAME was sufficient to cause a marked, significant



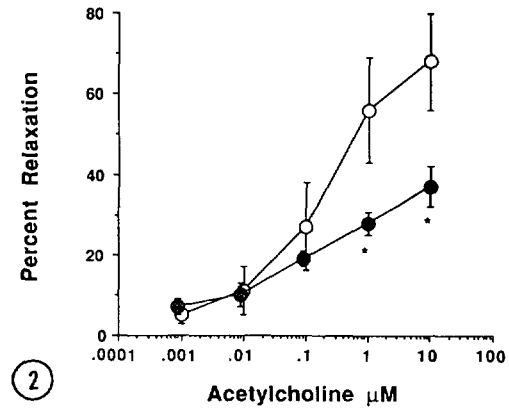


Figure 1. Effect of L-NAME treatment on mean blood pressure (MBP, upper panel) and heart rate (lower panel) in conscious rats. Columns and bars signify mean values± SEM, respectively. The asterisk denotes a significant difference between treatments. Columns on the left represent values obtained in rats dosed twice daily with 10 mg/kg po L-NAME (n=9) and those on the right represent values obtained in vehicle-treated rats (n=8). Rats had received the above treatments for 6 days prior to balloon injury and for 13 days thereafter. These values were recorded on the day prior to sacrifice.

Figure 2. Response of rat aortic rings to the endothelium-dependent vasodilator acetylcholine. Unfilled and filled symbols denote responses of rings obtained from vehicle treated rats (n= 5) and from L-NAME treated rats (n= 6), respectively. The asterisk denotes a significant difference between treatments.

elevation in mean blood pressure. We take this as evidence that there was sufficient inhibition of nitric oxide synthase to significantly suppress the vasodilator expression from NO formation. Furthermore, the inhibition of the vascular relaxant response to acetylcholine in aortic rings prepared from rats treated with L-NAME offers proof that there was effective inhibition of the vascular wall's ability to synthesize nitric oxide.

Table 1. Morphometric Analysis of Carotid Arterial Lesions 14 Days Post-Balloon Injury in Rats

Treatment	Lesion Level	Lesions Analyzed	Intimal Area mm^2	Medial Area mm^2	Ratio
Controls n=7					
	L-1	7	0.168±0.031	0.141±0.012	1.157±0.019
	L-2	7	0.130±0.027	0.103±0.017	1.273±0.160
	L-3	7	0.189±0.034	0.149±0.011	1.231±0.159
	Overall	21	0.161±0.016	0.130±0.008	1.222±0.077
L-NAME n=8					
	L-1	8	0.189±0.016	0.189±0.055	1.215±0.144
	L-2	8	0.162±0.024	0.126±0.007	1.289±0.181
	L-3	8	0.177±0.012	0.143±0.004	1.225±0.171
	Overall	24	0.176±0.012	0.153±0.018	1.243±0.088

Values represent means±SEM.

Two rabbit studies have recently reported that the L-arginine -NO pathway was an important mechanism for limiting the intimal proliferation in response to cholesterol feeding (5) or the intimal hyperplasia following balloon catheter injury (6). In the cholesterol-fed rabbits, descending thoracic aortic plaque surface area was reduced from 40% to 10% by L-arginine and intimal thickness was reduced (5). L-arginine (500 mg/kg daily) treatment resulted in a 39% inhibition of thoracic aortic intimal lesion size following balloon catheter injury (6).

One possible explanation for the different outcomes of the L-arginine -NO pathway manipulation we report here and those reported in rabbits (5,6) might well reflect a difference in the response of the two species. The ability of a given treatment to inhibit the intimal proliferative response induced by balloon injury is highly species dependent. For example, angiotensin converting enzyme (ACE) inhibitors cause substantial (70% or more) inhibition of intimal proliferation in the rat balloon catheter injury model (11). They do not cause significant inhibition of balloon catheter-induced lesions in swine (12-13) or primates (14) nor have they lessened the severity or rate of restenosis after angioplasty procedures in man (15).

When extrapolating from studies in any one small animal model, we urge caution in formulating general principles about the L-arginine -NO pathway's role in modulating the response of the arterial wall to injury . Additional studies in swine or primates would be very helpful in clarifying the role of the L-arginine -NO pathway as a control mechanism for vascular injury.

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