Effects of Acute Nitric Oxide Synthase Inhibition on Lower Leg Vascular Function in Chronic Tetraplegia

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Received December 12, 2008; accepted April 29, 2009

Abstract

Background/Objective: To improve our understanding of the lower-leg vascular responses of nitric oxide synthase inhibition in persons with tetraplegia.

Participants: Six people with chronic tetraplegia and 6 age-matched controls.

Methods: Lower-leg relative vascular resistance and venous volume variation were obtained by venous occlusion plethysmography and blood pressure by auscultation at baseline. Postintravenous infusion of the nitric oxide synthase inhibitor N^G-nitro-L-arginine-methyl-ester (1 mg·kg⁻¹) or placebo on separate days.

Results: At baseline in the group with tetraplegia compared with controls, mean arterial pressure and relative vascular resistance of the leg were significantly lower. After nitric oxide synthase inhibition, mean arterial pressure and lower leg vascular resistance were significantly elevated in both groups. There were no group or intervention differences in venous volume variation.

Conclusion: These preliminary results suggest that nitric oxide synthase inhibition with 1 mg·kg⁻¹ N^G-nitro-L-arginine-methyl-ester normalizes seated blood pressure and lower leg vascular resistance to control group baseline levels.

J Spinal Cord Med. November 2009;32(5):538–544

Key Words: Spinal cord injuries; L-NAME; Nitric oxide synthase; Tetraplegia; Vascular resistance; Vasoregulation

INTRODUCTION

Tetraplegia is associated with cardiovascular dysfunction, including a chronic relatively hypotensive state, altered diurnal fluctuation of blood pressure, and reflex bradycardia due to the loss of supraspinal control (1). The level and completeness of injury are strong predictors for the degree of sympathetic nervous system (SNS) impairment; people with a higher and more complete lesion tend to have a greater degree of SNS impairment (2,3). Control of vasomotor tone below the level of injury is

impaired due to the loss of SNS activity (1), interruption of the baroreflex (4), and associated diminished or absent release of norepinephrine from postganglionic SNS nerve terminals (5), despite evidence for preserved α -adrenergic tone of the lower leg vasculature (6). Impairments of vasomotor tone can result in reduced cardiac contractility and rate, decreased systemic vascular resistance, dilation of the venous capacitance vessels, and a reduction in cardiac preload (2,3), all of which can reduce blood pressure. Studies employing inhibitors of nitric oxide (NO) mediation have suggested that excess vascular NO production is a contributory mechanism to the hypotensive state in people with tetraplegia (7). In patients with autonomic failure, who also possess sympathetic nervous system dysfunction, excess vascular NO production was demonstrated to be responsible for orthostatic hypotension (8). These observations suggest that sympathetic vascular dysfunction in humans is associated with dysregulation of NO production that may affect vessel function and systemic blood pressure.

NO is synthesized in vivo from the amino acid L-arginine and oxygen by NO synthase (NOS), an enzyme that occurs in 3 distinct isoforms (9–11). The adminis-

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This research was supported by the Veterans Affairs Rehabilitation Research and Development Service (#B4335V, ARCD #3755 and #B4162C), James J. Peters VA Medical Center, and VIDD Foundation.

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Table 1. Characteristics of Study Participants

Characteristic	Control Group	Tetraplegia Group
Age (y)	35 ± 6	41 ± 10
Height (cm)	176 ± 5	170 ± 3 ^a
Weight (kg)	78 ± 6	76 ± 15
Body mass index (kg/m ²)	25 ± 2	27 ± 6
Calf circumference (cm)	35 ± 2	32 ± 5
Duration of injury (y)		19 ± 3
Complete lesion		3
Incomplete lesion		3

Values are presented as mean ± SD.

^a $P \leq 0.05$ vs control.

tration of NOS inhibitors specifically targeted to the endothelium (N^G -monomethyl-L-arginine, L-NMMA) results in a marked pressor effect and increase in leg vascular resistance (12–14). A limitation of L-NMMA is that it does not fully inhibit NOS activity and, thus, partially preserves NO synthesis. N^G -nitro L-arginine methyl ester (L-NAME) has been identified as an inhibitor of all 3 isoforms of NOS (12,15). The administration of L-NAME has the potential to serve as a therapeutic intervention to manage hypotension in people with cervical spinal cord injuries. Recently, studies of people with chronic tetraplegia demonstrated that L-NAME administered in the supine position resulted in a pressor effect that normalized blood pressure for at least 4 hours (7). These effects were independent of SNS activity (16).

The primary aim of this investigation was to improve our understanding of the effects of L-NAME administration on the peripheral vasculature in humans with cervical spinal cord injury. The following hypotheses were tested: the relative vascular resistance (RVR) response will be greater after NOS inhibition compared with placebo; the venous volume variation (VVV) will be smaller after NOS inhibition compared with placebo; the RVR response will be greater in the group with tetraplegia compared with controls; the reduction to VVV will be greater in the group with tetraplegia compared with controls. These hypotheses were tested by quantifying changes in lower leg vascular function after NOS inhibition using L-NAME compared with placebo administration in people with chronic tetraplegia and a matched neurologically intact control group.

METHODS

Subjects

Demographic data are presented (Table 1). Five men and 1 woman with chronic cervical spinal cord injury (C3–C8 with duration of injury >1 year) and 6 age-matched (± 5 years), neurologically intact male adult volunteers were recruited for study. Participants were excluded from the study if any of the following were present: acute medical

illness(es), active smoking or previous smoker within the past 5 years, myocardial infarction or stroke within the previous 3 months, hypertension, pregnancy, current medication therapy known to affect the cardiovascular system (eg, α - or β -adrenergic action, cholinesterase inhibitors), lack of mental capacity to provide informed consent. Using the ASIA classification of neurologic impairment (17), 3 individuals were previously diagnosed with complete injury (ASIA A) and 3 others with an incomplete injury (ASIA B and C). The study was approved by the James J. Peters VA Medical Center Institutional Review Board. Informed consent of each subject was obtained before the investigation was initiated.

Protocol

All subjects were required to abstain from the use of caffeinated and alcoholic beverages for 24 hours before testing and be well hydrated on the test day. Testing of all subjects was performed between 8:00 AM and 2:00 PM over 2 visits separated by no less than 7 days. Participants were studied in a seated upright semirecumbent position for the duration of the test day. Upon arrival to the testing center at each visit, the participants were seated for 15 minutes before instrumentation and placement of an intravenous catheter into the antecubital vein. The placebo infusion on test day 1 was composed of 100 mL of 0.9% sodium chloride (normal saline). Infusion on test day 2 consisted of L-NAME (Clnalfa, Switzerland) at a dosage of $1 \text{ mg} \cdot \text{kg}^{-1}$ delivered in 100 mL of normal saline. Study visits were open label and nonrandomized and adhered to the following timeline on each visit: instrumentation lasting 30 minutes (including seated rest period), baseline collection lasting 15 minutes (see below for measures), 60-minute infusion of placebo on test day 1 or L-NAME on test day 2, collection of postdata beginning 30 minutes after infusion and continuing for 15 minutes (see below for measures).

Data Acquisition

Blood pressure was measured by sphygmomanometry at the brachial artery of the right arm. Mean arterial pressure (MAP) was calculated as $(2 \times \text{diastolic blood pressure} + \text{systolic pressure}) / 3$. Venous occlusion plethysmography (VOP) was performed before the infusion and repeated 30 minutes after the infusion. During VOP, participants were seated with their right leg elevated on a stool to a height at which the ankle was at heart level. Blood pressure cuffs were placed on the mid-thigh and proximal to the right ankle (Figure 1). Circumference measurements of the right gastrocnemius muscle were taken to identify the largest circumference and determine an appropriate strain gauge size. A mercury in-silastic strain gauge (D.E. Hokanson, Inc, Bellevue, WA) measuring 4 cm less than the maximal circumference measurement was placed around the right calf at the point of its greatest diameter and secured with

Figure 1. Representation of cuff and strain gauge placement for venous occlusion plethysmography collection (strain gauge enhanced for visualization).

a piece of tape at its base (18). The plethysmograph (Model EC6, D.E. Hokanson) was calibrated according to the electrical method as previously described (19).

Before the measurements began, the distal cuff was inflated to a suprasystolic pressure of 220 mmHg (20). Data collection began with 60 seconds of baseline observation (21), during which the calibration spike was marked in the signal 10 seconds before mid-thigh cuff inflation. Occlusion of the mid-thigh cuff was performed with an automated rapid cuff inflation system (Models E20 and AG101, D.E. Hokanson) to a pressure of 50 mmHg (22). Occlusion was maintained for 2 minutes (23). After thigh cuff occlusion release, a 2-minute runoff period was collected to ensure adequate time for emptying of the capacitance bed. Two trials, separated by 2 minutes, were conducted, and the average values for the VOP parameters were reported for each data collection point. Control subjects were required to remain seated for the duration of the investigation.

The analog signal from the strain gauge was captured at a sampling rate of 500 Hz/channel with a 12-bit analog-to-digital converter (SCB 68, National Instruments, Austin, TX) on 1 input channel with a customized program created with LabVIEW (National Instruments). Converted digital data were stored as a text file. Analysis of the data files was completed offline using a customized LabVIEW program (National Instruments). The digitized data signal was filtered with a zero-lag, fourth-order Butterworth low-pass filter with a cutoff frequency of 1 Hz. Offline data analysis was performed using a calculation program created with LabVIEW (National Instruments) that was designed to quantify parameters of arterial and venous vascular function. The relative arterial inflow was calculated from the slope of the filling phase over 5 seconds after a 0.5-second delay from the onset of occlusion event marker (Figure 2). This delay was chosen to exclude from analysis the mechanical artifact from inflation of the thigh cuff (19). RVR was calculated using the methods of Kooijman et al. (24), wherein RVR is taken as the difference between MAP (mmHg) and thigh cuff occlusion pressure (50 mmHg) divided by relative arterial inflow ($\text{mL} \cdot \text{min}^{-1} \cdot 100 \text{ mL tissue}^{-1}$). RVR was expressed in arbitrary units of resistance. VVV was derived from the calculated differ-

Figure 2. Representation of the venous occlusion plethysmography parameters used for calculations.

ence between onset and end of occlusion, relative to the 1% calibration spike (25) (Figure 2), a change proportional to the increased circumference of the gastrocnemius. For calculation purposes, we assumed that central venous pressure was low and remained unchanged during the investigation.

Statistical Analysis

All data were analyzed using Statview 5.0 (SAS Institute, Inc, Cary, NC). Continuous data are reported as mean $\pm$ SD. An a priori level of significance was set at $P \leq 0.05$. Unpaired t tests were performed to identify group differences for demographic characteristics (age, height, weight, body mass index, and leg circumference) and baseline variables (MAP, RVR, and VVV). Single degree of freedom paired and unpaired t tests were performed as a posthoc analysis to explore significant relationships among the dependent variables. Three different mixed 2 by 2 (group: tetraplegia, control; visit: placebo, L-NAME) repeated measures (baseline, 30 minutes after) ANOVA were performed for each dependent variable (MAP, RVR, VVV).

RESULTS

There were no significant differences for age, weight, body mass index, or calf circumference (Table 1). Height was significantly greater in the control group ($P \leq 0.05$). At baseline, MAP ($P \leq 0.05$) and RVR ($P \leq 0.05$) were lower in the tetraplegia group compared with the control group; VVV was not different (Table 2). The coefficient of

Table 2. Combined Baseline Values of Dependent Variables

Variable	Control Group	Tetraplegia Group
Mean arterial pressure (mmHg)	86 $\pm$ 8	71 $\pm$ 12 ^a
Relative vascular resistance (arbitrary units)	20 $\pm$ 13	5 $\pm$ 4 ^a
Venous volume variation (%)	1.0 $\pm$ 0.7	0.9 $\pm$ 0.2

Values are presented as mean $\pm$ SD.

^a $P \leq 0.05$ vs control.

Table 3. Responses of the Dependent Variables to the Interventions

Variable	Placebo		L-NAME	
	Baseline	30 min after intervention	Baseline	30 min after intervention
Mean arterial pressure (mmHg)				
Control group	86 ± 8	88 ± 4	86 ± 11	98 ± 13 ^a
Tetraplegia group	71 ± 13	68 ± 12	72 ± 14	107 ± 21 ^a
Relative vascular resistance (arbitrary units)				
Control group	20 ± 15	30 ± 18	19 ± 12	28 ± 14 ^b
Tetraplegia group	7 ± 5	6 ± 6	4 ± 3	15 ± 11 ^b
Venous volume variation (%)				
Control group	0.9 ± 0.6	0.7 ± 0.4	1.2 ± 0.7	0.9 ± 0.4
Tetraplegia group	1.0 ± 0.2	0.9 ± 0.3	0.9 ± 0.3	0.9 ± 0.3

Values are presented as mean ± SD.

^a $P \leq 0.01$ vs baseline.

^b $P \leq 0.05$.

variation for VOP variables used for the calculation of outcomes is as follows: relative arterial inflow = 13.2%, VVV = 9.2%, total venous outflow = 21.0%.

Mean Arterial Pressure

Significant main effects were found for group ($F: 4.47$; $P \leq 0.05$), MAP ($F: 38.70$; $P \leq 0.0001$), and the interaction among MAP $\times$ group $\times$ visit ($F: 12.62$; $P \leq 0.01$). MAP changes after L-NAME were significant in both control ($P \leq 0.01$) and tetraplegia ($P \leq 0.01$) groups (Table 3). In the group with tetraplegia after NOS inhibition, MAP was not statistically different than the combined baseline MAP values from the control group.

Lower Leg Vascular Resistance

Significant main effects were found for group ($F: 12.25$; $P \leq 0.01$), RVR ($F: 43.67$; $P \leq 0.0001$), and the interaction among RVR $\times$ group $\times$ visit ($F: 8.96$; $P \leq 0.01$). Posthoc paired comparisons demonstrated that the RVR changes from baseline after L-NAME were significant in both the control ($P \leq 0.05$) and tetraplegia ($P \leq 0.05$) groups but not significant between groups (Table 3). A posthoc unpaired comparison demonstrated that the percent increase in RVR after NOS inhibition was significantly greater ($P \leq 0.01$) in the group with tetraplegia when compared with the control group (Figure 3).

Venous Volume Variation

Main effects for group, visit, and the interaction between VVV $\times$ group were not statistically different. The overall effect for VVV trended toward a significant decrease from observations before to observations after the study ($P = 0.07$) (Table 3).

DISCUSSION

The present investigation demonstrated the peripheral vascular responses to NOS inhibition with L-NAME via

changes to RVR and VVV of the lower leg in people with chronic tetraplegia. The influence of NOS inhibition in the lower leg arterial vascular tree was more profound than that in the venous system, wherein no effects were observed. The relative effect of L-NAME on RVR was not different between groups. The changes for lower leg RVR in those with tetraplegia approached baseline levels of vascular resistance observed in the control group, suggesting a normalization of vascular resistance. However, this increase after L-NAME, in terms of a percent change, was approximately 5 times greater in tetraplegia than controls (based upon group means), which is contrary to the responses reported after L-NMMA (14), in which no group differences were noted. These conflicting results were most likely the result of the specific dosage administered and action of the drug where L-NMMA is an endothelial-specific NOS inhibitor

Figure 3. Percent change of lower leg vascular resistance (arbitrary units) after nitric oxide synthase inhibition in both groups. $\dagger P < 0.01$.

(26) and L-NAME is a nonspecific NOS inhibitor. Their calculations included a normalization of outcomes to the vasoconstrictor responses of angiotensin II. The present finding supplements the postural and peripheral vascular effects of L-NAME administration to previous investigations in people with tetraplegia (7,16).

The administration of $1 \text{ mg} \cdot \text{kg}^{-1}$ body weight L-NAME in people with tetraplegia increased systemic blood pressure and lower leg vascular resistance to control group levels. Baroreceptor-dependent vasoregulation is an important mechanism for mitigating the potentially deleterious consequences of hypertensive episodes to the arterial endothelium (27–29). In the control group, buffering of the blood pressure response to NOS inhibition via the arterial baroreceptors and synergistic humoral pathways was present to modulate changes to vascular tone and, thus, vascular resistance (30,31); the observed arterial vasoconstrictor responses to NOS inhibition were the result of the sum of intact vasoregulatory pathways responding to the pressor effect of NOS inhibition. The contribution of such a compensatory baroreceptor response in the tetraplegic group was severely limited, which may explain why the pressor response was greater than control response for a comparable relative increase in vascular resistance.

It may be argued that NOS inhibition in people with tetraplegia permits unopposed vasoconstriction from an intact humoral pathway, such as angiotensin II, because catecholamine production and release are severely limited due to the SNS impairment (32,33). The renin-angiotensin system is activated in those with tetraplegia during orthostatic provocation and responsible for orthostatic tolerance (5,34–36). A recent investigation demonstrated that the pressor response of angiotensin II was enhanced after NOS inhibition due in part to increased vascular resistance (37). The opposing effects between NO and the renin-angiotensin system are counter-regulatory mechanisms that operate under normal physiologic conditions. Despite this, the SNS and renin-angiotensin system have been reported not to be involved in systemic vasoconstriction produced by L-NAME administration (38), contrary to evidence suggesting that α -adrenergic blockade with phentolamine administration attenuated the L-NAME pressor effect (12,39).

Despite evidence for a well-defined role of NO in the venous vasculature (14,40), NOS inhibition in either group did not result in differences in venous vascular function measured by the VVV. Deep veins of the legs have sparse SNS innervation; thus, their compliance is primarily controlled by surrounding skeletal muscles under normal physiologic conditions (41). As such, valves within these vessels ensure unidirectional blood flow with venous return to the heart with the assistance of external pressure from the skeletal muscle pump that works to prevent reflux at rest and during upright posture (42). In people with tetraplegia, a minimal

contribution from the skeletal muscle pump is present on the venous vasculature, increasing the potential for flaccid or fluid-distended vessels. We speculated that NOS inhibition would induce venous constriction, decreasing VVV, but this was not the case.

In neurologically intact humans, rapid activation of endothelial-derived NOS and production of NO in response to abrupt and dynamic fluctuations of blood flow are essential to enable adequate perfusion of metabolically active organs/tissue during exercise or stressful situations (27,28), as well as for hyperemic dilatation (43) and the protection against pressure-mediated injury to vital organs and tissues (29). The release of NO facilitates vasodilatation (43) and buffers the elevated arterial vascular resistance through a reduction of sympathetic nerve activity to the vascular smooth muscle (29). In people with tetraplegia, this mechanism is functionally impaired or absent due to SNS pathway interruption (4) and, in our study, pharmacologically inhibited through administration of L-NAME. Thus, the chronic administration of L-NAME and subsequent inhibition of NOS may impart deleterious consequences to both the vasculature and other biologic processes in which NO plays a role.

There are limitations and considerations to this investigation. We acknowledge the potential risks of L-NAME's use in this population (44) and closely monitored the individuals during and after the intervention. By design, our aim was not to determine if people with tetraplegia had an increased sensitivity to NOS inhibition versus controls but only to quantify the peripheral vascular effects of NOS inhibition with L-NAME. We chose to utilize noninvasive assessment measures to minimize participant discomfort. The functional differences between our 2 subject populations provided an excellent opportunity to investigate the action of the pharmacologic agent L-NAME. The dependent variables representing endothelium-dependent reactivity to NO, and thus vasoconstriction, selected for this investigation are subject to a number of humoral factors, such as catecholamines and angiotensin II, that were not quantified. The dosage of L-NAME used in this investigation was determined from a previous investigation in which individuals were in the supine position (7).

CONCLUSION

From these data, we conclude that NOS inhibition with $1 \text{ mg} \cdot \text{kg}^{-1}$ body weight L-NAME normalized seated blood pressure and lower leg vascular resistance to baseline control group levels.

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