L-Arginine improves endothelial vasoreactivity and reduces thrombogenicity after thrombolysis in experimental deep venous thrombosis

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Purpose: **Nitric oxide (NO) is important in regulation of platelet aggregation, endothelial function, and intravascular thrombosis. The purposes of this study were to assess the effect of thrombolysis on endothelial function in a porcine model of deep venous thrombosis (DVT) and to evaluate the effect of NO precursor L-arginine on endothelial function after thrombolytic therapy.**

Methods: **DVT was created in bilateral iliac veins by deploying a self-expanding stent-graft that incorporated an intraluminal stenosis, from a groin approach. Five pigs underwent sham operation. After 7 days of DVT, animals were randomized to three groups: saline pulse-spray (saline group, n = 5), thrombolytic pulse-spray with tissue plasminogen activator (alteplase, 8 mg; t-PA group, n = 5), and thrombolytic pulse-spray plus intravenous L-arginine (20 mmol/L; arginine group, n = 5). At 2 weeks iliac vein patency was evaluated at venography and intravascular ultrasound scanning. NO level was determined with a chemiluminescent assay of the nitrite and nitrate metabolites (NO_x). Thrombogenicity was evaluated with radiolabeled platelet and fibrin deposition. Veins were harvested and evaluated with light microscopy and scanning electron microscopy. Endothelial function was evaluated with organ chamber analysis.**

Results: **All iliac veins remained patent at 2 weeks. The luminal areas in the sham, saline, t-PA, and arginine groups were 53 ± 23 mm^2, 14 ± 11 mm^2, 34 ± 19 mm^2, and 42 ± 21 mm^2, respectively. No difference in endothelial cell structure was observed between the three treatment groups at light microscopy or scanning electron microscopy. Although no difference in fibrin deposition was noted among the three treatment groups, decreased platelet deposition occurred in the arginine group compared with the saline or t-PA groups ($P < .05$). The arginine group showed greater endothelial-dependent relaxation compared with the t-PA or saline groups (73% ± 23% vs 49% ± 18% and 32% ± 21%; $P < .05$). Local NO_x level in the arginine group was correspondingly higher compared with the saline or t-PA groups (1.8 ± 0.3 μmol/L vs 0.3 ± 0.05 μmol/L and 0.2 ± 0.04 μmol/L; $P < .05$).**

Conclusions: **NO precursor L-arginine supplementation enhances NO production at sites of venous thrombosis. Moreover, L-arginine preserves endothelial vasoreactivity and reduces platelet deposition after thrombolysis in iliac DVT. These data suggest that L-arginine may preserve endothelial function after thrombolysis and may reduce the likelihood of postthrombotic syndrome.** **(J Vasc Surg 2003;38:1396-403.)**

Deep venous thrombosis (DVT) is estimated to affect 20% to 30% of all patients who undergo major surgery, and, as a result of pulmonary embolism, is responsible for more than 60,000 deaths annually in the United States.[1,2] Catheter-directed thrombolytic therapy has been embraced by physicians as effective treatment of this disease. However, the physiologic response of the venous endothelium to thrombosis and thrombolytic therapy has not been widely investigated. The vascular endothelium produces a variety of vasoactive substances, including nitric oxide (NO), endothelin-1, and prostacyclin, that influence thrombogenicity and the vasomotor response of underlying smooth muscle cells.[3,4] Production of these endothelium-derived factors is significantly altered in venous thrombosis.[5] A recent study that analyzed cellular function in acute DVT after thrombotic therapy revealed that greater endothelial functions were preserved after thrombolysis compared with thrombectomy.[6] This phenomena may be mediated in part by endothelium-derived vasoactive substance such as NO, which is generated by enzymatic oxidation of the amino acid L-arginine into L-citrulline through the action of NO synthase.[7] L-arginine modulates the thrombotic process and inhibits platelet aggregation.[8,9] We recently characterized an experimental model of DVT created with an endovascular stent graft, and also demonstrated that this chronic DVT model was amenable to catheter-directed thrombolytic therapy.[10] The present study was designed to assess the effect of thrombolytic therapy on endothelial function in chronic DVT and to evaluate the effect of NO precursor L-arginine on endothelial function after thrombolytic therapy.

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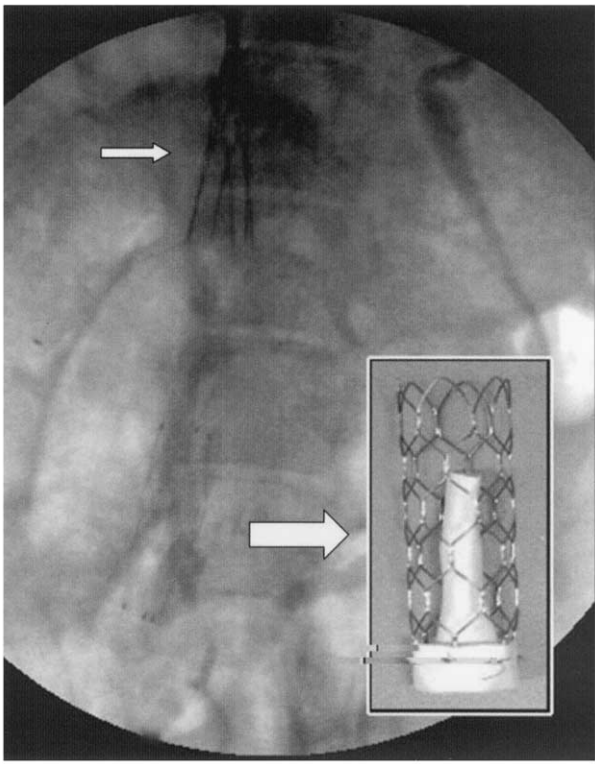

Fig 1. Tapered polytetrafluoroethylene graft was inverted within the nitinol stent to create intrastent stenosis, which was deployed in the common iliac vein to cause venous thrombus due to flow stasis. *Large arrow*, location of stent graft *(inset)* deployed to cause iliac venous thrombosis. A Greenfield filter was placed in the infrarenal vena cava to prevent pulmonary embolism (*small arrow*).

MATERIAL AND METHODS

Animal model and interventions. Twenty adult domestic swine, each weighing 45 to 50 kg, were used in all experiments. All animal procedures and care were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council. Washington, DC: National Academy Press; 1996). Animals were given intravenous thiopental sodium (10 mg/kg). Endotracheal intubation was performed, and anesthesia was maintained with 1% isoflurane. Under sterile conditions, bilateral groin cutdown incisions were made, to expose the superficial femoral veins, followed by placement of a 14F introducer sheath (Meditech, Watertown, Mass). Bilateral DVT was created with use of an endovascular model previously described.[10] In brief, a self-expanding nitinol stent graft (Symphony Stent; Meditech) that incorporated intrastent stenosis was deployed in bilateral common iliac veins. The intrastent stenosis was created with a tapered polytetrafluoroethylene graft (W. L. Gore & Associates, Flagstaff, Ariz), which caused flow stasis with resultant DVT (Fig 1). An infrarenal Greenfield vena cava filter (Meditech) was also placed in each animal, to prevent pulmonary embolism (Fig 1). Venography was performed, which documented the presence of DVT in bilateral iliac veins. The introducer sheaths were removed, and the venotomy was closed with 6-0 polypropylene suture, followed by layered groin wound closure. Endotracheal extubation was performed, and the animals were allowed to recover after stent-graft deployment.

The animals were divided into four groups, with 5 animals in each group. The first group underwent sham operation without stent-graft placement (sham group); the remaining 15 animals underwent bilateral stent-graft placement to induce DVT. After 7 days of DVT, the remaining 15 animals were divided into three groups: one group received saline pulse-spray thrombolysis (saline group); another group received thrombolytic pulse-spray with tissue plasminogen activator (t-PA; Alteplase, Genentech, San Francisco, Calif; t-PA group); and the third group received thrombolytic pulse-spray plus intravenous L-arginine (Sigma, St Louis, Mo; arginine group).

The thrombolytic pulse-spray procedure was performed with techniques previously described.[10-13] In brief, animals were given general anesthesia after endotracheal intubation, and bilateral groin incisions were made, followed by placement of 7F introducer sheaths (Meditech). A 0.035-inch Bentson guide wire (Meditech) was inserted through the introducer sheath in the femoral vein to cannulate the stent graft in the iliac vein. A 6F Mewissen infusion catheter (Meadox, Oakland, NJ) was placed over the guide wire and positioned across the tapered stent graft, and pulse-spray therapy was administered to iliac vein thrombus both above and below the stent graft. In the t-PA group, 8 mg of alteplase was mixed with 20 mL of sterile water and pulse-sprayed via the Mewissen catheter by manually injecting a 0.2-mL aliquot every 30 seconds with a tuberculin syringe. In the arginine group, 100 μg/kg of L-arginine was mixed with 10 mL of sterile water, which was pulse-sprayed in the iliac veins after t-PA thrombolysis. The L-arginine infusion dose was chosen from previous reports.[14,15] After pulse-spray thrombolysis, balloon angioplasty was performed with a 12-mm × 4-cm balloon (XXL; Meditech), to dilate the tapered stent graft, which eliminated the intrastent stenosis and restored venous flow. Iliac vein patency was assessed at venography and intravascular ultrasound scanning (IVUS).

Two weeks after pulse-spray thrombolytic therapy, animals in all groups were anesthetized. Bilateral femoral veins were exposed, followed by placement of 7F introducer sheaths (Meditech). Iliac vein patency was assessed at venography and IVUS. Laparotomy was performed to harvest the entire ileofemoral veins. Histologic analysis and organ chamber study were performed in one ileofemoral vein, and local thrombogenicity was evaluated in the contralateral vein before vein harvesting. All histologic and functional analyses of the iliac veins were performed in vein segments just below the stent-graft placement site, where thrombosis and pulse-spray thrombolytic therapy occurred.

IVUS. A 3.5F 20 MHz IVUS catheter was inserted over the guide wire with a ClearView Ultra Intravascular

Ultrasound System (Meadox). Before pulse-spray thrombolytic therapy, IVUS was performed to assess the iliac venous thrombus by a clinician blinded to the respective treatment groups. The IVUS catheter was first inserted in the proximal iliac vein just below the stent-graft, and five different images were obtained in the proximal 2 cm of each iliac vein for thrombus measurement. After thrombolysis, the IVUS catheter was again positioned within the proximal 2 cm of the iliac vein to obtain five different images for residual thrombus measurement. Spot fluoroscopic images were used to confirm the location of the IVUS transducer within the proximal iliac vein. The luminal dimensions before (Before) and after (After) pulse-spray thrombolysis were traced on the IVUS image with a tracking device, which allowed calculation of a cross-sectional area:

$$\textit{Efficacy of thrombolytic therapy}(\%) = 1 - (\textit{Area}_{\textit{After}}/\textit{Area}_{\textit{Before}}) \times 100$$

Organ chamber evaluation. Evaluation of endotheial vasoreactive functions with the organ chamber myograph system has been described in our laboratory.[16-18] Through an abdominal and bilateral groin exposure, one iliofemoral vein was carefully harvested in its entirety before injection of radiolabeled platelets and fibrinogen. The iliac vein proximal to the nitinol stent was sectioned into multiple 5-mm segments, which were incubated in Dulbecco modified Eagle medium at 37°C and 5% CO_2 in a cell culture incubator. After culture, rings were suspended between the wires of the organ bath myograph chamber (Organ Bath 700MO; Danish MyoTechnology, Aarhus, Denmark) in 8 mL of Krebs solution (NaCl, 120 mmol/L; $MgSO_4$, 1.17 mmol/L; KH_2PO_4, 1.18 mmol/L; $NaHCO_3$, 25 mmol/L; $CaCl_2$, 2.5 mmol/L; KCl, 4.7 mmol/L; glucose, 5.5 mmol/L) maintained at 37°C, and oxygenated with pure oxygen gas to maintain pH 7.4. Rings were subjected stepwise to a predetermined optimal tension of 2*g* and allowed to equilibrate for at least 60 minutes. After equilibration, each ring was precontracted with norepinephrine, 10^{-4} mol/L; Sigma) and allowed to re-equilibrate to produce the maximal contractile force (F_{max}) for the vessel. The organ baths were rinsed with Krebs solution, and the vessels were contracted to 75% of F_{max} with norepinephrine. The vessels were challenged with adenosine diphosphate (ADP; Sigma) in an incremental log concentration from 10^{-9} to 10^{-4} mol/L to determine endothelial-dependent relaxation.

In addition, vessels were challenged with calcium channel ionophore A23187 (10^{-9} to $10^{-6.5}$ mol/L), an endothelial-dependent vasodilator, and allowed to equilibrate for 10 minutes to assess endothelial-dependent relaxation. Relaxation dose-response curves were also determined with incremental additions of endothelium-dependent vasodilator bradykinin (10^{-7} to $10^{-4.5}$ mol/L). Endothelial-independent relaxation was assessed with addition of sodium nitroprusside (Sigma), from 10^{-7} to 10^{-5} mol/L, which influenced smooth muscle cell relaxation. Baths were rinsed, and vein segments were brought to 50% F_{max} with norepinephrine between each reagent. Contractility and percentage of relaxation were calculated on the basis of tension change.

Data for all iliac vein segments from each animal were averaged, and represented as 1 data point for statistical analysis.

Thrombogenicity. Blood samples from each animal were obtained and placed in anticoagulant-citrate-dextrose solution (ACD solution, modified; Squibb Diagnostics, New Brunswick, NJ) for centrifuge to acquire platelet pellet. Platelet sample was resuspended with ACD solution, followed by indium 111 oxyquinoline (Amersham Corp, Arlington Heights, Ill) to create radiolabeled platelets. Freeze-dried human fibrinogen labeled with iodine 125 (Amersham Corp) was reconstituted in 1.1 mL ACD solution. After harvesting of one iliofemoral vein for organ chamber analysis, the radiolabeled platelet and fibrinogen were injected into the animal and allowed to circulate for 3 hours before harvesting of the contralateral iliofemoral vein. The vein segment was cut into three sections with documented surface areas. Depositions of ^{111}In-labeled platelets and ^{125}I-labeled fibrinogen were analyzed in each iliac vein segment, in accordance with the surface area, with a gamma counter (Packard Instruments, Downers Grove, Ill). Hemocytometry was performed to determine platelet concentration. Formulas used to calculate platelet and fibrin deposition were chosen from published reports.[6,19]

Histologic analysis and immunohistochemistry. Segments of the iliac vein were fixed in 10% buffered formalin overnight, then transferred to 70% alcohol. The specimens were dehydrated with sequentially increasing concentrations of ethanol followed by xylene, and embedded in paraffin. Five-micrometer cross sections were cut and prepared as described.[20,21] Histologic staining with hematoxylin-eosin, methylene blue, and Verhoeff-Masson stains were performed. Immunohistochemical analysis was performed with the avidin-biotin complex immunoperoxidase procedure (LSAB Kit; DAKO, Carpenteria, Calif) as described.[20,21] Immunostaining for α-actin and factor VIII-related antigen was performed to identify smooth muscle cells and endothelial cells, respectively.

For scanning electron microscopy (SEM; Cambridge 360 SEM microscope, Cambridge, United Kingdom), the iliac vein segment was incised to allow en face imaging. This tissue specimen was placed in 2% glutaraldehyde and fixed overnight. The specimens were rinsed in phosphate-buffered saline solution three times, and dehydrated in a graded series of ethanol (30%-100%). Tissue samples were placed with a graded series of hexamethyldisilazane, air-dried, and gold sputter–coated before imaging. Endothelial loss based on SEM images was scanned and evaluated by a blinded observer using SEM image software (Bioview Image, Atlanta, Ga).

Nitric oxide evaluation. NO level was determined with a chemiluminescent detector. For systemic NO analysis, samples of venous blood from the superficial ear vein of each animal were assayed before pulse-spray therapy, 4 hours after pulse-spray therapy, and before tissue harvest.

Table I. Intravenous ultrasound evaluation of thrombus areas and thrombolytic efficacy after pulse-spray thrombolytic therapy

Group	*Pre-thrombolysis thrombus area (mm^2)*	*Post-thrombolysis thrombus area (mm^2)*	*Thrombolytic efficacy (%)*
Sham	14.4 ± 4.4	3.5 ± 1.7	79
Saline	71.4 ± 13.4	61.7 ± 16.7	14
t-PA	82.9 ± 15.5	32.4 ± 12.3	61*
Arginine	77.8 ± 18.9	16.9 ± 8.4	78*

t-PA, Tissue plasminogen activator.
*$P < .05$ compared with saline pulse-spray group.

Blood specimens were centrifuged at 3600 rpm to yield plasma, which was analyzed in a chemiluminescence detector (NO_x analyzer, Model 2108; Dosibi Environmental, Glendale, Calif). NO concentration was determined by reconverting its oxidation end products nitrite (NO_2) and nitrate (NO_3). Routine calibration of the chemiluminescence detector was performed with a known NO sample value during each sampling analysis. To determine local NO production, 1-cm segments of iliac veins were harvested and suspended in physiologic Krebs buffer for 10 minutes just before the animals were killed. One milliliter of buffer was drawn and refrigerated at 4°C for subsequent assay. All samples were performed in triplicate.

Statistical analysis. Values are expressed as mean ± SEM. Differences in vessel relaxation and contraction in organ chamber studies, and deposition of platelet and fibrin in thrombogenicity studies were determined with one-way analysis of variance or Student *t* test, where appropriate. A SAS statistical package was used for analysis (SAS Inc, Cary, NC). $P < .05$ was considered statistically significant.

RESULTS

All iliac veins remained patent at 2 weeks after pulse-spray treatment. A wide disparity of iliac venous thrombotic regions was detected with IVUS among the treatment groups. At 2 weeks after pulse-spray treatment, luminal area was 53 ± 23 mm^2 in the sham group, 14 ± 11 mm^2 in the saline group, 34 ± 19 mm^2 in the t-PA group, and 42 ± 21 mm^2 in the arginine group. Thrombolytic efficacy and thrombus areas calculated with IVUS are summarized in Table I.

Light microscopy and SEM. Histologic evaluation of thrombus revealed the presence of recanalized and organized thrombus in the saline group (60%, 3 of 5 animals), t-PA group (40%, 2 of 5 animals), and arginine group (60%, 3 of 5 animals). In contrast, the control specimen did not contain any recanalized thrombus (0%, 0 of 5 animals; $P < .05$). Immunohistochemistry revealed a similar pattern of factor VIII staining in all groups, without obvious evidence of endothelial cell loss. Immunohistochemical staining of α-actin showed a positive staining pattern in the thrombus and media, without evidence of intimal proliferation, in all groups. SEM evaluation revealed similar patterns of endothelial cell loss among the three treatment groups (saline, 34% ± 21%; t-PA, 36% ± 18%; arginine, 29% ± 16%). The control group showed minimal endothelial denudation (12% ± 8%) compared with the three treatment groups. When analyzing the inflammatory response on the basis of histologic findings, a significant increase in leukocytes, with a relatively equal presence of neutrophils and macrophages, was found in the saline, t-PA, and arginine groups compared with the sham control group.

Table II. Systemic and local NO_x in all groups

Group	*Systemic NO_x level (μmol/L)*	*Local NO_x level (μmol/L)*
Sham	32.3 ± 14.5	0.3 ± 0.1
Saline	35.6 ± 18.3	0.2 ± 0.1
t-PA	27.3 ± 17.8	0.4 ± 0.2
Arginine	32.6 ± 15.4	1.8 ± 0.5*

NO_x, Nitric oxide metabolites; *t-PA,* tissue plasminogen activator.
*$P < .05$ compared with sham, saline, and t-PA pulse-spray groups.

NO_x level. Systemic NO_x measurement based on a chemiluminescent assay immediately after pulse-spray thrombolysis showed no difference between groups ($P > .05$). Similarly, no differences in systemic NO_x levels were detected between groups at tissue harvest 14 days later. However, local NO_x level in the arginine group was correspondingly higher compared with the sham, saline, or t-PA groups ($P < .05$; Table II).

Thrombogenicity. No differences in preoperative hematocrit or platelet counts were noted between groups. Platelet count was 259,300/μL in the control group, 319,400/μL in the saline group, 284,500/μL in the t-PA group, and 331,600/μL in the arginine group. Hematocrit was 41.3% in the control group, 42.6% in the saline group, 49.3% in the t-PA group, and 46.7% in the arginine group. Two weeks after pulse-spray thrombolysis, radiolabeled fibrin deposition did not differ between groups. However, a decrease in platelet deposition was noted in the arginine group compared with either the t-PA or saline groups ($P < .05$; Table III).

Organ chamber studies. Maximal contraction curves in response to norepinephrine were similar in all tested groups at 10^{-4} mol/L, with mean F_{max} range 4.3 ± 1.2 to 5.3 ± 1.4 g. Calcium ionophore A23187 caused concentration-dependent relaxation in all tested groups. The arginine group exhibited a greater relaxation response at 10^{-6} mol/L compared with the t-PA or saline groups (75% ± 22% vs 52% ± 21% and 48% ± 16%; $P < .05$; Fig 2). No significant difference was found in endothelial-dependent relaxation in response to calcium ionophore A23187 between the arginine and control groups (Fig 2). Endothelial-dependent relaxation was also assessed by adding bradykinin in log escalating doses. The t-PA, arginine, and saline groups showed a similar response curve in a concentration-dependent fashion (Fig 3). ADP caused concentration-dependent relaxation in iliac venous segments in all groups. The arginine group exhibited greater relaxation compared with the t-PA or saline groups in response to ADP at 10^{-4}

Table III. Thrombogenicity of iliac veins 14 days after pulse-spray thrombolytic therapy

Group	*Platelet deposition (platelet/cm²)*	*Fibrin deposition (μg/cm²)*
Sham	4350 ± 2653	5.3 ± 3.4
Saline	19,862 ± 5852	8.7 ± 5.6
t-PA	21,505 ± 6550	11.3 ± 6.2
Arginine	8258 ± 3525*	9.6 ± 5.8

t-PA, Tissue plasminogen activator.
*$P < .05$ compared with saline and t-PA pulse-spray groups.

mol/L concentration (73% ± 23% vs 49% ± 18% and 32% ± 21%; $P < .05$; Fig 4). The control group exhibited the greatest degree of endothelial-dependent relaxation in response to bradykinin and ADP when compared with the arginine, t-PA, and saline groups (Figs 3, 4). Endothelial-independent relaxation was assessed by determining the relaxation response to log escalating doses of sodium nitroprusside. All vein segments responded similarly to sodium nitroprusside in a concentration-dependent manner (Fig 5).

DISCUSSION

Numerous clinical reports have shown that early resolution of deep venous thrombus is beneficial in improving clinical outcome, due in part to maintenance of valve competency and reduction of subsequent likelihood of post-thrombotic syndrome.[22-24] Resolution of venous thrombus can occur through spontaneous thrombolysis, mechanical thrombectomy, or catheter-directed thrombolytic therapy. Several experimental studies showed that catheter-based thrombolysis preserves greater venous endothelial function when compared with mechanical thrombectomy.[6,19] The endothelium is crucial in maintaining vasomotor reactivity, a function that is mediated in part by a vasoactive substance such as NO. The presence of venous thrombus affects production of NO, which in turn may influence thrombogenicity and vascular vasomotor response.[25] We recently characterized an endovascular model of DVT by creating an intraluminal stenosis that reduced venous flow, with resultant iliac venous thrombosis.[10] The intraluminal stenosis can subsequently be eliminated by means of balloon angioplasty to restore venous flow. The present study examined the venous structure and endothelial functions after experimental DVT and assessed the role of NO in ameliorating the venous endothelial function.

The data from this study demonstrate that supplementing catheter-directed thrombolytic therapy with L-arginine, the precursor of NO synthesis, results in improved thrombolytic efficacy, with ameliorated endothelial function, in a porcine model of DVT. This was evidenced by enhanced endothelial-dependent relaxation in the arginine group in response to both ADP and calcium ionophore A23187. The mechanism of endothelial-dependent relaxation was likely modulated by NO-mediated vasoreactive pathways, inasmuch as previous studies have shown such responses can be partially inhibited by the NO synthesis inhibitor N^G-monomethyl-L-arginine.[19,26]

Our findings also reveal that reduction in platelet aggregation occurred in the L-arginine–treated group, along with a correspondingly elevated local NO_x level, when compared with either the saline or t-PA group. NO, known as endothelium-derived relaxing factor, is synthesized by endothelial cells from the terminal guanidino nitrogen atoms of L-arginine. Under normal circumstances, endothelial cells constantly produce NO.[27] The synthesis and release of NO are also increased by increasing the amount of precursor, L-arginine.[27,28] In addition to its role as a major regulator of vessel tone, NO inhibits platelet activation in regulation of hemostasis and thrombosis.[7] Numerous studies have documented that blocking production of NO promotes mechanical injury–induced experimental thrombosis, whereas increasing synthesis of NO or providing NO precursors protects against intraluminal thrombosis.[15,29]

Several studies have examined the possible synergistic effect of NO and thrombolytic therapy in arterial thrombosis.[30,31] A clinical study was performed in patients with early myocardial infarction and coronary occlusion who underwent catheter-directed isosorbide dinitrate, an exogenous NO donor, and streptokinase thrombolysis.[30] Coronary artery recanalization was improved with the combination therapy compared with thrombolysis alone. In a study of a canine coronary thrombosis model, continuous catheter-based infusion of L-arginine at a concentration of 100 μg/kg per minute delayed coronary thrombosis and reduced recurrent thrombosis rates after thrombolytic therapy with t-PA. This study found that the improved thrombolytic efficacy as a result of combined L-arginine and t-PA infusion was associated with decreased collagen-induced platelet aggregation through increased concentration of NO.[14] Our findings are consistent with those of a recent report by Kashyap et al,[32] who examined the beneficial role of L-arginine with urokinase thrombolytic therapy in a rat arterial thrombosis model. Those authors reported that severe transient endothelial dysfunction, as evidenced by reduced endothelial-dependent relaxation, was associated with acute arterial thrombosis. However, administration of L-arginine in urokinase thrombolysis significantly improved endothelial-dependent relaxation after acute thrombosis when compared with urokinase thrombolysis alone. This improved endothelial function with L-arginine supplementation was associated with increased NO concentration.

Histologic and electron microscopic findings from our study revealed similar morphologic endothelial loss as a result of thrombus exposure or thrombolytic therapy. Endothelial loss in the three treatment groups ranged from 36% ± 18% to 29% ± 16%, whereas the control group demonstrated minimal endothelial denudation, at 12% ± 8%. Previous investigations of the effects of thrombus on the vascular endothelium have yielded mixed results. The presence of thrombosis in an arterialized vein graft significantly decreased endothelial function, as evidenced by decreased endothelial metabolism of prostacyclin.[33] Further-

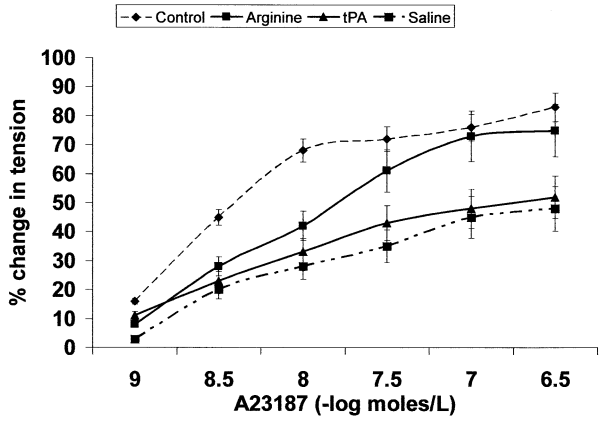

Fig 2. Endothelial-dependent response to calcium ionophore A23187 in porcine iliac veins 14 days after pulse-spray thrombolytic therapy.

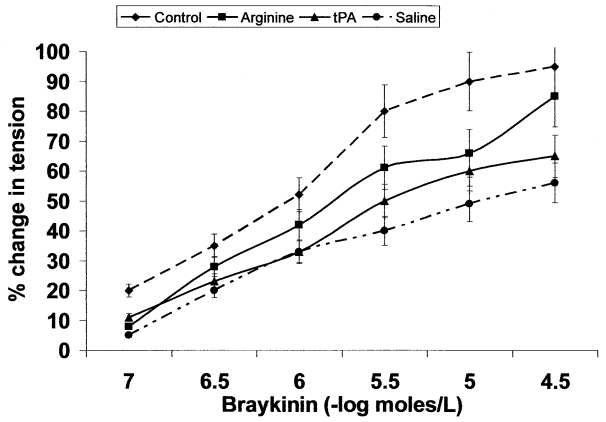

Fig 3. Endothelial-dependent response to bradykinin in porcine iliac veins 14 days after pulse-spray thrombolytic therapy.

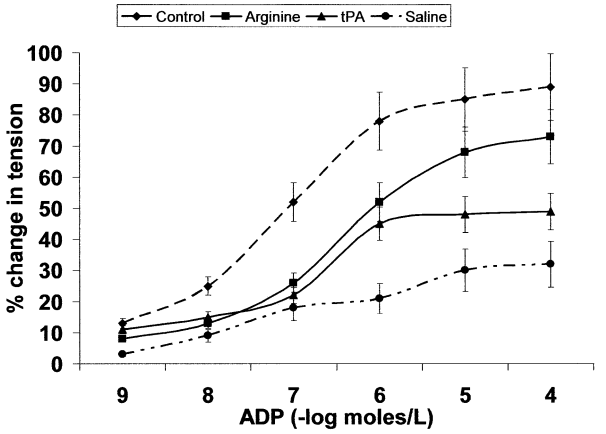

Fig 4. Endothelial-dependent response to adenosine diphosphate *(ADP)* in porcine iliac veins 14 days after pulse-spray thrombolytic therapy.

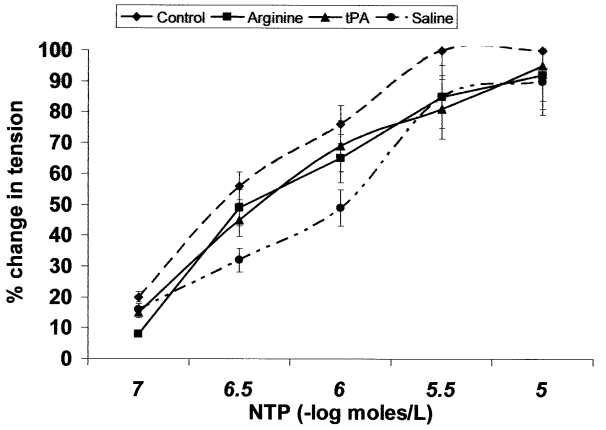

Fig 5. Endothelial-independent response to sodium nitroprusside *(NTP)* in porcine iliac veins 14 days after pulse-spray thrombolytic therapy.

more, the structural integrity of the vein graft showed morphologic damage to the endothelium after contact with thrombus for 5 days, but with subsequent partial structural recovery after thrombectomy. This finding was in contrast to a report by Whitley et al,[34] who studied the effect of thrombectomy and thrombolysis in a canine femoral arterial thrombosis model. These authors found no significant changes in endothelium-dependent relaxation or morphologic damage to the endothelium after exposure to thrombus for 24 hours. These observations were supported by a similar study that compared thrombolysis and balloon thrombectomy in a canine femoral thrombosis model, which found no significant changes in endothelial architecture after exposure to thrombus for 24 hours.[35] The effect of acute thrombus on venous endothelium was examined by Cho et al,[6] who compared thrombectomy and thrombolysis in a canine femoral vein thrombosis model. They reported that valve competence and endothelial anatomy were similarly preserved between the two treatment groups. However, thrombolysis preserved greater endothelial function than did thrombectomy. Moreover, endothelium treated with thrombolysis exhibited reduced thrombogenicity, as evidenced by decreased platelet deposition. The effect of chronic thrombus on venous endothelium was also studied by the same laboratory.[19] They noted venous endothelium had less residual thrombus 4 weeks after thrombolysis than with thrombectomy. In addition, venous thrombolysis maintained a greater degree of structural endothelial architecture and endothelial-mediated function, compared with thrombectomy.

Experimental arterial and venous thrombosis studies have reported increased inflammatory cytokine and adhesion molecule expression.[36-38] Moreover, NO-mediated platelet aggregation occurs in both the arterial and venous thrombosis models.[5,14] However, thrombosis of the arterial system differs significantly from that of the deep venous system in that the deep venous system is associated with a

greater degree of local inflammation and pain.[36,38,39] This disparity may be related to thrombus-induced inflammation that is partly mediated by leukocyte adhesion to the venous wall, which is supported in that inhibition of cytokines and adhesion molecules can inhibit the inflammatory response and recurrent thrombosis in the venous system.[39-41] A study by Broeders et al[8] compared the effect of NO-mediated thrombosis in a rabbit arterial and venous system, and found that intraluminal infusion of L-arginine resulted in significant reduction in thromboembolism in rabbit venules rather than arterioles. The authors reported that NO synthase was more important in the thrombotic process in venules than in arterioles. This finding has been supported by others who noted that inhibition of NO synthase induces spontaneous aggregation of platelets and leukocyte adhesion in venules but not in arterioles.[42,43]

The effect of L-arginine in modulating vascular pathophysiology has received increased investigative focus, particularly as a means of inhibiting atherogenesis and recurrent stenosis. Numerous studies have documented the efficacy of dietary L-arginine supplementation in reduction of arterial atherogenesis in animals with hypercholesterolemia.[44-46] Davies et al[47] studied the combined effect of oral L-arginine supplementation and dietary cholesterol reduction in rabbits with hypercholesterolemia treated with interposition vein bypass grafting. They noted that this combined therapy significantly reduced atheromatous progression in the vein grafts. In a study that examined the effect of L-arginine on intimal hyperplasia after iliac artery balloon angioplasty, Tarry et al[48] reported that rabbits fed an L-arginine–supplemented diet had significantly improved endothelial-dependent relaxation and decreased intimal hyperplasia. In a similar study that examined the effect of iliac balloon angioplasty in rabbits with hypercholesterolemia a single intramural local delivery of L-arginine significantly enhanced local NO production and endothelial-dependent relaxation at 1 week, and reduced intimal thickening 4 weeks later.[49] These findings were in contrast to a recent study by Landis et al,[50] who studied the effect of dietary L-arginine on flow-restricted vein graft in a nonhypercholesterolemic canine model. They found decreased vein graft vasoreactivity and no effect on intimal hyperplasia in animals receiving dietary L-arginine supplementation. The authors postulated that in a normal nondiseased artery, dietary L-arginine supplementation could result in a parallel increase in endogenous NO inhibitor, asymmetric dimethylarginine, and alter L-arginine metabolism through enzymatic pathways other than NO synthase. Irrespective of the vascular disease model, the collective findings from these studies underscore the potential therapeutic benefit of augmenting NO activity with L-arginine supplementation to improve endothelial function and luminal patency in diseased vasculature.

In summary, our findings suggest that the antiplatelet effect of NO may have therapeutic benefit in treatment of DVT. Supplementation of NO precursor, L-arginine, in catheter-directed thrombolysis improved endothelial vasoreactivity and reduced platelet deposition in our experimental DVT model. These data suggest a potential therapeutic role for L-arginine in standard thrombolytic therapy in treatment of DVT.

REFERENCES

1. Gupta R, Stouffer GA. Deep venous thrombosis: a review of the pathophysiology, clinical features, and diagnostic modalities. Am J Med Sci 2001;322:358-64.
2. Reinisch JF, Bresnick SD, Walker JW, Rosso RF. Deep venous thrombosis and pulmonary embolus after face lift: a study of incidence and prophylaxis. Plast Reconstr Surg 2001;107:1570-5; discussion 1576-7.
3. Cambria RA, Lowell RC, Gloviczki P, Miller VM. Chronic changes in blood flow alter endothelium-dependent responses in autogenous vein grafts in dogs. J Vasc Surg 1994;20:765-73.
4. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987;327:524-6.
5. Pawlak R, Chabielska E, Golatowski J, Azzadin A, Buczko W. Nitric oxide and prostacyclin are involved in antithrombotic action of captopril in venous thrombosis in rats. Thromb Haemost 1998;79:1208-12.
6. Cho JS, Martelli E, Mozes G, Miller VM, Gloviczki P. Effects of thrombolysis and venous thrombectomy on valvular competence, thrombogenicity, venous wall morphology, and function. J Vasc Surg 1998;28:787-99.
7. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1993;329:2002-12.
8. Broeders MA, Tangelder GJ, Slaaf DW, Reneman RS, Egbrink MG. Endogenous nitric oxide protects against thromboembolism in venules but not in arterioles. Arterioscler Thromb Vasc Biol 1998;18:139-45.
9. Broeders MA, Tangelder GJ, Slaaf DW, Reneman RS, Egbrink MG. Endogenous nitric oxide and prostaglandins synergistically counteract thromboembolism in arterioles but not in venules. Arterioscler Thromb Vasc Biol 2001;21:163-9.
10. Lin PH, Chen C, Surowiec SM, Conklin B, Bush RL, Lumsden AB. Evaluation of thrombolysis in a porcine model of chronic deep venous thrombosis: an endovascular model. J Vasc Surg 2001;33:621-7.
11. Lin PH, Surowiec SM, Conklin B, Chen C, Bush R, et al. Evaluation of thrombolysis and angioplasty in a porcine iliac artery thrombosis model: application of endovascular stent-graft-induced thrombosis. J Vasc Interv Radiol 2000;11:785-91.
12. Lin PH, Surowiec SM, Conklin B, Bush RL, Chen C, et al. An endovascular model of carotid stenosis for the evaluation of thrombolysis and angioplasty. J Endovasc Ther 2000;7:486-93.
13. Lin PH, Chen C, Surowiec SM, Conklin B, Bush RL, et al. A porcine model of carotid artery thrombosis for thrombolytic therapy and angioplasty: application of PTFE graft-induced stenosis. J Endovasc Ther 2000;7:227-35.
14. Yao SK, Akhtar S, Scott-Burden T, Ober JC, Golino P, et al. Endogenous and exogenous nitric oxide protect against intracoronary thrombosis and reocclusion after thrombolysis. Circulation 1995;92:1005-10.
15. Yao SK, Ober JC, Krishnaswami A, Ferguson JJ, Anderson HV, et al. Endogenous nitric oxide protects against platelet aggregation and cyclic flow variations in stenosed and endothelium-injured arteries. Circulation 1992;86:1302-9.
16. Surowiec SM, Conklin BS, Li JS, Lin PH, Weiss VJ, et al. A new perfusion culture system used to study human vein. J Surg Res 2000; 88:34-41.
17. Conklin BS, Surowiec SM, Ren Z, Li JS, Zhong DS, et al. Effects of nicotine and cotinine on porcine arterial endothelial cell function. J Surg Res 2001;95:23-31.
18. Chen C, Conklin BS, Ren Z, Zhong DS. Homocysteine decreases endothelium-dependent vasorelaxation in porcine arteries. J Surg Res 2002;102:22-30.
19. Rhodes JM, Cho JS, Gloviczki P, Mozes G, Rolle R, Miller VM. Thrombolysis for experimental deep venous thrombosis maintains valvular competence and vasoreactivity. J Vasc Surg 2000;31:1193-1205.

20. Chen C, Mattar SG, Lumsden AB. Oral administration of L-arginine reduces intimal hyperplasia in balloon-injured rat carotid arteries. J Surg Res 1999;82:17-23.
21. Chen C, Lumsden AB, Hanson SR. Local infusion of heparin reduces anastomotic neointimal hyperplasia in aortoiliac expanded polytetrafluoroethylene bypass grafts in baboons. J Vasc Surg 2000;31:354-63.
22. Comerota AJ, Aldridge SC, Cohen G, Ball DS, Pliskin M, White JV. A strategy of aggressive regional therapy for acute iliofemoral venous thrombosis with contemporary venous thrombectomy or catheter-directed thrombolysis. J Vasc Surg 1994;20:244-54.
23. Meissner MH, Manzo RA, Bergelin RO, Markel A, Strandness DE Jr. Deep venous insufficiency: the relationship between lysis and subsequent reflux. J Vasc Surg 1993;18:596-605; discussion 606-8.
24. Meissner MH, Caps MT, Zierler BK, Bergelin RO, Manzo RA, Strandness DE Jr. Deep venous thrombosis and superficial venous reflux. J Vasc Surg 2000;32:48-56.
25. Radomski MW, Vallance P, Whitley G, Foxwell N, Moncada S. Platelet adhesion to human vascular endothelium is modulated by constitutive and cytokine induced nitric oxide. Cardiovasc Res 1993;27:1380-2.
26. Miller VM, Barber DA. Modulation of endothelium-derived nitric oxide in canine femoral veins. Am J Physiol 1996;271:H668-73.
27. Kelm M, Schrader J. Control of coronary vascular tone by nitric oxide. Circ Res 1990;66:1561-75.
28. Panza JA, Casino PR, Badar DM, Quyyumi AA. Effect of increased availability of endothelium-derived nitric oxide precursor on endothelium-dependent vascular relaxation in normal subjects and in patients with essential hypertension. Circulation 1993;87:1475-81.
29. Willerson JT, Igo SR, Yao SK, Ober JC, Macris MP, Ferguson JJ. Localized administration of sodium nitroprusside enhances its protection against platelet aggregation in stenosed and injured coronary arteries. Tex Heart Inst J 1996;23:1-8.
30. Hackett D, Davies G, Chierchia S, Maseri A. Intermittent coronary occlusion in acute myocardial infarction: value of combined thrombolytic and vasodilator therapy. N Engl J Med 1987;317:1055-9.
31. Stamler JS, Vaughan DE, Loscalzo J. Synergistic disaggregation of platelets by tissue-type plasminogen activator, prostaglandin E_1, and nitroglycerin. Circ Res 1989;65:796-804.
32. Kashyap VS, Reil TD, Moore WS, Hoang TX, Gelabert HA, et al. Acute arterial thrombosis causes endothelial dysfunction: a new paradigm for thrombolytic therapy. J Vasc Surg 2001;34:323-9.
33. Manicone JA, Eisenbud DE, Hertz SM, Brener BJ, Creighton D, et al. The effect of thrombus on the vascular endothelium of arterialized vein grafts. Am J Surg 1996;172:163-6; discussion 167.
34. Whitley D, Gloviczki P, Rhee R, Tazelaar HD, Miller V. Urokinase treatment preserves endothelial and smooth muscle function in experimental acute arterial thrombosis. J Vasc Surg 1996;23:851-8; discussion 858-9.
35. Wengrovitz M, Healy DA, Gifford RR, Atnip RG, Thiele BL. Thrombolytic therapy and balloon catheter thrombectomy in experimental femoral artery thrombosis: effect on arterial wall morphology. J Vasc Interv Radiol 1995;6:205-10.
36. Tanaka H, Sukhova GK, Swanson SJ, Clinton SK, Ganz P, et al. Sustained activation of vascular cells and leukocytes in the rabbit aorta after balloon injury. Circulation 1993;88:1788-1803.
37. Downing LJ, Strieter RM, Kadell AM, Wilke CA, Brown SL, et al. Neutrophils are the initial cell type identified in deep venous thrombosis induced vein wall inflammation. ASAIO J 1996;42:M677-82.
38. Toursarkissian B, Schwartz D, Eisenberg PR, Rubin BG. Arterial thrombosis induces early upregulation of intercellular adhesion molecule in the media. J Vasc Surg 1997;26:663-9.
39. Wakefield TW, Strieter RM, Wilke CA, Kadell AM, Wrobleski SK, et al. Venous thrombosis–associated inflammation and attenuation with neutralizing antibodies to cytokines and adhesion molecules. Arterioscler Thromb Vasc Biol 1995;15:258-68.
40. Wakefield TW, Strieter RM, Downing LJ, Kadell AM, Wilke CA, et al. P-selectin and TNF inhibition reduce venous thrombosis inflammation. J Surg Res 1996;64:26-31.
41. Downing LJ, Wakefield TW, Strieter RM, Prince MR, Londy FJ, et al. Anti-P-selectin antibody decreases inflammation and thrombus formation in venous thrombosis. J Vasc Surg 1997;25:816-27; discussion 828.
42. Persson MG, Gustafsson LE, Wiklund NP, Hedqvist P, Moncada S. Endogenous nitric oxide as a modulator of rabbit skeletal muscle microcirculation in vivo. Br J Pharmacol 1990;100:463-6.
43. Kurose I, Kubes P, Wolf R, Anderson DC, Paulson J, et al. Inhibition of nitric oxide production: mechanisms of vascular albumin leakage. Circ Res 1993;73:164-71.
44. Wang BY, Singer AH, Tsao PS, Drexler H, Kosek J, Cooke JP. Dietary arginine prevents atherogenesis in the coronary artery of the hypercholesterolemic rabbit. J Am Coll Cardiol 1994;23:452-8.
45. Jiang J, Valen G, Tokuno S, Thoren P, Pernow J. Endothelial dysfunction in atherosclerotic mice: improved relaxation by combined supplementation with L-arginine-tetrahydrobiopterin and enhanced vasoconstriction by endothelin. Br J Pharmacol 2000;131:1255-61.
46. Boger RH, Bode-Boger SM, Kienke S, Stan AC, Nafe R, Frolich JC. Dietary L-arginine decreases myointimal cell proliferation and vascular monocyte accumulation in cholesterol-fed rabbits. Atherosclerosis 1998;136:67-77.
47. Davies MG, Fulton GJ, Huynh TT, Barber L, Svendsen E, Hagen PO. Combination therapy of cholesterol reduction and L-arginine supplementation controls accelerated vein graft atheroma. Ann Vasc Surg 1999;13:484-93.
48. Tarry WC, Makhoul RG. L-Arginine improves endothelium-dependent vasorelaxation and reduces intimal hyperplasia after balloon angioplasty. Arterioscler Thromb 1994;14:938-43.
49. Schwarzacher SP, Lim TT, Wang B, Kernoff RS, Niebauer J, et al. Local intramural delivery of L-arginine enhances nitric oxide generation and inhibits lesion formation after balloon angioplasty. Circulation 1997; 95:1863-9.
50. Landis ME, Ray EC, Gloviczki P, Miller VM. Effects of dietary L-arginine on structure and function of flow-restricted vein grafts. J Vasc Surg 2001;33:829-39.

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