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Rheolytic Pharmacomechanical Thrombectomy in Experimental Chronic Deep Vein Thrombosis: Effect of L-Arginine on Thrombogenicity and Endothelial Vasomotor Function

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Abstract

Purpose: Endovascular removal of intravascular thrombus using the AngioJet rheolytic thrombectomy (RT) system has been shown to be clinically effective. This system also permits the concomitant infusion of thrombolytic agent followed by thrombectomy, thus creating a novel strategy known as pharmacomechanical thrombectomy (PMT). Although these interventions have gained wide clinical application, little is known regarding the vessel wall response following thrombectomy therapy. The aims of this study were to assess the effect of thrombectomy interventions on endothelial function in a porcine model of deep venous thrombosis (DVT) and to evaluate the effect of nitric oxide (NO) precursor L-arginine on endothelial function following thrombectomy therapy.

Methods: Deep vein thrombosis was created in bilateral iliac veins by deploying a self-expanding stent-graft incorporating an intraluminal stenosis from a groin approach. Five pigs underwent sham operation. Following 14 days of DVT, animals were randomized to three groups: the first group received RT treatment (RT group, $n = 5$); the second group received pharmacomechanical thrombectomy (PMT) with tissue plasminogen activator (alteplase 10 mg; PMT group, $n = 5$); and the third group received PMT with tPA plus intravenous L-arginine (20 mmol/l) (arginine group, $n = 5$). Iliac vein patency was evaluated by venography and intravascular ultrasound at 1 week. Nitric oxide level was determined by a chemiluminescent assay of the nitrite/nitrate metabolites (NO_x). Thrombogenicity was evaluated by radiolabeled platelet and fibrin deposition. Veins were harvested and evaluated with light microscopy and scanning electron microscopy (SEM). Endothelial function was evaluated using organ chamber analysis.

Results: The luminal areas in the sham, RT, PMT, and arginine groups were 34 ± 10 mm^2, 21 ± 13 mm^2, 35 ± 18 mm^2, and 37 ± 16 mm^2, respectively. All iliac veins remained patent at 2 weeks. No difference in endothelial cell structure was observed between the three treatment groups by means of light microscopic or SEM examination. A decrease in platelet deposition

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occurred in the arginine group compared to the RT and PMT groups ($P < 0.05$). The arginine group also showed a greater endothelium-dependent relaxation compared to the RT or PMT groups in response to A23187, bradykinin, and ADP ($P < 0.05$). Local NO_x level was higher in the arginine group than in the RT or PMT group (2.6 ± 0.6 μmol/l versus 0.3 ± 0.1 μmol/l and 0.3 ± 0.2 μmol/l; $P < 0.01$).

Conclusions: AngioJet RT and PMT interventions resulted in similar attenuated endothelium-dependent vasoreactivity and morphologic effect. L-Arginine supplementation preserves endothelial vasoreactivity and reduces platelet deposition following PMT in iliac DVT. Additionally, L-arginine enhances NO production at sites of venous thrombosis. The NO precursor L-arginine may have a therapeutic potential in preserving endothelial function following mechanical thrombectomy.

Deep venous thrombosis (DVT) is estimated to affect 20%–30% of patients undergoing major surgical procedures, and it is responsible for more than 60,000 deaths annually in the United States as a result of pulmonary embolism.[1,2] Although systemic anticoagulation remains the mainstay of therapy, surgical thrombectomy with an embolectomy balloon catheter or catheter-directed thrombolysis is often indicated in symptomatic patients with severe DVT. While the technique of removing thrombus using an embolectomy balloon catheter is familiar to most surgeons, this procedure has never gained wide acceptance by physicians in the treatment of symptomatic lower extremity DVT, in part because of the mechanical trauma associated with the thrombectomy procedure. Numerous vessel-related complications have been described with the use of an embolectomy balloon catheter for thrombus removal, which included vessel rupture, endothelial denudation, and early vessel occlusion from accelerated intimal hyperplasia.[3–5]

In an effort to reduce procedure-related complications and enhance thrombectomy efficacy, researchers have reported the clinical benefit of percutaneous catheter-directed thrombolysis in the treatment of DVT.[6–8] When compared to an open balloon thrombectomy procedure, the advantage of catheter-directed pharmacological thrombolysis (PMT) is reduced mechanical trauma to the vessel wall. In addition, the administration of the pharmacological thrombolytic agent allows dissolution of thrombus in small-caliber vessels that otherwise are not accessible by a balloon thrombectomy catheter. Despite the perceived clinical advantages, however, numerous studies have demonstrated risks and complications related to PMT, including include stroke with intracranial hemorrhage, particularly in elderly patients.[9]

Over the past decade, percutanous mechanical thrombectomy has emerged as an effective treatment alternative to open surgical thrombectomy and pharmacological thrombolysis in patients with thrombotic occlusion.[10,11] The AngioJet rheolytic thrombectomy (RT) system (Possis Medical, Minneapolis, MN) is a commonly employed mechanical thrombectomy catheter that uses a complex mixture of rapid fluid streaming and hydrodynamic force to fracture thrombus, allowing extraction at the catheter tip using negative pressure based on the Bernoulli principle. A recent report highlighted the clinical advantage of this thrombectomy system because it allows simultaneous catheter-directed infusion of thrombolytic agents, thereby creating a PMT system.[11] This feature, also known as power-pulse spray, is initiated by shutting off the outflow fluid channel to permit infusion of a thrombolytic agent. The rheolytic thrombectomy mode is then resumed by opening the outflow channel to allow thrombus extraction. This technique combines the advantages of RT and PMT, in which the thrombolytic dosage can be reduced and the efficacy of mechanical thrombectomy can be maximized.[11,12]

Although numerous clinical studies have reported the effectiveness of the AngioJet RT system in the treatment of symptomatic DVT, the physiologic response of the venous endothelium and venous wall vasoreactivity to the rheolytic thrombectomy therapy remains unclear. A recent study analyzing the cellular function in acute DVT after thrombotic therapy revealed that more endothelial functions were preserved following thrombolysis when compared to thrombectomy therapy.[13] Our laboratory had previously reported the preservation of endothelial function and vasomotor response following manual PMT therapy in experimental chronic DVT.[14,15] In an effort to further understand the endothelial response to percutaneous thrombectomy therapy in a chronic DVT setting, we sought to evaluate the effect of the AngioJet RT system using the PMT technique on endothelial function and thrombogenicity in a porcine chronic DVT model.

MATERIALS AND METHODS

Animal Model and Interventions

Twenty adult domestic swine, each weighing 55–65 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 cut downs were performed to expose the superficial femoral veins, followed by placement of a 14 French introducer sheath (Meditech, Watertown, MA). Bilateral DVT was created using an endovascular model previously described.[14,16] Briefly, a self-expanding nitinol stent-graft (Symphony Stent, Meditech) that incorporated an intra-stent stenosis was deployed in bilateral common iliac veins. The intra-stent stenosis was created by a tapered polytetrafluorethylene (PTFE) graft (W. L. Gore, Flagstaff, AZ) which caused flow stasis resulting in DVT. An infrarenal Greenfield vena caval filter (Meditech) was also placed in each animal to prevent pulmonary embolism. Venography documented the presence of DVT in bilateral iliac veins, after which the introducer sheaths were removed, and the venotomy was closed with 6–0 polypropylene suture followed by layered groin wound closure. The animals were allowed to be extubated and recovered following the stent-graft deployment. The animals were divided into four groups (n = 5/group). The first group underwent sham operation without the stent-graft placement (sham group). The remaining 15 animals underwent bilateral stent-graft placement to induce DVT. After 3 weeks of DVT, the remaining 15 animals were divided into the following three groups: one group received AngioJet RT intervention (RT group); another group received AngioJet PMT power-pulse spray therapy using the tissue plasminogen activator alteplase (PMT group; Genentech, San Francisco, CA); and the third group received AngioJet PMT treatment plus intravenous L-arginine (arginine group; Sigma Chemical, St Louis, MO).

AngioJet RT Intervention

Rheolytic thrombectomy using the AngioJet system was performed according to techniques that we had previously described (Fig. 1).[17] Briefly, animals were given general anesthesia with endotracheal intubation, and bilateral groins were exposed for placement of the 7 F introducer sheaths (Meditech). A 0.035″ Bentson guidewire (Meditech) was inserted through the introducer sheath in the femoral vein to cannulate the stent-graft device in the iliac vein. An AngioJet Xpeedior Rheolytic Thrombectomy catheter (Possis, Minneapolis, MN) was inserted over the guidewire. Mechanical thrombectomy was performed by advancing the catheter across the iliac vein thrombotic segment at a rate of 1 mm/s. Catheter passage across the thrombotic segment was performed twice. After the RT intervention, balloon angioplasty using a 12 mm $\times$ 4 cm balloon (XXL, Meditech) was performed to dilate the tapered stent-graft, which eliminated the intra-stent stenosis and restored venous flow. Iliac vein patency was assessed by venogram and intravascular ultrasound (IVUS).

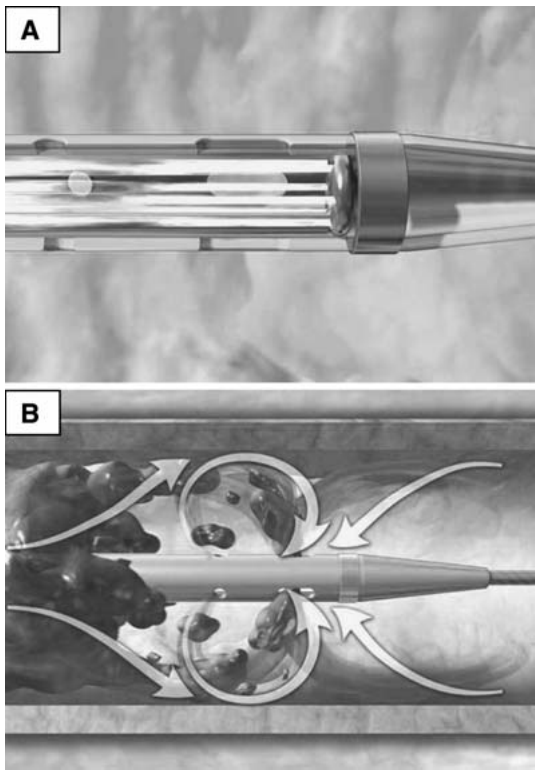

Figure 1. A. The AngioJet rheolytic thrombectomy (RT) catheter system emits high-velocity saline jets that are directed backward from the tip of the device to outflow channels in a coaxial fashion. **B**. In standard RT mode, this generates a vacuum force that draws the thrombus into the catheter, achieving the rheolytic thrombectomy effect.

AngioJet PMT Intervention

Detailed techniques in the application of AngioJet PMT treatment have been described in our previous report

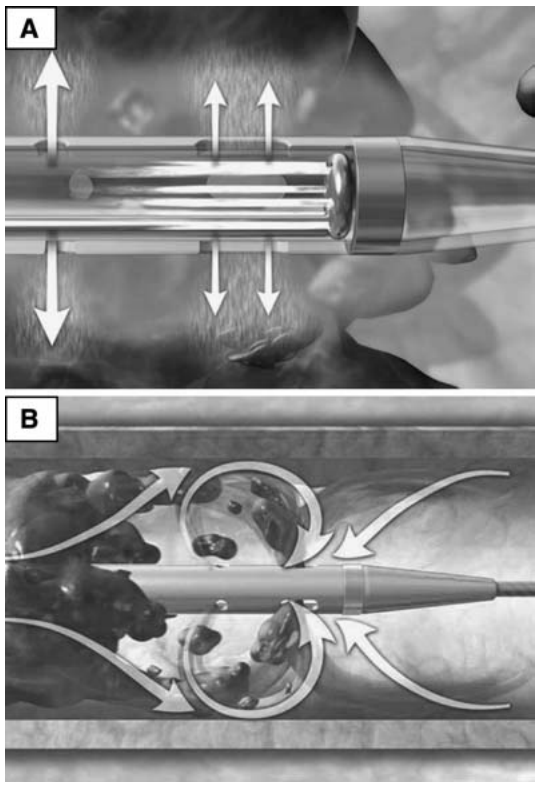

Figure 2. A. In the pharmacomechanical thrombectomy (PMT) therapy, the thrombolytic agent is first administered into the venous thrombus by shutting off the outflow channel. **B**. After a period of 15 min to allow thrombolytic therapy to take effect, the outflow channel is reopened, placing the catheter system once again in rheolytic thrombectomy mode.

(Fig. 2).[17] Briefly, an AngioJet Xpeedior catheter (Possis) is inserted over a 0.035″ Bentson guidewire (Meditech). A three-way stopcock is connected to the outflow lumen of the AngioJet catheter (Possis) to close the fluid outflow, after which 10 mg of tPA (Genentech) is mixed in 50 ml of normal saline. That solution is then connected to the Power Pulse Delivery kit (Possis), and the thrombolytic agent is delivered to the thrombosed iliac vein while the outflow channel is turned off. This maneuver enables the thrombolytic agent to be mechanically delivered to the venous thrombus. Fifteen minutes after completion of the thrombolytic infusion, the outflow channel of the AngioJet catheter is switched open and rheolytic thrombectomy is performed as described. The combination of transient thrombolytic infusion followed by rheolytic thrombectomy has a result equivalent to pharmacomechanical thrombectomy. Following the PMT intervention, balloon angioplasty using a 12 mm $\times$ 4 cm balloon (XXL, Meditech) is performed to dilate the tapered stent-graft, thereby eliminating the intra-stent stenosis and restoring venous flow. Completion venogram and IVUS assessment are then performed to determine thrombectomy efficacy.

With regard to the arginine treatment group, 100 μg/kg of L-arginine was mixed in 10 ml of sterile water, and the solution was pulsed-spray into the iliac veins following tPA thrombolysis. The L-arginine infusion dose was chosen from previously published reports.[18,19] Following the PMT therapy, balloon angioplasty using a 12 mm $\times$ 4 cm balloon (XXL, Meditech) was performed to dilate the tapered stent-graft, thereby eliminating the intra-stent stenosis and restoring venous flow. Iliac vein patency was assessed with venogram and intravascular ultrasound (IVUS).

One week after the thrombectomy intervention, animals in all groups were anesthetized. Bilateral femoral veins were exposed followed by the placement of 7 F introducer sheaths (Meditech). Once again, iliac venous patency was assessed by venogram and IVUS. Laparotomy was performed to harvest complete ileofemoral veins. Histologic analysis and an organ chamber study were performed on one ileofemoral vein, and local thrombogenicity evaluation was performed on the contralateral vein prior to vein harvest. All histological and functional analyses of the iliac vein were performed in vein segments just below the stent-graft placement site, where thrombosis and thrombectomy therapy took place.

Intravascular Ultrasound

A 3.5 F 20 MHz IVUS catheter was inserted over the guidewire using a ClearView Ultra Intravascular Ultrasound System (Meadox). Prior to the pulse-spray thrombolytic therapy, IVUS was used to assess the iliac venous thrombus. The IVUS exam was performed by an individual who was blinded to the respective treatment groups of the animals. 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 thrombectomy intervention, 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 imaging was used to confirm the location of the IVUS transducer within the proximal iliac vein. The luminal dimensions before (Before) and after (After) thrombectomy interventions were traced on the IVUS image with a tracking device, which allowed calculation of a cross-sectional area as follows:

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

Organ Chamber Evaluation

The evaluation of endothelial vasoreactive functions using the organ chamber myograph system has been previously described in our laboratory.[20–22] Through an abdominal and bilateral groin exposure, one iliofemoral vein was carefully harvested in its entirety prior to the injection of radiolabeled platelets and fibrinogen. The iliac vein proximal to the nitinol stent was sectioned into multiple 5-mm segments that were incubated in DMEM 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 (Danish MyoTechnology Organ Bath 700MO, Aarhus, Denmark) in 8 ml of Kreb's 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 the pH at 7.4. Rings were subjected stepwise to a predetermined optimal tension of 2 g and allowed to equilibrate for at least 60 min. Following equilibration, each ring was precontracted with norepinephrine (NE, 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 the Krebs solution, and the vessels were contracted to 75% of F_{max} with NE. The vessels were then challenged with ADP (Sigma) in an incremental log concentration from 10^{-9} to 10^{-4} mol/l to determine EDR. Additionally, vessels were challenged with calcium channel ionophore A23187 (10^{-9}–$10^{-6.5}$ M), an endothelium-dependent vasodilator, and allowed to equilibrate for 10 min to assess EDR. Relaxation dose–response curves were also determined by incremental additions of endothelium-dependent vasodilator bradykinin (10^{-7} to $10^{-4.5}$ M), as well as serotonin (10^{-9} to 10^{-4} M). Endothelium-independent relaxation (EIR) was assessed with the addition of sodium nitroprusside (NTP; 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 NE between each reagent. Contractility and percentage of relaxation were calculated based on the tension changes. For statistical analysis, the data of all iliac vein segments from each animal were averaged and represented as one data point.

Thrombogenicity

Blood samples were obtained from each animal and placed in anticoagulant-citrate dextrose solution (ACD solution Modified; Squibb Diagnostics, New Brunswick, NJ) for centrifugation to acquire a platelet pellet. The resulting platelet sample was resuspended with ACD solution followed by indium (In) 111 oxyquinoline (Amersham Corporation, Arlington Heights, IL) to create radiolabeled platelets. Freeze-dried human fibrinogen labeled with radioactive iodine (^{125}I; Amersham Corporation) was reconstituted in 1.1 ml ACD solution and 1.9 μCi/kg. Following the harvesting of one ileofemoral vein for organ chamber analysis, the radiolabeled platelet and fibrinogen were injected into the animal and allowed circulation for 3 h prior to harvesting of the contralateral iliofemoral vein. The vein segment was cut into three sections with documented surface areas. Depositions of ^{111}In-platelets and ^{125}I-fibrinogen were analyzed in each iliac vein segment in accordance with the surface area with a gamma counter (Packard Instruments, Downers Grove, IL). A hemocytometer was used to determine the platelet concentration. The formulas used to calculate platelet and fibrin deposition were chosen from reports that were published earlier.[13,23]

Histologic and Immunohistochemical Analysis

Segments of the iliac vein were fixed in 10% buffered formalin overnight and then transferred to 70% alcohol. The specimens were dehydrated using sequentially increasing concentrations of ethanol followed by xylene and embedded in paraffin. Five-micrometer cross sections were cut and prepared as previously described.[24,25] Histological staining with hematoxylin and eosin, methylene blue, and Verhoeff-Masson stain was then performed. Immunohistochemical analysis was accomplished using the avidin-biotin complex immunoperoxidase procedure (LSAB Kit, Dako Co, Carpenteria, CA) as previously described.[24,25] 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), 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 PBS three times and then dehydrated in a graded series of ethanol (30%–100%). The tissue samples were placed with a graded series of hexamethyldisilazane, air dried, and gold sputter coated before imaging (Cambridge 360 SEM microscope). Endothelial loss based on the SEM image was scanned and evaluated by a blind observer who used an SEM image software (Bioview Image, Atlanta, GA).

Table 1.
IVUS evaluation of thrombus areas and thrombectomy efficacy after thrombectomy interventions

Treatment group	Preintervention thrombus area (mm^2)	Postintervention thrombus area (mm^2)	Thrombectomy efficacy (%)
Sham	14.4 ± 4.4	3.5 ± 1.7	85
RT	74.6 ± 15.7	38.9 ± 14.2	46
PMT	86.5 ± 16.9	24.9 ± 11.5	71*
Arginine	83.6 ± 17.3	22.3 ± 9.8	73*

RT: rheolytic thrombectomy; PMT: pharmacomechanical thrombectomy.
*$P < 0.05$ compared to the RT treatment group.

Statistical Analysis

Values are expressed as mean ± standard error of the mean. Differences in vessel relaxation and contraction in the organ chamber study were determined by one-way analysis of variance or Student's *t*-test where appropriate. Unless otherwise stated, *n* refers to the number of arteries. As a normal distribution cannot be assumed for both morphologic parameters and percent changes in vasoreactivity, the Kruskal-Wallis, and Friedman or Wilcoxon signed rank sum tests were used to compare mean group values. An SAS statistical package was used for analysis (version 5.0, Abacus Concepts, Berkeley, CA). Significance was considered when the *p* value was less than 0.05.

RESULTS

Radiological Evaluation

Iliac veins from all three groups remained patent based on both angiographic and IVUS assessment at 1 week after thrombectomy treatment. The IVUS evaluation revealed a wide disparity of residual iliac venous thrombosis following interventions among various groups. The luminal areas in the sham, RT, PMT, and arginine groups were 34 ± 10 mm^2, 21 ± 13 mm^2, 35 ± 18 mm^2, and 37 ± 16 mm^2, respectively, at 1 week after chronic DVT interventions. The thrombectomy efficacy and the thrombus areas calculated by IVUS are summarized in Table 1.

Thrombogenicity

The preoperative hematocrit or platelet counts were similar among all groups. The platelet counts were 268,900/μl, 306,600/μl, 302,4500/μl, and 313,400/μl, and the hematocrit counts were 39.6%, 43.5%, 44.2%, and 48.4%, in the sham, RT, PMT, and arginine group, respectively. One week after the thrombectomy interventions, radiolabeled fibrin deposition did not differ among the groups. However, a decrease in platelet deposition was noted in the arginine group, compared to either the RT group or the PMT group. ($P < 0.05$, Table 2).

Table 2.
Thrombogenicity of the iliac veins following thrombectomy interventions

Treatment group	Platelet deposition (platelets/cm^2)	Fibrin deposition (ng/cm^2)
Sham	4,645 ± 1,875	3.6 ± 4.2
RT	26,854 ± 6,895	7.2 ± 6.1
PMT	27,954 ± 5,648	8.6 ± 5.4
Arginine	6,324 ± 2,235*	7.2 ± 7.2

Light Microscopy and Scanning Electron Microscopy

Histological evaluation of the thrombus revealed the presence of recanalized and organized thrombus in the RT (70%, 7/10 vessel specimens), PMT (60%, 6/10 vessel specimens), and arginine groups (60%, 6/10 vessel specimens). In contrast, the control specimen did not contain any recanalized thrombus (0%, 0/10 vessel specimens, $P < 0.01$). Immunohistochemical evaluation revealed a similar pattern of factor VIII staining in all groups, but without obvious evidence of endothelial cell loss. Immunohistochemical staining of α-actin showed a positive staining pattern in the thrombus as well as the media, without evidence of intimal proliferation in all groups. The SEM evaluation revealed similar patterns of endothelial cell loss among the three treatment groups (RT, 36% ± 15%; PMT, 39% ± 21%; arginine, 31% ± 18%), whereas the control group showed minimal endothelial denudation (11% ± 9%). With further histological assessment in regard to the inflammatory response, a significant increase in leukocytes, with a more or less equal presence of neutrophils and macrophages,

Table 3.
Levels of NO_x in all treatment groups

Treatment group	Systemic NO_x level (μmol/l)	Local NO_x level (μmol/l)
Sham	33.1 ± 15.2	0.2 ± 0.1
RT	35.6 ± 17.5	0.3 ± 0.1
PMT	25.9 ± 13.8	0.3 ± 0.2
Arginine	26.7 ± 14.2	2.6 ± 0.6*

* $P < 0.01$ compared to the sham, RT, or PMT interventions.

was found in the RT, PMT, and arginine groups compared to the sham control group.

NO_x Level

Systemic NO_x measurement based on a chemiluminescent assay immediately following thrombectomy interventions showed no difference between all groups ($P > 0.05$). Similarly, no differences in systemic NO_x levels were detected between all groups at the time of tissue harvest 7 days later. However, the local NO_x level in the arginine group was correspondingly higher compared to the sham, RT, or PMT groups ($P < 0.05$; Table 3).

Vasoreactivity Evaluation

Maximal contraction curves in response to NE were similar in all tested groups at 10^{-4} mol/l, with a mean F_{max} ranging from 2.6 ± 1.7 to 5.5 ± 1.6 g. The calcium ionophore A23187 caused concentration-dependent relaxations in all tested groups, but the arginine group exhibited a greater relaxation response at $10^{-7.5}$ mol/l compared to the PMT or RT groups (61 ± 23% versus 43 ± 22% and 35 ± 16%, $P < 0.05$, Fig. 3). No significant difference was found in the EDR in response to calcium ionophore A23187 between the arginine group and the control group (Fig. 3). Endothelium-dependent relaxation was also assessed by adding bradykinin in log escalating doses (Fig. 4). The arginine group displayed a greater relaxation response at 10^{-5} and $10^{-4.5}$ mol/l compared to the PMT and RT groups ($P < 0.05$; Fig. 4). Endothelial dependent relaxation was also assessed by adding serotonin in log escalating doses, which displayed a classical dose–response curve (Fig. 5), although no significant difference was noted among the RT, PMT, or arginine groups. The presence of ADP caused a concentration-dependent relaxation in iliac venous segments in all groups. The arginine group showed a greater relaxation than the RT or PMT groups in response to ADP at 10^{-6}, 10^{-5}, and 10^{-4} mol/l concentration ($P < 0.05$, Fig. 6). The control group exhibited the greatest degree

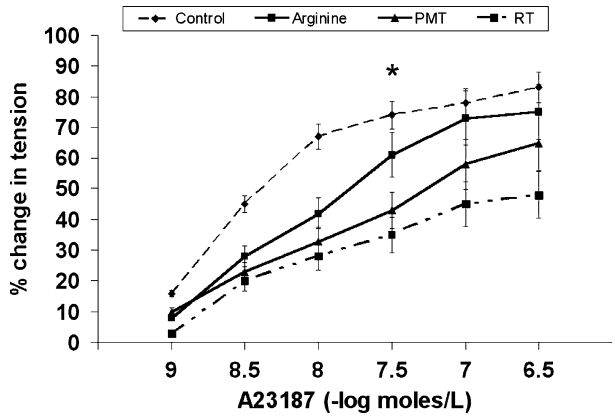

Figure 3. Endothelium-dependent (EDR) relaxation response to calcium ionophore A23187 in porcine iliac veins 7 days after thrombectomy interventions. (*$P < 0.05$ arginine group versus RT and PMT groups, Kruskal-Wallis test).

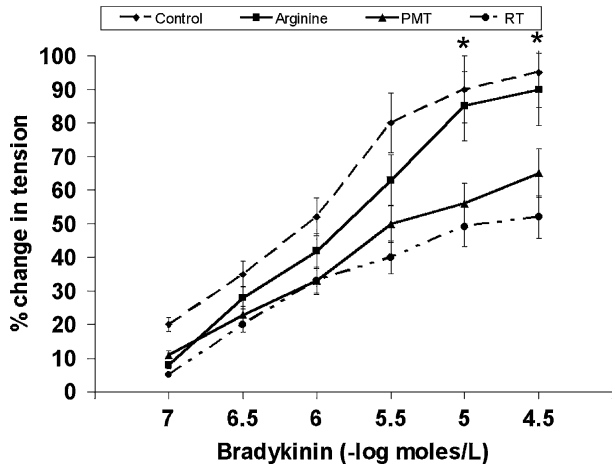

Figure 4. Endothelium-dependent relaxation response to bradykinin in porcine iliac veins 7 days after thrombectomy interventions. (*$P < 0.05$ arginine group versus RT and PMT groups; Kruskal-Wallis test).

of EDR in response to A23187, bradykinin, serotonin, and ADP when compared to the RT, PMT and arginine groups (Figures 3–6). Endothelium-independent relaxation was assessed by determining the relaxation response to log escalating doses of NTP. All vein segments responded similarly to NTP in a concentration-dependent manner (Fig. 7).

DISCUSSION

The clinical significance of DVT is incontrovertible as it is estimated that more than 300,000 patients are hospitalized each year in the United States annually due to this condition.[1,2] Furthermore, DVT is responsible for more

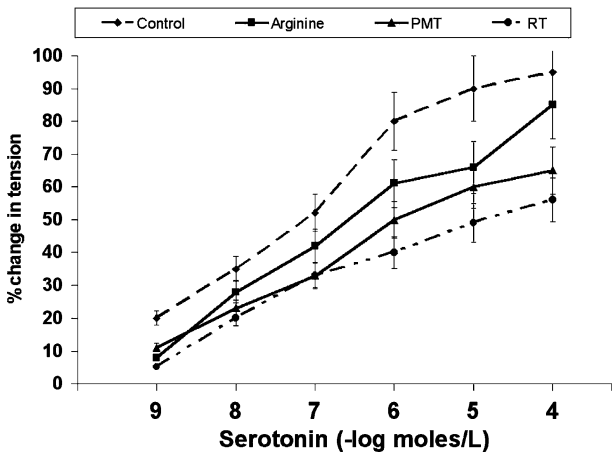

Figure 5. Endothelium-dependent relaxation response to serotonin in porcine iliac veins 7 days after thrombectomy interventions. (*$P < 0.05$ arginine group versus RT and PMT groups; Kruskal-Wallis test).

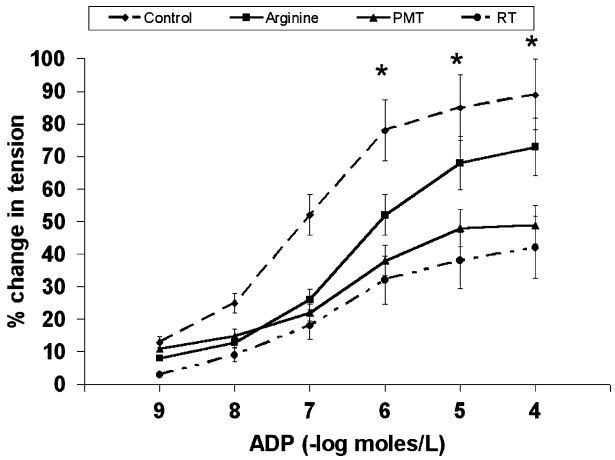

Figure 6. Endothelium-dependent relaxation response to ADP in porcine iliac veins 7 days after thrombectomy interventions. (*$P < 0.05$ arginine group versus RT and PMT groups; Kruskal-Wallis test).

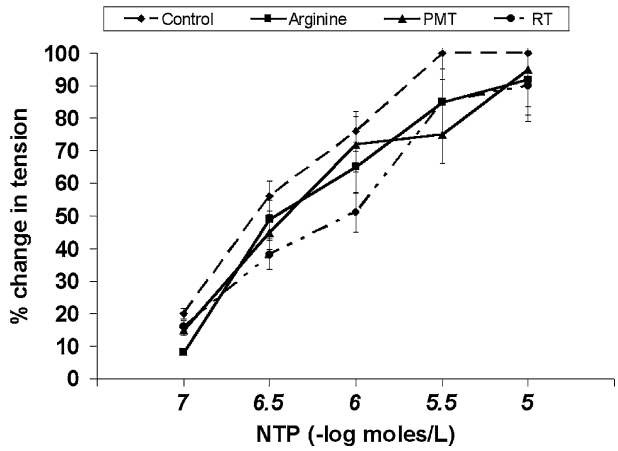

Figure 7. Endothelium-independent relaxation response to sodium nitroprusside (NTP) in porcine iliac veins 7 days after thrombectomy interventions.

than 60,000 deaths from pulmonary embolism each year in the United States.[1,2] The long-term sequalae of DVT involve the valve dysfunction caused by the venous thrombus, which can result in valvular incompetence leading to chronic venous insufficiency. Recent advances in endovascular interventions have led to a variety of catheter-based therapeutic strategies for removing large venous thrombus to alleviate symptoms and maintain luminal patency.[26,27] Among these therapeutic tools, the concept of PMT using the AngioJet RT system has gained a wide clinical acceptance, in part because of its ability to permit transient pharmacological thrombolysis followed by rapid mechanical thrombus extraction.[11] It has been hypothesized that rapid and complete removal of thrombus would not only eliminate the risk of pulmonary embolism but also reduce the likelihood of valvular incompetence. Despite many clinical reports documenting the clinical efficacy of PMT in patients with venous thrombosis,[17,27] very little is known regarding venous wall function and thrombogenicity following PMT therapy. Our study is notable because it represents the first study to analyze endothelial vasoreactivity after mechanical thrombectomy or PMT in an in vivo chronic DVT model. In addition, our study demonstrates an equivalent endothelial vasoreactivity with reduced thrombogenicity following PMT when compared to rheolytic mechanical thrombectomy intervention.

Thrombus formation is a complex process culminating in a common final pathway involving thrombin cleavage of fibrinogen to fibrin. Fibrin strands then bind with activated and aggregated platelets and red blood cells to form thrombus. Plasmin cleaves fibrin strands, leading to thrombus dissolution. Plasminogen is converted to plasmin by naturally occurring tPA, which is secreted by normal vascular endothelium. Therefore, endogenous t-PA is essential in achieving the delicate balance between hemostasis and fibrinolysis. The administration of exogenous tPA during mechanical thrombectomy forms the basis of the theoretical advantage of a PMT intervention, which mechanically delivers the thrombolytic agent under high pressure to allow maximum thrombus penetration. As a result, enhanced thrombolytic exposure and pharmacological thrombolysis can take place in a rich clot-bound fibrin thrombin environment.[28] The jet streams within the AngioJet RT catheter system generate an effective pressure of 2,000 psi at a flow velocity of 360 km/h within the catheter.[29] These high-velocity jets create a localized low-pressure zone based on the Bernoulli

effect,[30] and this leads to thrombus maceration and aspiration. The jets also provide the driving force for evacuation of thrombus particulate debris through the catheter. During PMT, the outflow channel of the jet streams is shut off, which permits the thrombolytic agent to be delivered radially into the venous thrombus. Although clinical studies have demonstrated therapeutic efficacy of PMT involving the delivery of the thrombolytic agent via the radially-administered jet streams into the vessel wall,[11,17] the risks of intimal injury or vessel wall dysfunction as a result of PMT remain a subject of debate.

The findings from our study reveal that there was no difference in endothelial vasoreactivity between the RT and PMT treatment groups in a porcine chronic DVT model. However, both of these treatment groups showed significantly attenuated endothelial vasoreactivity to bradykinin, serotonin, A23187, and ADP compared to the sham control group (Fig. 3–6). The arginine group, in contrast, showed improved endothelial vasoreactivity compared to the RT and PMT groups in response to A23187, bradykinin, and ADP (Fig. 3, 4, and 6). These findings of attenuated endothelial vasoreactivity following either rheolytic mechanical or pharmacomechanical interventions are consistent with other reports that have reported diminished endothelial function following in vivo mechanical thrombectomy.[13,23] The endothelium serves a crucial function in maintaining vasomotor reactivity, an activity that was partially mediated by a vasoactive substance such as NO. The presence of venous thrombus has been shown to affect the production of NO, which in turn may influence the thrombogenicity and vascular vasomotor response.[31] Our study further notes that administering L-arginine, the precursor of NO synthesis, along with PMT intervention, results in improved thrombolytic efficacy with ameliorated endothelial function when compared to the RT or PMT group. This was evidenced by the enhanced endothelium-dependent relaxation of the arginine group in response to ADP, calcium ionophore A23187, and bradykinin. The mechanism of the endothelium-dependent relaxation was likely modulated by nitric oxide–mediated vasoreactive pathways, as researchers have previously reported that such responses could be partially inhibited by the nitric oxide synthesis inhibitor NG-monomethyl-L-arginine (L-NMMA).[23,32]

With regard to thrombogenicity evaluation, our findings showed decreased platelet aggregation in the arginine group, along with a correspondingly elevated local NO_x level when compared to either the RT or PMT group. Also known as endothelium-derived relaxing factor, NO is produced by endothelial cells from the terminal guanidino nitrogen atoms of L-arginine. Under normal circumstances, endothelial cells constantly produce NO.[33] The synthesis and release of NO can be augmented by increasing the amount of precursor, L-arginine.[33–35] In addition to its role as a major regulator of vessel tone, NO also inhibits platelet activation in the regulation of hemostasis and thrombosis.[33,35,36] Researchers have previously reported that blocking NO production would promote mechanical injury–induced experimental thrombosis. In contrast, increasing the synthesis of NO or providing NO precursors protects against intraluminal thrombosis.[18,37,38]

The findings of our experiments were consistent with a recent in vivo study that examined the beneficial role of L-arginine with urokinase thrombolytic therapy in a rat arterial thrombosis model.[39] The authors reported that a severe transient endothelial dysfunction, as evidenced by the reduced endothelium-dependent relaxation, was associated with acute arterial thrombosis. However, the administration of L-arginine in urokinase thrombolysis significantly improved endothelium-dependent relaxation following acute thrombosis when compared to urokinase thrombolysis alone. This improved endothelial function with L-arginine supplementation was associated with an increased NO concentration.[39] A study by Davis and colleagues similarly noted that a combined treatment with L-arginine and thrombolysis using tPA significantly ameliorated endothelial dysfunction in a porcine iliac artery thrombosis model.[40] The authors reported improved vasoreactivity in vessels treated with L-arginine and tPA with corresponding elevated local NO levels when compared to treatment vessels received tPA therapy alone.[40] These findings, along with our study, underscore a potential protective role of NO precursor L-arginine in maintaining endothelial function following thrombolytic therapy.

The histological analysis of vessel wall specimens showed comparable endothelial loss among the RT, PMT, and arginine groups. The degrees of endothelial loss in these treatment groups were $37 \pm 15\%$, $33 \pm 13\%$, and $28 \pm 16\%$, respectively. In contrast, the control group showed a reduced endothelial loss of $26 \pm 13\%$. Because of the similar degree of endothelial denudation in the three treatment groups, we postulated that circumferential infusion of thrombolytic agents directly into the venous thrombus in the PMT treatment did not incur an added injurious effect to the vessel wall compared to the RT treatment modality. This observation is further supported by the similar degree of vasoreactivity to endothelium-dependent vasorelaxants between

the two groups. Previous studies assessing the effect of thrombus on venous endothelium yielded similar findings.[41,42] In a canine interposition vein graft model, the exposure to vein graft thrombosis caused significant endothelial injury and dysfunction, as evidenced by the decreased endothelial production of 6-keto-prostaglandin F_1 alpha in response to arachidonic acid.[41] The study noted that severe structural loss of the endothelium occurred as early as 5 days after thrombus induction, but with partial structural recovery following thrombectomy. In a similar study, the researchers reported that although the endothelium exhibited structural recovery following thrombus exposure, the endothelial function never fully recovered to the baseline level following chronic vein graft thrombosis.[42] The effect of acute thrombus on venous endothelium was examined in a canine DVT model where the effects of thrombectomy and thrombolytic therapy were compared.[13] The study showed that valvular competence and endothelial morphology were similarly preserved between the two treatment groups. However, thrombolysis preserved greater endothelial function than thrombectomy. Additionally, endothelium treated with thrombolysis had reduced thrombogenicity as evidenced by a decrease in platelet deposition. The effect of chronic thrombus on venous endothelium was also studied by the same researchers, who noted that the venous endothelium had less residual thrombus 4 weeks after thrombolysis than it did 4 weeks after thrombectomy.[23] Lastly, venous thrombolysis maintained a greater degree of structural endothelial architecture as well as endothelium-mediated function compared to thrombectomy.[23]

Admittedly, there are several limitations to our study. First, the clinical applicability of the RT and PMT results remains uncertain. However, the similar morphological and functional analyses between these treatment groups support the conclusion that PMT exerts an improved thrombolytic effect without conferring an added injurious effect to the endothelium. Additionally, the study has some statistical limitations because of the relatively small sample size per treatment group. Nonetheless, the overall consistency in the vasoreactivity dose responses to various vasorelaxants supports the data accuracy in our study. Regarding the L-arginine treatment, variation in the treatment dose was not analyzed in the present study. Whether additional functional improvement can be elicited with different dosing or timing regimens remains to be analyzed. Lastly, questions may be raised regarding the structural stability and bioactivity of the tPA molecule administered through a high shear force created by the AngioJet catheter. A recent in vitro study that analyzed the biological and structural integrity of tPA after Angiojet rheolytic thrombectomy showed that the molecule remained structurally stable and biologically active.[43] It is believed that the thrombolytic molecule would retain full bioactivity in the PMT treatment mode.

In summary, our study showed that L-arginine administration attenuated endothelial dysfunction following rheolytic and pharmacomechanical thrombectomy. This result supports a potential therapeutic benefit of NO in preserving vasoreactivity following DVT intervention. Our study further noted a superior thrombolytic efficacy of PMT when compared to RT treatment alone without damaging the endothelium. Further investigations are undoubtedly needed to elucidate the role of L-arginine in the treatment of DVT.

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