The Effects of a Mechanical Thrombolytic Device on Normal Canine Vein Valves

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PURPOSE: To determine if the Arrow-Trerotola Percutaneous Thrombolytic Device (PTD) causes damage to normal vein valves.

MATERIALS AND METHODS: Ten lateral saphenous veins in five dogs were studied with descending venography with use of a wedge balloon catheter positioned above 48 valves (demonstrating 51 valves) before and after five antegrade passes each with an over-the-wire (0.025-inch), 6.5-F, 9-mm-diameter PTD. Vein diameters were 3.2–11.4 mm (mean, 5.9 mm). Contrast matter was injected at incremental rates from 3 to 15 mL/min during continuous pressure monitoring. Imaging was performed with digital subtraction angiography at a rate of 1 frame/sec. The time to valve reflux was determined by noting the frame at which reflux was first seen through the valve. The time to reflux and pressure required to reflux were compared before and after the PTD passes. All vessels were explanted and evaluated histologically for presence or absence of endothelial loss, thrombus formation, inflammation, or valve degeneration. Four veins in two animals were studied with venography to determine the variability of the venographic method. These veins thrombosed during venography and therefore served as positive pathologic controls. In two animals, one vein was studied with venography and one was not studied to provide pathologic controls.

RESULTS: With use of two physiologic tests of valve function, 77% of valves had minimal or no damage as assessed by valve competency and 80% had minimal or no damage as demonstrated by the change in the pressures the valve can withstand before reflux. Twenty-six of 51 valves (51%) had no difference or later reflux after PTD use. Thirteen (26%) refluxed 1 second earlier after PTD use and 12 (23%) refluxed ≥2 seconds earlier (six at 2, four at 3, and two at 4). Four of the six valves with more than a 2-second difference in reflux times were in valves with diameters less than 4.2 mm. All these vessels were smaller than 7 mm in diameter. Twenty-one of 48 valve levels (44%) had no difference or sustained higher pressures before reflux after PTD use. Seventeen (36%) had a pressure drop of <10 mm Hg; five (10%) had drops of 12–24 mm Hg; and five (10%) had drops of more than 40 mm Hg. There was a significant difference in endothelial loss, thrombus formation, and inflammation between experimental veins, the veins with thrombus, the venography controls, and the normal vein controls. There was significant difference only in terms of inflammation when the experimental group was compared to the thrombosis group.

CONCLUSION: The antegrade use of the PTD across normal canine vein valves does not cause physiologically significant damage in valves 7 mm or larger in diameter in this animal model.

THE incidence of deep vein thrombosis (DVT) has been reported as 1.6 per 1,000 people in an urban population (1). The current standard of care for the management of DVT is 6 months of anticoagulant therapy (2). The incidence of postthrombotic syndrome in patients with DVT is approximately 60% (3,4). The goal of aggressive therapy for DVT has been to remove thrombus, thereby restoring flow, and preserving vein valve function. To this end, thrombus removal has been attempted with use of thrombectomy (5–9), catheter directed thrombolysis (10–13), and percutaneous mechanical thrombectomy (14–19). Because the development of postthrombotic syndrome is related to recurrent venous obstruction and venous insufficiency secondary to valve reflux (4, 20, 21), there is concern about the effect of mechanical thrombolytic devices on valve function. Previous studies have evaluated the effect of mechanical thrombolytic devices or catheteriza-

a. **b.**
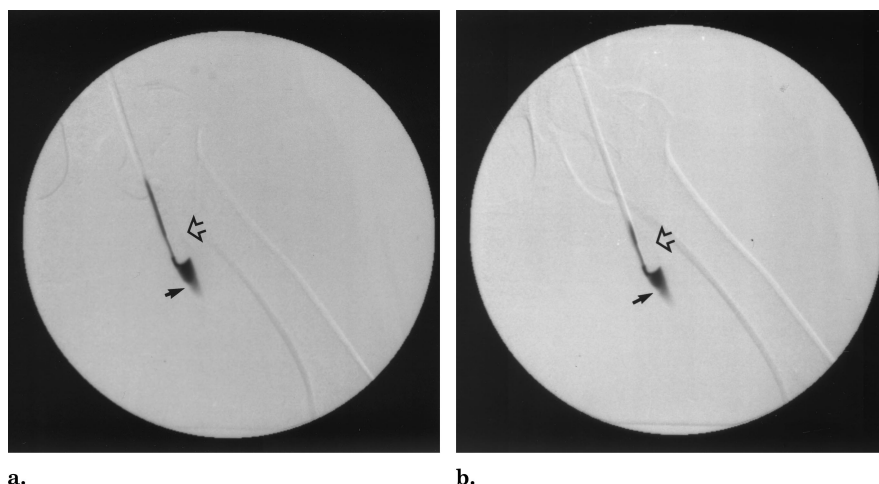
Figure 1. Venography was performed with the wedge balloon inflated (open arrow). Filming was started at the same time the injection was started with a Harvard pump. The pressure was continuously monitored as the pressure built up behind the valve. When the pressure resistance of the valve was overcome, contrast matter would reflux across the valve leaflets (arrow). The time it took to reflux across the valve was determined by looking at the frame on which reflux was first seen (white circle). Before PTD use, reflux was seen at 6 seconds **(a)**. After PTD use, reflux was seen at 5 seconds **(b)** for a difference of 1 second.

tion on vein valves (22–24). In these studies, acute changes were evaluated by means of pathologic grading of acute damage. In one study, valve function was extrapolated from the contractile response of the smooth muscle in organ chambers (24).

The purpose of this study was to determine if the Arrow-Trerotola Percutaneous Thrombolytic Device (PTD) causes damage to normal vein valves that acutely limit their function. To do this, we devised a means for measuring the time it takes for a venous valve to reflux and the pressure the valve can withstand before reflux. This study evaluates valve damage in terms of these two physiologic measures of valve function and correlates these data with pathological findings.

MATERIALS AND METHODS

All aspects of the care and handling of the animals in this study conform to the recommendations of the National Institutes of Health's Guide for the Care and Use of Laboratory Animals, as well as state and institutional guidelines. Also, the experimental procedures were conducted under the Food and Drug Administration's Good Laboratory Practice for Nonclinical Laboratory Studies Regulations (21 CFR, Part 58).

All animals were anesthetized with 20–30 mg/kg of sodium thiopental (Fort Dodge Animal Health, Fort Dodge, IA). The animals were intubated and anesthesia was maintained with 3%–4% isoflurane (Schering-Plough, Rochester, NY). In five nonconditioned mixed-breed dogs (25 kg ± 2), lateral saphenous veins were exposed by cutdown just above the ankle and cannulated with a 6-F sheath, and a venogram from the lateral saphenous vein to the common femoral vein was obtained with use of digital subtraction venography and a graduated marker wire (Magic Torque; Boston Scientific/Medi-tech, Watertown, MA). The animals were then injected with 100 U/kg of heparin (American Pharmaceuticals Partnership, Los Angeles, CA). A 0.035-inch Bentson wire (Cook, Bloomington, IN) was then advanced into the intrahepatic inferior vena cava. The Bentson wire was snared with a 20-mm right-angle snare (Microvena, Great Bear Lake, MN) and withdrawn through an 8-F vascular sheath (Boston Scientific/Medi-tech) placed via cutdown in the right internal jugular vein. A 5-F wedge balloon catheter (Arrow International,

Reading, PA) was advanced over the wire and positioned below the level of the knee. The guide wire was then removed. A Y adapter was created by cutting a 16-gauge Angiocath short and positioning it within a rotating hemostatic valve. This adaptor was attached to the end of the wedge balloon catheter and the Angiocath was attached to a 20-cm^3 contrast syringe in a Harvard pump. The side port of the rotating hemostatic valve was connected to a pressure transducer to allow continuous pressure monitoring during contrast injection. Venography was performed above each visualized valve with use of digital subtraction angiography at a rate of 1 frame/sec before and after five antegrade passes with an over-the-wire (0.025-inch) 6.5-F, 9-mm Arrow-Trerotola PTD (Arrow International, Reading, PA). Contrast material was injected with a Harvard pump at flow rates between 3 cm^3/min and 15 cm^3/min during continuous pressure monitoring. The flow rate was optimized before PTD use by increasing the flow rate until the reflux across the valve was seen within 10 seconds. The optimized flow rate was used for the venogram obtained after PTD use. Venograms were analyzed by filming the frame in which valve reflux is first observed at the optimized injection rate. The frame on which reflux is observed after PTD use was subtracted from the frame on which reflux was noted before PTD use to determine the difference in the time to reflux as a result of PTD use (**Fig 1**). The pressure within the vein was monitored during injection. As the injections progressed, the pressure built up until reflux occurred. At this point, the pressure reached a plateau. By subtracting the maximum pressure from the baseline pressure, we determined the pressure that the valve could withstand before reflux. This pressure withstood before PTD use was subtracted from the pressure withstood after PTD use to determine the difference in the amount of pressure the valve could withstand before reflux. The diameter of the vein at each valve was measured on the preprocedure venogram with use of the marking wire as a calibration guide. The animals were then killed by intravenous injection (Beuthanasia; 0.2 cm^3/kg, Schering-Plough) and the veins were dissected, opened longitudinally,

and pinned out for fixation in 10% formalin. After fixation, the valves were visually identified and the segment of the vein containing the valve was cut out. The valves were embedded in paraffin, step sectioned, and stained with hematoxylin and eosin. The sections were evaluated by assessing the presence or absence of premortem thrombus, inflammation, infiltration of the vessel wall, cellular degeneration in the valve, and loss of endothelium.

Venographic Controls

Two additional animals were used to determine the variability of our venographic methods. In these animals, four venograms were obtained as previously described in each lateral saphenous vein without the animals undergoing heparin injection. Because these venograms were performed without heparinization, thrombus formed in each vein during the third and fourth venogram. After sacrifice, these veins were harvested and evaluated as positive pathologic controls to determine the effect of thrombus on the pathologic measures.

Pathologic Controls

Two additional animals were used as pathological controls. In these animals, venography was performed once on one side and the other side underwent no intervention. The veins were then harvested, pinned, and studied in a manner similar to the experimental animals. Therefore, pathologic data was generated for four groups of valves: the experimental group that underwent venography before and after PTD use, the normal valves that were not exposed to venography or PTD use, the control valves that underwent venography once and were not exposed to PTD use, and the thrombosis valves that were exposed to thrombus as part of the venographic control group.

Statistical Analysis

The difference in reflux time and pressure withstood was compared from before to after PTD use with use of a one-sample t-test. The mean and SD of pressure change and reflux time change were calculated. Linear regres-

sion was used to determine if vessel size was a predictor of change in reflux time or pressure. As a descriptive tool, the reflux data were categorized into six categories: negative (post-PTD refluxed later than pre-PTD) and 0- (no change in reflux time), 1-, 2-, 3-, and 4-second changes, and the pressure data were categorized into four groups: Positive (post-PTD withstood more pressure than pre-PTD), <10 mm Hg, 12–24 mm Hg, and >40 mm Hg pressure loss.

A t-test was used to determine if there is a significant difference in vessel size between the vessels that refluxed earlier (1, 2, 3, and 4 seconds) and those that did not (0 or negative). The variability of the time to reflux and pressure measurements in the venographic controls was analyzed with a one-way analysis of variance and the root mean square error (RMSE) for reflux time and pressure measurements was determined. Fisher's exact tests were used to compare the four groups of pathologic data and to compare the thrombosis group to the experimental group.

RESULTS

The RMSE of reflux times was 1.3 seconds. The RMSE of pressure measurements was 37.4 mm Hg. Fifty-one valves were identified with the catheter positioned at 48 levels (3–8 valves per vein). Several veins had multiple veins below the level of the knee within 1 cm of each other. This prevented the catheter from being placed above each valve so the pressure withstood could only be measured with respect to the most proximal valve with the catheter in this position. Each valve was evaluated for time to reflux and each valve level was evaluated for the amount of pressure the proximal valve withstood before reflux. Seventy-seven percent of valves demonstrated no significant (1 second or less) change in reflux time. Twenty-six of 51 valves (51%) had no difference or later reflux after PTD use. Thirteen (26%) refluxed 1 second earlier after PTD use and 12 (23%) refluxed 2 or more seconds earlier (six at 2, four at 3, and two at 4). Four of the six valves with more than a 2-second difference in reflux times were in valves with diameters less than 4.2 mm. No excessive reflux was seen in vessels 7 mm or larger.

Table 1
Reflux Times (sec)

	Negative	0	1	2	3	4
No. of valves	15	11	13	6	4	2
Ave size (mm)	5.0	6.9	5.0	4.6	4.4	5.5

Table 2
Pressure Differences (mm Hg)

	Positive	0	<10	12–24	>40
No. of valves	17	4	17	5	5
Avg size (mm)	5.6	5.3	5.4	6.6	4.6

The mean change in reflux time was 0.49 seconds with a SD of 1.6 seconds. This difference was statistically significant ($P = .03$). The reflux time data are summarized in **Table 1**. Eighty percent of valves demonstrated no significant (<10 mm Hg) change in pressure that they withstand. Twenty-one of 48 valves whose pressure was measured (44%) had no difference or sustained higher pressures before reflux after PTD use. Seventeen (36%) had a pressure decrease of <10 mm Hg; five (10%) had decreases between 12 and 24 mm Hg; and five (10%) had decreases of more than 40 mm Hg. The mean change in pressure was 0.54 mm Hg with a SD of 38 mm Hg. No significant change in pressure was measured ($P = .922$). Pressure data are summarized in **Table 2**. Regression analysis of the valve size with respect to reflux time and pressure change indicated that vessel size was not a predictor of change in pressure ($P = .59$) or reflux time ($P = .48$). However, valves that refluxed earlier were significantly smaller than those that refluxed at the same time or later (4.8 mm vs 5.8 mm; $P = .03$). None of the valves that refluxed 2 seconds or earlier after PTD use measured 7 mm or more; only two measured more than 6 mm.

Pathologic evaluation was conducted on 48 valves in the experimental group and compared to 15 valves from normal veins not subjected to venography, eight valves subjected to

Table 3
Pathologic Data

Group	No. of Valves	Endothelial Loss	Clot	Inflammation	Degeneration
Experimental	48	46 (96)	23 (48)	17 (35)	5 (10)
Control	8	0	2 (25)	2 (25)	0
Normal	15	0	0	0	0
Thrombosed	23	22 (96)	10 (43)	19 (83)	1 (4)

Note.—Numbers in parentheses are percentages.

venography by not subjected to PTD use, and 23 valves exposed to thrombus without the use of the PTD. Four identifiable pathologic findings were seen and the number of valves demonstrating each finding is tabulated in **Table 3**. Endothelial loss was present if any portion of the luminal surface was not covered with endothelial cell and the study pathologist felt that the finding could not be attributed to processing artifact. **Figure 2a** demonstrates intact endothelium. If thrombus was present in the section as in **Figure 2b**, clot was present. Inflammation was identified by the presence of an inflammatory cellular infiltrate as in **Figure 2c**. Valve degeneration was defined as valve leaflet thickening and degeneration associated with tearing as seen in **Figure 2d**. The four groups differ significantly ($P < .01$) in the proportions of findings shown except for degeneration ($P = .667$). When comparing the thrombosis and experimental groups, no significant differences ($P > .05$) were found for any findings except inflammation.

DISCUSSION

The goal of treatment for deep venous thrombosis (DVT) is to restore flow in thrombosed veins, prevent pulmonary embolus, and preserve vein valve function to prevent chronic venous insufficiency that can lead to postthrombotic syndrome. Previous studies of valve damage from mechanical devices (22), catheterization (23), and thrombolysis (24) have used histologic and pathologic grading scales to assess valvular damage. One major issue confronting the use of a device for the removal of clot is to assess the effect of the device on valve function. Our study assessed the acute effects of

the PTD on valve function using two quantifiable measures of valve function—reflux time and pressure required to cause reflux. On the basis of reflux time, 77% of the valves studied demonstrated 1 second change in reflux time or less. We consider a 1-second change to be normal because the RMSE of the method is 1.3 seconds. Regression analysis demonstrated that small vessel size was not a predictor of early reflux time or pressure change. However, when we compare the size of the vessels that refluxed after PTD use with those that did not, we found a significant difference: the valves that refluxed earlier had an average diameter of 4.8 mm and the valves that did not averaged 5.8 mm. No valves that refluxed 2 seconds or earlier after PTD use measured 7 mm or more; only two measured more than 6 mm. This represents two of 15 valves studied that measured 6 mm or more (13%). Because the PTD has a 9-mm basket, its use in a 6-mm vessel represents a 50% oversize, and, in a 7-mm vessel, it is a 30% oversize.

The pressure changes were more variable than the reflux times. We consider a pressure change of <10 mm Hg to be normal but the RMSE of 37.4 mm Hg would include the 12–24 mm Hg group. If we were to include this group in the normal category, 95% of the valve levels would show no change after PTD use. To be conservative, we include this group as abnormal and report that 80% of valve levels show no change after PTD use. No significant difference in valve sizes was noted between groups based on pressure measurements.

Our pathologic methodology differs from previous studies in which an attempt at subjective grading was performed (22,23). We minimized the subjectivity of our pathologic

analysis by assessing only the presence or absence of acute findings. These findings are illustrated in **Figure 2**. In doing so, we were able to make some general comments about the effect of our venographic method on valves and illustrate the acute effects of the PTD. In our control groups, the venography and normal groups demonstrate no endothelial loss or degeneration. Only two of the venography valves had evidence of clot or inflammation. When we compare the four groups of valves, there is a significant difference in the amount of endothelial loss, thrombus formation, and inflammation. There is no significant difference in the amount of valve degeneration ($P = .667$). When the experimental group is compared to the thrombosis group, there is no difference in endothelial loss, infiltration, degeneration, or thrombus formation, but there is significantly more inflammation in the thrombosis group ($P < .001$). The high incidence of acute changes in specimens that had thrombosis but did not have PTD use (venographic control group) raises the question of whether the PTD would worsen the damage inflicted by thrombosis. This question is not answered by this study. From this evaluation, we conclude that the PTD caused acute endothelial loss, thrombus formation, and inflammation compared to normal controls. However, the effect of the PTD was no worse than that seen with vessel thrombosis and PTD use was associated with less inflammation than exposure to thrombus. One limitation of this study is that it is limited to an evaluation of the acute effects of the device on vein valves. Although we were able to preserve valve function acutely in 77% of valves, it is unclear whether the acute pathologic changes would result in delayed changes in valve function. Future study should be directed toward the use of the functional measures we have developed to assess the delayed effects of mechanical thrombolytic on valve function.

In conclusion, the use of the PTD in the direction of flow does not cause enough damage to cause a change in valve function in this animal model.

a.

b.

c.

d.
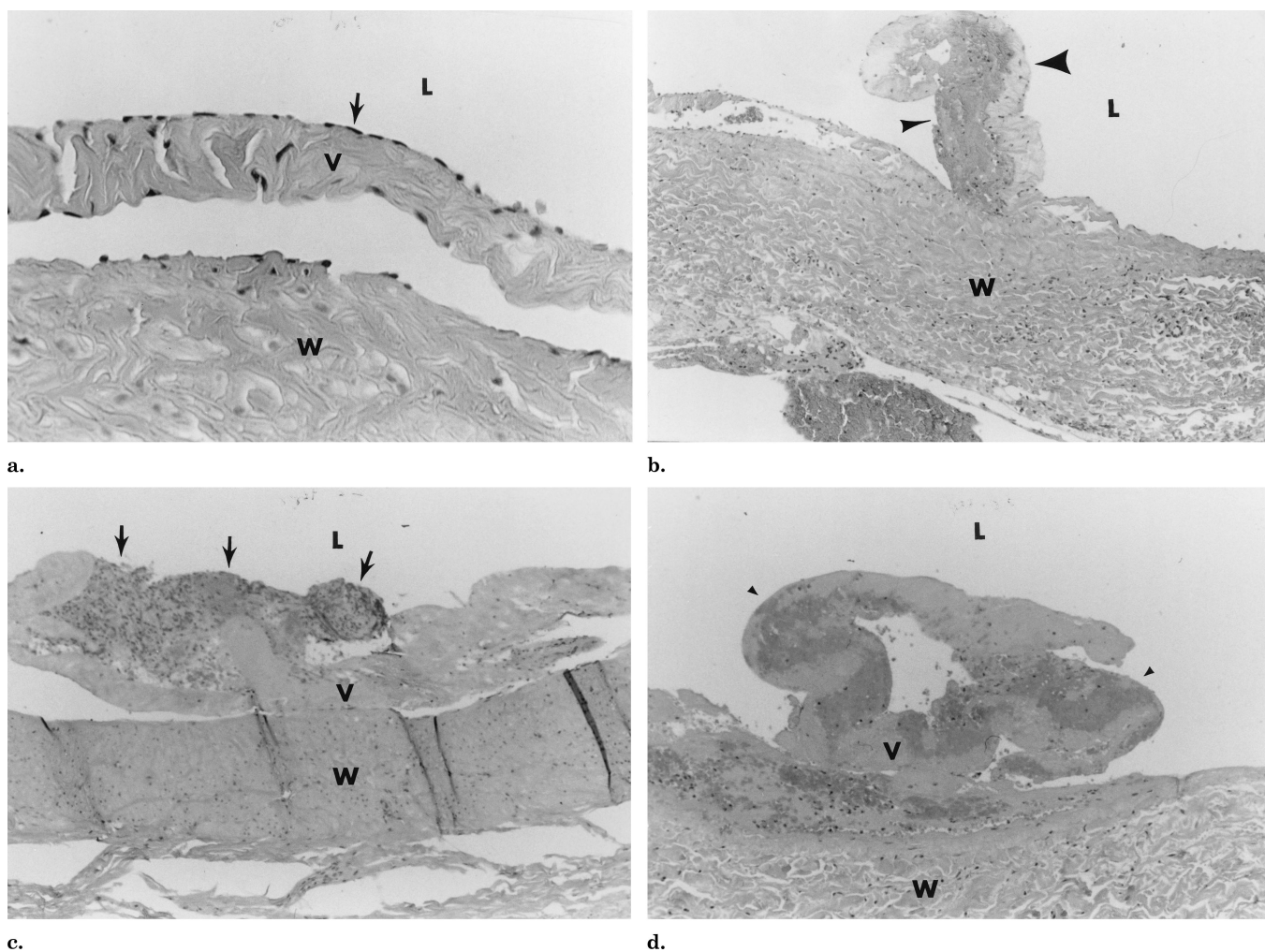
Figure 2. Pathologic changes: all specimens were stained with hematoxylin and eosin. The vessel lumen *(L)*, valve *(V)*, and vein wall *(W)* are identified. **(a)** Normal vein with intact endothelium (arrow); 66× magnification. **(b)** Thrombus (small arrowhead) is seen under a valve (large arrowhead); 33× magnification. **(c)** Inflammation on the surface of a valve (arrows) with multiple inflammatory cells and a small amount of thrombus; 33× magnification. **(d)** Degenerated valve with thickening and tearing (arrowheads); 66× magnification.

Pathologic changes are no worse than seen with veins exposed to thrombus.

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