BASIC RESEARCH STUDIES

From the Midwestern Vascular Surgical Society

Experimental pulmonary embolism: Effects of the thrombus and attenuation of pulmonary artery injury by low-molecular-weight heparin

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Background: **Pulmonary embolism (PE) is a life-threatening condition that is associated with the long-term sequelae of chronic pulmonary hypertension. Prior experimental work has suggested that post-PE inflammation is accompanied by pulmonary artery intimal hyperplasia. This study evaluated the effect of the thrombus and tested the hypothesis that thrombolytic, antiplatelet, and anticoagulant agents would decrease pulmonary injury.**

Methods: **Male Sprague-Dawley rats (n = 267) underwent laparotomy and temporary clip occlusion of the infrarenal inferior vena cava for the formation of endogenous thrombus or placement of an inert silicone "thrombus." Two days later, repeat laparotomy was performed, the clip removed, and the thrombus or silicone plug was embolized to the lungs. The endogenous thrombus group received normal saline, low-molecular-weight heparin (LMWH), tissue plasminogen activator (tPA), or a gIIB/IIIA antagonist (abciximab). Lung tissue was harvested at various times over 21 days and assayed for total collagen, monocyte chemoattractant protein-1 (MCP-1), interleukin-13 (IL-13), and transforming growth factor-β (TGF-β). Fixed sections were stained with trichrome for intimal hyperplasia determination and ED-1 monocytes and α-actin-positive staining.**

Results: **The overall survival for rats undergoing PE was 90%, was not affected by treatment, and 84% of all PE localized to the right pulmonary artery. The PE significantly reduced Pa_{O_2} in all groups. Compared with controls, the silicone emboli group had an increased level of IL-13 on day 1, an increased level of MCP-1 on day 4, and an increase in the levels of all inflammatory mediators on day 14 ($P < .05$). Accompanying these differences were greater pulmonary artery intimal hyperplasia at days 4 and 21 in the silicone group compared with controls ($P < .05$). LMWH treatment in the thrombus of PE rats significantly decreased IL-13 levels at all time points, whereas treatment with abciximab or tPA significantly increased IL-13 levels compared with controls. TGF-β levels were significantly increased by LMWH at day 4 and 14, and abciximab was associated with lower TGF-β at day 14. Only LMWH was associated with less pulmonary artery intimal hyperplasia at day 14 compared with controls and the other treatment groups.**

Conclusions: **Persistent pulmonary artery distention by an inert material is sufficient to invoke significant inflammation and intimal hyperplasia independent of the thrombus itself. Compared with nontreated PE, LMWH is the only therapy associated with a significant reduction in late intimal hyperplasia and, with the exception of TGF-β, lower profibrotic growth-factor production. (J Vasc Surg 2006;43:800-8.)**

Clinical Relevance: **Pulmonary embolism is a highly fatal disease and may be associated with long-term pulmonary hypertension. Few good animal models exist for this condition, and the aim of this report was to evaluate the role of the thrombus itself on the pulmonary artery injury and to assess the effect of currently available therapies. Our data suggest that rapid treatment of pulmonary embolism with low-molecular-weight heparin is associated with least injury response, and that persistent occlusion of the pulmonary artery is associated with significant injury.**

Pulmonary embolism (PE) is a life-threatening condition with an acute mortality of 30% that is estimated to occur in 3.5 of every 1000 hospitalized patients.[1] Most often, PE arises from a lower-extremity deep venous thrombosis (DVT) and is a potential long-term sequelae of pulmonary hypertension,

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for which therapy is only palliative.[2] Although the risk of DVT and associated PE has decreased with improved anticoagulant prophylaxis, little true decrease in incidence has occurred over the last several years.[3] Heparin, in either the unfractionated or low-molecular-weight form, remains the mainstay of acute treatment for PE. Clinical trials of systemic thrombolytic therapy have failed to demonstrate a significant reduction in early mortality and were associated with a significant risk of bleeding while infused; however, certain clinical scenarios warrant its use.[4,5] Effectiveness of gIIB/IIIA antiplatelet therapy for acute treatment of PE is currently unknown, but platelets play an important role in thrombosis and vascular injury.[6]

At present, little is known about the pathobiology of PE at the cellular and molecular level. Resolution of PE in humans in may take >30 days,[7] and is dependent on native fibrinolytic mechanisms. For example, inhibition of the urokinase-type plasminogen activator was associated with increased fibrin in hypoxemic lung tissue.[8] The PE may also cause bilateral pulmonary vasoconstriction and injury outside of the affected pulmonary artery (PA) bed.[9] Several large-animal models of PE have been developed, but these models rely upon exogenously formed thrombus or air emboli and do not mimic the human clinical disease. Prior experimental work in our laboratory has shown that post-PE inflammation is associated with early monocyte influx, elevated monocyte chemotactic protein-1 (MCP-1), and PA intimal hyperplasia (PAIH).[10] Importantly, how the thrombus itself, and whether currently available therapies affect the PA and distal lung parenchyma is not known.

The aim of this study was (1) to determine the effect of PA distention independent of the thrombus proper on PA and lung parenchymal injury, and (2) to determine if PA and parenchymal injury is affected by currently available anticoagulant therapies.

METHODS

This study was approved by the University of Michigan Institutional Animal Care and Use Committee, and all animal care guidelines were strictly followed. Male Sprague-Dawley rats (n = 224), all approximately 10 weeks old and weighing 300 g (Charles River Laboratories, Wilmington, Mass), underwent laparotomy under isoflurane inhalational anesthesia. Inferior vena cava (IVC) thrombosis was produced by temporary clip occlusion of the infrarenal IVC and ligation of IVC tributaries.[10] Of these, 27 procedures were technically unsuccessful, resulting in 197 rats being used during the course of this study.

A transverse caval venotomy was created above the confluence of the iliac veins in a separate experiment (n = 40), and a preformed medical-grade silicone plug approximately 2.38 mm in outer diameter and 3 mm in length (Tygon, Beaverton, Mich) was placed in the IVC, below the renal veins, with temporary clip control. We chose a diameter of 2.38 mm for the silicone plug based upon the average diameter of thrombus that is created within the IVC using this model and the average diameter of 2.5 mm for the rat main pulmonary vein.[11] The venotomy was closed with interrupted 9-0 nylon sutures.

Table I. Surgical model outcomes

Outcome	*Numbers (affected/total)*	*Percentage*
Successful PE	178/197	90.5
PE in right PA	165/197	84
Unable to embolize thrombus	3/197	1.5
No PE in IVC	9/197	4.5
Death	15/197	7.6
Death from PE	8/15	53

PE, Pulmonary embolism; *PA,* pulmonary artery; *IVC,* inferior vena cava.

Two days later, a repeat laparotomy was performed, the clip was removed, and the thrombus or silicone plug was embolized to the lungs. Of note, silicone was compared with intravenous tubing and a rubber plug material and found to generate the least inflammatory cell response when placed in the subcutaneous position (data not shown).

At the time after embolization and after closure of the laparotomy, a pulmonary angiography with iodinated contrast (Hypaque Parenteral, Amersham Health, Princeton, NJ) was performed via a left internal jugular approach to confirm PE and determine the location of the embolus within the PAs. This allowed identification of affected lung tissue for analysis. Oxygen saturation measurements were taken via the ventral tail artery in all rats at the time of the initial laparotomy, at PE, and at harvest.

Treatment groups received one-time subcutaneous low-molecular-weight heparin (LMWH) (450 IU/kg, n = 27) (Dalteparin, Pharmacia and Upjohn Co., Kalamazoo, Mich), intravenous tissue plasminogen activator (tPA) (1 mg/kg, n = 27) (Retavase, Centocor, Malvern, Penn), or the gIIB/IIIA antagonist abciximab (1 mg/kg, n = 26) (Eli Lilly and Co, Indianapolis, Ind) via tail vein injection immediately after the pulmonary angiography (within 30 minutes after the PE). Doses of the various treatment agents were derived from the available literature.[12-14] No treated rats died from hemorrhagic complications. Control rats (n = 61) were injected with an equivalent volume of normal saline either subcutaneously or in the dorsal tail vein, as appropriate.

At days 1, 4, and 14, the rats were sacrificed, and the lungs affected with PE and the contralateral lungs were harvested. The affected lung lobe that was identified on pulmonary angiogram at the time of embolization was snap frozen in liquid nitrogen or perfusion-fixed with formalin for trichrome staining and histologic processing. Lung tissue from rats that received inert silicone emboli was harvested at 1, 4, 14, and 21 days post-emboli. The silicone emboli were removed from the lung tissue before processing.

The saline-injected control rats used for comparison in the above two experiments were completed concurrently with both the silicone inert thrombus and the venous thromboembolism treatment groups. The data from the saline control rats were pooled to increase the power of the comparison

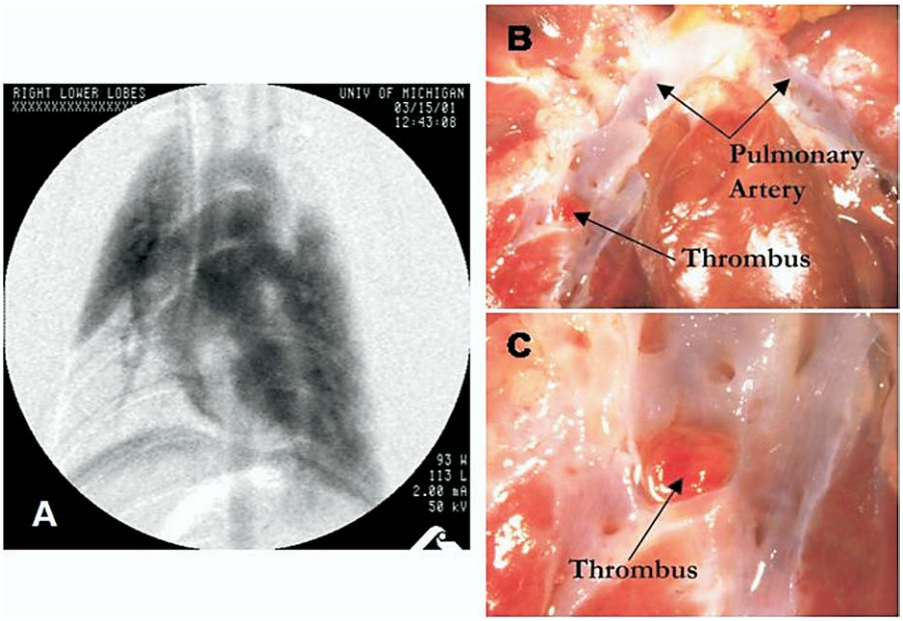

Fig 1. Example of pulmonary angiogram and pulmonary embolus. **A,** Pulmonary angiogram in a study rat after embolization of the inferior vena cava thrombus. Note lack of perfusion of the right lower lobe distal to embolus. **B** and **C,** Necropsy pulmonary specimens show the thrombus within the right lower lobe branch of the pulmonary artery at different magnifications (B = 4× and C = 10×).

with both the silicone inert thrombus and the venous thromboembolism groups.

Collagen and glycosaminoglycan assay. Lung collagen-glycosaminoglycan content was estimated by a commercially available kit according to manufacturer's instructions (BioColor LTD, Belfast, North Ireland).[15] This collagen assay uses a quantitative method by which anionic Sirius red (Direct Red) dye binds to the side chains of basic amino acids found in collagen types I to IV. Levels were corrected to milligrams of protein in the sample.

Histologic analysis and staining. Sections of affected and contralateral lungs were stained for structural analysis by trichrome processing as described.[15] Then, intimal hyperplasia was determined by analyzing five sections of main PA to account for asymmetry and assessed in a blinded fashion with the aid of computer imaging quantification.[10]

Permanent, fixed 10-μm sections were analyzed for cellular content by immunohistochemical staining for ED-1 (1:100) (Santa Cruz Biotechnology, Inc, Santa Cruz, Calif) and smooth muscle α-actin (1:200) (Serotec, Raleigh, NC). A species-specific autoantibodies to collagen (ABC) alkaline phosphatase or peroxidase kit for rabbit, goat, or mouse (Vector Laboratories, Inc, Burlingame CA) was used according to the manufacturer's instructions for the secondary antibody and subsequent steps. The slides were then counterstained with hematoxylin.

For cell counting, a Zeiss Axioplan 2 microscope (Thornwood, NY) connected to a Zeiss AxioCam HRc camera was used to obtain ×20 images of PE-affected and contralateral unaffected PAs in α-actin– and ED-1–stained lung tissue slides. Images were analyzed with AxioVision, release 4.3, (Zeiss) using the automatic measurement program, in which α-actin– and ED-1–positive staining was defined, and a field-specific measurement was set to analyze densitometric mean value red. A measurement frame selecting the PA was defined for each image, and densitometric values were obtained.

Table II. Inert embolus inflammatory mediators, day 1

Treatment group (day 1)	*IL-13 (ng/mL)*	*MCP-1 (ng/mL)*	*TGF-β (ng/mL)*
Saline (n = 18)	66 ± 6	312 ± 106	232 ± 36
Silicone (n = 18)	268 ± 47*	489 ± 69	244 ± 15

All data are presented as mean ± SEM.
*$P < .05$.

Chemokine/cytokine enzyme-linked immunoabsorbent assay. After thawing, the pulmonary tissue was placed in complete lysis buffer at 0°C (Boehringer Mannheim, Indianapolis, Ind), homogenized, sonicated for 10 seconds, centrifuged at 10,000*g* for 5 minutes, and the supernatant was collected. Quantification of peptide mediators was normalized to total protein in the sample by a modified Bradford assay according to the manufacture's instructions (Pierce, Inc, Rockford, Ill), with serial dilutions of bovine serum albumin (Sigma Chemical, St. Louis, Mo) as standards. Lung tissue from the affected and contralateral lung homogenate was assayed for MCP-1, IL-13, and TGF-β by using species-specific primary antibodies as

Table III. Inert embolus inflammatory mediators and collagen, day 4

Treatment group (day 4)	*IL-13 (ng/mL)*	*MCP-1 (ng/mL)*	*TGF-β (μg/mg protein)*	*Lung collagen*
Saline (n = 19)	90 ± 8	40 ± 8	241 ± 23	64 ± 8
Silicone (n = 12)	98 ± 16	97 ± 13*	220 ± 29	70 ± 16

IL-13, Interleukin-13; *MCP-1,* monocyte chemotactic protein-1; *TGF-β,* transforming growth factor-β.
All data are presented as mean ± SEM.
*$P < .05$.

Table IV. Inert embolus inflammatory mediators and collagen day 14

Treatment group (day 14)	*IL-13 (ng/mL)*	*MCP-1 (ng/mL)*	*TGF-β (μg/mg protein)*	*Lung collagen*
Saline (n = 24)	92 ± 7	32 ± 3	154 ± 14	136 ± 28
Silicone (n = 10)	153 ± 24*	89 ± 16*	231 ± 20*	58 ± 6

IL-13, Interleukin-13; *MCP-1,* monocyte chemotactic protein-1; *TGF-β,* transforming growth factor-β.
All data are presented as mean ± SEM.
*$P < .05$.

described.[10,12] Standards were log dilutions of the cytokines from 10 pg/mL to 100 ng/mL with a sensitivity ≥50 pg/mL.

Statistical analysis. All data are presented as mean ± SEM. Comparisons between treated and control groups were done with analysis of variance and secondary Student's *t* test or Mann-Whitney rank sum test where the data were not normally distributed. SigmaStat, version 2.03, statistical software (SPSS, Chicago Ill) was used to calculate probability values.

RESULTS

The overall survival for the 267 rats undergoing PE was 90% and was not affected by treatment. Most PE were localized to the right PA (Table I, Fig 1). Pao_2 measurements at the time of initial laparotomy and at lung harvest were similar between all groups at 95% to 97%. Mean Pao_2 for the treated groups at the time of PE was 88% for saline, 87% for LMWH, 86% for abciximab, 86% for tPA, and 90% for rats undergoing silicone PE ($P < .05$ compared with baseline Pao_2) (P = NS between all groups). No significant differences in PE presence and resolution were found amongst the treatment groups at the time points analyzed, with all fully lysed by 4 days.

Effects of inert pulmonary emboli on lung injury

Day 1 results. In the rats treated with inert silicone emboli, IL-13 levels were markedly elevated at day 1 compared with controls that underwent venous thromboembolism ($P = .001$). MCP-1 and TGF-β level were similar between groups at this time point (Table II).

Day 4 results. At day 4, IL-13, TGF-β, and lung collagen levels in affected lung tissue were similar between silicone and thrombi emboli groups (Table III). MCP-1 was elevated twofold in the silicone emboli group compared with controls ($P = .002$).

Day 14 results. At day 14 after PE, IL-13 was significantly elevated in rats with silicone emboli compared with thrombus controls ($P = .002$) (Table IV); MCP-1 and TGF-β were also significantly elevated compared with controls ($P = .004$). Although lung collagen trended lower in the silicone group, this did not reach significance ($P = .15$).

Pulmonary artery intimal hyperplasia results. PAIH was evaluated in the silicone and thrombus control PE groups. At day 4, PAIH was threefold greater in the silicone emboli group than in the thrombus group ($P = .003$). At day 21 after embolization, the difference between groups was even greater, nearly fourfold. ($P = .007$). Also, the IH present was qualitatively more asymmetric in the silicone-embolized rats compared with the other groups (Figs 2 and 3).

Contralateral lung results. In the contralateral unaffected lung at day 1, silicone-treated rats had significantly elevated levels of IL-13 (282 ± 55 ng/mL vs 87 ± 9 ng/mL, $P = .008$). There was no difference in IL-13 between the groups at days 4 or 14. There were no differences in MCP-1 or TGF-β between the two groups at any time point, and total lung collagen was similar between the silicone and thrombus emboli groups at day 4 and 14. The amount of PAIH was minimal and not significantly different between the unaffected lungs of silicone and thrombus PE groups at days 1, 4, and 21 (data not shown).

Effect of treatment of pulmonary embolism on inflammation in the affected lung

Day 1 results. Compared with controls, levels of IL-13 in the LMWH-treated rats at day 1 were threefold lower ($P = .001$) and were nearly twofold greater in rodents treated with abciximab and tPA ($P < .001$) (Fig 4). MCP-1 levels were similar in all groups at day 1 with the exception of rats treated with tPA, which had significantly lower levels compared with saline controls ($P < .001$) (Fig 5).

Day 4 results. At day 4, there were no significant differences in TGF-β levels or PAIH between treatment groups and control (Figs 2, 6, and 7). The LMWH-treated rats showed fourfold lower levels of IL-13 compared with the saline-treated rats ($P < .001$) (Fig 4). IL-13 levels in

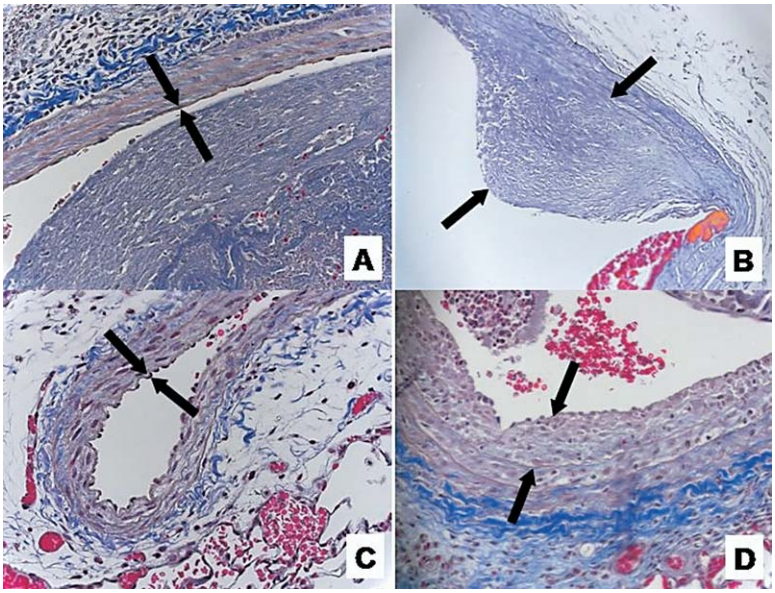

Fig 2. Examples of pulmonary artery intimal hyperplasia. Representative trichrome photomicrographs (×400 magnification) of affected pulmonary arteries in **(A)** day 1 saline-treated intraluminal thrombus, **(B)** day 21 silicone embolus, **(C)** day 14 low-molecular-weight heparin, and **(D)** day 14 saline-treated rats. *Arrows* denote distance between the inner most elastic lamina and the endothelial lining.

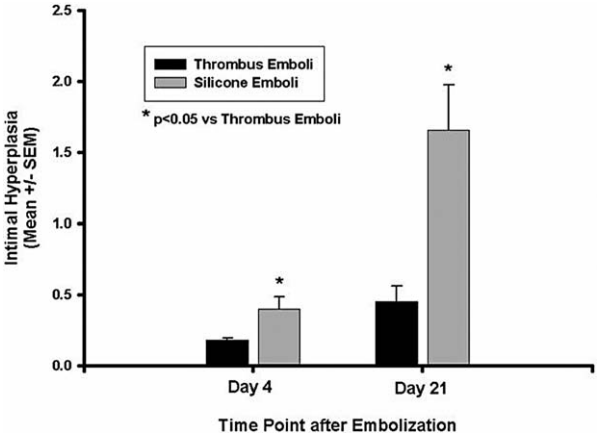

Fig 3. Pulmonary artery intimal hyperplasia in thrombus and silicone emboli groups at 4 and 21 days after embolization. The effects of inert silicone emboli in the affected pulmonary artery compared with rats with thrombus emboli. Rats undergoing silicone embolization have a greater amount of pulmonary artery intimal hyperplasia compared with venous thrombus embolization. This effect is seen at both 4 and 21 days after embolization.

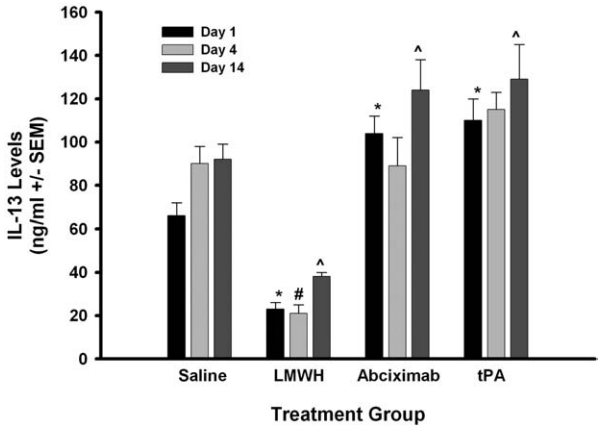

Fig 4. Interleukin *(IL-13)* levels in saline control and treatment rats 1, 4, and 14 days after pulmonary embolism. Graft shows the effect of treatment with saline, low-molecular weight heparin *(LMWH)*, abciximab, and tissue plasminogen activator *(tPA)* on pulmonary tissue levels of IL-13. Saline, n = 18; LMWH, n = 10; abciximab, n = 10; and tPA, n = 12 for day 1. Saline, n = 21; LMWH, n = 10; abciximab, n = 10; and tPA, n = 10 for day 4. Saline, n = 24; LMWH, n = 12; abciximab, n = 10; and tPA, n = 9 for day 14. $^{*}P < .05$ compared with saline controls for day1. $^{\#}P < .05$ compared with saline controls for day 4. $^{\wedge}P < .05$ compared with saline controls for day 14.

abciximab and tPA rats were similar to controls. MCP-1 levels in all groups were similar to the control group with the exception of abciximab-treated rats. Rats treated with abciximab showed a 4.2-fold increase in MCP-1 levels compared with controls ($P = .003$) (Fig 5). TGF-β levels at day 4 were similar between the saline, abciximab, and tPA groups, but significantly elevated in the LMWH group ($P = .005$) (Fig 7). Lung collagen remained similar between the saline control group and in the tPA and abciximab treatment groups. However, lung collagen was 35% greater in the LMWH treatment group than in the thrombus controls at day 4 (99 ± 12 vs 64 ± 8 μg/mg protein,

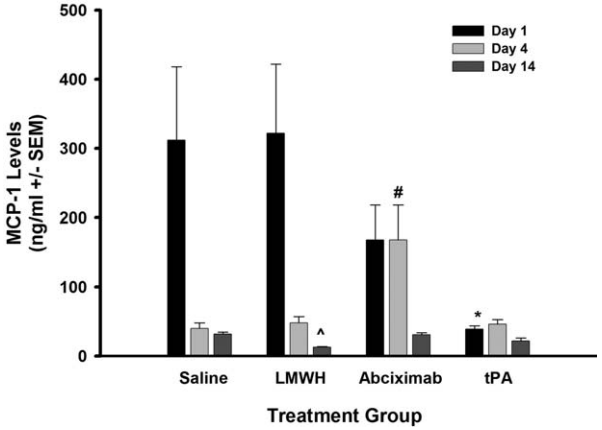

Fig 5. Monocyte chemotactic protein-1 *(MCP-1)* levels in saline control and treatment rats 1, 4, and 14 days after pulmonary embolism. Graph shows the effect of treatment with saline, low-molecular-weight heparin *(LMWH)*, abciximab, and tissue plasminogen activator *(tPA)* on pulmonary tissue levels of MCP-1. Saline, n = 18; LMWH, n = 10; abciximab, n = 10; and tPA, n = 12 for day 1. Saline, n = 21; LMWH, n = 10; abciximab, n = 10; and tPA, n = 10 for day 4. Saline, n = 24; LMWH, n = 12; abciximab, n = 10; and tPA, n = 9 for day 14. $^{*}P < .05$ compared with saline controls for day 1. $^{\#}P < .05$ compared with saline controls for day 4. $^{\wedge}P < .05$ compared with saline controls for day 14.

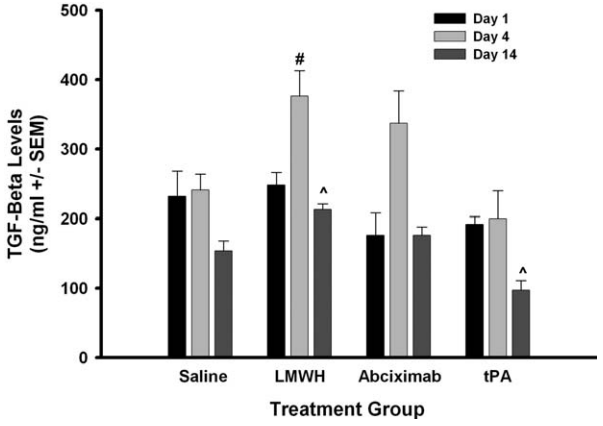

Fig 7. Transforming growth factor-β *(TGF-β)* levels in saline control and treatment rats 1, 4, and 14 days after pulmonary embolism. Graph shows effect of treatment with saline, low-molecular-weight heparin *(LMWH)*, abciximab, and tissue plasminogen activator *(tPA)* on pulmonary tissue levels of TGF-β. Saline, n = 18; LMWH, n = 10; abciximab, n = 10; and tPA, n = 12 for day 1. Saline, n = 21; LMWH, n = 10; abciximab, n = 10; and tPA, n = 10 for day 4. Saline, n = 24; LMWH, n = 12; abciximab, n = 10l and tPA, n = 9 for day 14. $^{*}P < .05$ compared with saline controls for day 1. $^{\#}P < .05$ compared with saline controls for day 4; $^{\wedge}P < .05$ compared with saline controls for day 14.

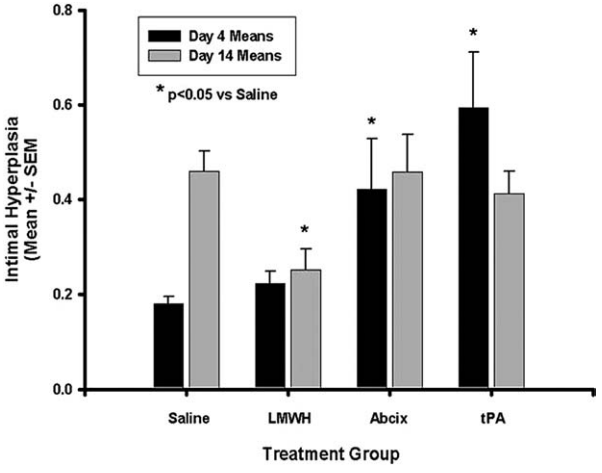

Fig 6. Pulmonary artery intimal hyperplasia (PAIH) in rat treatment groups. The effects of treatment with saline, low-molecular-weight heparin *(LMWH)*, abciximab, and tissue plasminogen activator *(tPA)* on rat PAIH 4 and 14 days after treatment. LMWH significantly reduced IH at day 14 compared with saline control. At day 4, abciximab and tPA groups had significantly more IH than the day 4 saline control.

n = 9 to 18; $P = .003$). PAIH was similar between the saline and LMWH groups but significantly elevated in the abciximab and tPA groups compared with the saline controls ($P < .05$) (Fig 6).

Day 14 results. At day 14, the LMWH-treated group exhibited significantly lower levels of IL-13 with in the tissue of the affected lung compared with saline control rats ($P < .001$) (Fig 4). IL-13 levels were elevated by 25% compared with saline controls in the abciximab- and tPA-treated groups ($P = .05$). MCP-1 levels at this time point were similar between the saline, abciximab, and tPA groups. However, LMWH-treated rats had significantly lower MCP-1 levels compared with saline-treated rats ($P = .001$) (Fig 5). TGF-β levels were 38% lower in the tPA-treated group at day 14 compared with the saline control group ($P = .027$) and elevated by 28% in the LMWH-treated group ($P = .007$). TGF-β levels in the abciximab-treated group were similar to the saline control group (Fig 7). PAIH was decreased by 54% in the LMWH-treated group ($P = .015$), but the abciximab and tPA treatment groups were similar to controls (Fig 3). Total lung collagen levels at day 14 ranged from a mean of 14 to 35 μg/mg of protein and were not significantly different between treatment groups (data not shown).

Cell count results. The number positive ED-1 and smooth muscle α-actin cells within the affected pulmonary arteries of rats treated with LMWH, abciximab, and tPA were similar compared with PE controls. The only significant difference was that tPA-treated rats had more ED-1–positive staining cells within the PA wall compared with saline controls at day 1 (148 ± 19 vs 47 ± 17 cells/area, tPA vs control, $P = .029$). At day 14, only abciximab-treated rats had significantly more smooth muscle α-actin–positive cells within the PA wall than saline controls (157 ± 11 vs 133 ± 9 cells/area, $P = .047$).

Effect of pulmonary embolism on the contralateral

nonaffected lung. To determine if any bilateral pulmonary inflammation occurred after PE and its treatment, the same measures of inflammation were analyzed in the contralateral, unaffected lungs of all rat groups in this model. As with the affected pulmonary tissue, IL-13 levels at day 1, 4, and 14 were significantly lower in the contralateral lungs of rats treated with LMWH (day 1: 17 ± 4 ng/mL vs 87 ± 9 ng/mL, n = 10, $P < .001$; day 4: 22 ± 6 ng/mL vs 103 ± 10 ng/mL, n = 10, $P < .001$; day 14: 36 ± 2 ng/mL vs 133 ± 17 ng/mL, n = 12, $P < .001$ for LMWH vs saline, n = 14, 30, and 24 at days 1, 4, and 14, respectively). IL-13 levels at day 14 were significantly elevated in the contralateral lungs in the abciximab group (140 ± 11 ng/mL, n = 9) and in the tPA group (147 ± 16 ng/mL, n = 7) compared with saline controls (113 ± 17 ng/mL, n = 24; both $P < .01$).

MCP-1 levels were significantly lower in the contralateral lungs of rats treated with tPA at day 1 compared with saline-injected rats (31 ± 4 ng/mL, n = 12 vs 88 ± 15 ng/mL, n = 8, $P < .001$). MCP-1 levels did not differ between groups at day 4; however, at day 14, the LMWH group had lower levels of MCP-1 compared with the saline rats (12 ± 0.8 ng/mL, n = 12 vs 32 ± 4 ng/mL, n = 20, respectively, $P < .001$).

TGF-β levels in the contralateral lungs of all groups were similar at day 1. Interestingly, lung TGF-β levels were markedly elevated in the LMWH and tPA groups compared with saline PE controls (271 ± 48 ng/mL, n = 10; 258 ± 29 ng/mL, n = 10 vs 183 ± 21 ng/mL, n = 10; both $P < .05$) at day 4. Abciximab-treated rats had lower levels of TGF-β (103 ± 18 ng/mL; n = 10) compared with saline-treated rats ($P = .024$) at day 4. At day 14, TGF-β levels remained significantly elevated in LMWH-treated rats (223 ± 9 ng/mL, n = 12 vs 161 ± 18 ng/mL, n = 24; $P = .026$), but no differences in levels in the rats treated with tPA or abciximab remained at day 14.

Total collagen levels were similar in the contralateral lungs of all groups at day 14. No significant IH was found in the contralateral PAs of rats in any treatment group at any time point compared with control rats (data not shown). Counts of ED-1 and smooth muscle α-actin-positive cells were also similar in all unaffected lungs in all groups compared with the saline control group at days 1, 4, and 14 (data not shown).

DISCUSSION

PE remains a significant cause of death in hospitalized patients.[1] Increased awareness of venous thromboembolism risk factors and prophylaxis is now a Center for Medicare Services quality process variable. Better understanding of the molecular and cellular pathophysiology may allow improved therapies to lessen the early mortality and long-term morbidity of PE. Although experimental, the current report suggests that persistent PA occlusion may be more injurious than the contents of the lysing thromboembolism, and that the current standard of care for PE treatment may be better than other agents for preventing delayed PA injury. It is also likely the observed responses are a result of local humoral and globally neuronal-mediated effects from the PA stretch and hypoxemia.

The PA and parenchymal response to the persistent stretch of a silicone plug was striking, with marked PAIH in the affected lung through 21 days, and elevation of IL-13 levels in the contralateral lung. Although our prior report[10] suggested that the formed thrombus may primarily direct the PA and lung responses via MCP-1, it is clear that the occlusion of the PA also increases local MCP-1, as observed on day 4 and 14. Although not identical to our model, styrene microsphere embolization to the PA produced an intense inflammatory response,[16] including elevation of CCR2, the primary receptor for MCP-1. MCP-1 has been shown in histologic sections of patients with pulmonary hypertension secondary to PE and is a direct monocyte chemoattractant.[17]

Monocytes were not significantly altered by the silicone embolus insult or the different anticoagulant treatments. This suggests that the local environment may direct monocyte activity but not necessarily increase the overall number. Moreover, the current data support that hypoxic pulmonary vasoconstriction may play a role in the observed PAIH, as the silicone is inert and would not be a direct source of MCP-1 or IL-13. Modulation of PAIH secondary to persistent pulmonary vasoconstriction is well supported[18] but is speculative in this model, because we did not directly measure PA pressures.

Rapid administration of LMWH was the most effective agent for reducing both PAIH and, possibly, lung injury despite no gross acceleration of venous thromboembolic resolution in this model at the time points examined in this study. The affected and contralateral lung tissue of LMWH-treated rats also had lower profibrotic growth factor levels of IL-13 and MCP-1 than control PE rats, suggesting an effect of the regional pulmonary systemic injury after PE.

The unique anti-inflammatory and antifibrotic effects of LMWH may be due to its effect on cell adhesion molecules. For example, in a rat model of DVT, both P-selectin inhibition and LMWH equally inhibited vein wall fibrotic injury.[19] Similarly, LMWH has direct anti-inflammatory properties independent of its anticoagulant effects with less vein wall inflammatory cellular influx after DVT in a rat model.[20] Interestingly, LMWH was associated with increased levels of TGF-β, a cytokine associated with lung fibrosis after pulmonary injury in other models.[21,22] However, these are direct parenchymal chemical injury models and different than a PE-induced injury. Further, TGF-β has known anti-inflammatory properties, depending on the local environment, and has antiproliferative effects on some cell types.[21] Our data, however, do not definitely prove a direct cause-and-effect relationship between these mediators and the different phenotypic responses observed.

These experiments also suggest an important role for IL-13 in the pathophysiology of acute PE. Consistent with the observed PAIH effects, IL-13 is a profibrotic cytokine that causes fibroblast and smooth muscle cell proliferation and can promote organ fibrosis indepen-

dent of TGF-β.[23] Similarly, IL-13 can directly stimulate fibroblast proliferation, increase extracellular matrix production, and cause decreased production of vasorelaxant mediators such as prostaglandin E_2.[24,25] In a infectious model of pulmonary injury, IL-13 directly stimulated MCP-1 expression.[26] Our data did not show this consistent relationship and may be due to the timing of mediator analysis. Despite marked differences in PAIH, no difference in α-actin–positively stained cells was observed between the treatments, or between the silicone and venous thromboemboli, and suggests that a profibrotic phenotype was promoted rather than fibroblast proliferation. This observation is consistent with the activities of both MCP-1 and IL-13.[23,25]

Treatments with tPA and abciximab were not associated with reduced PAIH development compared with control PE rats. In fact, both of these agents seemed to stimulate earlier PAIH than that which occurred in controls. Although platelets are rich in growth factors, the lack of a protective effect of abciximab suggests that the platelet may have less of a role in promoting PAIH, perhaps because the formed venous thromboembolus is fibrin rather than platelet rich. It is possible that abciximab may have stimulated release of platelet-derived growth factor.[27] Moreover, activated platelets increase local MCP-1 release from endothelium,[28] and lung levels of MCP-1 were significantly greater on day 4 in abciximab-treated rats than in controls and the other groups. None of the agents seemed to affect pulmonary fibrosis within the time frame analyzed, although a trend toward lesser total lung collagen was noted with all treatments.

Limitations of the current study include only single-agent dosing. This was done in part for logistic reasons, and our data may underestimate a greater effect with dosing every day or more frequently. The PE themselves lysed at similar rates (all by day 4), however, and our data support that the effects of these agents are independent of thrombus resolution. This observation is limited in that thrombus lysis may have been accelerated and occurred earlier in the treatment groups at a time point between our day 1 and day 4 study groups, and that thrombus might have been present around the silicone embolus for some duration of time, although this was not noted at harvest. Also, the rat seems to have a survival that is greater than humans, in part because it lacks comorbid cardiac and pulmonary disease, and direct extrapolation of our findings should be done with caution. We also did not determine the cellular source of these mediators and cannot comment on whether these mediator responses were primarily from the parenchyma or the PAs. This will require further study.

Finally, whether the beneficial effect of certain treatments such as LMWH would remain with treatment several hours after PE is unknown. The fact that the rats were rapidly dosed may also not reflect the typical clinical situation, but does suggest that rapid therapy maybe associated with better long-term outcomes.

CONCLUSION

The data we have presented suggest a complex interplay of both affected and contralateral lung profibrotic mediators that may be attenuated with rapid LMWH therapy. Conversely, persistent PA obstruction seems more important in promoting pulmonary injury than does the release of venous thromboembolism mediators. Although other agents such as tPA are clinically beneficial in certain cases of massive PE,[29] this agent and abciximab are not as effective against later PA injury, and in fact, may stimulate PAIH.

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AUTHOR CONTRIBUTIONS

Conception and design: JR, KBD, PS, DM, CL, TW, PH
Analysis and interpretation: JR, KBD, DM, GU, TW, PH
Data collection: JR, KBD, DM, PS, EL, AM, CL, ND
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Final approval of the article: JR, KBD, PS, EL, AM, DM, ND, CL
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