Chronic Pulmonary Thromboembolism in Dogs Treated With Tranexamic Acid

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Background. Many questions remain regarding the pathogenesis, natural history, diagnosis, and treatment of chronic thromboembolic pulmonary hypertension in patients. To answer such questions, we developed an animal model of this disorder. The brisk thrombolytic response of canines to acute embolism has, previously, prevented the establishment of such a model.

Methods and Results. The fibrinolytic inhibitor tranexamic acid was given orally to canines before, and for intervals after, pulmonary emboli were released from venous thrombi formed in vivo in femoral veins or the inferior vena cava. Preliminary studies disclosed that embolic residuals from femoral vein thrombi were not sufficient to cause significant, persistent pulmonary hypertension. With repetitive, larger thrombi embolized from the inferior vena cava, however, persistent pulmonary hypertension was achieved in most animals.

Conclusions. Resolution of emboli in the canine can be inhibited by tranexamic acid. As in humans, a spectrum of embolic residuals is encountered, and the perfusion lung scan consistently underestimates the extent of embolic residuals. Studies of this animal model continue. (Circulation 1991;83:1371-1379)

The broad spectrum of long-term outcomes among patients surviving an acute pulmonary embolic event is slowly becoming elucidated. At one end of this spectrum appear to be the great majority of patients who, when treated, resolve their emboli and emerge with little or no significant residual vascular obstruction as determined by perfusion lung scan, clinical status, or hemodynamic assessment.1-7 At the other end of this spectrum lies that minority of patients who retain embolic obstruction sufficient to cause chronic pulmonary hypertension.8-11 How many patients lie at intermediate points along this spectrum is, at present, unknown. However, that various outcomes can occur, with respect to extent of residual obstruction and its consequences, is clear.12 Furthermore, webs, bands, and other chronic emboli residuals are commonly encountered at autopsy13; but how often such findings are associated with residual perfusion scan, pulmonary hemodynamic aberrations, or clinical symptoms is not known.

Our interest in defining this spectrum has been stimulated by the expanding number of patients being recognized at the chronic pulmonary hypertensive end of the spectrum. Some 300 such patients now have undergone surgical pulmonary thromboendarterectomy for relief of this condition.14-18 Such patients have caused multiple questions to be raised about this disorder, including its pathogenesis, natural history, optimum selection criteria for surgery, and certain aspects of postoperative management.

These questions are difficult to resolve by investigations restricted to the patients themselves. Therefore, we have devoted considerable effort to establishing a large-animal model of chronic pulmonary embolism.19 Prior studies disclosed that the efficient fibrinolytic system of the canine made it impossible to establish such a model by repetitive embolization of venous thrombi induced in vivo.20 However, using the competitive thrombolytic inhibitor tranexamic acid,21,22 we have now established such a model. Although our primary objective was to achieve a model of chronic, large-vessel thromboembolic hypertension, preliminary studies in which these objectives were not met are also reported here because they may provide some insights regarding other aspects of the postembolic spectrum.
Methods

Preliminary Studies

Seventeen conditioned mongrel dogs were studied. All were given 75 mg/kg tranexamic acid orally three times a day beginning 72 hours before embolization. On the day of embolization, the dogs were anesthetized with intravenous surital followed by intubation and inhalation of halothane. Baseline chest radiographs and baseline four-view perfusion lung scans (using technetium-99m–labeled macroaggregated albumin) were normal. A Swan-Ganz catheter was inserted into the pulmonary artery by way of the right external jugular vein, and an arterial line was inserted into the left femoral artery. Baseline right atrial, mean pulmonary artery, mean systemic, and pulmonary capillary wedge pressures and arterial blood gases and cardiac output were measured. pH was maintained between 7.33 and 7.38, PaCO₂ between 35 and 45 mm Hg, and O₂ saturation greater than 90%.

Next, venous thrombi were induced by insertion of a modified Swan-Ganz catheter through saphenous vein cutdowns into both femoral veins. One hundred units of topical thrombin were injected through a port below the inflated balloon. Thirty minutes later, the balloon was deflated, and the thrombi were aged for 2 hours. The thrombi were embolized by massaging the hindlegs of the animal along the course of the femoral vein and by gently exercising the legs. This procedure was repeated twice so that a total of four femoral vein thrombi were embolized in each dog.

One hour after embolization, perfusion lung scans, chest radiographs, pulmonary and systemic hemodynamic measurements, and arterial blood gas analyses were repeated. The animal was then extubated and allowed to recover. At 3 days, under light anesthesia, chest radiographs and perfusion lung scans were repeated; three of the first six animals that were studied were killed and autopsied at this juncture (dogs 2, 4, and 6).

At 8 days, the animals were again anesthetized, intubated, and mechanically ventilated. All baseline studies were repeated. Then, three additional animals were killed (dogs 1, 8, and 10) and autopsied.

Of the remaining 10 animals, tranexamic acid was continued for 10 days in five and for 30 days in five. At 30 days, all baseline studies were repeated, and the animals were given heparin, were killed, and were autopsied.

In additional animals, a series of other observations was made. First, because the tranexamic acid feeding schedule three times daily was cumbersome and often associated with nausea, other schedules were explored in nonembolized dogs. A dose of 110 mg/kg twice daily was found to be better tolerated, and it achieved serum levels of tranexamic acid sufficient to achieve marked inhibition of thrombolysis. Previous studies of tranexamic acid serum levels in human subjects used the high voltage paper electrophoresis technology available at that time.

We performed studies of tranexamic acid serum clearance in canines using the assay methods described below, which were developed in our laboratories. The kinetics of tranexamic acid clearance and the tranexamic acid levels achieved in canines with this assay were comparable to those previously reported (Table I).

Second, to develop larger emboli, we specially designed a double-balloon catheter. Placed in the inferior vena cava, with thrombin injected between the balloons, large emboli were induced that, after balloon deflation and leg exercise, consistently resulted in recovery of large (main, lobar, and segmental) pulmonary artery emboli at autopsy. Third, we found that animals (with rare exceptions) tolerated three or four such emboli without demise.

Based on observations made in these preliminary studies, the protocols followed in the remainder of the animals were developed.

Final Protocols

In 22 dogs, the sequence of study was essentially as described above. Animals were fed tranexamic acid, 110 mg/kg b.i.d., for 72 hours. Then, the animals were anesthetized, intubated, and mechanically ventilated. A Swan-Ganz catheter was passed into the pulmonary artery, and an arterial line was placed. A baseline chest radiograph and perfusion lung scan were obtained. Next, the double-balloon catheter was inserted into a saphenous vein cut down and placed into the inferior vena cava, with the proximal balloon below the renal veins. After balloon inflation, 100 units topical bovine thrombin was injected between the balloons. Thirty minutes later, the balloons were deflated, and the thrombus was embolized by exercising the dog’s legs and gently pushing the catheter proximally in the inferior vena cava. This procedure was repeated three times in phase 1 and four times in phases 2 and 3. At 1 hour after embolization, lung scan, hemodynamic measurements, and arterial blood gas measurements were repeated. All lines were removed, and the animal was allowed to recover. One dog succumbed immediately after embolization.

Table I. Serum Levels of Tranexamic Acid After Oral Dose of Tranexamic Acid

<table>
<thead>
<tr>
<th>Hours after dose</th>
<th>Tranexamic acid serum levels (µg/ml)</th>
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<tbody>
<tr>
<td>0</td>
<td>0.47±1.05</td>
</tr>
<tr>
<td>1</td>
<td>7.11±2.60</td>
</tr>
<tr>
<td>2</td>
<td>19.47±6.19</td>
</tr>
<tr>
<td>4</td>
<td>24.92±3.18</td>
</tr>
<tr>
<td>6</td>
<td>20.10±6.57</td>
</tr>
<tr>
<td>10</td>
<td>9.11±2.62</td>
</tr>
<tr>
<td>24</td>
<td>2.50±1.98</td>
</tr>
</tbody>
</table>

Values are mean±SD. n=6 dogs. Values obtained after 110 mg p.o. tranexamic acid.
On day 3, chest radiograph and perfusion lung scan were repeated under light anesthesia. On day 8, the animal was again anesthetized and intubated, and the lung scan, hemodynamic measurements, and arterial blood gas measurements were obtained. In the first five dogs (phase 1), no additional emboli were formed; one of these dogs succumbed after anesthetization. In the next 16 dogs, four additional inferior vena cava thrombi were formed and released. Instruments were removed from all animals, and animals were allowed to recover; one died after reembolization.

Tranexamic acid feedings were continued for 30 days in nine dogs (four phase 1 dogs not reembolized, five phase 2 dogs reembolized) and for 40 days in the remaining 10 dogs (phase 3). At that time, lung scan, hemodynamics, and arterial blood gases were repeated under anesthesia and mechanical ventilation; then, all animals were given heparin, were euthanized, and were autopsied. In the four phase 1 and five phase 2 dogs, serum tranexamic acid levels were measured at 3, 8, and 30 days (Table 2).

Last, while these studies were in progress, six control dogs were administered tranexamic acid according to the above schedule, and all elements of the phase 2 protocol were performed except embozization. The dogs were killed at 30–40 days and were then autopsied.

In all animals, the cardiac chambers were opened, and the pulmonary arteries were dissected to the subsegmental level. Gross findings were recorded and, in some instances, photographed. Multiple sections from each lobe and the heart block, plus selected pulmonary arterial sections, were submitted for microscopic examination.

All hemodynamic data were analyzed statistically by paired t tests in which baseline values were compared with subsequent values. A value of \( p < 0.05 \) was accepted as significant. Pulmonary vascular resistance was calculated as \( [\text{mean pulmonary artery pressure minus wedge pressure}] \) divided by cardiac output] multiplied by 80.

**Calculation of Tranexamic Acid in Serum**

Blood samples (10 ml) were collected in siliconized glass vacutainer tubes before and at various time periods after starting the tranexamic acid treatment regimen. The blood was allowed to clot at room temperature, and the serum was separated by centrifugation at 700g for 10 minutes. Serum samples were stored at 20°C until analyzed. Tranexamic acid levels in serum were determined by quantitative ion exchange thin-layer chromatography after an initial extraction of tranexamic acid from the serum. Each sample was treated as follows: 2 ml serum was mixed with an equal volume of distilled water, and serum proteins were precipitated by adding 2 ml ice-cold 100% (wt/vol) trichloroacetic acid. After incubating 20 minutes on ice, the sample was centrifuged at 2,000g for 15 minutes, and the supernatant was applied to a 3-ml disposable filtration column (J.T. Baker Chemical Co., Phillipsburg, N.J.) packed with 1 ml Dowex-50W ion exchange resin (Sigma Chemical Co., St. Louis, Mo.). The column was washed with 10 ml distilled water, and absorbed material (including tranexamic acid) was eluted with 5 ml 0.4N NH₄OH. The eluate was evaporated to dryness in a speed-vac concentrator (Savant Industries, Farmingdale, N.Y.), and the residue was dissolved in 0.1 ml distilled water. Extracted samples were then analyzed directly or stored at −20°C for later analysis. Extracted samples (10 μl each) were applied as small spots (<5 mm diameter) to an ion-exchange thin-layer chromatography plates (Polygram Ionex-25-SA-Na, 20×20 cm, Macherey-Nagel Co., FRG) 3 cm from the bottom edge and 2 cm apart. Tranexamic acid was resolved by discontinuous descending chromatography with 0.2 M sodium acetate (pH 5.2) until the solvent front had migrated about 5 cm beyond the origin and, thereafter, with 0.1 M Na₂HPO₄ (pH 7.0) until the solvent front reached the other end of the plate. Plates were dried and sprayed with 0.2% ninhydrin in absolute ethanol, and spots were visualized by heating the plates in a 60°C oven for 10–20 minutes. Tranexamic acid in extracted serum samples was identified based on its mobility relative to tranexamic acid standard solutions. After scoring a 1-cm-diameter circle around each tranexamic acid spot with a cork borer, the resin within each circle was carefully scraped from the plate and collected in separate 1.5-ml microfuge tubes. A sample of resin adjacent to the tranexamic acid standards was taken to serve as a blank. N-Propanol and water (1:1 vol/vol) (0.6 ml) were added to each tube, and after vortexing, the tubes were centrifuged at 700g for 5 minutes. The absorbance at 570 nm of each supernatant was recorded with an Ultrospec Spectrophotometer (LKB Instruments, Gaithersburg, Md.), zeroing the machine against the blank. Micrograms of tranexamic acid in the unknown samples were determined from a standard curve constructed from the tranexamic acid standard solutions. If the absorbance of an unknown exceeded the absorbance of the highest point on the standard curve, an appropriate dilution of the supernatant was made with N-propanol and water. Tranexamic acid concentrations in serum were finally calculated by taking into account overall dilution and concentration factors. Recovery of tranexamic acid from serum was assessed by processing serum samples to which tranexamic acid had been added (final concentration, 50 μg/ml) and found to be 82±11%
TABLE 3. Hemodynamic Data in 10 Dogs That Were Studied at 1 Hour, 8 Days, and 30 Days After Femoral Vein Thromboembolism

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 Hour</th>
<th>8 Day</th>
<th>30 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAm (mm Hg)</td>
<td>13.2±2.0</td>
<td>15.6±2.4*</td>
<td>15.9±2.8*</td>
<td>14.0±2.0</td>
</tr>
<tr>
<td>PVR (dynes/sec · cm²)</td>
<td>218±43</td>
<td>262±99</td>
<td>259±106</td>
<td>225±54</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>2.72±0.6</td>
<td>2.9±0.5</td>
<td>2.83±1.0</td>
<td>2.49±0.5</td>
</tr>
<tr>
<td>PCW (mm Hg)</td>
<td>5.2±1.1</td>
<td>5.9±1.4</td>
<td>6.7±2.2</td>
<td>5.1±0.7</td>
</tr>
</tbody>
</table>

Data are mean±SD. 
PAm, mean pulmonary artery pressure; PVR, cardiac output; CO, cardiac output; PCW, wedge pressure. 
There were no differences in these measurements between dogs that received tranexamic acid for 10 vs. 30 days.
*p<0.05 vs. baseline.

(n=28). Values reported are not corrected for recovery. Sensitivity of the assay was found to be 1.75 μg tranexamic acid/ml (±1.04, n=28).

Results

Preliminary Studies

All dogs given four femoral vein emboli demonstrated a modest, but significant (p<0.05), elevation of mean pulmonary artery pressure at 1 hour after embolization (Table 3). Cardiac output, wedge pressure, and calculated pulmonary vascular resistance were not significantly altered, although mean pulmonary vascular resistance was modestly elevated. Perfusion scans disclosed more than two lobar or segmental-sized defects in all dogs.

At 3 days, the scan defects were essentially unchanged in all dogs, and chest radiographs were normal. In the four dogs killed at this time, autopsy disclosed emboli in several lobar or segmental arteries. On gross examination, these were red and spongy and were easily detached from the arterial wall. On microscopic examination, very early organization was present. In addition, two dogs had similar emboli attached loosely to right ventricular chordae tendineae.

At 8 days, segmental or larger perfusion scan defects persisted in all dogs (Figure 1A). Chest radiographs were normal. The mean pulmonary artery pressure remained modestly, but significantly, elevated in the group. Again, cardiac output, wedge pressure, and calculated pulmonary vascular resistance were not significantly altered, although pulmonary vascular resistance remained modestly elevated. In the three dogs that were killed, autopsy demonstrated several lobar or segmental chronic emboli. On gross examination, these were found to be whitish and were firmly attached to the arterial wall. On microscopic examination, they showed significant organization. In one animal, similar organized embolic material was attached to right ventricular chordae.

By 30 days, mean pulmonary artery pressure and other hemodynamic values were not significantly different from baseline values. Of the five dogs in which tranexamic acid had been discontinued at 10 days, the perfusion scan was normal in two; the other dogs demonstrated several subsegmental defects, substantially smaller than those on day 8. Autopsy disclosed no embolic residual in the dogs with normal scans; in the others, small, well-organized thrombi were present in subsegmental vessels that firmly attached to the arterial wall. The five dogs that were continued on tranexamic acid to 30 days demonstrated several residual subsegmental or segmental scan defects, although they had decreased in size and number since day 8 (Figure 1B). Autopsy disclosed organized, whitish thrombi in lobar and segmental vessels in each, which were tightly attached to the arterial wall. In three dogs, similar organized thromboemboli were attached to right ventricular chordae.

Thrombus was always present at autopsy in vessels corresponding to scan perfusion defects. However, substantial organized and recanalized (although not totally occlusive) chronic thrombus was frequently found in vessels supplying areas that were normal by perfusion scan.

Final Protocols

Of the 22 animals studied, one died after the initial embolization. (Hemodynamic values obtained shortly before demise disclosed a mean pulmonary artery pressure of 26 mm Hg, cardiac output of 1.6 l/min, wedge pressure of 6 mm Hg, and calculated pulmonary vascular resistance of 900 dynes/sec · cm²). Autopsy disclosed fresh emboli occluding nearly all lobar vessels.

All surviving animals demonstrated both lobar and segmental perfusion defects, and a significant elevation of mean pulmonary artery pressure at 1 hour after embolization (Table 4). At 3 and 8 days after embolization, lobar and segmental defects persisted in all dogs. Chest radiographs were normal. At 8 days, mean pulmonary artery pressure remained elevated, but not significantly, in all dogs (Table 4). Pulmonary vascular resistance remained elevated above baseline but was significant only in phase 3 animals. Cardiac output and wedge pressure did not vary significantly from baseline except in phase 3 animals that demonstrated a significant decline in cardiac output.

One of the five phase 1 dogs not reembolized on day 8 died during recovery from anesthesia. Extensive and well-organized whitish thrombi were present in multiple lobar and segmental vessels.

Of the 16 dogs (phases 2 and 3) that were reembolized on day 8, all demonstrated a further rise in
mean pulmonary artery pressure and pulmonary vascular resistance, a fall in cardiac output, and new scan defects. One dog died shortly after reembolization. Autopsy disclosed whitish, organized emboli in several lobar and segmental branches and large, red emboli occluding all lobar branches, including two in which whitish emboli were present distally.

At 30 days, the four phase 1 nonreembolized dogs maintained an elevated mean pulmonary artery pressure and pulmonary vascular resistance, although neither elevation was significant. At 30 days, the five phase 2 reembolized dogs maintained a significant elevation of mean pulmonary artery pressure and a nonsignificant increase in calculated pulmonary vascular resistance. Multiple perfusion scan defects, which had decreased in size to a variable degree, persisted in all animals.

At 40 days, the 10 phase 3 dogs maintained a significant elevation in mean pulmonary artery pressure and calculated pulmonary vascular resistance and a significantly reduced cardiac output.

At autopsy, all phase 1 and phase 2 dogs had reddish white, organized, recanalized thrombi in several lobar or segmental vessels that were very firmly attached to the arterial wall. On gross examination, there appeared to be more extensive residuals in the phase 2 (reembolized) than in the phase 1 (nonreembolized) dogs. Again, thrombi were found in vessels supplying all areas that were abnormal by lung scan. However, substantial amounts of organized thrombi were also present in multiple vessels supplying areas that were normally perfused by scan (Figure 2). Among the 10 reembolized phase 3 dogs, the autopsy findings were identical to those seen in phase 2 dogs. Again, disparities between scan defects and the presence of chronic thrombi were frequent.

In the six control animals that received tranexamic acid, hemodynamic values were unchanged from baseline at 8, 30 (three dogs), and 40 days (three dogs). Chest radiographs and perfusion scans remained normal. Autopsies disclosed no venous thrombi or pulmonary emboli.

**Discussion**

Prior investigations disclosed that in canine models subjected to pulmonary embolism from venous thrombi induced in vivo in the absence of tranexamic acid, substantial resolution occurs within 24–48 hours. Furthermore, even when venous thrombi are aged in vivo for 1 week or more before embolization, residual emboli recovered beyond 4 weeks are minimal. The studies reported here have demonstrated, for the first time, that inhibition of the thrombolytic system in canines can induce chronic thromboembolic obstruction of the pulmonary vascular bed sufficient to cause pulmonary hypertension at 30–40 days after release of fresh thromboemboli.

However, our data also indicate that despite substantial thrombolytic inhibition, a spectrum of outcomes is encountered, from nearly complete resolu-
TABLE 4. Hemodynamic Data in the Four Phase 1 Dogs That Survived to 30 Days, the Five Phase 2 (Reembolized) Dogs, and the 10 Phase 3 (Reembolized) Dogs

<table>
<thead>
<tr>
<th>Postembolism</th>
<th>Baseline</th>
<th>1 Hour</th>
<th>8 Day</th>
<th>Postembolism</th>
<th>8 Day</th>
<th>30 Day</th>
<th>40 Day</th>
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<tbody>
<tr>
<td>PAm (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Phase 1</td>
<td>13.0±2.9</td>
<td>19.5±3.5*</td>
<td>16.0±3.2</td>
<td>—</td>
<td>16.8±3.4</td>
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<tr>
<td>Phase 2</td>
<td>15.0±1.2</td>
<td>18.6±2.5*</td>
<td>17.8±4.4</td>
<td>20.5±3.0*</td>
<td>18.0±2.4*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Phase 3</td>
<td>16.5±3.2</td>
<td>22.7±4.8*</td>
<td>19.1±3.8</td>
<td>23.1±4.1*</td>
<td>—</td>
<td>20.3±2.0*</td>
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<tr>
<td>PVR (dynes/sec·cm²)</td>
<td></td>
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</tr>
<tr>
<td>Phase 1</td>
<td>192±67</td>
<td>412±206</td>
<td>339±224</td>
<td>—</td>
<td>253±108</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Phase 2</td>
<td>205±109</td>
<td>324±238</td>
<td>404±341</td>
<td>420±216*</td>
<td>371±210</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Phase 3</td>
<td>199±32</td>
<td>339±121*</td>
<td>331±130*</td>
<td>551±235*</td>
<td>—</td>
<td>335±99*</td>
<td>—</td>
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<tr>
<td>CO (l/min)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Phase 1</td>
<td>2.7±0.4</td>
<td>2.6±0.9</td>
<td>2.58±0.6</td>
<td>—</td>
<td>2.91±0.2</td>
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<tr>
<td>Phase 2</td>
<td>3.1±1.0</td>
<td>3.6±1.4</td>
<td>2.8±1.1</td>
<td>2.6±0.8*</td>
<td>3.3±1.2</td>
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<td>—</td>
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<tr>
<td>Phase 3</td>
<td>3.2±0.5</td>
<td>3.1±0.6</td>
<td>2.8±0.7*</td>
<td>2.6±0.6*</td>
<td>—</td>
<td>2.8±0.7*</td>
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<tr>
<td>PCW (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Phase 1</td>
<td>6.8±1.9</td>
<td>7.0±2.2</td>
<td>6.3±0.9</td>
<td>—</td>
<td>7.5±1.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Phase 2</td>
<td>8.2±1.9</td>
<td>7.0±1.0</td>
<td>6.6±1.9</td>
<td>6.8±1.9</td>
<td>5.0±2.0*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Phase 3</td>
<td>8.6±1.0</td>
<td>9.9±1.8</td>
<td>8.2±1.6</td>
<td>9.1±3.5</td>
<td>—</td>
<td>8.6±1.9</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are mean±SD. PAm, mean pulmonary artery pressure; PVR, cardiac output; CO, cardiac output; PCW, wedge pressure.

There were no differences in these measurements between dogs that received tranexamic acid for 10 vs. 30 days. *p<0.05 vs. baseline.

These results appear to replicate those observed among patients surviving acute pulmonary embolism. Follow-up scans and hemodynamic or clinical observations indicate that most patients resolve their emboli well and, after weeks to months, are left with a modest perfusion scan and little or no hemodynamic abnormality. Unlike our canines, the vast majority of these patients were treated for their embolic

Figure 2. Panel A: Photograph of autopsy specimen in a dog treated with tranexamic acid for 30 days demonstrating large organized thrombus in a major branch of the right descending pulmonary artery. A similar thrombus was found in the left upper lobe. Panel B: Anterior perfusion lung scan performed just before autopsy shows no corresponding defect in right lower lobe. Marked reduction in left upper lobe perfusion persists.
events. Nevertheless, despite the generally excellent outcomes, there clearly is a spectrum of resolution outcomes among patients.\textsuperscript{12} For example, small webs, bands, and other residuals have been commonly discovered when detailed postmortem examination of the pulmonary vessels is performed.\textsuperscript{13} These residuals closely resemble those we found in our preliminary femoral vein embolism studies. Furthermore, in phase 1 of the National Institutes of Health Urokinase Pulmonary Trial, scan residuals averaging 25\% of the initial magnitude still persisted at 1 year, regardless of whether urokinase or heparin was the initial therapy.\textsuperscript{26} Clearly, within these patients there was a spectrum of resolution, as was also apparent in subsequent extensions of these thrombolytic trials.\textsuperscript{27} It also has become evident that a small number of patients, whether or not treated initially, retain a sufficient degree of pulmonary thromboembolic obstruction to result in resting pulmonary hypertension.\textsuperscript{8–12} Indeed, although such patients are uncommon, more than 400 of them now have been discovered and more than 300 have undergone surgery for relief of this obstruction.\textsuperscript{14–18}

Thus, there is a spectrum of chronic residuals among patients with prior emboli. Some have modest residuals, have no or nominal symptoms, and have no or minimal elevations of resting mean pulmonary artery pressure and pulmonary vascular resistance. Others come to attention with severe pulmonary hypertension and in florid right ventricular failure. Our animal models appear to reflect this same broad spectrum of outcomes. Despite tranexamic acid administration, some animals completed the study period with minor chronic thrombotic residuals at autopsy, modest or no scan abnormalities, and no resting hemodynamic abnormalities. Others completed study with significant residuals and persistent pulmonary hypertension.

Some of the variables that conditioned these different outcomes are indicated by our study. First, the size of the initial emboli had an effect. The smaller femoral vein thrombi released in preliminary studies induced less-extensive and less-persistent abnormalities than did the large inferior vena caval thrombi released in the final protocols. Also, the longer-term consequences of three inferior vena cava emboli (phase 1) were less than when four emboli (phases 2 and 3) were released. That the size of the initial embolic event can condition the extent of embolic residuals in canines has been observed by other investigators\textsuperscript{24,25} and has been suggested by Riedel et al\textsuperscript{12} in patient studies. Another factor conditioning residual extent appeared to be reembolization. Repetition of major embolization at 8 days (phases 2 and 3) led to greater ultimate residuals (anatomic, hemodynamic, and scan) than observed in canines embolized only once (phase 1).

Our preliminary studies also suggested that embolic resolution was less complete when tranexamic acid was continued for 30 days rather than interrupted 10 days after embolization. The potential influence of persistent inhibition of thrombolysis also is suggested by the somewhat greater hemodynamic compromise among the phase 3 40-day canines than among the phase 2 30-day canines. However, although substantial inhibition of thrombolytic activity was achieved by the tranexamic acid doses administered, complete and continuous inhibition cannot be ensured at the doses tolerated, and some animal-to-animal variability in tranexamic acid serum levels occurred (Tables 1 and 2).

Yet, some of the variables that conditioned embolic outcome remain undefined. For example, highly organized whitish thrombi were found in animals autopsied at 8 days. Our initial assumption, given the extent of organization present at 8 days, was that these thrombi would persist relatively unchanged. That assumption proved incorrect. Instead, most animals demonstrated hemodynamic and scan improvement by 30 days, and residual obstruction at autopsy was considerably less than anticipated from the animals at 8 days. Clearly, then, even though organized, apparently stable residuals were present at 8 days, the process of organization and recanalization continued, and pulmonary vascular obstruction moderated. There was also considerable individual variation in this process among dogs, with a spectrum from "efficient resolvers" to "poor resolvers." The reasons for such variation are unknown. They do not relate to the age of the emboli before embolization because this was constant. Variations in thrombus composition or in factors involved in promoting organization and recanalization remain to be explored.

In patients undergoing thromboendarterectomy, we frequently observe whitish, fibrotic obstructions more distally and reddish, less-organized thrombus more proximally. This has led us to speculate that there may be in situ proximal growth of thrombi, perhaps from local activation of coagulation components induced by turbulence due to distal obstruction.\textsuperscript{15} The canines rarely demonstrated this phenomenon at autopsy. However, other investigators have made the same observation of more recent thrombus associated with chronic embolic residuals at autopsy in patients,\textsuperscript{13} and Riedel et al\textsuperscript{12} suggested this as one mechanism for late postembolic deterioration in patient status.\textsuperscript{12} How often and extensive such local propagation may occur at sites of embolic residuals remains to be defined, as does the differentiation between in situ propagation and embolic recurrence. Only a small minority of the patients we have encountered have had evidence of embolic recurrence by lung scan when they deteriorate clinically.\textsuperscript{11,15} However, in our canines subjected to reembolization at 8 days, the increase in mean pulmonary artery pressure, pulmonary vascular resistance, and anatomic residuals at 30–40 days were all greater than those in the group not reembolized. Thus, perhaps undetected embolic recurrences do contribute to the development of worsening of thromboembolic pulmonary hypertension in some patients.
One observation that appears particularly relevant to the human counterpart is the consistent underestimation of the extent of embolic residuals by the perfusion scan in these canines, an observation previously reported in patients.28 Specifically, the scans commonly demonstrated either no abnormality or a modest decrement in perfusion to lung areas supplied by vessels containing extensive proximal residual thrombus. Such disparity also was noted previously by Sabiston and Wolfe29 in studies of the natural history of canine embolism. Several possibilities may bear on this disparity. First, when subsegmental vessels are occluded, gamma radiation from the nonoccluded lung areas can obscure small potential deficits. Second, and probably more important clinically, is that pulmonary vessels, like renal and coronary arteries, continue to have normal distal flow until there is 80% or more luminal occlusion. Thus, perfusion scans, which detect only flow abnormalities, will not detect proximal residual thrombi that cause lesser degrees of luminal compromise. Thus, as shown in this and prior studies,29 recanalization and organization may allow relatively normal flow despite significant residuals apparent angiographically or at autopsy. Third, in our canines and in patients, true scan defects (no flow) appear only when total occlusion is present; often, near-total occlusion is manifested by scan as a gray (reduced flow) zone (Figure 2B).

Another observation made in this study was the frequency with which some emboli may become entrapped in the right cardiac chambers, an observation made in prior canine studies24-25 and by recent echocardiographic studies in patients.30,31 Such emboli may represent a potential source of embolic recurrence, despite therapy or placement of a vena caval filter.

Last, the experiments reported here may provide some insight into the pathogenesis of chronic, major vessel thromboembolic pulmonary hypertension. Our prior attempts to achieve chronic obstruction with repetitive emboli failed because the canine fibrinolytic system led to rapid embolic resolution. Tranexamic acid serves as a potent inhibitor of thrombolytic activity by attaching to lysine-binding sites on plasminogen, thus inhibiting plasminogen binding to fibrin. Tranexamic acid is seven to 10 times more potent than e-aminocaproic acid in achieving such inhibition and has a longer biological half-life, making it particularly suitable for the purposes of this study.21

The demonstration that tranexamic acid inhibition of thrombolysis can induce chronic thrombosis in this animal model adds some experimental evidence to support the postulate that defects in the thrombolytic system may contribute to the outcome of acute thrombotic states in humans, including acute pulmonary embolism.32-34

Another interesting observation in patients with chronic thromboembolic pulmonary hypertension has been that pulmonary artery pressures that are moderately elevated at resting (and often low) cardiac outputs rise sharply when cardiac output is augmented by exercise.35 Our phase 3 dogs had a residual mean pulmonary artery pressure of just more than 20 mm Hg but had a reduced cardiac output and a substantial residual elevation of pulmonary vascular resistance. Recently, in additional animals with chronic embolism, we augmented cardiac output with incremental dobutamine infusions and found sharp elevations in pulmonary artery pressure.36 Thus, in detecting the severity of residual thrombotic obstruction, a resting measurement of pulmonary artery pressure may be deceptive.

Further studies with this model are proceeding, with our particular interest focused on the hemodynamic, pulmonary vascular, and right ventricular changes that occur over periods well beyond 40 days and on the responses of animals with chronic hypertension to thromboendarterectomy.

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- pulmonary hypertension  
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