Imaging Thromboembolism with Fibrin-Avid ^{99m}Tc-Peptide: Evaluation in Swine

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A pentapeptide, Gly-Pro-Arg-Pro-Pro, with high affinity for α-chain-fibrin was labeled with ^{99m}Tc (^{99m}Tc-TP850) and evaluated in swine to image experimental venous thromboembolism (deep vein thrombosis [DVT]) and pulmonary embolism (PE). **Methods:** Scatchard analysis was performed to determine fibrin affinity for TP850 and the number of binding sites (receptors) per milligram of fibrin. DVT was induced in the left jugular vein and PE was induced by introducing a preformed autologous blood clot into the right atrium using a 7-French introducer sheath inserted into the right jugular vein. ^{99m}Tc-TP850 was injected at 4, 24, 48, 72, 96, or 120 h later. Animals were imaged for up to 4 h after injection, heparinized, and sacrificed. Lungs were extirpated, radiographed, and imaged, and the PE was removed. Other tissues, including blood and normal lungs, were harvested and, concomitantly, ^{99m}Tc was counted for determination of target-to-tissue ratios and the percentage injected dose per gram of tissue. **Results:** The affinity for human fibrin was 10^{-9} mol/L and there were >10^{15} receptors per milligram of fibrin. DVT and PE were visualized for up to 4 h after injection with high DVT/blood (7.9–22.6), DVT/muscle (31.1–89.4), PE/blood (1–155), and PE/lung (0.8–245) ratios. Thereafter, the PEs fragmented spontaneously below the spatial resolution of the γ-camera and, despite the high associated radioactivity, could not be localized in vivo. The fragmented clots were detectable by scintigraphy on excised lungs and provided excellent concordance with radiograms. **Conclusion:** ^{99m}Tc-TP850 with its modest affinity (10^{-9} mol/L), rapid blood clearance, and high DVT and PE uptake is a promising agent for imaging vascular thrombosis.

Key Words: imaging vascular thrombosis; imaging pulmonary embolism; imaging venous thrombosis; ^{99m}Tc-antifibrin peptide

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The incidence of deep venous thrombosis (DVT) and pulmonary embolism (PE), in high-risk populations, in hospitalized patients, as well as in the general population remains remarkably high, yet an accurate diagnosis of DVT and PE continues to be unreliable (*1*).

The development of radioactive agents for scintigraphic imaging of DVT and PE is centered on the use of ^{99m}Tc-labeled peptides specific for resting or activated platelets (*2–7*). Peptides are smaller in size, easier to produce than monoclonal antibodies, expected to clear more rapidly from circulation than radiolabeled proteins, are less likely to induce any immunologic reaction, and yet, in most cases, they enjoy receptor specificity and binding constants as high as those of the monoclonal antibodies. ^{99m}Tc is inexpensive, easy to obtain worldwide, and decays with emission of γ-rays (140 keV, 90%) that can be efficiently detected by γ-cameras. Its half-life (6 h) is long enough to perform examinations before excessive radioactive decay has occurred, yet not too long to persist in the body long after the examinations have been performed. One peptide, ^{99m}Tc-P280 (*5*), is approved by the Food and Drug Administration under the trade name AcuTect (^{99m}Tc-P280; Diatide, Inc.) (*8*), which can image acute thrombi but not old clots or PE.

Fibrin, a trimeric molecule consisting of α-, β-, and γ-chains, is a major constituent of a clot, fresh or old, and, when dislodged, could cause a PE. The actual quantity of fibrin content in a clot may vary from clot to clot, but generally it is the same as the fibrinogen content of the blood, which in most adults is as high as 5 g/100 g of plasma proteins (*9*). Fibrin not only forms a large component of a clot but also exists on the surface of clots that are slowly dissolving, either spontaneously or under the influence of therapeutic intervention. The development of a ^{99m}Tc-labeled peptide, specific for fibrin, as an agent for imaging vascular thrombosis is therefore appealing. One peptide of particular interest is the N-terminus fibrin α-chain peptide, H-Gly-Pro-Arg-OH, an inhibitor of fibrinogen/thrombin clotting (*10*). Kawasaki et al. prepared several more analogs and found that a pentapeptide, H-Gly-Pro-Arg-Pro-Pro-OH (GPRPP), had the highest fibrinogen/thrombin clot-inhibiting activity (*11*). We have labeled this peptide with ^{99m}Tc and evaluated it in vitro and in rabbits, with experimental venous thrombosis and PE. However, we could not evaluate the agent in rabbits for imaging thrombosis or PE older than 24 h, because these clots dissolved spontaneously (*12*). This is because of a high fibrinolitic activity in rabbits, where plasminogen concentration is about 2 times higher than that in humans (*13–17*). The plasminogen concentration in pigs, on the other hand, is much smaller than that in rabbits and in humans and,

thereby, has lesser fibrinolytic potential (*17,18*). Craig et al. (*18*) have shown that experimentally induced clots in pigs could be examined for up to 7 d. This observation led us to the conclusion that the swine model would be most suitable for studying localization of ^{99m}Tc-GPRPP (TP850) in up to 96-h-old DVT and PE.

MATERIALS AND METHODS

Preparation of Peptide

To label GPRPP with ^{99m}Tc, we have chosen a group of 4 amino acids, Gly-(D)-Ala-Gly-Gly- (GAGG), as a chelating moiety that provided an N$_4$ configuration for strong chelation of ^{99m}Tc. Rather than the conventional postsynthesis conjugation, the tetrapeptide chelating moiety permitted modification of the primary peptide at the C terminus during the synthesis, with an additional amino acid, Aba (4-aminobutyric acid), inserted as a spacer between the chelating moiety and the primary peptide to minimize any possible steric hindrance resulting from the ^{99m}Tc-GAGG complex. The synthesis of this modified peptide was one hybrid process, which eliminated the multistep, lengthy, and frequently inefficient conjugation procedure yet provided a chelating group for a strong chelation of ^{99m}Tc- (*19,20*). The resultant decapeptide, Gly-Pro-Arg-Pro-Pro-Aba-Gly-(D)-Ala-Gly-Gly, which has an expected molecular weight of 850, is hereafter referred to as TP850.

The peptide was custom synthesized (PeptidoGenic Research Co.) using a Shimadzu solid-phase synthesizer and separated using a HATsil (Higgins Analytical Inc.), C$_{18}$, 5-μm preparative high-performance liquid chromatography (HPLC) column. Ion spray mass analysis was performed using a Sciex APZ I mass spectrometer (Perkin Elmer).

Radiolabeling and Quality Control

Fifty micrograms of TP850 were dissolved in 10 μL of 10% acetonitrile in water; then 200 μL of 0.1 mol/L Na$_3$PO$_4$ were added, followed by 370–1,110 MBq (10–30 mCi) ^{99m}Tc in 200 μL isotonic saline previously reduced with 100 μg SnCl$_2$ in 10 μL of 0.05 mol/L HCl. Lately, with a new batch of high-purity SnCl$_2$ (Sigma-Chemical), we have been able to reduce the SnCl$_2$ to 10 μg. The reaction mixture was then incubated for 30 min in a water bath at 90°C. The product was examined by HPLC (Rainin) using a reverse-phase C$_{18}$ column and gradient solvents of 0.1% trifluoroacetic acid (TFA) in water (solvent A) and 0.1% TFA in acetonitrile (solvent B). The gradient was such that at 0 min solvent A was 90% and at 30 min solvent B was 100%. The flow rate was 1 mL/min. The HPLC was equipped with an ultraviolet detector set at 278 nm, a 2″ NaI(Tl) γ-counter, and a rate meter.

Determination of TP850 Affinity for Human Fibrin By Scatchard Plot Analysis

Human fibrin was obtained from Sigma-Chemical. Ten milligrams of fibrin were dissolved in 400 μL of 2 mol/L NaOH and the solution was diluted to 4 mL by the addition of 3.6 mL of 0.05 mol/L Na$_3$PO$_4$ solution, pH 12. To each of 6 borosilicate test tubes, 400 μL (1 mg of fibrin) of the above solution were dispensed, followed by 375 μL of the 0.05 mol/L Na$_3$PO$_4$ solution, pH 12. ^{99m}Tc-TP850 (185 MBq/50 μg [5 mCi/50 μg] TP850; specific activity, 3.15 TBq/mmol [85 Ci/mmol]) was prepared and analyzed by HPLC using the methods described earlier. Free ^{99m}Tc and colloid formation combined amounted to <2%. Forty microliters (1 μg TP850) of this solution were added to test tube 1 and 200 μL (5 μg TP850) were added to each of the test tubes 2 through 6. Sixty-five micrograms of TP850 were dissolved in 65 μL of 0.05 mol/L acetate buffer, pH 5.6, of which 5, 10, 15, and 25 μL were added to test tubes 3, 4, 5, and 6, respectively, making a total TP850 content of 1, 5, 10, 15, 20, and 30 μg in test tubes 1–6, respectively. A calculated quantity of 0.05 mol/L Na$_3$PO$_4$ solution was then added to each test tube to make a total reaction volume of 0.5 mL.

Each test tube was then placed in a water bath at 37°C and the reaction mixture was incubated for 30 min. Fibrin was then separated using a Centricon 30 molecular filtration device (Amicon), and radioactivity associated with fibrin and radioactivity that remained unbound were determined using a Capintec calibrated dosimeter. The experiment was repeated 3 times and data were subject to Scatchard plot and regression analysis to determine the best fit for the intercepts at the *x*- and y-axes. Dissociation constant (K$_d$) values and the number of binding sites per milligram of fibrin were determined.

Swine Anesthesia

The swine were anesthetized with 0.5 mL Telazol (Fort Dodge, Wyeth Pharmaceuticals) and 1 mL of atropine, both administered intramuscularly. In the first animal, anesthesia was maintained using intravenous propofol at 10–16 mg/kg/h. This animal had irregular respiration and it was found at dissection that the animal had collapsed lungs, leading to darkened lungs due to formation of microemboli. Therefore, in the second animal, anesthesia was maintained using 1.5%–2% isoflurane in 98%–98.5% oxygen, delivered through an endotrachael tube. No lung damage was observed in this animal at sacrifice. Isoflorane was therefore used in subsequent animals.

Blood Clearance

All animal protocols were approved by the Institutional Animal Care and Use Committee and were strictly followed. Blood clearance of the agent was examined in 5 swine. A 23-gauge catheter was inserted in the right ear artery and connected to a Luer-Lok (Burron Med. Inc.). The patency of the catheter was maintained by the administration of heparin at 6 IU/mL of sterile 0.9% NaCl administered through the Luer-Lok. This catheter was used for drawing 0.5-mL blood samples in duplicate at 12 time points between 0.5 min and 4 h after radionuclide injection. Before each sample collection, enough blood was withdrawn to replace saline, which avoided the dilution of each blood sample collected.

The marginal vein of the contralateral ear was used for injecting radioactive agents. The radioactivity in the syringe was measured before and after injection to determine the dose injected, and a suitable ^{99m}Tc standard solution was prepared. Blood samples were weighed, radioactivity was counted, and results were expressed as the percentage injected dose per gram (%ID/g) of blood and plotted as a function of time.

Inducing Venous Thrombosis

Each of the 27 swine (male or female; average body weight, 17.5 kg) was anesthetized as described, the right cubital vein or jugular vein was exposed, and a stimulating electrode was inserted (*21*). The electrode was constructed from a 20-gauge stainless steel hypodermic needle bent at a 90° angle and attached to a 30-gauge; Teflon-insulated (DuPont) silver-coated copper wire. The needle was inserted into the vessel and then gently pulled so that it was in contact with the endothelial lining of the vessel and secured

in place with a flared sleeve inserted over the copper wire. The second electrode was applied to the hind leg of the swine. The stimulating electrode was attached to the anode and the other electrode was attached to the cathode of a power supply. A 750-μA current was then applied for 45 min.

Inducing PE

Pulmonary emboli were induced in all animals. Radioopaque pulmonary emboli were prepared by drawing 0.5–0.75 mL blood, through a 21-gauge butterfly needle inserted in the marginal ear vein, into a 1-mL syringe containing 15 mg tantalum powder and 6 IU of thrombin. The contents of the syringe were then mixed gently and a clot was allowed to form and harden for 18 h. The clot was removed from the syringe and 1-cm-long pieces of the clots were drawn in a 7-French introducer sheath (Pinnacle; MediTech), which was then inserted into a previously isolated jugular vein and advanced into the right atrium; the clots were then flushed from the sheath with isotonic saline. The position of the tantalum-containing clots was confirmed by recording a chest radiograph of the animals before the administration of ^{99m}Tc-TP850 and a radiograph of the excised lungs after sacrifice. After clot administration and the confirmation of its localization by x-ray, ^{99m}Tc-TP850 was injected and the animals were imaged as described.

Imaging and Tissue Distribution

This procedure was initiated either immediately or at approximately 20, 44, 68, 92, or 116 h after the induction of DVT and PE. Seventy-four megabecquerels (2 mCi) of ^{99m}Tc-TP850 (average specific activity, 12.95 TBq [350 Ci/mmol]) in 1 mL phosphate buffer (5 μg peptide), pH ~7, was injected through a marginal ear vein. The radioactivity in each dose was measured before and after administration and recorded. A suitable reference solution with a known quantity of ^{99m}Tc was also prepared. Serial scintigraphic images were obtained in the supine or lateral position using a Starcam γ-camera (GE Healthcare) coupled to a low-energy, parallel-hole collimator and 20% energy window. For each image, a total of 350,000 counts were collected. After 4 h of imaging for PE or DVT, each animal was given an intravenous injection of heparin (1,000 IU) and then was euthanized with sodium pentobarbital (100 mg/kg). At sacrifice, the PE and DVT were 4, 24, 48, 72, 96, and 120 h old in vivo. A blood sample was drawn, and then the lungs and heart were excised, washed free of blood, radiographed, and imaged scintigraphically and the clots were

carefully harvested. Lungs were sliced into 0.5-cm-thick transverse sections to facilitate the visualization of clots dark in color. The clots and blood were weighed, the radioactivity associated with them was counted, normalized for a gram of weight, and the clot-to-blood, clot-to-muscle, and clot-to-lung ratios were determined. The number of animals entered into each time point is given in Table 1.

RESULTS

Peptide Radiolabeling, Quality Control, and Stability

HPLC analysis showed that the peptide was >95% pure. The expected molecular weight of the peptide was 850 Da and the molecular weight observed by mass spectroscopic analysis was 849.4 Da. The proposed structure of ^{99m}Tc-TP850, given previously (12), shows that ^{99m}Tc is bound to the chelating moiety with N_4 configuration. The ^{99m}Tc labeling consistently produced >95% yield. HPLC analysis indicated that >95% of that activity was eluted in a single peak at a retention time (R_t) of 7 min. A small quantity (<5%) of radioactivity was eluted at a R_t of 6.2 min and any unbound ^{99m}Tc was eluted at a R_t of 3.5 min. A typical elution profile and the preparations of ^{99m}Tc-TP850 that were stable at 22°C for 24 h were reported previously (12).

Affinity of TP850 for Human Fibrin

Results of the Scatchard plot analysis are shown in Figure 1. The data indicate that the affinity for TP850 to human fibrin was 8×10^{-9} mol/L and that there were 3.8×10^{15} binding sites per milligram of fibrin. This is a large pool of binding sites and, thereby, enhances the probability of imaging vascular thrombosis.

Blood Clearance and Tissue Distribution

Our initial goal was to include 4 swine in each of the 6 groups. However, because we had to experiment with several different methods of preparing clots to minimize or eliminate fragmentation at 24 h and beyond after induction of clots (PE), we had to recruit more animals than we had initially planned: 24-, 48-, and 96-h groups. Restricted by the number of swine that could be entered into the protocol, fewer than 4 animals in the 2-, 72-, and 96-h group were

TABLE 1
Tissue Distribution of ^{99m}Tc-TP850 in Swine (%ID/g)

Tissue	4 h (n = 2)	24 h (n = 5)	48 h (n = 6)	72 h (n = 3)	96 h (n = 9)	120 h (n = 1)
Muscle	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00
Myocardium	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.008	0.00
Lungs	0.00 ± 0.00	0.01 ± 0.003	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.008	0.00
Blood	0.00 ± 0.00	0.03 ± 0.003	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.008	0.00
Spleen	0.00 ± 0.00	0.01 ± 0.005	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.008	0.00
Kidneys	0.03 ± 0.01	0.12 ± 0.10	0.05 ± 0.03	0.09 ± 0.08	0.08 ± 0.04	0.08
Liver	0.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.00
Urine	0.05 ± 0.04	0.32 ± 0.29	0.29 ± 0.10	0.15 ± 0.05	0.33 ± 0.27	0.16
Bone	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00

Each animal in each group was sacrificed at ~4 h after administration of ^{99m}Tc-TP850. Time in hours indicates the period at which animals were sacrificed after inducing DVT and PE.
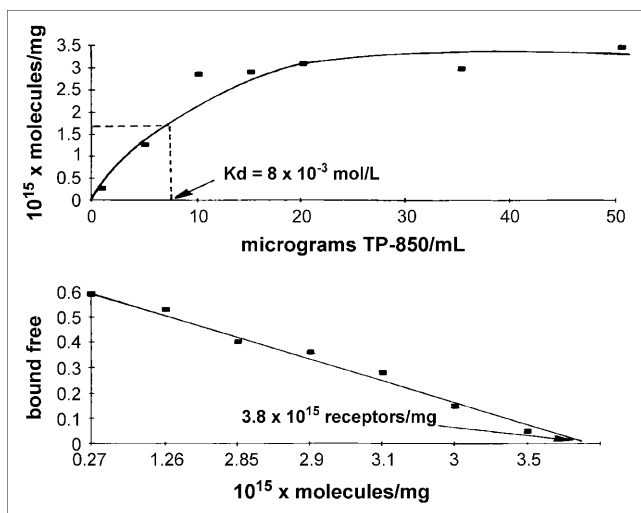
FIGURE 1. Scatchard plot curves determining the K_d value for the affinity of TP850 to human fibrin (top) and the number of binding sites (receptors)/mg of human fibrin to which TP850 binds (bottom).

studied. However, this did not adversely influence the outcome of this feasibility study. Blood clearance was biphasic with an α-phase half-life of 4 ± 0.2 min (20%) and a β-phase half-life of 13 ± 1.0 min (80%). All animals in all groups were sacrificed at ~ 4 h after injection. The tissue distribution data (%ID/g) are given in Table 1. As expected, the data within the groups are not statistically significantly different except in the urine, for which the 4-h radioactivity content is dependent on voiding of the animal spontaneously during the 4-h experimental time. The highest radioactivity was in the kidneys, as the kidneys were the primary routes of excretion. Hepatic uptake was lower than that of the kidneys, followed by the spleen and blood. This small proportion of radioactivity in circulating blood facilitated the imaging of vascular thrombi. Radioactivity in all other tissues was remarkably low, falling into a third decimal, not included in Table 1.

DVT/PE Ratios

The DVT/blood, DVT/muscle, and PE/blood, PE/lung, and PE/muscle ratios of the respective %ID/g, for each time period of the study, are tabulated in Table 2. Generally, in most cases, these ratios were higher than those reported previously in many studies (2,4,5).

However, the range of ratios was very wide. The lower ratios resulted from contamination of small thrombi with normal tissue, which could not be separated without losing the clots. The tissue had very little to no activity associated with it, but it accounted for the weight and thereby decreased the counts (cpm/g) of this tissue, and apparently decreased the ratios, which contributed to this variation. Therefore, Table 2 reports not the averages but, rather, the range of ratios as calculated for each DVT or PE found in the animals studied in each group.

Fragmentation

The PEs that, on the average, were 1 cm in length and 3 mm in diameter when administered, were found to be fragmented in almost every animal that was sacrificed at 48 h after administration or beyond. This fragmentation was evident even in animals that were sacrificed at 24 h after administration of the PE. Examples of this fragmentation are given in Figures 2A and 2B, which provide only partial evidence, because only the part of the PE that contained tantalum was evident on radiography. The PE portions that had no tantalum were also broken into small pieces, as evidenced on lung dissection and imaging of intact excised lungs. We tried several different ways to prevent this fragmentation. These attempts are reviewed in the Discussion.

Scintigraphic Images

The tiny fragmented clots were beyond the resolution of the γ-camera and were not detectable in vivo. However, clots up to 24 h old that had not yet fragmented to smaller pieces were imaged in vivo. Examples of this are shown in Figures 3 and 4. Generally, these images were diagnostic at ~ 90 min after injection of ^{99m}Tc-TP850. Also, as a function of time, the radioactivity on these lesions continued to increase (Fig. 3). The typical corroboration of scintigraphic images of the clots and their location by radiography in the lungs is given in Figures 5–7.

TABLE 2
DVT and PE Radioactivity Ratios as a Function of Time (Range)

Age (DVT/PE) (h)	n	V/B	V/M	P/B	P/L	P/M
4	2	7.9–22.6	31.1–89.4	1.0–155.0	0.82–245.2	3.6–654.4
24	5	1.0–5.8	1.9–20.2	0.07–4.8	0.74–7.9	0.9–14.6
48	6	1.1–4.8	2.4–75.1	0.68–6.5	0.15–63.7	4.1–23.9
72	3	0.4–13.4	3.4–53.7	1.3–5.1	2.6–10.1	5.9–22.7
96	9	0.5–1,261.9	2.3–5,961.9	0.5–35.0	0.9–53.2	2.4–155.1
120	1	2.51	8.0	1.6–13.8	2.1–18.8	5.2–43.7

n = number of animals used; V = DVT; P = PE; M = muscle; B = blood; L = lung.
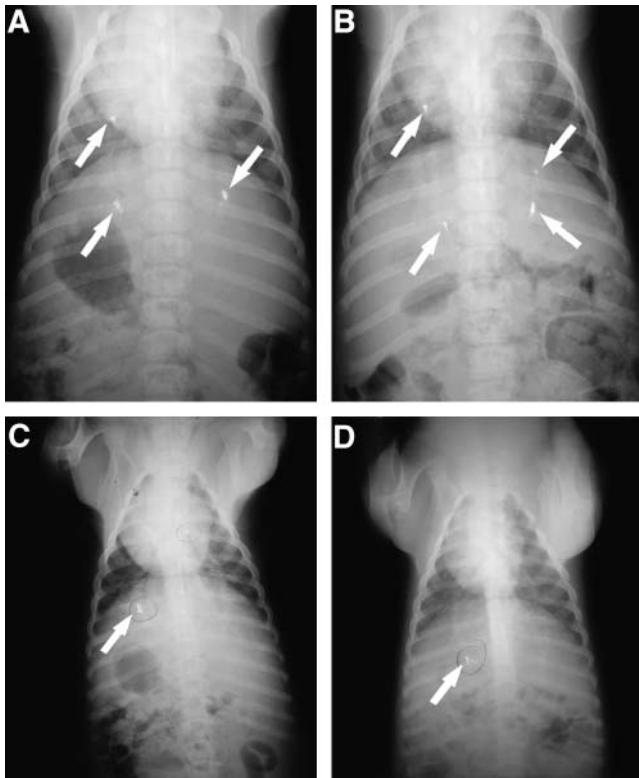Total number of animals used was 27. One animal died during the experiment and is not included.

FIGURE 2. Two (anterior) radiograms of the chest of a swine show PE on the day of induction (A, arrows) and their fragmentation 96 h later (B, arrows) (portion of a clot or clots that did not have tantalum is also fragmented but cannot be located in intact animals). Orientation is such that cephald is on top and caudad is on bottom. (C and D) Two additional (anterior) radiograms of a different swine that show the fragments of PE had moved to the lower part of the lung lobes. Orientation as in A and B.

FIGURE 3. A composite of sequential (anterior) images of a swine (A–D) inoculated with clots (PE) 24 h earlier. Orientation is such that animal is lying on its back, cephald is at right, and caudad is at left. Hot spot in the ear is the site of injection and the one in the center of the neck (C and D) is from the esophageal injury from the ventilation tubing. In image A (45 min after injection), no abnormal accumulation in the lungs is seen. In image B (57 min after injection), 1 hot spot (arrow) is seen and in images C (180 min after injection) and D (240 min after injection), 4 hot spots (arrows) developed. Note the increasing activity in these PEs as a function of increasing time after injection. (A–D). Diminishing cardiac blood-pool activity is noticeable in all images and is consistent with the rapid blood clearance.

DISCUSSION

PE is a relatively common and potentially a fatal disorder; yet it remains underestimated, underdiagnosed, and undertreated disease despite the continued evolution of diagnostic imaging techniques over several decades (22,23). While developing novel ways to detect DVT or PE noninvasively by scintigraphic imaging, agents that will target the glycoprotein IIb·IIIa complex expressed on activated platelets have been evaluated. However, activated platelets—most of which acreed on a thrombus during its early phase, and a lesser quantity on preformed blood clots—prevent these agents from accumulating enough radioactivity required for visualization of that clot. Fibrin, on the other hand, remains a significant component of a clot, at all times irrespective of whether the clot is fresh or several days old. However, fibrin as a target for imaging DVT or PE has drawn relatively less attention.

We hypothesized that a ^{99m}Tc-peptide that may have an avid affinity for fibrin may facilitate a noninvasive localization of DVT or PE. Our data showed that the radiolabeled peptide TP850 has a modest ($K_d = 8 \times 10^{-9}$ mol/L) affinit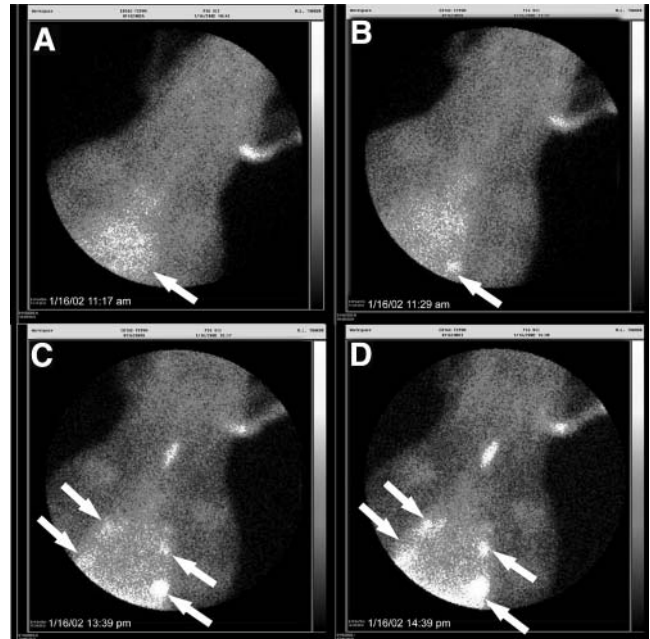y for fibrin and there are $>10^{15}$ binding sites per milligram of fibrin. On an average, each gram of a clot may have 50 mg of fibrin. Even if the ^{99m}Tc-TP850 molecules bind to only a small fraction of these binding sites (e.g., 10^9 binding sites, all bound to ^{99m}Tc-TP850, will account for 370 kBq [10 μCi] ^{99m}Tc), a sufficient quantity of radioactivity would accumulate for a reliable scintigraphic detection of a thrombus. The rapid blood clearance of this agent reduces background activity and is expected to facilitate imaging of vascular thrombosis. We believe it is for these reasons that we were able to image both DVT and PE in vivo within 90 min after injection (Figs. 3 and 4). As can be seen on subsequent images, which at times were >24 h after administration of the agents, the thrombi fragmented into small pieces, frequently weighed only ≤1 mg, and, despite the high radioactivity that they had, were not visualized by external detection. This inability may have been compounded by the low spatial resolution of the γ-camera as well as by the high attenuation of the radioactivity that may have resulted from the relatively thick pyramidal chest wall of the swine, particularly in the anterior position. These clots were detectable by imaging of excised lungs and supported this notion (Fig. 7). On many
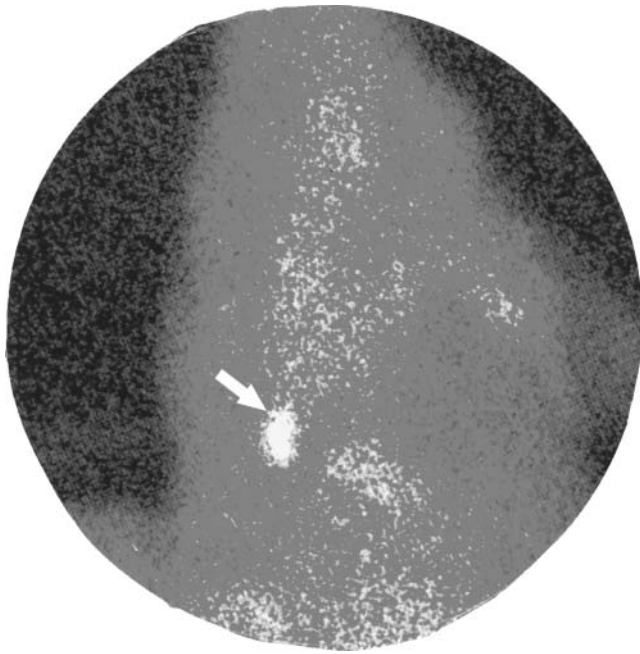
FIGURE 4. A 24-h-old thrombus in right jugular vein induced by alteration of intima. Focal hot spot corresponds to injury (arrow). Orientation is such that animal is lying on its back with cephald at top and caudad at bottom.

FIGURE 5. The 2 bottom images are the scintigram (left) and the radiogram (right) of excised lungs and heart from a swine in which PE was induced 24 h earlier. The hot spot (arrow) in the scintigram corroborates with the radiographic image of the clot in the right lung. The 2 in vivo images at the top do not discern the clot, probably because it was too small and beyond the resolution of the γ-camera. Although the average liver uptake was only 0.02%ID/g (Table 1), the liver was visible as it was the only organ in the field of view that had this high radioactivity.

occasions the scintigraphic location of these clots corroborated very nicely with their anatomic locations as depicted by corresponding radiographs (Figs. 5 and 6).

These imaging results (Figs. 3 and 4) are consistent with our previous evaluation of ^{99m}Tc-TP850 in rabbits (12). However, because of high fibrinolytic activity in rabbits, all induced clots spontaneously dissolved after 24 h and restricted our ability to demonstrate the feasibility that the agent may be useful in imaging DVT or PE older than 24 h.

The report by Craig et al. (18) demonstrates that, in swine experiments, PE can sustain for up to 7 d as the plasminogen concentration in swine is much less than that in rabbits. In our case, the PEs, though sustained for our observation period of up to 5 d and lysed, were fragmented and made no difference even when clots were produced and induced on multiple occasions using a procedure exactly identical to that described by the authors. Assuming that the addition of tantalum might have rendered the clots to be fragile and susceptible either to physiologic lysis or to the stress caused by hemodynamics, clots were prepared without tantalum. There was no avail. In fact, many clots recovered at necropsy contained tantalum and a few did not. Most of these clots weighed <2 mg but contained up to 0.03% injected radioactivity per gram of the clot. All these were visualized by scintigraphic imaging of the excised lungs. Many of these hot spots corroborated with radioopaque spots seen on radiographs.

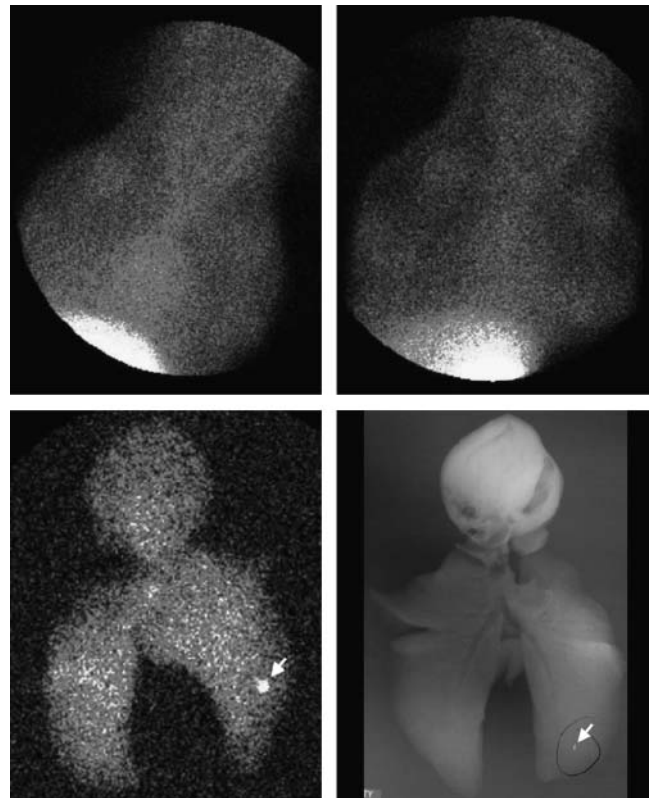Many of the DVTs were bodily adhered to the vessel wall into which they were induced and could not be separated clean of the contaminating tissue. A similar difficulty was also observed in separation of the little clots in the lungs. These resulted in large variation in associated radioactivity normalized as a percentage of administered dose per unit weight of the clot. We have, therefore, chosen to present the range of ratios without the SEM, as such statistical analysis, in this case, will not be valid.

Various investigators have used different agents and different animal models previously, but none have evaluated the efficacy of their agents for imaging experimental PE (2,4,5). DVT/blood and PE/normal lung radioactivity ratios provide a realistic measure of the detectability of these blood clots by external scintigraphy. Although ^{99m}Tc-TP850 shows the highest ratios, all agents demonstrate promising results and the ultimate choice of the agent will be determined by the ability of the agent to detect clots, DVT, or PE in humans.

^{99m}Tc-TP850 has a high affinity for human fibrin, which is an integral part of DVT as well as PE, and has an in vivo stability. It can be prepared in a high specific activity, and no more than 10 μg (~10 nmol) will be required to inject into humans, which is unlikely to induce any detectable
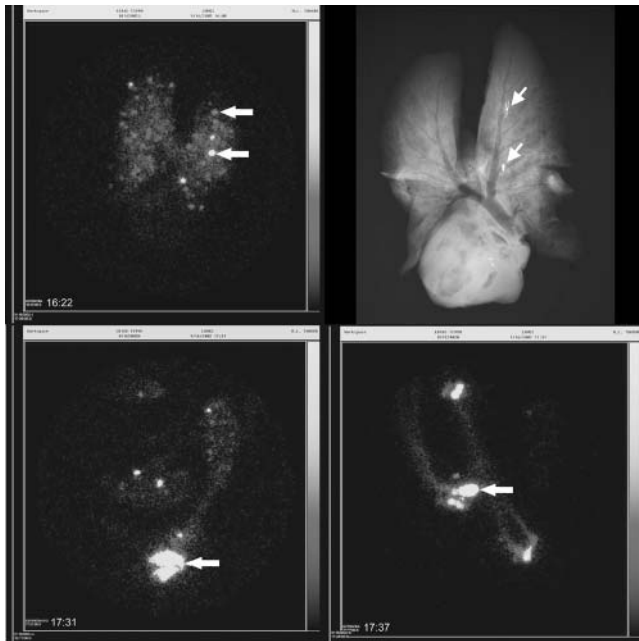
FIGURE 6. The 2 top images are the scintigram (left) and the radiogram (right) of another swine in which PE was injected, also 96 h earlier. Fragmented clots are seen in the radiographic image (top right) and many hot spots in the scintigraphic image (top left). Some hot spots (arrows) corroborate with the anatomic location of the PE seen on radiographic image (arrows) and some hot spots may be attributed to the fragmented PE that did not have tantalum and cannot be located by radiographic imaging. Several small clots were retrieved from these 2 lungs/lobes and had a high quantity of radioactivity associated with them. The 2 bottom panels show the scintigraphic images of some of the clots recovered from the bronchioles (arrows). They were imaged with a string of mucus attached to them. When separated from mucus, each weighed <1 mg.

FIGURE 7. Another scintigram of a pair of excised lungs from a third swine injected with PE 96 h earlier. Several hot spots indicate the fragmented PE.

pharmacologic effects. These qualities, together with its rapid blood clearance, make the agent a promising radiopharmaceutical for imaging DVT and PE.

In recent years, much attention has been drawn to scintigraphic detection PET. This modality offers high resolution and, therefore, may provide a better sensitivity than the corresponding SPECT imaging that can be performed with ^{99m}Tc. We have labeled peptides with a positron-emitting ^{64}Cu (t$_{1/2}$ = 0.7 h, β+ = 5 keV (17.4%)) and this radionuclide can be considered to label TP850 to enhance the ability of this agent to image fragmented, smaller clots in vivo that may not be imagable with ^{99m}Tc-TP850 (*24*).

CONCLUSION

Fibrin is an integral part of a vascular thrombosis. A fibrin-binding peptide, Gly-Pro-Arg-Pro-Pro, was synthesized with Aba as spacer and Gly-(D)-Ala-Gly-Gly as a chelating moiety. The peptide has a high affinity for fibrin and was able to localize experimental DVT and PE induced in swine. The agent has a rapid blood clearance and pro-
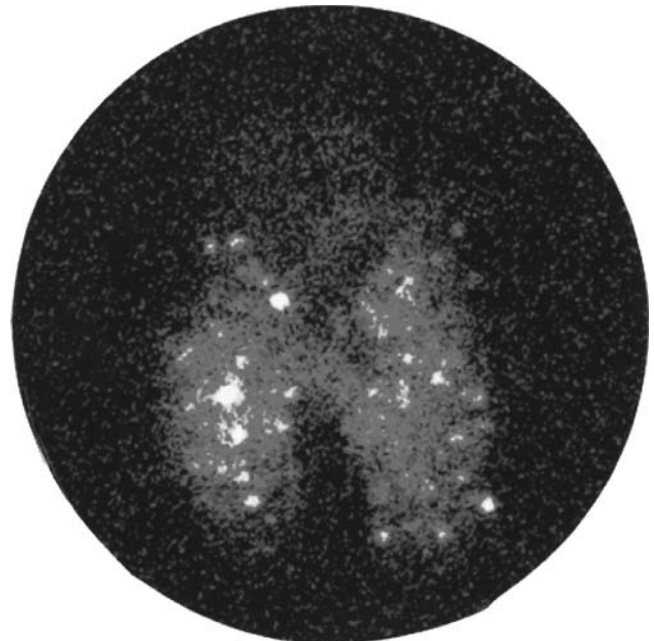
vided high DVT/blood and PE/normal lung radioactivity ratios. However, at 24 h after induction, PE and DVT were fragmented, were smaller than the spatial resolution of a γ-camera, and were detectable only in excised lungs. The PE and DVT were clearly visualized in vivo for up to 24 h after induction and rendered ^{99m}Tc-TP850 a promising agent for imaging vascular thrombosis.

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