Detecting Deep Venous Thrombosis with Technetium-99m-Labeled Synthetic Peptide P280

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Scintigraphy, using small, thrombus-avid, synthetic peptides labeled with gamma-emitting nuclides is an innovative approach to the noninvasive detection of acute deep venous thrombosis (DVT). The goal of this study was to evaluate clinically ^{99m}Tc-P280 for imaging DVT. The peptide P280 is a 26 amino acid dimer that binds with high affinity to the GPIIb/IIIa receptor expressed on activated platelets and can be labeled with ^{99m}Tc. **Methods:** Scintigraphy with ^{99m}Tc-P280 (10–22 mCi) was performed in nine patients with clinical suspicion and diagnostic evidence of DVT. Planar and tomographic images of the legs, abdomen/pelvis, chest and head were obtained immediately, 1, 2, 4 and 24 hr after injection. **Results:** No adverse effects were noted after ^{99m}Tc-P280 administration in any patient. Positive visualization of thrombi occurred in eight of nine cases with confirmed DVT within 1 hr of tracer injection. The majority of the patients had recent onset of DVT symptoms (less than 3 wk), while the only negative case was diagnosed 42 days earlier and was likely related to an accident 7 mo earlier. Thrombi-to-background ratios were essentially constant over the study. Technetium-99m-P280 accumulation was also discernible in two patients with pulmonary embolism, while in a third patient the radiotracer concentrated in a cerebellar hemangioblastoma. **Conclusion:** These human studies indicate that ^{99m}Tc-P280 is a potentially safe and sensitive procedure for diagnosing DVT and pulmonary embolism. It also may have substantial utility in monitoring active venous thrombosis.

Key Words: deep venous thrombosis; technetium-99m-labeled peptides; receptor imaging

J Nucl Med 1995; 36:1384–1391

The clinical diagnosis of deep venous thrombosis (DVT) can often be problematic because only one-third of symptomatic patients have objective evidence of the disease, and more than 50% of the patients with DVT may be asymptomatic (*1*). Among several invasive and noninvasive procedures currently used to detect DVT (*2*), none is optimally accurate, particularly in patients with relatively limited disease (*3–16*). In addition, all these procedures share the inability to differentiate acute or active thrombosis from the residual of previous episodes of DVT.

Because of these drawbacks, several radionuclide-based probes, highly specific for components of thrombi, have been investigated for the identification of newly formed thrombi. In clinical studies, one of these agents, ^{111}In-oxine-labeled autologous platelets (*17*), has been reported to identify fresh thrombi in asymptomatic patients with a specificity of 97% and a sensitivity of 93%. These rates, however, were significantly reduced when thrombi were more than 1 day old, even in symptomatic patients (*18*). In addition, the radiolabeling procedure for this method is technically complex and requires additional equipment within the radiopharmacy.

More recently, radiolabeled monoclonal antibodies (MAbs), particularly those directed against fibrin and platelets, have been used to identify acute thrombi (*15,16,20–27*). Small synthetic peptides, however, which also bind with high affinity to components of thrombi, should be cleared from the bloodstream more rapidly than the considerably larger MAb molecules, enabling earlier detection of thrombi as well as facilitating the enhancement of target-to-background ratios. These smaller molecules also should eliminate potential HAMA responses and foreign protein or virus contamination. Similar to natural fibrinogen, synthetic peptides containing the RGD sequence have been shown to bind specifically to the GPIIb/IIIa receptor expressed on activated platelets (*28–31*).

Preliminary studies in a canine venous thrombus model have shown that ^{99m}Tc-P280, which contains an RGD mimetic sequence, selectively accumulated in fresh thrombi (*32*). The thrombus-to-background ratios, measured by region of interest (ROI) analysis, averaged 2.3 at 4 hr postinjection, and external gamma imaging was positive as soon as 1 hr postinjection. Therefore, this study was conducted to determine the feasibility of externally imaging DVT with

Received June 1, 1994; revision accepted Apr. 3, 1995.

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TABLE 1
Patient Demographics, Thrombus Location and Age, Medications and Scintigraphic Results

Patient No.	Sex	Age (yr)	Body surface area (m^2)	^{99m}Tc-P280 dose (mCi)	Thrombus age (days)	Medication	Thrombus location	Foci of ^{99m}Tc-P280 uptake
1	F	34	2.0	10.7	40	Warfarin	Left popliteal and posterior tibial	Left calf
2	M	42	1.99	11.0	20	Nitrates, β-blocker, Ca channel blockers	Right posterior tibial	Right ankle and calf
3	M	34	1.80	22.1	42*	Warfarin	Left femoral Bilateral pulmonary emboli	Bilateral pulmonary emboli (Left femoral)*
4	M	30	1.97	21.9	2	None	Left iliac and femoral	Left ankle and calf Cerebellar hemagioblastoma
5	M	55	2.14	20.7	5	None	Right femoral	Right thigh
6	M	51	2.02	15.0	1	None	Left iliac, femoral, popliteal	Left thigh and calf
7	F	42	1.68	21.0	1	None	Left tibial	Left calf
8	M	38	1.97	21.0	5	Heparin	Right posterior tibial	Right calf
9	F	60	1.72	10.0	2	None	Left posterior tibial Right pulmonary embolism	Left calf Right lung pulmonary embolism

*Thrombus age and pulmonary embolism were estimated to be 42 days old, but Patient 3 also had an earlier (7 mo) episode of pulmonary embolism.

^{99m}Tc-P280 in humans and to conduct a preliminary exploration of the relationship between clinically determined thrombus age and its effect on DVT identification.

MATERIALS AND METHODS

Patients

Nine patients (3 men, 6 women; aged 30–60 yr; mean age 43 yr) participated in the study. Each patient had clinical and objective evidence of DVT and presented with clinical symptoms suggestive of DVT, which was then documented by Doppler ultrasound and/or ascending contrast venography, using the clinical impression of the interpreting radiologist. Patients were excluded if they were pregnant or lactating, if hepatic or renal function were impaired, if renal vein thrombosis was present, if they reported a history of or currently manifested thrombocytopenia, collagen vascular disease, bone marrow impairment, leukemia, polycythemia vera, thrombocytosis or myelofibrosis and if extreme obesity or any other condition was present that would interfere with the imaging procedure. The study was approved by the Ethical Committee on Studies Involving Human Beings of the National Cancer Institute of Naples, Italy. All patients provided written informed consent before study participation.

Admission to the study was designed to include three groups of patients according to the age of the thrombi or, more precisely, to the onset of clinical symptoms. Three patients were imaged within 36 hr of the initial diagnosis of the thrombus (Group 1), three patients were studied between 48 hr and 1 wk after diagnosis (Group 2) and three patients were studied more than 1 wk after diagnosis (Group 3). For seven patients, the diagnosis of DVT was made by ultrasound, in one patient by contrast venography and by ultrasound and venography for the remaining patient.

At study entry, four patients were on concomitant medications, including three receiving anticoagulant therapy. Two patients were on warfarin sodium and one was on heparin sodium. The remaining patient on medication was taking nitrates, a beta-blocker and a calcium channel blocker for hypertension and a prior anterolateral myocardial infarction. The other five patients were not being treated with any medications at the time of the scintigraphic study. Pulmonary emboli were present in two patients, documented in both patients by ventilation/perfusion lung scanning. Patient data, concomitant medications and thrombus age and location are summarized in Table 1.

P280 Synthesis

P280 [Peptech (Europe) A/S, (formerly Carlbiotech Ltd., Copenhagen, Denmark)] is a 3021.4-Da peptide composed of two identical linked 13 amino acid cyclic peptide monomers and produced by solid-phase synthesis. The final step in its synthesis was purification by preparative high-pressure liquid chromatography on a C-18 reverse-phase chromatography column using a gradient elution of acetonitrile in water containing 0.1% trifluoroacetic acid (TFA). The purified peptide TFA salt was then lyophilized to a dry white powder. The identity of the peptide was confirmed by amino acid analysis and by electrospray mass spectroscopy. The structure of P280 is shown in Figure 1.

P280 Toxicity

Four toxicology studies, two in rats and two in rabbits, were performed using P280 reconstituted with decayed ^{99m}Tc generator eluate. A reference dose of 2 μg peptide/kg permits identification of the multiple over maximum human dose (MHD).

The rat studies consisted of a 4-day multi-injection protocol at 62.5× and 125× MHD and 14-day multi-injection protocol at 75× and 250× MHD. Daily clinical observations were recorded, and necropsies were performed in all studies. Selected blood chemistries, coagulation panels, hematology and urinalyses also were performed.

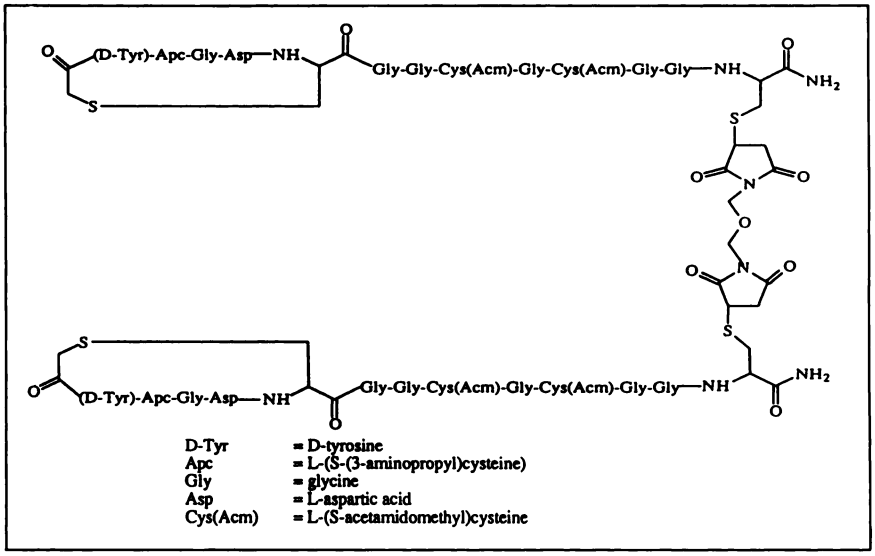

FIGURE 1. Structure of synthetic peptide P280.

No treatment-related effects could be attributed to ^{99m}Tc-P280 at any dosing level. No treatment-related gross or histopathologic lesions were observed. Blood and urine chemistries and hematology parameters showed no significant changes when compared to the control groups. The results indicate that single or multiple injections of ^{99m}Tc-P280 at doses up to 500 μg/kg (250× MHD) do not produce any significant toxicologic effects.

Peptide Dose Rationale

The dose of P280 peptide was selected to be the minimum amount necessary to provide a consistently high radiochemical yield of ^{99m}Tc-P280. No dose response was observed in any preclinical studies ranging from 2 to 50 μg/kg peptide. The single bolus dose of 250 μg P280 peptide, the maximum injected dose, is equivalent to 5 μg/kg in a 50-kg subject. Preclinical studies showed no physiologic effect at this level and toxicity studies support a 100-fold margin of safety.

P280 Labeling Procedure

The peptide was radiolabeled by a transfer reaction using the pure P280 TFA salt and ^{99m}Tc-gluceptate. Briefly, a vial of gluceptate (approximately 200 mg sodium gluceptate and 0.06 mg stannous chloride) was reconstituted with 1.0 ml of freshly eluted [^{99m}Tc]sodium pertechnetate containing approximately 100 mCi; the contents were gently mixed for 15 min. The peptide was dissolved in 0.9% sodium chloride to achieve a final concentration of 0.25 mg/ml. To facilitate P280 dissolution, the peptide solution was warmed slightly in a hot water bath. To each milliliter of peptide solution (0.25 mg), 0.25 ml ^{99m}Tc-gluceptate was added. The mixture of P280 and ^{99m}Tc-gluceptate was incubated for 15 min at 100°C in a boiling water bath and then allowed to cool. Before administration, the preparation was aseptically filtered through a low-protein-binding, sterile 0.22 μm Millex-GV filter (Millipore, Bedford, MA).

Quality control was performed using two 10-cm instant thin-layer chromatography strips for each preparation. A 10-μl aliquot of ^{99m}Tc-P280 was placed on each strip. Phosphate-buffered saline and saturated sodium chloride solutions were used as solvents to measure the percentage of ^{99m}Tc-colloid and ^{99m}Tc-gluceptate and/or pertechnetate, respectively. The solvents were allowed to migrate to the top of the strip, then the strips were cut at R_f 0.5 and the activity associated with each portion was measured in a dose calibrator. Product quality was considered acceptable for administration if net labeling was ≥95%.

Imaging Technique

The injected dose of ^{99m}Tc-P280 ranged between 10 and 22 mCi and contained approximately 125–250 μg P280 peptide. The dose was delivered by intravenous bolus administration. Multiple planar images of the lower extremities, abdomen/pelvis, chest and head were obtained immediately and 1, 2–4 and 24 hr postinjection, using a LFOV gamma camera equipped with a high-resolution collimator (photopeak 140 keV, window ± 20%). Region of interest analysis was performed on the images stored by a dedicated computer. SPECT images of the legs and chest were acquired 2–4 hr postinjection; 64 images were acquired, one every 5°6′ with an acquisition time of 40 sec. The counts per view ranged from 30,000 to 60,000. Reconstruction was performed by a back-projection algorithm using a Shepp-Logan-Hanning filter. Attenuation correction was performed using Sorenson's method, and the correction coefficient was 0.15 cm^{-1}.

The most representative slices from reconstructed studies were summed together and ROI analysis was applied to measure the thrombus-to-background ratios and to make preliminary assessment of the target organ for radiation dosimetric purposes.

RESULTS

Technetium-99m-P280 Safety

The ^{99m}Tc-P280 imaging procedure was well tolerated by all nine patients. No adverse reactions were observed after peptide injection or during the study period. In addition, no laboratory values (clinical chemistry and hematology parameters) reflected any significant or otherwise unexplainable changes.

Technetium-99m-P280 Biodistribution

In the two patients who gave consent to repeat blood sampling, less than 5% of the injected dose was still circulating 1 hr after intravenous injection of the agent. Rapid blood clearance also is well documented by whole-body serial images obtained at varying times after injection (Fig. 2 is representative). As predicted from the animal model,

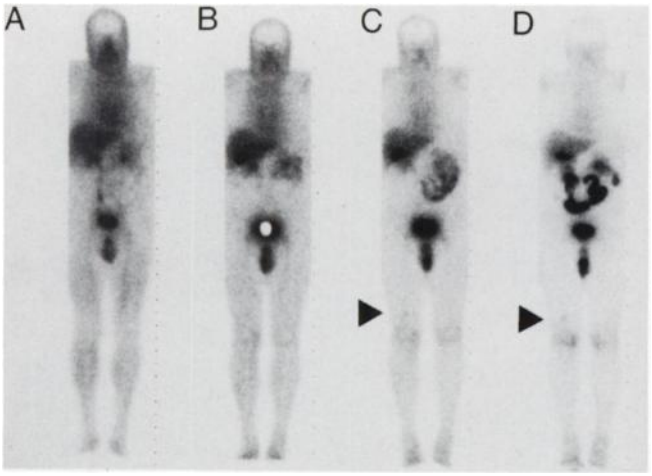

FIGURE 2. Anterior whole-body images (1000 kcts) collected at (A) 15, (B) 60, (C) 120 and (D) 240 min after intravenous injection of ^{99m}Tc-P280. Excretion occurred rapidly after injection through both the renal and hepatobiliary systems. The thrombus, located in the right thigh (arrowheads), became diagnostic between 120 and 240 min after injection.

the renal system was the main route of excretion (60%–75% ID). Thus, repeat voiding was required between imaging procedures to preclude activity in the urinary bladder from potentially masking iliac thrombi. Between 10% and 30% of radioactivity was excreted by the liver. The intrahepatic biliary ducts and gallbladder were visualized within 10 to 30 min after ^{99m}Tc-P280 administration. Activity was present in the small bowel as early as 30 min. The pharmacokinetic behavior of labeled peptide was quantified by ROI analysis. Figure 3 presents the pharmacokinetic profile of a representative patient.

Thrombus Imaging

Thrombus visualization was evident by planar imaging as early as 15 to 30 min after injection. The thrombus-to-background ratios in the images collected between 1 and 4 hr were essentially constant. The foci of agent uptake are summarized in Table 1. Uptake correlated well with DVT localization obtained by conventional techniques in eight of nine patients. Technetium 99m-P280 did not localize in the femoral thrombus of Patient 3. Deep venous thrombosis was diagnosed by ultrasound in that patient 42 days before the scintigraphic study, at the time of a second known pulmonary embolic event. The patient's clinical history suggested that the DVT had first developed 7 mo before and contributed to an earlier initial pulmonary thromboembolic event. This patient was on chronic warfarin sodium treatment. Notably, ^{99m}Tc-P280 was able to detect two pulmonary emboli in this patient. Although the thrombi may have been of more recent origin than the event of 42 days earlier, we could not confirm this possibility. The agent also visualized a known recent (within 36 hr) pulmonary embolism in Patient 9. Peptide uptake within the right lung correlated with the photopenic area seen on the lung perfusion scan (Fig. 4). In addition, ^{99m}Tc-P280 serendipitously detected a cerebellar hemangioblastoma, also documented by other modalities, in Patient 4 (Fig. 5). This uptake was considered to be in the tumor. No contiguous thrombosis, only edema, was felt to be present by CT.

SPECT reconstruction provided no advantage in DVT detection compared with that available from planar views. An example of the similarity in available data from the two methods is shown in Figure 6. Conversely, SPECT was pivotal in the detection of pulmonary emboli, which were not visible in the planar views. The thrombus-to-background ratios from 1 to 24 hr postinjection measured from planar images ranged between 1.1 and 2.6 (Table 2). The lowest overall ratios were measured for the two oldest thrombi (40 and 42 days, respectively). Ratios did not vary widely among thrombi less than 120 hr old; only one patient with a thrombus less than 120 hr old received anticoagulant drugs. Nevertheless, the highest ratios tended to be found in patients studied earliest after the clinical event,

Time	Kidney	Liver	Heart	Thrombus	Background	Lower Abdomen	Bladder
0	1600	1300	1600	610	320	1200	1700
1	1800	1400	1200	710	380	1100	1900
2	1600	1900	1009	560	376	1500	1700
4	1575	1700	930	480	310	1000	1100
24	845	1000	501	399	218	758	423

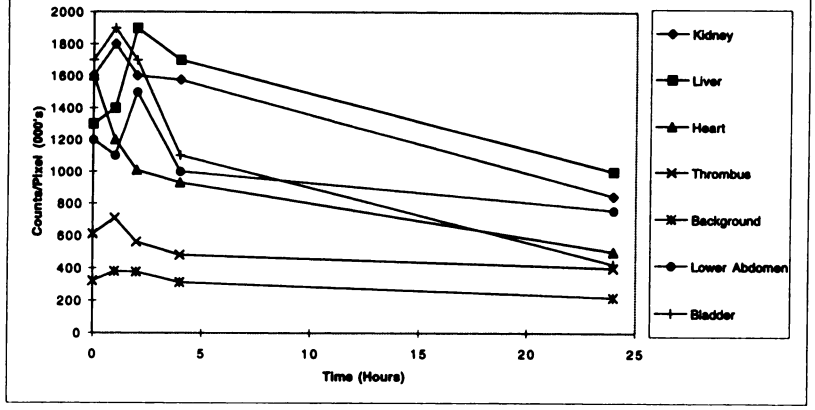

FIGURE 3. Technetium-99m-P280 pharmacokinetics from ROI analysis.

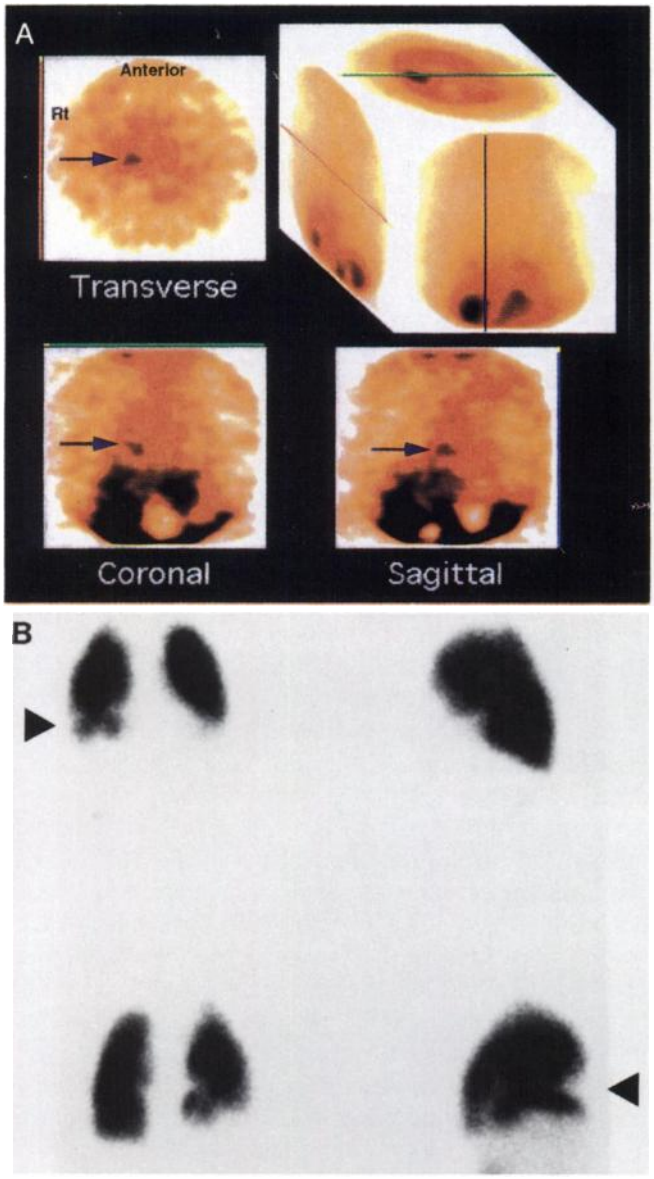

FIGURE 4. (A) SPECT reconstructions performed 2 hr postinjection detected a small pulmonary embolus in the lower lobe of the right lung (arrows). (B) Anterior, left lateral, right lateral and posterior planar views of the lung perfusion scan, in which a photopenic area is evident in the same pulmonary segment as the focus of ^{99m}Tc-P280. Pulmonary angiography results also corroborated the peptide study results.

while they were yet unmedicated. Although some scans were diagnostic as early as 15 min postinjection, definition of the thrombus generally improved over the first hour as adjacent blood-pool activity cleared. The uptake of ^{99m}Tc-P280 within thrombi at different times postinjection (Figs. 7 and 8), indicated that the optimum time for imaging was between 1 and 3 hr; delayed scans were not generally necessary for diagnosis. The apparent nonvascular uptake seen in these figures is characteristic of this agent. The explanation is unknown, but may include localized edema, involvement of activated platelets or vascular uptake in small vessels (below the resolution of the imaging system).

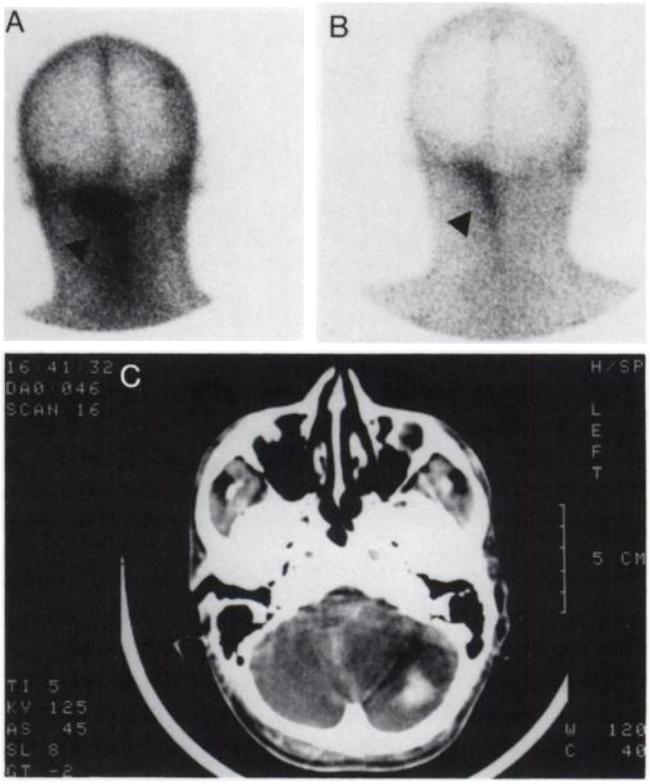

FIGURE 5. Cerebellar hemangioblastoma serendipitously detected by ^{99m}Tc-P280 in a patient with DVT. Posterior views of the skull acquired (A) 15 and (B) 240 min after peptide injection clearly indicate the lesion, subsequently confirmed by (C) CT.

DISCUSSION

Platelet activation and deposition are the initial events in acute thrombus formation and may continue for a variable period as thrombus organization progresses. Activated platelets express the cell surface integrin glycoprotein IIb/IIIa (GPIIb/IIIa) in a form that binds the peptide sequence arginine-glycine-aspartate (RGD) present in several vascular proteins, including fibrinogen, von Willebrand factor, vitronectin and fibronectin (*28–31*). Nonactivated platelets express virtually none of the receptor in its active conformation. Therefore, the GPIIb/IIIa receptor makes an excellent target for a thrombus-imaging radiopharmaceutical.

The peptide P280 is a small oligopeptide made of two identical, linked, cyclic, 13 amino acid monomers. It can be labeled with ^{99m}Tc and binds with high affinity to the GPIIb/IIIa receptor of activated platelets. In an assay for inhibition of aggregation of ADP-activated human platelets to assess receptor-binding affinity, ^{99m}Tc-P280 has an IC_{50} (concentration at which platelet aggregation is inhibited by 50%) of 0.087 μM. In comparison, echistatin, a small snake venom protein that is one of the most potent, naturally occurring GPIIb/IIIa binding agent yet identified, has an IC_{50} of 0.03 μM. Technetium 99m-P280 also has been shown to provide images of experimentally induced acute thrombi in a canine model of DVT as early as 60 min after injection (*32*).

Our results indicate that peripheral venous thrombi can

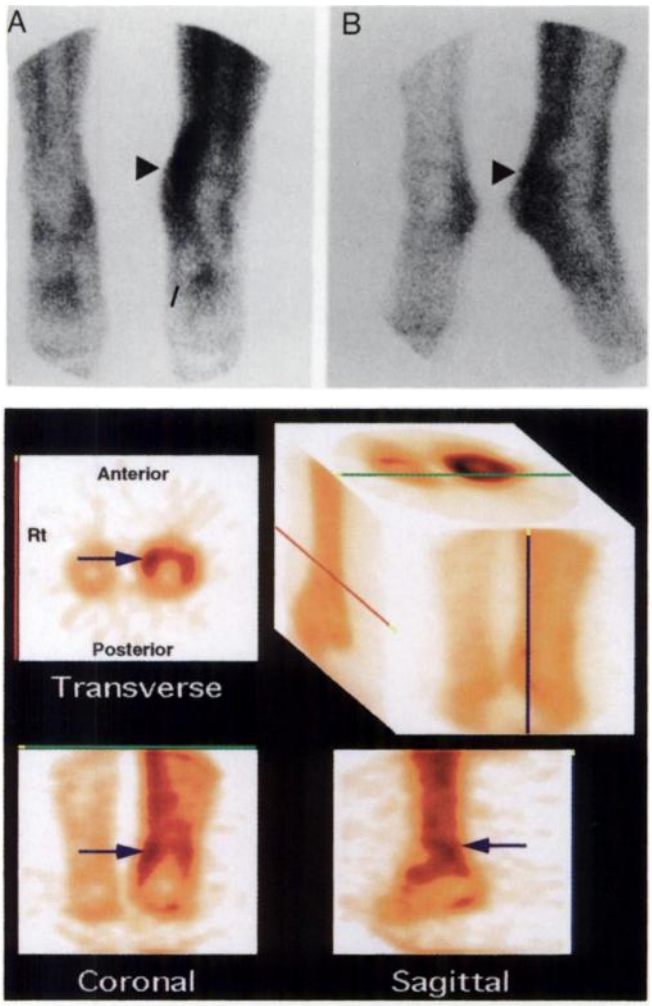

FIGURE 6. Thrombus uptake of ^{99m}Tc-P280 is evident in both scans of the ankles acquired (A) 5 and (B) 180 min postinjection. Tomographic reconstruction (C) did not facilitate thrombus diagnosis.

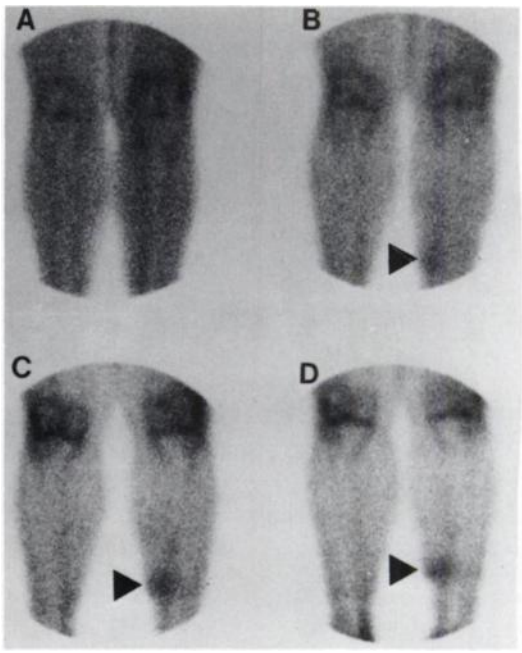

FIGURE 7. Serial images of the calves acquired (A) 30, (B) 60, (C) 120 and (D) 240 min after ^{99m}Tc-P280 administration. The thrombus, located in the left calf, is visualized by 1 hr but is better defined in the later scans when the background activity has cleared.

be visualized noninvasively when ^{99m}Tc-P280 is administered intravenously in association with external gamma camera imaging. The small number of patients evaluated in this initial human study with the agent precludes both the expectation of statistical reliability in determining diagnostic sensitivity and the inference that our population truly was representative of the larger ambient population with venous thrombi. Nonetheless, identification of thrombi in eight of nine patients in whom thrombi had been detected by conventional methods is consistent with the premise that ^{99m}Tc-P280 is efficient in venous thrombus detection. This finding suggests the appropriateness of further study in evaluating the agent's diagnostic accuracy over a wide range of patients, thrombus ages and apparent thrombus sizes and locations, both alone and in comparison with other noninvasive methods.

A primary clinical advantage of ^{99m}Tc-P280, as suggested by our results, is the rapid visualization of venous thrombi in comparison with radiolabeled antifibrin antibodies. This characteristic is likely to be related predominantly to the low molecular weight and pharmacokinetic behavior of ^{99m}Tc-P280 in addition to its avid receptor binding affinity. It is possible that peptide uptake within thrombi, and consequent visualization, may be due to hypervascularization of the area or other nonspecific phenomena rather than to specific binding to the thrombus. In the present study, we did not use a nonspecific peptide as a negative control. Knight et al. (*31*), however, have shown that a nonspecific peptide failed to concentrate at the site of the thrombus in a canine model of DVT. Thus, it is likely that the observed localization of ^{99m}Tc-P280 at the thrombus is attributable to a specific process.

In theory, platelet activation and consequent exposure of the GPIIb/IIIa receptor should be of relatively short duration, particularly after administration of anticoagulants such as heparin and warfarin. In two of our patients, however, one on heparin and one on warfarin, thrombi were visualized several days after diagnosis and treatment, suggesting that efficacy may be preserved even if therapy has begun, as is often necessary in the clinical environment. This observation needs further evaluation in an appropriately selected population.

Thrombi were seen in two patients 20 and 40 days after clinical diagnosis, suggesting the possibility of noninvasive identification of thrombi even in the subacute stage or of an ongoing thrombogenic potential in these patients. The maximum age at which a thrombus can be imaged with ^{99m}Tc-P280, the factors which modify this characteristic and the likelihood of detecting a thrombus as a function of its age remain to be determined.

None of the patients experienced adverse reactions to

TABLE 2
In Vivo Thrombus-to-Background Ratios

Patient no.	Thrombus age	Time after Injection (hr)					
		0.1	1	2	3	4	24
1	40 d	ND	1.3	1.06	ND	1.4	ND
2	20 d	ND	1.5	1.5	1.8	ND	ND
3	42 d	ND	1.0	ND	0.9	1.0	1.0
4	48 hr	2.0	1.5	ND	1.8	ND	1.57
5	5 d	1.1	1.1	1.5	1.6	1.7	1.38
6	24 hr	2.2	2.1	1.9	1.8	2.3	1.58
7	24 hr	1.4	1.4	1.8	1.4	2.1	1.63
8	5 d	1.9	1.7	1.9	2.0	1.9	1.36
9	36 hr	3.34	2.6	2.6	ND	2.3	ND
Mean ± s.d.	13 ± 17	2 ± 0.76	1.6 ± 0.5	1.8 ± 0.4	1.6 ± 0.4	1.7 ± 0.5	1.4 ± 0.5

ND = not done.

the peptide. In humans, as in animal models, the main route of excretion for ^{99m}Tc-P280 was the renal system. A fraction of the dose, ranging from 10% to 30%, was excreted by the hepatobiliary system. This secondary route of excretion is probably related to the lipophilicity of the compound. From the biodistribution data, the bladder is likely the critical organ for human dosimetry; in an animal model, the estimated dose to the bladder is 140 mrem/mCi (Stubbs J, *personal communication*, 1994).

The ability to image pulmonary emboli or neovascular tumors with ^{99m}Tc-P280 would substantially broaden the diagnostic scope of this agent. Pulmonary embolism is the most frequent and serious complication in patients afflicted with DVT. Thus, a noninvasive imaging procedure permitting detection both of pulmonary embolism and of the DVT from which it originated would be highly desirable (*33–35*). Further study will be needed to define the capacity of ^{99m}Tc-P280 to enable such imaging. Similarly, our intriguing serendipitous identification of a cerebellar hemangioblastoma indicates the need for further study, particularly involving in vitro assessments to distinguish between nonspecific trapping of the agent in the modified capillary network of the tumor versus binding to GPIIb/IIIa receptors expressed on neoplastic cells or neovasculature (*36*).

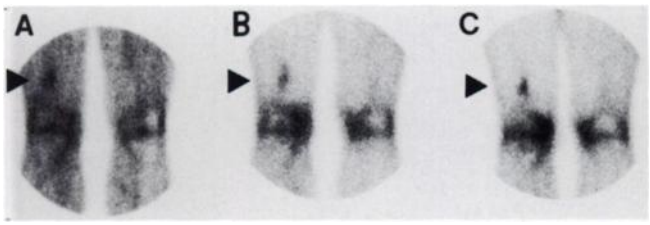

FIGURE 8. Anterior views of the knees collected (A) 30, (B) 120 and (C) 240 min after ^{99m}Tc-P280 administration clearly indicate the thrombus in the right thigh. The decrease in background activity provides better contrast in the later scans, but the images are diagnostic in the early views.

CONCLUSION

Early after intravenous administration, ^{99m}Tc-P280 can provide diagnostic images of DVT. Because of its binding affinity and molecular size, which facilitates rapid blood clearance, ^{99m}Tc-P280 imaging may overcome some of the inaccuracies and practical disadvantages of other imaging modalities. Further clinical trials are warranted to evaluate the full potential of this agent, including not only its clinical utility in DVT but also in the detection of pulmonary embolism, arterial thrombi and vascular tumors.

ACKNOWLEDGMENTS

Financial support for this study was provided by Diatech Inc., Londonderry, NH.

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