Thrombus Imaging with Technetium-99m Synthetic Peptides Based upon the Binding Domain of a Monoclonal Antibody to Activated Platelets

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Monoclonal antibodies which recognize fibrin or platelets have enabled imaging of vascular thrombi, however, early imaging has been difficult because of the slow blood disappearance of even small antibody fragments. It was theorized that it might be possible to synthesize peptides which possess the same thrombus affinity as monoclonal antibodies, but which would leave the blood pool much more rapidly. **Methods:** In this study, peptides were synthesized with amino acid sequences based on the primary binding region of the platelet glycoprotein IIb/IIIa-directed monoclonal antibody PAC1. Both termini of the peptides were blocked to prevent rapid proteolysis and a metallothionein-derived sequence was incorporated as a chelating agent for reduced technetium. **Results:** Technetium-99m-labeled peptides produced images of fresh clots in the jugular veins of rabbits and day-old thrombi in the femoral veins of dogs within 2 hr after injection. In control experiments, a ^{99m}Tc-labeled nonspecific peptide failed to produce focal images of thrombus. Another control compound, ^{99m}Tc-glucoheptonate, did produce images of fresh clots in rabbits but failed to produce focal images of day-old thrombi. As was hoped, blood clearance of the ^{99m}Tc peptides was rapid, with excretion through the kidneys, however, none of the peptides studied had better thrombus-to-blood ratios than iodinated fibrinogen and all had significantly lower deposition in the thrombus. **Conclusion:** Using labeled synthetic peptides appears to be technically feasible but the absolute binding to thrombus is not yet sufficient for reliable imaging of preexisting thrombi.

Key Words: thrombus imaging; ^{99m}Tc peptides; platelet binding

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Most radiopharmaceuticals which have been investigated for their ability to bind to thrombi have been based on native proteins, antibodies and cells. Native proteins have long lifetimes in the blood which preclude imaging thrombus-bound tracer over the blood background within the first few hours after injection (*1–4*). Monoclonal antibodies (Mabs) raised against fibrin or platelets (*2,4–12*) have received attention recently because they have highly specific molecular recognition regions which can be targeted to specific sites on preformed thrombi. In principle, Mabs can be proteolytically degraded to fragments to increase the rate of blood disappearance, but experience with antifibrin antibodies has shown that even Fab fragments usually leave the blood pool too slowly to provide a reliably rapid diagnosis (*8*). As a potential solution to this problem, it was theorized that synthetic oligopeptides, with amino acid sequences mimicking the active binding regions of Mabs or native glycoproteins, might be used to carry a radiotracer to thrombi. The synthetic peptides would be expected to exhibit more rapid blood clearance than the larger proteins and thus potentially permit earlier imaging of a thrombus. In these studies, peptides based on the active binding region of Mab PAC1, which binds to activated platelets (*13*) were evaluated for their ability to bind to thrombi in animal models.

MATERIALS AND METHODS

Source of Peptides

The peptides tested in this project were prepared to order (Bachem California, Torrance, CA) by conventional solid-phase peptide synthesis (Table 1) (*14*). Briefly, a polypeptide chain was constructed one amino acid at a time, beginning with the carboxy-terminal amino acid, which was covalently bound to a solid polymer resin to facilitate washing away of byproducts. Using a carbodiimide reaction, the carboxy group of an added amino acid formed a peptide bond with the free amino (NH_2) group on the end of the growing peptide chain. For this to work efficiently, each amino acid added to the chain had its own NH_2 group blocked with a *t*-butyloxycarbonyl (BOC) ester so that the only NH_2 groups available for reaction were those on the end of the peptide attached to the solid support. After each cycle of adding an amino acid to the chain, the BOC-protecting groups were removed, preparing the growing peptide to react with the next amino acid in the sequence. The addition of succeeding amino acids was per-

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TABLE 1
Peptides Tested in Vivo

Peptide	Amino acid sequence	IC_{50}* (μM)
PAC-2	ac-ARRSPSYYRGDGAPYYAMDYKCTCCA-am	9
PAC-3	ac-ARRSPSYYRYYDGAPYYAMDYKCTCCA-am	>500
PAC-8	ac-SYGRGDVRGDFKCTCCA-am	12
PAC-15	ac-KCTCCAPSYYRGDGAPSYYRGDGAPSYYRGDA-am	3.5
PAC-19C	ac-SGAYGSRGDGKCTCCA-am	82
PAC-25	ac-PSYYRYDGAKCTCCA-am	68
PAC-26	ac-PSYYRYDGAPSYYRYDGAKCTCCA-am	47

*IC_{50} = concentration of peptide required to inhibit rate of human platelet aggregation by 50%.
ac = acetyl; am = amide; A = Ala; C = Cys; D = Asp; F = Phe; G = Gly; R = Arg; S = Ser; K = Lys; M = Met; P = Pro; T = Thr; V = Val; and Y = Tyr.

formed one at a time in a stepwise manner until the desired sequence was assembled. After the last amino acid was added to the chain, the final step was the removal of the peptide from the solid support by treatment with hydrogen fluoride which resulted in protection of the carboxy terminus with an amide group. The NH_2-terminus was protected by reaction with acetic anhydride, which served to acetylate the NH_2-terminus. The purity of the peptides was reported to be greater than 95% (as judged by 280-nm area % on a Dynamax 300 Å C-4 column with a 0–80% acetonitrile gradient in 5% acetic acid over 30 min).

Peptide Testing for Inhibition of Platelet Activation

The activity of the peptides with respect to binding to the fibrinogen receptor on platelets was evaluated by adding various concentrations of a peptide to human platelet-rich plasma and measuring the turbidimetric response to 10 μM ADP in a Payton aggregometer (Buffalo, NY) stirred at 1000 RPM (*15*). The concentration required to halve the initial rate of aggregation (IC_{50}) was determined.

Radiolabeling

The peptides all contained the amino acid sequence KCTCCA (Lys-Cys-Thr-Cys-Cys-Ala) in order to complex reduced technetium. Labeling was accomplished by transchelation from the technetium glucoheptonate complex. Technetium-99m-glucoheptonate was prepared using a commercial kit (Glucoscan, DuPont, N. Billerica, MA), which was reconstituted at a concentration of 40 mCi/ml. Typically, equal volumes of ^{99m}Tc-glucoheptonate and KCTCCA-containing peptides (approximately 0.5–1 mg/ml in isotonic saline) were combined and incubated for 1 hr at room temperature or 37°C. The radiochemical purity was assessed by instant thin-layer chromatography on ITLC-SG media, developed in 0.9% NaCl. Iodine-125-fibrinogen was prepared by radiolabeling human fibrinogen (Kabi Diagnostika, Stockholm, Sweden) with $Na^{125}I$ (ICN) using the iodine monochloride method (*3*).

Stability In Vitro

Samples of labeled peptide were incubated at 37°C with citrated human blood for 24 hr. To evaluate stability, samples of the incubate were removed at various times and centrifuged in an Eppendorf microfuge at 14,000 rpm for 5 min to separate cells. The cell pellet and plasma fractions were counted to determine the percentage of total counts associated with the cells. The resultant plasma was subjected to several types of analyses: instant thin-layer chromatography (ITLC) as described above; gel filtration HPLC (TSK-3000 column, eluted with phosphate-buffered saline, pH 6.0); and reverse-phase HPLC on a C-6 column eluted with an acetonitrile gradient of 0 to 60% in 20 mM sodium phosphate, pH 6.0. Both columns were attached to Beckman 170 in-line radiation detectors.

Thrombus Imaging Studies In Vivo

The ability of the ^{99m}Tc-labeled peptides to image thrombi was assessed in animal models (36 rabbits and 7 dogs). These studies were reviewed and approved by the Institutional Animal Care and Use Committee before the work was begun.

A model of fresh thrombi was created in rabbits by a method similar to that of Collen (*16*). Thirty-six New Zealand white rabbits weighing 1.8–3.2 kg were preanesthetized by intramuscular injection of 40 mg/kg ketamine hydrochloride and 0.4 mg/kg acepromazine maleate. For surgery, sodium pentobarbital (10 mg/kg) was administered intravenously. Additional doses of anesthetic were administered as needed to maintain anesthesia. In each rabbit, a clot was induced in the left jugular vein and the right jugular vein was used as a control. The surgical procedure described by Collen was used, except that a silk thread was used to anchor the clot, and the jugular vein was not cannulated nor was the blood removed from the vein. Ten units of bovine thrombin (Parke-Davis) in 0.1 ml of saline were injected into a segment of the left jugular vein using a 27-ga needle. After 30 min, the clamps were removed from the vein and the incision was sewn closed. Within 30 min after closing the wound, the radiotracers (74–259 MBq [2–7 mCi] ^{99m}Tc-peptide and 0.93–1.85 MBq [25–50 μCi] ^{125}I-fibrinogen) were administered through a butterfly infusion set placed in a marginal ear vein on the contralateral side. Isotonic saline was used to flush the butterfly after injecting each tracer. The usual dose of peptide was 50 μg per animal, however, in the case of PAC-8, animals were studied with three different dose levels (5, 50 and 500 μg).

In order to assess the behavior of some of the peptides in a model with more mature lesions, thrombi were induced in seven mongrel dogs (11–17 kg) by transcatheter placement of embolization coils in their femoral veins, as previously described (*9*). A radiograph without contrast was obtained to document the location of each coil after placement. The thrombi were allowed to age on the coils for 24 hr before administration of the radiotracers. Then 148–407 MBq (4–11 mCi) ^{99m}Tc-peptide and 1.48–2.59 MBq (40–70 μCi) ^{125}I-fibrinogen were injected into a foreleg vein and were flushed in with isotonic saline.

Before injection, each animal was positioned for an anterior view of the chest, using a large field of view gamma camera (MaxiCamera, General Electric, Milwaukee, WI). The camera was fitted with a low-energy, all-purpose collimator and was set to acquire the 140-keV photopeak of ^{99m}Tc with a 20% window. A Macintosh IIx computer was interfaced to the camera using a NucLear Mac acquisition board and software (Scientific Imaging, Denver, CO). Initially, the computer was set to acquire a dynamic series of 10-sec frames for a total of 10 min. The acquisition was begun just before injection of the radiotracers. At hourly intervals, additional 10-sec static anterior views of rabbits' chests were acquired. These images were used to determine the rate of blood disappearance. In each frame, a region of interest (ROI) was drawn around the heart. The counts in the region of the heart were decay-corrected and expressed as a percentage of the maximum counts in the heart region.

Immediately after completion of the initial dynamic acquisition, and at approximately hourly intervals thereafter, rabbits were repositioned to obtain anterior views of the head and neck, and dogs were repositioned to obtain an anterior view of both hind legs. Static images were acquired in a 256 × 256 matrix and 500,000 counts were accumulated in each image.

As negative controls, ^{99m}Tc-glucoheptonate or peptide PAC-3 were administered in place of the ^{99m}Tc-peptide in additional rabbits and dogs with induced thrombi. The rest of the experiment was carried out in the same way. Iodine-125-fibrinogen was used as a positive control in each animal.

At 4-hr postinjection, a blood sample was drawn and animals were euthanized with a bolus intravenous injection of T-61 euthanasia solution (American Hoechst, Animal Health Division, Somerville, NJ). The vein segment containing the thrombus was removed and the thrombus was separated from the vessel wall and coils (dogs) or threads (rabbits). Samples of the control vessel (uninjured vein from the same site on the contralateral side) and skeletal muscle were also taken. All samples were weighed and counted for ^{99m}Tc content in a NaI(Tl) well counter. An aliquot of the injected dose was diluted and a portion was counted with the tissue specimens in order to relate the counts in each specimen to the injected dose. Samples were re-counted 1 wk later (after ^{99m}Tc had decayed) to determine ^{125}I content of the samples.

Image Analysis

Irregularly shaped ROIs were drawn on the images to encompass the area of thrombus. The average counts per pixel in the ROI was determined. Similarly sized ROIs were applied to the adjacent background muscle area on the thrombus side (= bkg) and to the area corresponding to the thrombus area on the contralateral side (= contr). The average counts per pixel were also determined for these ROIs. These results were reported as ratios of thrombus/bkg and thrombus/contr.

Statistical Analysis

Results of the in vivo studies were compared for all the test compounds using factorial analysis of variance (ANOVA). This statistical test is appropriate for comparing three or more test situations in which a different group of animals was used for each test situation. For each determined quantity (e.g., %ID/g thrombus), the values were grouped according to the peptide tested, and each group compared with all other groups ($p \leq 0.05$ was considered significant). This analysis was performed on a Macintosh IIcx personal computer using StatView II software (Abacus Concepts, Berkeley, CA).

RESULTS

In Vitro Studies

The activity of the peptides with respect to inhibition of platelet aggregation (IC_{50}) is reported in Table 1. Lower IC_{50}s indicate higher potency for inhibiting platelet aggregation. PAC-2, which is based on the sequence of the PAC1 antibody, had much better activity than PAC-3 in which the RGD sequence was disrupted by substituting RYYD. PAC-19C, which is a much shorter peptide than PAC-2, lost some of its potency. Although also short, PAC-8 was synthesized to contain two RGD subunits, which improved its activity about sevenfold over PAC-19C. Peptide PAC-15 which included three RGD subunits had even better activity.

Radiolabeling

Technetium-99m-glucoheptonate consistently had a radiochemical purity of >99%. After reaction with the peptides, the radiochemical purity of labeled peptide averaged 95.7% ± 0.5% (mean ± s.e.m.). No further purifications were performed.

Stability In Vitro

Approximately 5% of radioactive peptide added to citrated blood was found in the cell pellets and approximately 10% was found in the high molecular weight region of the gel filtration column. These percentages did not change with longer incubation times, indicating that binding of peptide to resting platelets or other cells, or binding to plasma proteins were not major in vivo fates of the labeled peptide. Therefore the primary stability concerns related to the approximately 85% of counts present as free species in the plasma.

Both ITLC and reverse-phase HPLC indicated "release" of up to 20% of plasma counts in the first 4 hr and 30% by 24 hr. The "released" counts ran to the solvent front on ITLC (whereas initial labeled peptide remained at the origin) and on HPLC the "released" counts eluted within 3 min (versus 19 min retention for the labeled conjugate). The chemical nature of these "released" counts was not established, however, it is unlikely to be free pertechnetate, since thyroid and stomach activity were not observed in the in vivo imaging studies. It is possible that these counts represented labeled peptides smaller than the original peptide, because in our experience, peptides shorter than seven residues can migrate on ITLC.

Thrombus Imaging Studies in Rabbits

With all RGD-containing peptides tested, the radioactivity left the blood pool quickly (Fig. 1) and appeared in the kidneys. Figure 2F shows the distribution of radiotracer in a rabbit's body at 10 min postinjection. Although the cardiac blood pool was still visible at this time, the kidneys were even more prominent and the urinary bladder contained excreted activity. The percent administered dose in the blood decreased to about 2%–3% by 4 hr. When peptides containing the RGD sequence were used, clots were often visible within 1 hr, and were focally positive by 2 hr

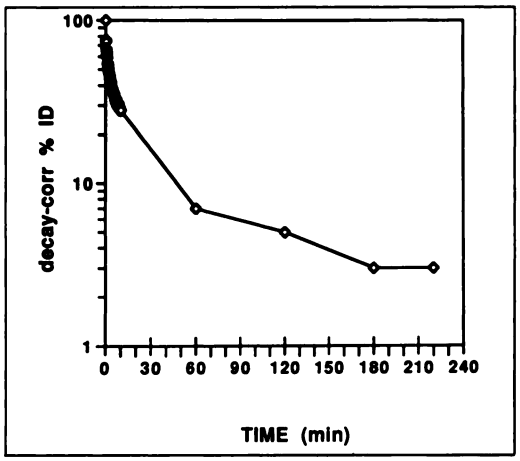

FIGURE 1. Curve showing blood disappearance of ^{99m}Tc activity following injection of ^{99m}Tc-PAC-8 peptide into a rabbit.

postinjection (Fig. 2). The area of uptake often extended into the jaw and ear, possibly related to clot progression in the vein. PAC-3, which contains an RYYD sequence in place of the RGD sequence, did not provide focal clot images (Fig. 2D). Technetium-99m-glucoheptonate, however, did produce images which appeared to show focal uptake along the jugular vein (Fig. 2E).

The uptake data obtained from counting excised tissue specimens are given in Table 2. Of the PAC peptides, PAC-8 appeared to have the highest uptake (%ID/g), and this was significantly different from all peptides except PAC-15. Iodine-125-fibrinogen had absolute uptake which was at least tenfold higher than any of the peptides including PAC-8 ($p < 0.0001$). Clot-to-blood ratios, however, were low for fibrinogen because of its slow blood disappearance. For the more rapidly clearing peptides, clot-to-blood ratios were all less than 2:1. Glucoheptonate, used as a negative control, had clot-to-blood and clot-to-muscle ratios which were significantly higher than many of the peptides tested. This is related to its extremely low blood and muscle levels, since the %ID/g in the clot was low.

The administered dose of PAC-8 appeared to have an effect on the uptake of the peptide. Table 3 shows that the lowest dose (5 μg per rabbit) resulted in the highest %ID/g bound to the clot, and the highest clot-to-muscle ratios.

Table 4 shows the results of image analysis for all the peptides and glucoheptonate. Glucoheptonate, PAC-8 and PAC-19C appeared to have the best clot-to-contralateral side ratios, however differences between PAC-8 and the other peptides did not reach statistical significance.

Thrombus Imaging Studies in Dogs

As in the rabbits, blood clearance of PAC-8 was rapid, with only about 2% of the administered dose remaining in the blood by 4 hr (corrected for decay). The kidneys were the primary organ of excretion (Fig. 3D).

Images with PAC-8 were generally focally positive within 1–2 hr after injection (Fig. 3A). In control experiments, PAC-3 did not produce an image of thrombus (Fig.

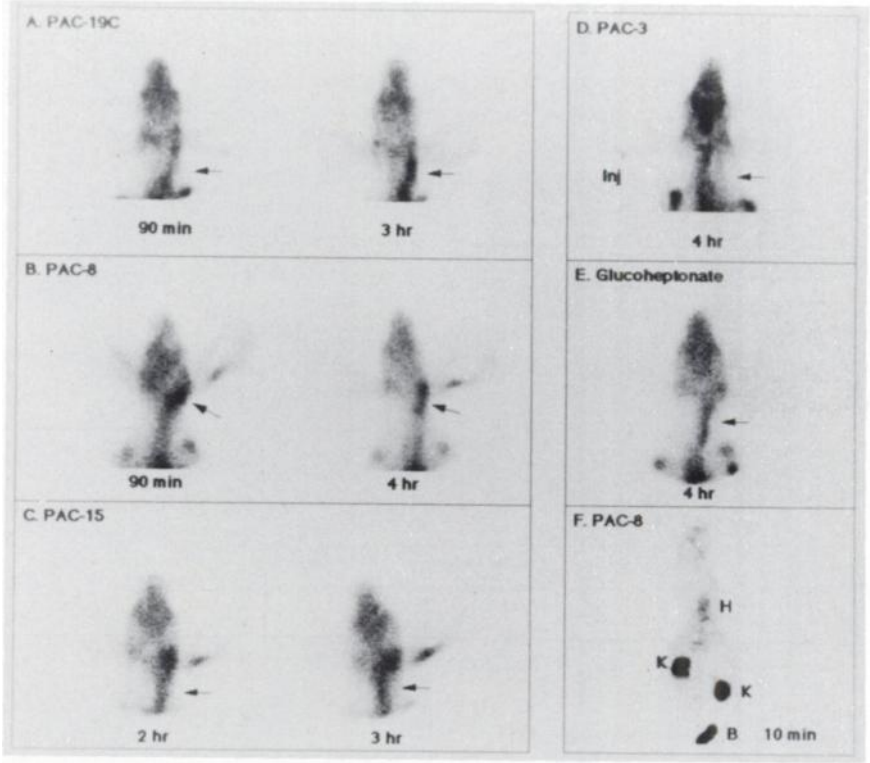

FIGURE 2. Panels A–E show anterior (head and neck) images of rabbits after injection of various labeled compounds. A clot had been induced in the left jugular vein of each rabbit (arrow). (A) Technetium-99m-PAC-19C peptide showed moderate uptake in the ends of the clot by 90 min and focal uptake throughout the clot by 3 hr postinjection. (B) Technetium-99m-PAC-8 peptide showed focal uptake in the jaw by 90 min which persisted at 4 hr as the background partially cleared. Tracer uptake was also seen along the lower margin of the ear on the clot side. (C) Technetium-99m-PAC-15 peptide showed uptake in the left jugular vein and jaw at 2 hr and 3 hr postinjection. Uptake was also seen along the lower margin of the ear on the clot side. (D) Technetium-99m-PAC-3 peptide failed to produce more than a faint suggestion of uptake in the left jugular vein at 4 hr postinjection. A small amount of extravasation occurred at the injection site (Inj). (E) Technetium-99m-glucoheptonate showed uptake in the left jugular vein at 4 hr postinjection. (F) Anterior image of the body of a rabbit 10 min after injection of ^{99m}Tc-PAC-8 peptide shows tracer biodistribution. There was rapid clearance from the blood as judged by the low counts in the heart (H), and there was rapid excretion by the kidneys (K) into bladder (B).

TABLE 2
Thrombus Uptake of Peptides in Rabbit Model

Peptide	%ID/g in clot	Clot-to-blood	Clot-to-muscle	n
PAC-2	*†0.016 ± 0.006	*‡0.78 ± 0.08	*‡7.58 ± 1.44	4
PAC-8	*0.077 ± 0.016	‡1.08 ± 0.13	*15.3 ± 6.9	5
PAC-15	*0.045 ± 0.029	1.65 ± 0.84	*20.9 ± 15.8	2
PAC-19C	*†0.012 ± 0.007	*0.86 ± 0.09	*‡7.02 ± 1.83	3
PAC-25	*†0.020 ± 0.001	*0.73 ± 0.12	*‡3.16 ± 0.70	2
PAC-26	*†0.034 ± 0.022	‡0.76 ± 0.01	*‡3.36 ± 0.80	2
^{125}I-fbg	0.924 ± 0.108	1.65 ± 0.17	206 ± 97	5
PAC-3	*†0.018 ± 0.005	1.27 ± 0.41	*14.5 ± 5.54	4
^{99m}Tc-GHA	*†0.019 ± 0.008	2.08 ± 0.58	*31.4 ± 16.2	3

*$p < 0.05$ versus fibrinogen.
†$p < 0.05$ versus PAC-8.
‡$p < 0.05$ versus Tc-GHA.
GHA = glucoheptonate and fbg = fibrinogen.

3B). In the dog model, ^{99m}Tc-glucoheptonate exhibited only diffuse uptake in the area of the thrombus (Fig. 3C).

Table 5 shows the tissue uptake and target-to-background ratios for the dog studies. PAC-8 was not significantly different from glucoheptonate or PAC-3. Thrombus-to-muscle ratios and %ID/g thrombus for PAC-8 were significantly lower than for fibrinogen.

DISCUSSION

Glycoproteins IIb and IIIa, transmembrane proteins on the surface of platelets, comprise a heterodimeric complex which changes conformation in response to platelet-stimulating agents. Before stimulation, this complex does not bind fibrinogen, but after stimulation it can bind fibrinogen which is necessary for cell-cell attachment during the process of platelet aggregation. This glycoprotein IIb-IIIa complex (GPIIb-IIIa) belongs to the family of cytoadhesive receptors which have been called the integrin, or cytoadhesin, family. The subfamily of compounds which recognize the GPIIb-IIIa receptor generally contain the tripeptide sequence Arg-Gly-Asp (RGD). Fibrinogen contains this tripeptide sequence in two locations in the alpha chain: the sequence RGDS is found in residues $\alpha 572$–$\alpha 575$ and the sequence RGDF is found in $\alpha 95$–$\alpha 97$.

PAC1 is a pentameric IgM which binds to agonist-stimulated platelets with an apparent Kd of 5 n*M*, but does not bind to unstimulated (resting) platelets (*13*). In these respects, it behaves much like fibrinogen. Preliminary thrombus-imaging studies were performed with radiolabeled PAC1 (*17*). However, because PAC1 is an IgM, it was not considered practical for development as a thrombus imaging agent.

Taub et al. (*18*) performed studies to identify the regions of PAC1 that interact with the fibrinogen receptor. The molecular recognition region of antibody molecules is contained within the variable regions of the heavy and light chains, which are subdivided into four framework regions separated by three hypervariable or complementarity-determining regions (CDRs). In the case of PAC1, CDR-3 of the heavy chain was found to contain a sequence Arg-Tyr-Asp (RYD), which might behave similarly to the RGD sequence in fibrinogen. Taub et al. further demonstrated that a 21-residue synthetic peptide encompassing the H-CDR3 region, Ala-Arg-Arg-Ser-Pro-Ser-Tyr-Tyr-Arg-Tyr-Asp-Gly-Ala-Pro-Tyr-Tyr-Ala-Met-Asp-Tyr-amide (ARRSPSYYRYDGAPYYAMDY-am), inhibited fibrinogen-dependent platelet aggregation as well as PAC1 binding to platelets (*18*). In addition, it was shown that substitution of Gly for Tyr in the 21-residue peptide (to convert the se-

TABLE 3
Dose Response of ^{99m}Tc-PAC-8 in Rabbits

Dose	%ID/g in clot	Clot-to-blood	Clot-to-muscle
5 μg	0.077 ± 0.016	1.08 ± 0.13	15.34 ± 6.89
50 μg	*0.015 ± 0.002	0.88 ± 0.11	6.69 ± 1.55
500 μg	*0.018 ± 0.004	1.04 ± 0.20	4.44 ± 0.89

*$p < 0.05$ compared with 5-μg dose.
Each value represents the mean ± s.e. of five animals.

TABLE 4
Analysis of Clot-to-Background Contrast in Rabbit Images

Peptide	Clot area/contralateral side	Clot area/adjacent bkg
PAC-2	*1.89 ± 0.13	*1.46 ± 0.08
PAC-8 (5 μg)	*†1.46 ± 0.07	*1.26 ± 0.05
PAC-8 (50 μg)	2.15 ± 0.16	1.88 ± 0.20
PAC-8 (500 μg)	*†1.82 ± 0.19	*1.38 ± 0.07
PAC-19C	2.45 ± 0.42	1.86 ± 0.20
PAC-25	*†1.61 ± 0.14	1.64 ± 0.22
PAC-26	*†1.62 ± 0.05	1.81 ± 0.15
PAC-3	*†1.60 ± 0.20	*1.46 ± 0.16
^{99m}Tc-GHA	2.56 ± 0.25	2.05 ± 0.37

*$p < 0.05$ vs. Tc-GHA.
†$p < 0.05$ vs. PAC-19C.

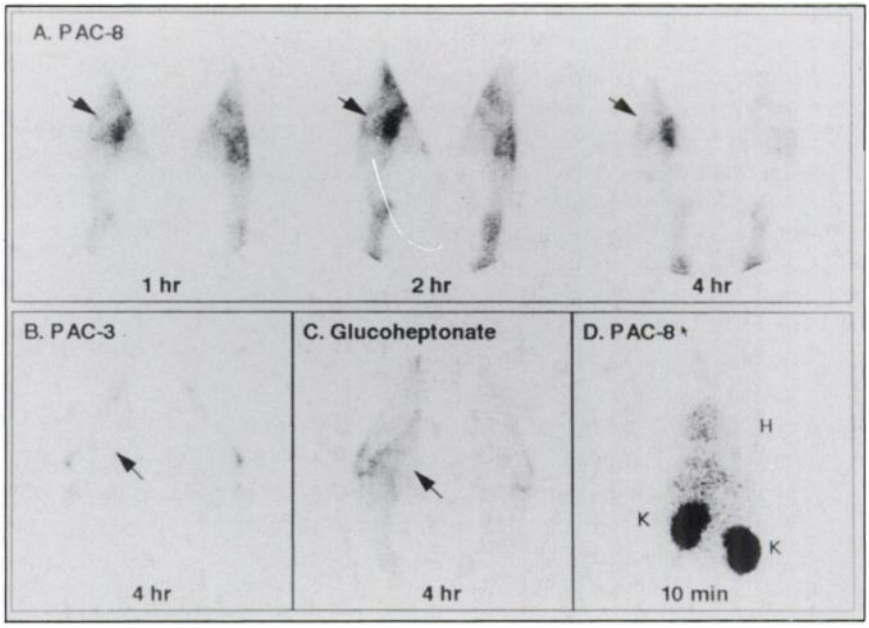

FIGURE 3. Panels A–C show anterior images of the hind legs of a dog after injection of various compounds. Thrombi were induced in the right femoral vein of each dog by placement of a coil (arrows indicate the location). In each case, lead was used to shield the bladder. (A) Technetium-99m-PAC-8 peptide showed uptake in the region of the coil 1 hr postinjection. The uptake became more prominent as time progressed. (B) Technetium-99m-PAC-3 peptide showed no uptake in the region of the coil. (C) Technetium-99m-glucoheptonate showed only diffuse uptake in the region of the thrombus. (D) Anterior image of the body of a dog 10 min after injection of ^{99m}Tc-PAC-8 peptide shows tracer biodistribution. There was rapid clearance from the blood as judged by the low counts in the heart (H). There was rapid uptake by the kidneys (K).

quence RYD to RGD) increased its antiplatelet potency tenfold (*18*).

In these studies, the 21 amino acid sequence from CDR-3H was used as a starting point for design of other synthetic peptides. The peptides were initially screened to select those with the highest potency for inhibition of platelet aggregation. It was assumed that high potency would correlate with high binding to activated platelets in vivo. The rabbit studies did demonstrate that PAC-8 and PAC-15, the two peptides with lowest IC_{50}s (Table 1), appeared to have the highest %ID/g and clot-to-muscle ratios (Table 2). Beyond these two compounds, the %ID/g clot and clot-to-background ratios did not appear to relate directly to IC_{50} for inhibition of platelet aggregation. The in vitro inhibition of aggregation in an isolated situation only gives an estimate of a compound's ability to compete with fibrinogen for sites on the platelet, and cannot predict uptake in normal background tissues.

Labeled fibrinogen was used as a positive control in these studies, because it has been well-characterized as to its binding to thrombi (*19–23*). Furthermore, in these studies we were testing an agent which binds to the fibrinogen receptor on platelets. Fibrinogen thus has a dual function in that it can bind to platelets via its receptor, and it can bind to thrombi by taking part in the coagulation pathway. Thus, comparison of the peptides with fibrinogen is a very stringent test. However, fibrinogen has been shown to have limited clinical utility for rapid imaging of pre-existing thrombi, in part because of its slow blood clearance. Thus, if thrombus-to-background ratios for a new tracer are not as good as fibrinogen by 4 hr postinjection, the new compound is probably inadequate.

The amino acid sequence KCTCCA was used in these studies as a chelator for reduced technetium. This sequence is the carboxy terminal hexapeptide of mouse metallothionein, a cysteine-rich protein that binds many metals and has particularly strong affinity for mercury, cadmium and technetium (*24*). Five peptides prepared from this protein were examined for binding of cadmium and KCTCCA was found to function well (*25*).

Various antibodies have been directed at the GP IIb-IIIa site on platelets. Of these, some recognize only activated

TABLE 5
Thrombus Uptake of Peptides in Dog Model*

	Tissue uptake			Image contrast	
Peptide	%ID/g in thrombus	Thrombus-to-blood	Thrombus-to-muscle	T/contr	T/bkg
PAC-3	0.0032 ± 0.001	1.55 ± 0.19	†6.90 ± 0.71	1.92	2.64
PAC-8	†0.0056 ± 0.001	2.25 ± 0.12	†6.47 ± 1.05	1.69	2.18
Tc-GHA	†0.0035 ± 0.001	2.27 ± 0.49	†4.12 ± 1.40	1.67	1.35
I-Fbg	0.156 ± 0.044	3.00 ± 0.74	71.0 ± 15.9	NA	NA

*at 4 hr postinjection.
†$p < 0.05$ vs. I-Fbg.

platelets (PAC1) while others bind to resting platelets. Antibody P256 has been shown to selectively recognize the IIb component (*26*). In imaging studies, ^{111}In-labeled P256 effectively bound to circulating platelets and provided the equivalent of ex vivo platelet labeling. Antibody 7E3 also appears to bind primarily to resting platelets (80% to human platelets and 60%–70% to canine platelets in whole blood) (*11*). Thus, neither of these antibodies is directly comparable in properties to the peptides in this report.

The labeled peptides tested in this study had very rapid blood disappearance rates, in part because they did not bind significantly to resting platelets. Although rapid blood disappearance is a major requirement for a tracer for rapid imaging of thrombi, this alone is not sufficient for reliable thrombus imaging. To date, Mabs have produced much better images and target-to-background ratios (*2,9,11*) although they have slower blood disappearance. Further development of peptides should be pursued to find peptides which can achieve higher uptake by thrombus.

In conclusion, it appears to be feasible to use synthetic peptides directed against activated platelets as radiopharmaceuticals for imaging thrombi in vivo. Such peptides have the advantages of rapid disappearance from blood and muscle background. The peptide sequences evaluated in this study, however, demonstrated thrombus-to-blood ratios which were too low for imaging in areas with a large blood volume. Our findings suggest that further peptides need to be developed which have a higher %ID/g in thrombus in order to improve target-to-background ratios.

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