Radioiodinated Plasminogen: An Imaging Agent for Pre-Existing Thrombi

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We have reinvestigated radioiodinated plasminogen as an agent for localizing preformed thrombi. Canine plasminogen was isolated from fresh plasma by the affinity chromatography technique on a lysine–sepharose 4B column and tagged with I-123 or I-131, at less than one iodine atom per molecule of enzyme, by the conventional ICl method. When injected into dogs more than 2 days after thrombus induction, radioiodinated plasminogen produced thrombus-to-blood activity ratios of 7.8 ± 2.4. Thrombi as old as 6 days can be visualized in 80% of the cases. Both the weight of the thrombus and the thrombus-to-blood ratio are more variable for 1-day-old thrombi; this may be associated with plasminogen release accompanying thrombus retraction. The results suggest that radioiodinated plasminogen has potential as an imaging agent for pre-existing thrombi.

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Techniques for radioactive detection of deep-vein thrombi can be divided into two categories: those useful in localizing fresh thrombi, in which fibrin deposition is actively occurring, and those useful in localizing preformed thrombi, in which net fibrin deposition has slowed. Several agents have been developed recently for imaging thrombi of the first type. These include fibrinogen labeled with Tc-99m (*1,2*) or various iodine isotopes (*3–7*) and radioiodinated fibrinogen-derived species (*8,9*). For the detection of pre-existing thrombi, much attention has been focused on the fibrinolytic agents urokinase (UK) and streptokinase (SK) (*10–15*). These activators are responsible for converting plasminogen into its active form, plasmin. Both UK and SK have been labeled with Tc-99m and with iodine isotopes, but the results obtained in animal and human studies have been extremely variable. Radioiodinated plasminogen has also been studied as a thrombus-localizing agent (*16*), but the preliminary results were discouraging and the idea was abandoned. Careful study of this previous work indicated that the unsuccessful results with radioiodinated plasminogen were probably due to technical problems, such as an impure source of protein and poor iodination technique. Further evaluation of this agent for thrombus detection therefore appeared warranted. This paper describes our experience with I-131- and I-123-labeled plasminogen in thrombus uptake experiments in dogs with thrombi of different ages. The potential of radioiodinated plasminogen for imaging preformed thrombi with a scintillation camera is also evaluated.

MATERIALS AND METHODS

Isolation of canine plasminogen. Canine plasminogen was isolated from fresh dog plasma by the affinity chromatography technique on a sepharose 4B–lysine column (*17*). The purified canine plasminogen had a specific enzyme activity of 7–9 CTA units per milligram of protein, as measured by the amount of tyrosine released from α-casein following the action of SK-activated plasminogen on the substrate (*18*).

Radioiodination of canine plasminogen. Canine plasminogen was iodinated with I-131 or I-123 by

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the conventional ICl method (*19*). The I-123 was produced in the Washington University 54-in. cyclotron by the $^{121}Sb(\alpha,2n)^{123}I$ reaction and isolated in a protein-iodination grade by a previously described method (*9*). After labeling, the protein was separated from any unbound radionuclides by dialysis against 0.05 *M* Na_2HPO_4 buffer, pH 8.0. In some cases, ^{125}I-canine fibrinogen, prepared as previously reported (*20*), was used for comparison studies.

Physicochemical properties of labeled plasminogen. SDS gel electrophoresis (*21*) was performed on plasminogen with 7.5% polyacrylamide gel in Tris–glycine buffer, pH 8.3, before and after iodination. The gels were then stained with Coomassie brilliant blue and scanned at 750 nm in a spectrometer.* Duplicate iodinated plasminogen samples were examined electrophoretically: one was stained while the other was sliced, and the radioactivity in each slice was counted in an automatic well counter.

The enzymatic activity of the radiolabeled plasminogen was compared with the parent nonlabeled plasminogen by the caseinolytic assay described above.

Animal studies. A deep-vein thrombus was induced in each of 17 dogs by the technique of intimal alteration (*22*). At 24–196 hr after thrombus induction, 1 mCi of ^{123}I- or ^{131}I-plasminogen and 400 μCi of ^{125}I-fibrinogen were injected through a catheter inserted into the opposite leg. Scintillation imaging was performed in some cases at 16–24 hr after injection. A venogram was then obtained to establish the location of the thrombus, after which the thrombus was removed. A blood sample was taken at the time of thrombus removal, and the thrombus and blood sample were weighed and counted. The results were expressed as (radioactivity/gm thrombus)/(radioactivity/gm blood).

RESULTS

The radioiodinated canine plasminogen retained its full enzymatic activity as compared with the nonlabeled parent canine plasminogen. Upon SDS gel electrophoresis the radioactivity scan could be superimposed on the spectroscopic absorbance scan of the sample before iodination (Fig. 1), indicating an absence of any essential covalent-structural change.

Table 1 summarizes the thrombus-uptake experiments in dogs. When injected at 2 or more days after induction of femoral vein thrombosis, radioiodinated plasminogen produced thrombus-to-blood activity ratios of 7.8 ± 2.4, excluding the one case in which a completely occlusive thrombus was observed. For 1-day-old thrombi, both the weight and thrombus-to-blood activity ratios were more variable. Higher thrombus-to-blood ratios were obtained for ^{123}I- or ^{131}I-plasminogen compared to ^{125}I-fibrinogen in eight dogs having thrombi more than 2 days old.

Figure 2 is a scintigram taken at 16.5 hr after the injection of 1.5 mCi of ^{123}I-plasminogen into a dog with a 100-hr-old thrombus. A medium-energy collimator was employed to reduce high-energy penetration from a small amount of I-124 contamination (*9*). The removed thrombus weighed 54 mg. The

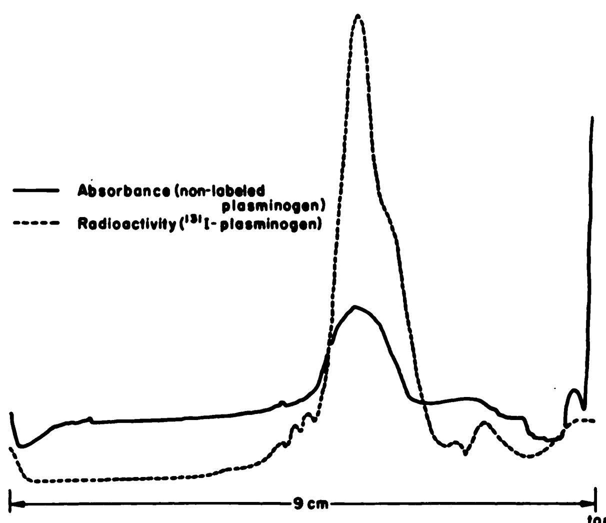

FIG. 1. SDS polyacrylamide gel electrophoresis of canine plasminogen before and after iodination.

TABLE 1. SUMMARY OF THROMBUS-UPTAKE EXPERIMENTS WITH RADIOIODINATED CANINE PLASMINOGEN

		Thrombus-to-blood ratios		
Thrombus age (days)	Thrombus weight (mg)	Plasminogen* (A)	Fibrinogen† (B)	A/B
1	87	2.1	2.9	0.70
1	109	2.0	1.4	1.4
1	27	9.6	4.6	2.1
1	79	1.9	2.4	0.79
2	20	9.5	5.9	1.6
2	24	7.5	5.0	1.5
2	291‡	1.3	—	—
3	25	12.6	4.5	2.8
3	16	5.8	2.9	2.0
4	14	10.5	3.1	3.4
4	18	10.0	3.3	3.0
4	54	5.0	1.8	2.8
4	39	5.8	—	—
5	15	7.5	3.4	2.2
6	16	8.4	—	—
6	6	5.0	—	—
8	6	6.0	—	—

* Labeled with I-123 or I-131.
† Labeled with I-125.
‡ Complete occlusion.

FIG. 2. Scintigram obtained 16.5 hr after injection of 1.5 mCi of ^{123}I-plasminogen in dog with 100-hr-old thrombosis of right femoral vein. Removed thrombus weighed 54 mg.

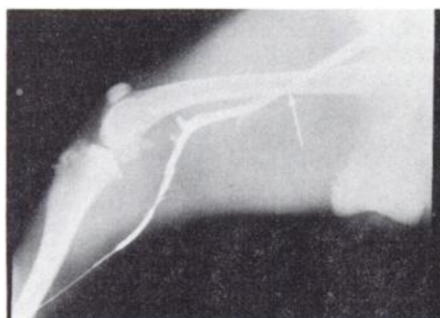

FIG. 3. Venogram of right thigh of same dog as in Fig. 2, showing filling defect in femoral vein.

corresponding venogram is shown in Fig. 3. Imaging was also performed with ^{131}I-plasminogen, but the unfavorable emission characteristics of the I-131 isotope reduced the quality of the images obtained. Imaging was performed in a total of nine dogs, and the thrombi were well visualized in seven of these despite the small thrombus size.

DISCUSSION

Several studies have been made of detection of preformed thrombi by radioactive techniques (*10–15*). Most of these studies have used radiolabeled UK and SK on the theory (*23*) that plasminogen is incorporated during thrombus formation and that this gel-phase plasminogen is the prime factor for fibrin degradation. Activators such as UK and SK are thought to diffuse subsequently into the thrombus to activate plasminogen and initiate thrombus dissolution. However, other theories have also been proposed. In 1960, Ambrus and Markus (*24*) suggested that a plasmin–antiplasmin complex functions as a carrier and/or reservoir for plasmin. The fibrin in the thrombus competes with plasma antiplasmin for plasmin. In 1972, Chesterman et al. (*25*) proposed that the extrinsic plasminogen can penetrate into, or adsorb onto the thrombus to be activated by the intrinsic activators. Our approach, using radiolabeled plasminogen as a localizing agent for preformed thrombi, is based on this third theory, and also on indirect immunofluorescence data obtained by Gottlob et al. (*26*) in 1973 which showed an increasing plasminogen content in older thrombi removed from patients. This latter conclusion was based on qualitative comparison of fluorescence intensity between thrombi of different ages. No comparison was made with blood.

The idea of using radiolabeled plasminogen as a thrombus-localizing agent is not new. As early as 1963, Gomez et al. (*16*) tested ^{131}I-plasminogen as a potential tracer for thrombus localization. Their preliminary results were not encouraging and the idea was abandoned. In 1973, Strachan et al. (*27*) reexamined uptake of radioiodinated plasminogen in preformed thrombi and emboli in dogs. They were able to show increased uptake of radiolabeled plasminogen in smaller emboli, but imaging was not attempted.

Several explanations exist for the poor results obtained by Gomez et al. (*16*). First, their plasminogen showed a relatively unstable protein–tracer bond and a 50% loss of enzymatic activity after iodination. Second, Gomez et al. employed human plasminogen that was probably contaminated with other plasma proteins. The techniques of isolating plasminogen have been greatly improved over the past decade. Strachman et al. (*27*) used a plasminogen preparation that was highly purified and more carefully iodinated. Their results with in vivo emboli were quite encouraging. The low thrombus-to-blood ratios with 3-day-old thrombi may have been due to their model of deep-vein thrombosis, which suffered from completely occlusive thrombi. Any circulating thrombus-localizing agents would probably show decreased uptake in a completely occlusive thrombus. However, human venous thrombi often only partially occlude vessels. We employed a different model of deep-vein thrombosis in which thrombi are only rarely completely occlusive. Our results are very encouraging in that a mean value of 7.8 $\pm$ 2.4 for the thrombus-to-blood ratio is obtained for thrombi more than 2 days old. The greater thrombus-to-blood ratios for plasminogen, as opposed to fibrinogen, may be related to its greater affinity for older thrombi and the decreased blood background with plasminogen due to its shorter blood $T_{1/2}$. The fact that the thrombus weights and the thrombus-to-blood ratios are more variable for 1-day-old thrombi remains to be explained. The variability may be related, in part, to thrombus retraction and subsequent plasminogen release (*28*).

The potential of radioiodinated plasminogen for thrombus imaging, indicated by the thrombus-to-

blood ratios in the older thrombi, is emphasized by the results of the scintigraphic studies. Plasminogen labeled with I-123 or I-131 provides documented visualization of thrombi as old as 6 days in 80% of the animals studied. For human use I-123 would be the more desirable isotope, due to its superior physical characteristics and lower radiation dose. Recent techniques for producing I-123 of protein-iodination grade with a small biomedical cyclotron (*9*) and the availability of large amounts of I-123 produced by spallation (*29*) make the preparation and use of I-123-labeled plasminogen feasible. Human plasminogen prepared by the affinity chromatographic technique may result in a much lower hepatitis risk. Further experimental studies and, ultimately, clinical trials are needed to evaluate the applicability of this new agent in a patient population.

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FOOTNOTE

* Model 25, Beckman (Fullerton, Calif.).

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