Rapid Localization of Indium-111-Labeled Inhibited Recombinant Tissue Plasminogen Activator in a Rabbit Thrombosis Model

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The thrombus localizing properties of indium-111-recombinant tissue plasminogen activator (^{111}In-rt-PA) have been investigated in an effort to achieve prompt and accurate detection of thrombi. Unlike previous studies with rt-PA, the active plasminogen catalytic site was permanently inhibited with peptides of chloromethyl ketone so that the radiotracer binds to fibrin without causing fibrinolysis. Thrombi were created in the external jugular vein of 14 male New Zealand white rabbits followed by injection of ^{111}In-rt-PA. The agent cleared rapidly in vivo with a half-time of 4.6 min. The thrombus: blood ratio in nonheparinized rabbits (n = 7) was 6.39 ± 0.86. The ratio in heparinized rabbits (n = 4) was 3.11 ± 0.23. Thrombi were clearly visible in the planar images of both groups 1 hr postinjection. The combination of rapid thrombus localization and positive images, especially in the presence of anticoagulation, suggests that further work is warranted with rt-PA thrombus imaging.

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There have been numerous attempts over the last two decades to devise a radiopharmaceutical that would accumulate in deep venous thrombi and thus allow their scintigraphic detection. Techniques have included labeling various plasma proteins [fibrinogen (*1*), fibrin (*2*), fragment E-1 (*3*)], cells [leukocytes (*4*), erythrocytes (*5*), platelets (*6*)], monoclonal antibodies (*7*) and their fragments (*8*), and components of the fibrinolytic system [plasminogen (*9*), streptokinase (*10*), urokinase (*11*), tissue plasminogen activator (t-PA) (*12*)]. The latest efforts with radiolabeled monoclonal antibodies have shown promising results. One of the main limitations of these radiopharmaceuticals is their relatively slow blood clearance, which can be hours to days depending on the antibody fragments and the isotope. This precludes early imaging and thus restricts clinical applications. In an attempt to overcome this limitation, we have investigated the thrombus localizing properties of indium-111-labeled recombinant tissue plasminogen activator (^{111}In-rt-PA).

Tissue plasminogen activator is a naturally occurring serine protease enzyme that is intimately involved in vascular homeostasis. This enzyme is produced in the vascular endothelium (*13*) and continuously released into the circulation. It binds to fibrin and is relatively inactive without fibrin binding (*14–16*). Thus, for fibrinolysis, binding to fibrin is the initial step. The circulatory half-life of t-PA is approximately 3–4 min in humans. Recently, a recombinant DNA t-PA (rt-PA) has been produced (*17*) and is available commercially (Activase, GENENTECH, Inc., San Francisco, CA). The enzyme has at least two functional sites: a fibrin binding site(s) and a catalytic site that is responsible for the conversion of plasminogen to plasmin (*18*). As with other serine proteases, such as thrombin, plasma kallikrein, and urokinase, this active site is able to be permanently inhibited with peptides of chloromethyl ketone (*19–21*).

In this study, a rabbit model of thrombosis has been used to evaluate the thrombus localizing characteristics of ^{111}In-labeled rt-PA following inhibition of the active site with d-phenylalanine-l-proline-l-arginine chloromethyl ketone (P-PACK).

MATERIALS AND METHODS

Radioactive Labeling

Aliquots (50 mg each) of sterile lyophilized rt-PA powder, (GENENTECH., Inc., San Francisco, CA) were dissolved in 10.0 ml sterile 0.05M HEPES (pH 7.00) and stored at −20°C. Protein labeling was carried out with slight modifications of the method of Hnatowich et al. (*22*). First, 2.5 mg of rt-PA was dialyzed against 0.2 *M* NH_4HCO_3 (pH 8.2) for 6 hr. Diethylenetriaminepentaacetic acid (DTPA) dianhydride (Aldrich Chemicals, Milwaukee, WI) then was dissolved in dimethyl sulphoxide (DMSO) [Aldrich Chemicals] with

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DTPA being added in a 5-15-DTPA:1 rt-PA ratio. The mixture was incubated at room temperature for 60 min and then dialyzed for 16–20 hr against 0.15 *M* arginine hydrochloride (pH 7.00) (Sigma Chemical Co., St. Louis, MO). The rt-PA-DTPA then was labeled with ^{111}In-111 (Amersham Corp., Arlington Heights, IL) at pH 3–4, and incubated for 60 min at room temperature. Subsequently, it was purified on a 30 × 1.0 cm column of Sephadex G-25 (Pharmacia Inc., Piscataway, NJ) and eluted with 0.15 *M* arginine (pH 7.00). The radiolabeling achieved yields as high as 78% (mean = 45%). Protein concentration was determined by Bradford assay (*23*) (Bio-Rad, Richmond, CA) using unaltered rt-PA as the reference protein.

Active Site Inhibition

Indium-111-rt-PA in 0.15 *M* arginine buffer (pH 7.00) was mixed with P-PACK (Bachem Bioscience, Inc., Philadelphia, PA) in a 15-P-PACK:1 rt-PA molar ratio then incubated for 16–18 hr at 4°C. Extensive dialysis against 0.15 *M* arginine was performed over 6–8 hr to remove excess P-PACK.

Fibrin Binding Assay

Indium-111-rt-PA (0.05–0.50 μg) in both its inhibited and uninhibited forms was added to a solution of 1.0 mg human fibrinogen (Kabi Vitrum, Stockholm, Sweden) and 100 IU Aprotonin (Mobay Chemical Corporation, New York, NY) in 1.0 ml HEPES 0.05 *M* (pH 7.00). After 10 min of incubation at 37°, 5 U Thrombin (Parke Davis, Lilitz, PA) was added and incubated at 37°C for 60 min. The resulting clot then was carefully wound onto a wooden applicator stick. The clot and the remaining solution were counted in a NaI well counter. As a control, the identical assay was performed with 0.05–0.50 μg of ^{111}In-labeled monoclonal antibody (TP61.4 IgG directed against high molecular weight melanoma-associated antigen).

Amidolytic Activity Assay

The ability of the inhibited ^{111}In-rt-PA to block the conversion of plasmin to plasminogen was tested using an amidolytic assay. Unlabeled rt-PA (control) and ^{111}In-rt-PA (2.5 ng to 10 ng) was added to 400 μg human fibrinogen (Kabi Vitrum, Stockholm, Sweden) and 0.2 IU plasminogen (Kabi Vitrum, Stockholm, Sweden) in 100 μl 0.005M Tris buffer (pH 7.40) and incubated for 10 min. A 100-μl aliquot of 5 m*M* S-2251 dye (d-Ile-pro-arg-p-nitroaniline) [Kabi Vitrum] then was added and the absorbance at 405 nm was determined after a further 10-min incubation. The identical assay was performed on the inhibited ^{111}In-rt-PA except that approximately ten-fold higher quantities were assayed to allow more rigorous evaluation of the presence of inhibition.

Gel Electrophoresis and Autoradiography

Indium-111-rt-PA and its inhibited form were analyzed by electrophoresis according to the method of Laemmli (*24*) using 7.5% polyacrylamide and a Phast-System (Pharmacia, Piscataway, NJ). No reducing agent was used. The gel then was processed for autoradiography according to the method of Laskey et al. (*25*) using Kodak AR film (Rochester, NY).

Animal Model and Biodistribution

Male New Zealand white rabbits (Hare Marland, Hewitt, NJ) weighing 2.0–5.0 kg were anesthetized with 35 mg/kg ketamine (Parke Davis, Lilitz, PA) and 5 mg/kg xylazine (Mobay Corp., Shawnee, KA) given intramuscularly. Supplemental doses of xylazine were given as needed. Hydration was maintained by a slow infusion of 0.9% NaCl through a marginal ear vein. The model of Collen et al. (*26*) was used with minor modifications. This model was chosen for several reasons. First, t-PA has been shown to be thrombolytic in rabbits (*26*), and the thrombolytic activity in rabbits is greater than either dogs or rats (*27*). Second, the fibrinolytic system in the rabbit appears to be more similar to the human system than that of the dog (*28,29*).

The external jugular vein was exposed through a 6–8-cm paramedian incision in the neck. The vein was mobilized over a distance of 4 cm. A cotton thread was introduced into the vein with an ordinary needle, and then the vein was clamped proximally and distally. In addition, all collaterals were either clamped or tied off. A 10 U aliquot of thrombin (Parke Davis) then was injected directed into the clamped portion of the vein. After 30 min, all clamps were released. Five groups of animals were studied. In Group 1 (n = 7), inhibited ^{111}In-rt-PA (0.25–0.60 mg with activities of 0.6–11.1 MBq) was administered by bolus injection over 5–10 sec through a marginal ear vein in the contralateral ear. In Group 2 (n = 3), inhibited ^{111}In-rt-PA (0.25–0.60 mg with activities of 1.3–1.7 MBq) was infused over a 30-min infusion through a marginal ear vein in the contralateral ear. In Group 3 (n = 4), animals received 350 U bolus of heparin (Organon Inc., W. Orange, NJ) 30 min prior to the bolus administration of inhibited ^{111}In-rt-PA (0.25–0.60 mg with activities of 1.4–13.9 MBq). In Group 4 (n = 3), ^{111}In-hydrochloride (17.9 ± 4.6 MBq, Amersham Corp.) was injected directly into a marginal ear vein in the contralateral ear. In Group 5 (n = 3), an ^{111}In-labeled nonspecific monoclonal antibody (0.40 mg with activities of 13.6–16.8 MBq of 763.47 IgG directed against melanoma-associated antigen) was injected.

Sixty minutes following the bolus dose or the termination of the infusion, the rabbits were euthanized with pentobarbitol 150 mg/kg (Butler Company, Columbus, OH). Sixty minutes was chosen as the imaging and sampling time because previous data using related agents (^{125}I-labeled rt-PA) had been modestly successful at this time, and because it would be a clinically convenient interval between injection and imaging. The segment of the jugular vein containing the thrombus was excised and cut open. The thrombus adherent to the thread was carefully removed, blotted dry and counted in a NaI well counter. Blood and various specimens of different organs were taken, blotted dry, and counted with appropriate standards.

In Vivo Imaging

Two animals from Group 1 and three animals from Group 3 were euthanized at 60 min and then images of the animals were obtained using a gamma camera (Searle Pho Gamma HP) interfaced to a dedicated nuclear medicine computer (Medical Data Systems A-2) using a pinhole collimator. Images were obtained using the 173-keV photopeak and a 64 × 64 matrix., with 30-50K counts acquired in each image. Each image took 20–30 min to acquire.

Blood Clearance Studies

An arterial catheter was placed in the central ear artery ipsilateral to the thrombus site or in a femoral artery and serial blood sampling at 2-min intervals was performed following the bolus injection of inhibited ^{111}In-rt-PA. The percentage

of injected activity recovered in whole blood samples from each time point was calculated assuming 100% recovery at the earliest time point (i.e., 2 min). This was then plotted against time and then analyzed using Cricket Graph analysis program (Cricket Software, Malvern, PA) for a biphasic exponential clearance in the form:

$$A = A_1e^{-\beta 1^t} + A_2e^{-\beta 2^t}$$

The half-life of each component was calculated by dividing lne2 by β_1 and β_2.

Statistical Analysis

Statistical analyses were performed using an unpaired Student's t-test. Results are presented as the mean ± 1 s.d. unless otherwise noted.

RESULTS

Fibrin Binding and Amidolytic Assay

The mean fibrin binding for ^{111}In-rt-PA was 46% (±16%), for inhibited ^{111}In-rt-PA, it was 59% (±10%), and for ^{111}In-IgG, it was 12% (±5%). Binding of the inhibited rt-PA was higher than the non inhibited ^{111}In-rt-PA ($P < 0.05$) with ^{111}In-IgG binding being significantly lower than both ^{111}In-rt-PA and inhibited ^{111}In-rt-PA ($p < 0.0001$).

The mean amidolytic activity of ^{111}In-rt-PA was 53% (±7%) of the unlabeled rt-PA. Even at ten-fold higher concentrations, the inhibited ^{111}In-rt-PA showed no significant amidolytic activity using this assay.

Gel Electrophoresis and Autoradiography

Figure 1 depicts the electrophoretic pattern of ^{111}In-rt-PA and inhibited ^{111}In-rt-PA. Greater than 90% of the activity is in the 50,000–75,000 dalton band with two smaller bands at approximately 20,000 and 40,000 daltons. The principal band corresponds to the radiolabeled rt-PA, which has a molecular weight of 63,000 to 65,000 daltons. Both ^{111}In-rt-PA and inhibited ^{111}In-rt-PA demonstrate identical patterns of migration except that there is qualitatively less of the 20,000 dalton band in the inhibited ^{111}In-rt-PA. No significant polymerization is seen.

Biodistribution

The biodistribution of inhibited ^{111}In-rt-PA at 6 min postinjection is detailed in Table 1. The mean thrombus-to-blood ratios (mean ± s.e.m.) were 6.39 ± 0.861 for Group 1, 4.31 ± 1.89 for Group 2, 3.11 ± 0.231 for Group 3, 0.438 ± 0.140 for Group 4, and 0.141 ± .028 for Group 5. The thrombus-to-blood ratio in Group 3 was significantly lower than in Group 1 ($p = 0.021$), but there was no significant difference between Groups 1 and 2. No significant difference was found in the absolute radiotracer uptake of any single tissue type in Groups 1, 2 or 3. Thrombus-to-blood ratios in Groups 1, 2, and 3 were significantly higher than in Group 4 and 5. The sizes for the thrombi in each group are as follows: 100 mg ± 70 (Group 1), 110 mg ± 40 (Group 2), 70 mg ± 60 (Group 3), 70 mg ± 30 (Group 4), and 30 mg ± 10 (Group 5).

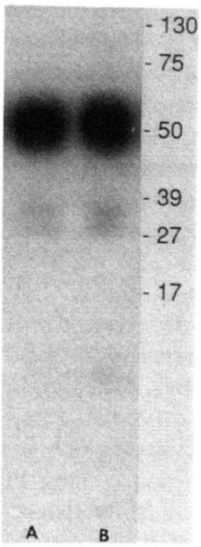

FIGURE 1
Gel electrophoresis of ^{111}In-rt-PA (A) and ^{111}In-rt-PA in its inhibited form (B).

In Vivo Imaging

All thrombi were visualized in the 60-min image. Typical images from Group 1 and Group 3 animals are depicted in Figure 2A–B. Blood samples for the calculation of biodistribution was taken immediately prior to euthanasia of the animal with the remainder of the tissue samples taken following imaging. This was performed so that no further blood clearance of tracer would be possible after the 60-min time point. The quality of the images were somewhat disappointing considering the high thrombus to background ratios. This is due to the characteristics of the gamma camera used at our institution for animal imaging as well as the small size of the thrombi that are obtained in this animal model. Although the average size of these thrombi was only 80 mg, this still did not prevent reliable scintigraphic visualization in the 60-min image.

Blood Clearance

Analysis of the blood clearance curve (Fig. 3) revealed satisfactory curve fitting (coefficient of determination $R^2 > 0.95$) with a two component biexponential analysis in the form:

$$y = 92.5e^{-0.153\times} + 20.7\ e^{-0.021\times},$$

where y is the activity/ml blood (expressed as a percentage of the first blood sample at 2 min) and × is the time in minutes. This gives blood clearance half-times for the fast component of 4.6 min and for the slow component of 33 min.

DISCUSSION

We have demonstrated that inhibited ^{111}In-rt-PA has specific clot localizing properties both in vitro and in

TABLE 1
Biodistribution at 60 Minutes Postinjection of Inhibited ^{111}In-rt-PA Controls*

	Group I (no heparin bolus) (n = 7)	Group 2 (no heparin 30 min) infusion (n = 3)	Group 3 (heparin bolus) (n = 4)	Group 4 (^{111}In-transferrin) (n = 3)	Group 5 (^{111}In-Mab) (n = 3)
Thrombus	0.260 ± 0.097	0.220 ± 0.046	0.160 ± 0.049	0.113 ± 0.038	0.082 ± 0.020
Blood	0.037 ± 0.009	0.064 ± 0.011	0.054 ± 0.010	0.349 ± 0.205†	0.570 ± 0.056†
Lung	0.029 ± 0.019	0.052 ± 0.007	0.051 ± 0.006	0.246 ± 0.064†	0.243 ± 0.056†
Muscle	0.0014 ± 0.0005	0.0013 ± 0.0001	0.0009 ± 0.0001	0.023 ± 0.004†	0.006 ± 0.0003
Bone cortex	0.004 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.042 ± 0.009†	0.011 ± 0.001
Bone marrow	0.168 ± 0.028	0.282 ± 0.053	0.186 ± 0.024	0.108 ± 0.050	0.154 ± 0.046
Liver	0.996 ± 0.073	1.358 ± 0.159	0.919 ± 0.006	0.154 ± 0.054†	0.222 ± 0.038†
Spleen	0.758 ± 0.133	1.080 ± 0.117	0.636 ± 0.082	0.091 ± 0.018†	0.144 ± 0.0002†
Kidney	0.104 ± 0.011	0.149 ± 0.042	0.107 ± 0.013	1.849 ± 0.471†	0.147 ± 0.0002
Small intestine	0.087 ± 0.065	0.026 ± 0.012	0.042 ± 0.019	0.173 ± 0.071	0.064 ± 0.003
Large intestine	0.008 ± 0.004	0.005 ± 0.001	0.025 ± 0.020	0.077 ± 0.017†	0.025 ± 0.002†
Thrombus/ Blood	6.39 ± 0.861	4.31 ± 1.89	3.11 ± 0.231†	0.438 ± 0†	0.141 ± 0.028†
Thrombus/ Muscle	206 ± 42	188 ± 38	189 ± 62	4.61 ± 1.37†	105 ± 11

* Data presented as percent of injected dose per gram of tissue ± s.e.m.
† Represents significant difference from Group 1.

vivo. In addition, owing to the rapid blood clearance of this radiotracer, high thrombus-to-blood and thrombus-to-muscle ratios are achieved within 60 min postinjection. This allows early scintigraphic detection of thrombus and thus greatly enhances the clinical potential of such a radiopharmaceutical.

Binding of inhibited rt-PA to fibrin is relatively specific as evidenced by the significantly higher binding of these agents as compared to a nonspecific radiolabeled IgG. Fibrin binding of the inhibited ^{111}In-rt-PA is slightly, though significantly, higher than that of ^{111}In-rt-PA. After inhibition with P-PACK, the inhibited ^{111}In-rt-PA is extensively dialyzed to remove the P-PACK. Any small molecular weight radiolabeled component would also be dialyzed and hence fibrin binding would appear to improve. That this occurs is demonstrated in the gel electrophoretograms with significantly less of the 20,000 molecular weight band in the inhibited ^{111}In-rt-PA as opposed to the ^{111}In-rt-PA.

One of the theoretical limitations of using radiolabeled rt-PA to identify thrombus in vivo is the thrombolytic ability of the agent. The rt-PA may successfully bind to the thrombus only to quickly lyse the thrombus and hence break off from the thrombus. It is an extremely potent thrombolytic agent (*26*) and that infusing 100,000 IU over a 4-hr period results in a 70%–80% thrombolysis in the same model of thrombosis as used here. The rt-PA used in this study has a specific activity of approximately 600,000 IU per milligram (*18*). The uninhibited ^{111}In-rt-PA had its activity re-

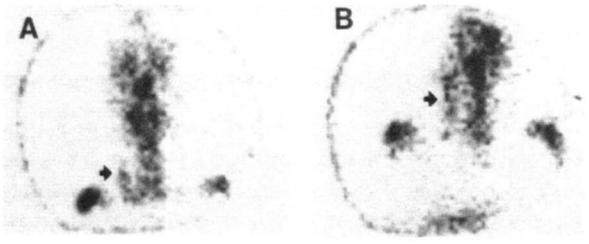

FIGURE 2
(A) Planar anterior image of the neck of a nonheparinized rabbit with a right-sided external jugular vein thrombus (arrow) imaged 60-min postinjection. (B) Planar anterior image of the neck of a heparinized rabbit with a right-sided external jugular vein thrombus (arrow) imaged 60-min postinjection.

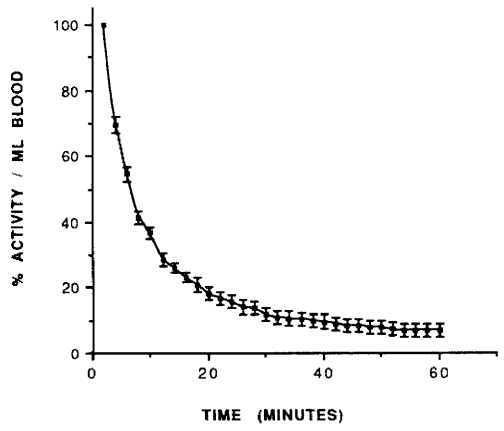

FIGURE 3
Blood clearance data expressed as percent activity in first sample at 2-min postinjection, mean ± s.e.m. There were five animals per data point.

duced by half by the radiolabeling process. If this agent were used to localize clots in the dosage used here (0.25–0.6 mg), thrombolysis in the 70%–80% range would be anticipated. Using ^{111}In-rt-PA in an inactivated form overcomes this problem. Fry et al. have recently worked with an active site mutant of t-PA (*30*) that is thrombolytically inactive. Binding of this tracer to clot was however significantly lower than the clot binding of P-PACK inhibited rt-PA. This tracer is not commercially available at present and the radiolabel, ^{125}I, makes the agent less desirable.

The rapid blood clearance of inhibited ^{111}In-rt-PA in rabbits reported in this study is similar to previous reports using ^{125}I-t-PA (*31,32*) and ^{131}I-t-PA (*33*). In contrast to these studies, however, clearance of radioactivity from the blood in our studies continued to decrease with time. The previously reported secondary peak seen approximately 30–60 min postinjection was not seen in this study. Radioiodinated t-PA is rapidly cleared from the circulation by the liver, and it is postulated that the previously reported second peak is caused either by enterohepatic recirculation of partly metabolized iodinated t-PA or there is deiodination of the tracer in the in the liver and that iodine in small molecular weight forms re-enters the circulation. Such a phenomenon is regularly seen with radioiodinated monoclonal antibodies. Those disadvantages apparently do not exist with ^{111}In-rt-PA.

Due to the rapid blood clearance of ^{111}In-rt-PA, optimization of thrombus localization was attempted by infusing the tracer over a 30-min period rather than in a bolus injection. This was not successful; absolute thrombus uptake was unchanged from the bolus administration and, thrombus-to-blood ratios were lower due to the higher blood levels.

It has been proposed that a thrombus-to-blood ratio of 3:1 or greater is needed for the scintigraphic detection of thrombus (*34*). In the present study, such ratios are achieved even in the presence of heparin. There are several reasons for the decreased uptake of tracer in the presence of heparin. First, the heparin binding site to t-PA appears to be identical to or at least related to the fibrin binding site on t-PA (*35*). Second, heparin has also been found to enhance endogenous t-PA release in rats (*36*) and endogenous t-PA may compete with ^{111}In-rt-PA for binding sites on the thrombus.

The thrombus localizing ability of ^{111}In-rt-PA has been investigated previously (*12*) using a canine model of thrombosis. In that study using active rt-PA in a canine arterial model of thrombosis, thrombus-to-blood ratios of approximately 5:1 were obtained in less than 60 min postinjection. Although the thrombus-to-blood ratios are similar to those reported in this study, the absolute uptake of tracer by thrombus was only 0.04%–0.05% of the injected dose, approximately five-fold less than reported here. In addition, the arterial model of thrombosis used in that study would favor uptake as almost the entire dose of tracer would come in contact with the thrombus during its first pass through the heart and down the aorta. Technetium-99m-labeled t-PA has also been used with suboptimal results (*37*).

One significant limitation of the application of radiolabeled monoclonal antibodies to thrombus imaging is the prolonged blood clearance of the tracer, which precludes early imaging. In an attempt to overcome this, various antibody fragments have been used. Rosebrough et al. (*38*), using a canine model of thrombosis, showed rapid clearance with an ^{111}In-labeled antifibrin Fab (T2GIs). However, relatively poor thrombus localization with an absolute uptake of 0.0023% of injected dose per gram of thrombus was obtained at 48 hr postinjection—nearly a hundred-fold less than the present study. Knight et al. (*39*) using similar techniques (^{111}In-Fab 59D8 and a canine thrombosis model) showed a blood clearance of approximately 11 hr with an improved absolute uptake of approximately 0.08% of injected dose per gram of thrombus. Thrombus-to-blood ratios of 7:1 at 24 hr postinjection were obtained. However, in the same study, their rabbit model of thrombosis showed high residual blood activity with thrombus-to-blood ratios of 2:1 being obtained even at 24 hr. Knight et al. (*8*), using a canine model of venous thrombosis and a ^{99m}Tc-labeled antifibrin antibody Fab fragment (T2G1s) showed a mean thrombus-to-clot ratio of 4:1 at 4 hr after injection and an absolute uptake of 0.089% ± 0.064% of injected dose per gram of thrombus. While marked improvement has occurred in monoclonal antibody target-to-background ratios, and the clearance has become more rapid by the use of smaller fragments, inhibited ^{111}In-rt-PA provides faster blood clearance and better absolute uptake.

Another limitation of using radiolabeled monoclonal antibodies is the induction of human anti-mouse antibodies that is well recognized to occur following administration of murine protein to humans (*40*). In addition, direct allergic effects attributable to the administration of foreign protein have been reported (*41*). There are no reported instances of acute allergic reactions to the administration of rt-PA nor is there any a priori reason to suspect any immune response to the administration of rt-PA.

Applying results achieved in animal models to the human situation is always hazardous, but there is reasonable evidence that the results reported here in rabbit thrombosis may be applicable to human thrombosis. The blood clearance of rt-PA in humans in very similar to the data presented here in rabbits (*18*). The thrombolytic activity of t-PA is approximately 50% greater in humans than in rabbits (*27*), which implies that t-PA-fibrin binding may be higher in humans than in rabbits. In addition, significant thrombolysis is exerted by t-PA on both fresh thrombi and aged thrombi (*26*), which

suggests that there would be significant t-PA-fibrin binding in both fresh and aged thrombi. These data and the current findings suggest that the application of this tracer in humans may allow early scintigraphic detection of fresh and aged thrombi, even in the presence of heparin. With such a rapid blood clearance and with improved methods of ^{99m}Tc labeling now available (*42*), future efforts towards ^{99m}Tc-labeling of inhibited rt-PA seem warranted.

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15 30

Selected manuscripts from the issues of *The Journal of Nuclear Medicine* published 15 and 30 years ago.
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MARCH 1961

On Being Well Named: A Standard Nomenclature of Radioisotope Procedures

Ervin Kaplan

The use of radioisotopes as radioactive labels in medical and paramedical procedures has become widespread in recent years. The scope of application of these labeled substances has cut across the conventional compartments of specialization and has become involved not only in the various fields of medicine, but also in the physical manifestations of chemistry, physics, and engineering as applied to medicine. As a consequence of the heterogenous applications in this field, information is scattered in a large variety of literature. It is written by, published for, and read by a polygot group of many disciplines.

A reasonable approach to classifying this burgeoning knowledge, particularly in the biologic sciences, would be the development of a comprehensive system of nomenclature.

Assuming the necessity of a Standard Nomenclature of Radioisotope Procedures (SNORP), a scheme is necessary that conveys the maximum information and yet is brief and accurate. Accepting the need for physiologic criteria as an important aspect of terminology, an appropriate generic classification scheme may be developed. One such example is as follows.

Scheme for Diagnostic Nomenclature

A. Isotope
1. Compound 2. Physical state

B. Organism
1. Organ 2. Tissue 3. Cell, etc.

C. Function measured
1. General 2. Specific

D. Method
1. Detector 2. Assay material
3. Assay technique

E. Author

Examples of Diagnostic Nomenclature

1. Selenium-75-selenomethionine pancreatic localization by scintillation detector (Blau).
2. Iodine-131-hippuric acid cerbral hemisphere differential circulation volume (Oldendorf).

Scheme for Therapeutic Nomenclature

A. Route of Administration

B. Isotope
1. Compound
2. Physical state

C. Therapy

D. Disease or pathologic state

E. Method

F. Author

Examples of Therapeutic Nomenclature

1. Interstitial iridium-192 metallic pellet therapy for pelvic tumor by implant (Liegner).
2. Intracavitary yttrium-90-chelate therapy of malignant effusions by injection (Greenberg). ■

MARCH 1976

Rat Model for Acute Myocardial Infarction: Applications to Technetium-Labeled Glucoheptonate, Tetracycline, and Polyphosphate

Norman Adler, Leopoldo L. Camin, and Peter Shulkin

The need for a specific diagnostic agent for myocardial infarcts and the potential of radiopharmaceuticals to solve this problem have long been recognized. Two main lines of inquiry have been explored. One approach is based on the fact that potassium and metabolites of the cardiac muscle are selectively concentrated in healthy heart muscle.

The second line of inquiry is to seek radioactive agents that are preferentially concentrated in the myocardial lesion as opposed to normal myocardium.

Subsequent application of the research tool that we developed led to the discovery that ^{99m}Tc-glucoheptonate and other metal complexing agents show promise as agents for the detection of acute myocardial infarcts.

Procedure

Rats were anesthetized with ether, secured to a dissection board, and a 3-cm longitudinal incision was made in the thorax, 2 cm to the left of the midline. The heart was exposed by subperiosteal resection of the fourth and fifth ribs. Several overlapping burns were made on the left ventricular wall by gently applying a 2-mm tipped soldering iron to the epicardium. The incisions were then closed by stapling together the muscle and then the skin. The entire operation must be completed in under two minutes.

The syringe containing the radiopharmaceutical was counted in an ionization chamber immediately before injection. The animal was anesthetized and secured and 0.25 ml of the radiopharmaceutical was injected into an exposed femoral vein. The syringe was then recounted and the injected dose was determined from the difference. After an appropriate interval, the animal was decapitated and the blood was collected in a pre-weighed cup. The area of infarction was excised carefully using microscalpels. A slice of myocardium approximately 1 mm thick was cut around the periphery of the excised section to measure the activity concentration over the infacted-to-normal transition zone.

Results

The cauterized rat model was initially assessed by trial with Tc-tetracycline, an agent known to concentrate in myocardial infarcts in man. The results showed that the agent is preferentially concentrated in the experimentally-produced lesion. ■