

Determinants of the distribution of radiolabeled congeners of tissue-type plasminogen activator and its modification for improved clot imaging

Joseph P. Hasapes, MD, Alan Daugherty, PhD,
Jeffrey E. Saffitz, MD, PhD, and Burton E. Sobel, MD

Background: This study was performed to elucidate determinants of the distribution in diverse tissues of radiolabeled congeners of tissue-type plasminogen activator (t-PA) and to modify the distribution to enhance clot imaging.

Methods: Uptake and degradation studies in rabbit alveolar macrophages in culture were performed with native t-PA rendered enzymatically inactive with PPACK (D-Phe-L-Pro-L-Arginyl-Chloromethyl Ketone) and conjugated to a residualizing label, radioiodinated dilactitol tyramine, (¹²⁵I-DLT). Pharmacokinetics and biodistribution of radiolabeled t-PA congeners were evaluated in rabbits.

Results: Concentration-dependent and saturable uptake and degradation in macrophages of ¹²⁵I-DLT-t-PA and ¹²⁵I-DLT-PPACK-t-PA were demonstrated. Uptake and degradation of ¹²⁵I-DLT-t-PA were inhibited by mannosylated bovine serum albumin (BSA) or mannan, specific ligands having high affinity for mannose fucose receptors. *In vivo*, clearance in rabbits was rapid after intravenous injection of both t-PA and its congeners. A high mannose- deficient t-PA congener was cleared only slightly less rapidly. Results of autoradiographic, histologic, and immunocytochemical analyses showed that clearance was associated with uptake of PPACK-t-PA in macrophages in bone marrow, spleen, and liver. Accordingly, we hypothesized that interference with high mannose-mediated macrophage uptake would reduce uptake by tissues that can interfere with clot imaging. Compared with PPACK-t-PA, accumulation of the high mannose- deficient PPACK-t-PA congener in bone marrow and in spleen was reduced by an average of 61% and 59% respectively. Results of experiments with ligands competing for mannose fucose receptors were consistent with these observations. Uptake by liver macrophages was decreased substantially with high mannose- deficient PPACK-t-PA. Accumulation was prominent, however, along edges of hepatic sinusoids adjacent to endothelial cells.

Conclusions: Thus, inhibition of the interaction of PPACK-t-PA with high mannose receptors in macrophages reduces tissue uptake that can interfere with clot imaging without compromising rapid clearance from the blood pool mediated by mannose fucose receptor-independent mechanisms in the liver.

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Keywords: t-PA, mannose fucose receptor, macrophages, residualizing labels, imaging of thrombi, biodistribution

The utility of congeners of tissue-type plasminogen activator (t-PA) for imaging thrombi has been demonstrated *in vitro* [1] and *in vivo* [2]. Tissue uptake and clearance of congeners and endogenous t-PA are similar [3-8]. Uptake is prominent in the liver [3,4,9] in parenchymal [4,10-15] and endothelial cells [11-13,16-19], as well as Kupffer cells

(macrophages) [11,13,20]. Uptake in endothelial cells and macrophages appears to be mediated by mannose fucose receptors [11-13,17-20]. Uptake in hepatocytes appears to be mediated by galactose (asialoglycoprotein) receptors [12,13,18] and by a novel receptor that recognizes t-PA complexed to plasminogen activator inhibitor (PAI-1) [21-23]. In our studies

From the Cardiovascular Division, Washington University School of Medicine, St. Louis, MO, USA.

Requests for reprints to Dr. Joseph P. Hasapes, Washington University School of Medicine, Cardiovascular Division, 660 S. Euclid Avenue, Campus Box 8086, St. Louis, MO 63110, USA.

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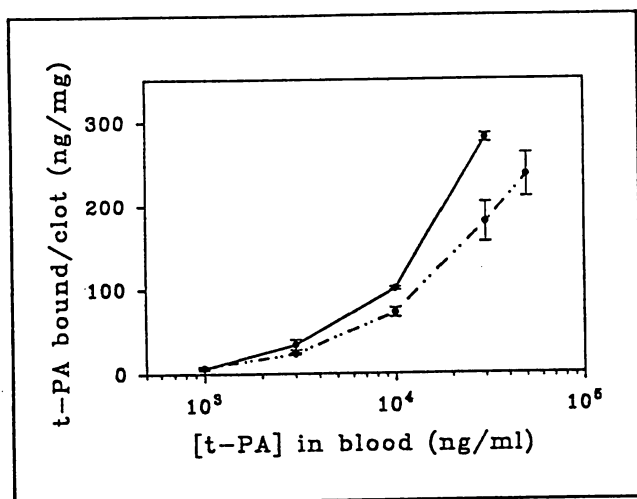


Fig. 1. Binding of ^{125}I -DLT-PPACK-t-PA (solid line) and ^{125}I -DLT-PPACK-high mannose-deficient t-PA (dotted line) to clots formed from human blood *in vitro* for 1 h before exposure to each tracer. Radiolabeled t-PA was incubated with clots for 1 h at 37°C. Points are means of three observations; bars are standard error of the mean.

of congeners of t-PA for clot imaging [2], it became clear that imaging could be improved by preferential shunting of the tracer to clots and away from bone marrow and spleen. Accordingly, we performed the present study to delineate and to favorably modify determinants of the biodistribution of radiolabeled congeners of t-PA for clot imaging.

Methods

Materials

Recombinant human type 1 wild type t-PA (high mannose-rich) [24] and asparagine 117 (ASN-117) high mannose-deficient t-PA purified from Chinese hamster ovary cell lines were supplied by Genentech (South San Francisco, CA). The serine protease inhibitor, D-Phe-L-Pro-L-Arginyl-Chloromethyl Ketone (PPACK), was obtained from Calbiochem (La Jolla, CA). A synthetic, tripeptide chromogenic substrate, S-2288, used for assay of amidolytic activity of proteases, was obtained from Kabi Vitrum (Stockholm, Sweden). Reagents used for measurement of t-PA activity in an enzyme-linked immunosorbent assay (ELISA) were obtained from American Diagnostica (Greenwich, CT). Centricon microconcentrators were obtained from Amicon (Danvers, MA). Carrier-free Na^{125}I was obtained from ICN Radiochemicals (Irvine, CA) and Amersham (Arlington Heights, IL). Bovine serum albumin (BSA) was obtained from United States Biochemical (Cleveland, OH); 2,3,4,6-Tetra-O-acetyl 1-thio-beta-D-mannosepyranoside (CNM) from E.Y. Laboratories Inc. (San Mateo, CA); and sodium methoxide and anhydrous methanol from Aldrich (Milwaukee, WI).

For use in receptor competition studies, purified 60 kd mannan was kindly provided by Dr. Philip Stahl of Washington University (St. Louis, MO). An antibody to rabbit alveolar macrophages RAM11, was supplied by Dr.

Allen M. Gown of the University of Washington (Seattle, WA) [25].

Calcium magnesium-free serum (CMFS) containing heparin (Sigma, St. Louis, MO, USA), and Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL, Grand Island, NY, USA) containing penicillin and streptomycin were obtained from the Washington University Diabetes Research and Training Center Tissue Culture Facility. All other reagent-grade chemicals were obtained from Sigma Chemical (St. Louis, MO).

Alveolar macrophages, used in binding and degradation studies as described previously [26,27], were harvested from New Zealand white rabbits obtained from Doe Valley, Arkansas.

Tissue-type plasminogen activator and its congeners

Recombinant type 1 wild-type t-PA (high mannose-rich) (4 mg/mL) and high mannose-deficient t-PA (0.4 mg/mL) were bound to PPACK as described previously [2] and were concentrated to 40 mg/mL with Centricon 30,000 mW cut-off centrifugal microconcentrators (5000 g, SS34 rotor, at 4°C for 5 hours). Complete inactivation of serine protease activity was verified by amidolytic assay with S-2288 [1].

Radioiodination procedures

Tissue-type plasminogen activator, PPACK-t-PA, and PPACK high mannose-deficient t-PA were conjugated to the radioiodinated residualizing label, DLT, as previously described [2]. Incubations were performed at 42°C. The free aldehyde groups on ^{125}I -DLT were quenched with 0.1 M NH_4HCO_3 after conjugation to t-PA. Characterization of binding of labeled t-PA to clots *in vitro* was accomplished with clots formed from whole human blood in Chandler

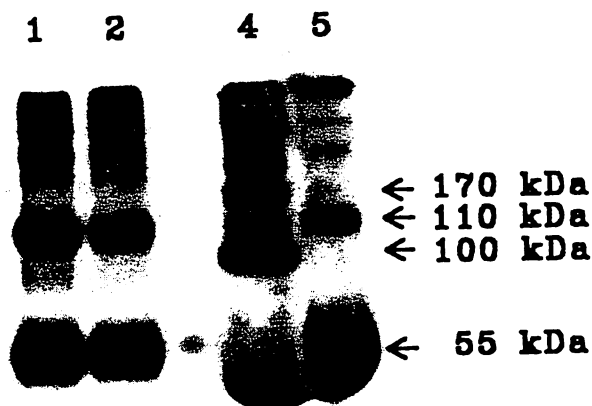


Fig. 2. Autoradiogram of ^{125}I -DLT-t-PA (55 kd monomer band in lane 1) and ^{125}I -DLT-PPACK-t-PA (lane 2) after SDS-PAGE under nonreducing conditions. t-PA congeners were incubated for 10 minutes at room temperature in 1:2 molar ratios with PAI-1 from LPS-enriched rabbit plasma (lanes 4 and 5). Two bands are absent in lane 5 (100 kd and 170 kd), reflecting lack of complex formation of t-PA with PAI-1 and C1-esterase.

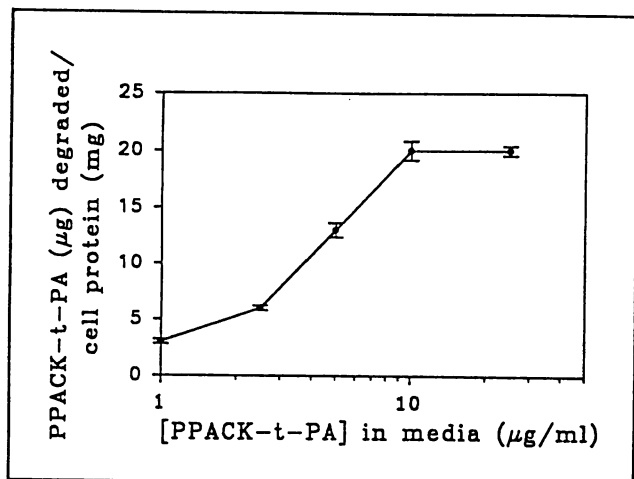


Fig. 3. Degradation of ^{125}I -DLT-PPACK-t-PA by rabbit alveolar macrophages incubated for 8 hours at 37°C . Points are means of three observations; bars are standard error of the mean.

tubes constructed of Tygon (Fischer, St. Louis, MO) tubing [2,28,29].

Concentrations of t-PA and t-PA congeners were determined with a modified Lowry protein assay procedure [30] and an enzyme-linked immunosorbent assay (ELISA) with specific, affinity-purified goat antihuman t-PA immunoglobulin G as the capture antibody [31].

Binding of t-PA to PAI-1 was assayed with the use of plasma with augmented concentrations of PAI-1 from rabbits injected intravenously with lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4 reconstituted with phosphate-buffered saline. Doses of 0.1 to 10 $\mu\text{g}/\text{kg}$ elicited peak elevations of PAI-1 activity in 5 hours. Whole blood was collected into polystyrene tubes containing 150 μL of 3.8% sodium citrate (1.5 mL) and placed into Eppendorf tubes containing PPACK (2 μM) and aprotinin (200 KIU/mL). Samples were centrifuged at 600 g at 4°C to separate the PAI-1-enriched plasma for storage at -80°C .

For use in binding experiments, PAI-1 in the enriched plasma was incubated for 10 minutes at room temperature in 1:1 to 3:1 molar ratios with either ^{125}I -DLT-t-PA or ^{125}I -DLT-PPACK-t-PA in 0.2 M arginine phosphate, pH 7.2.

Free t-PA congeners and congeners complexed to PAI-1 were separated under nonreducing conditions by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE). Samples and high molecular weight markers were run through a 4% stacking gel and 7.5% separating gel at 120 V and 200 V, respectively. Gels were stained with Coomassie blue, destained, dried, and placed in a cassette on radiographic film (Eastman Kodak, Rochester, NY, USA), stored at -80°C , and the film developed after 16 hours. A standard curve was constructed with high molecular weight standards.

Synthesis of mannose-bovine serum albumin

Mannose residues were linked covalently to primary amines on lysine residues in BSA with a 2-imino-2-methoxyethyl thiomannopyranoside (IME) [32], formed by reacting, under nitrogen, 100 mg CNM in 2.5 mL anhydrous methanol with 0.01 M sodium methoxide in a mo-

lar ratio of 1:10 for 48 hours. Excess methanol was removed by sublimation at -150°C under vacuum. The oily pellet was constituted in 1 mL of 0.1 M disodium tetraborate buffer pH 9.3 containing 50 mg BSA (molar ratio of IME to BSA, 250:1). The reaction mixture was incubated and rotated at 37°C for 3 hours and was dialyzed three times (1:1000) against 0.2 μm sterile, filtered 0.1 M NaCl, and pH 6.2 at 4°C . The concentration of mannose-BSA was determined with the Bensadoun-modified Lowry assay and was approximated 20 mannose residues per molecule BSA. Mannose-BSA was snap-frozen and stored at -80°C .

Pharmacokinetics and biodistribution of radiolabeled tissue-type plasminogen activator congeners

Fasted female rabbits (3.5 to 4 kg) were anesthetized with 60 mg/kg ketamine and 10 mg/kg xylazine intramuscularly, and anesthesia was maintained with ketamine as needed (10 mg/kg). Animals were ventilated with room air with a Harvard apparatus ventilator (South Natick, MA, USA). Femoral arteries and veins were catheterized, and a thyroid-blocking dose of sodium iodide (0.25 mL of Lugol's solution) was administered intravenously. ^{125}I -DLT-t-PA, ^{125}I -DLT-PPACK-t-PA, or ^{125}I -DLT-PPACK high mannose-deficient t-PA, (2 mCi/mg) was diluted in 20 mL sterile 0.9% saline and injected (0.1 mg IV over 20 s) for biodistribution studies. For autoradiography, 2 mg aliquots were injected intravenously over 20 seconds via the right femoral vein. ^{125}I -DLT-PPACK-t-PA was given alone or after mannosylated BSA (20-fold greater molar ratio), followed by infusion of 0.125 times the initial mass per minute for 30 minutes to rapidly attain and sustain steady-state plasma levels [33].

For monitoring clearance of radioactivity from plasma, 1-mL aliquots of arterial blood were collected at selected intervals, placed in tubes containing 100 μL 0.129 M sodium citrate (Becton Dickinson Labware, Lincoln Park, NJ, USA), were mixed, and were placed on ice. Plasma trichloroacetic acid precipitable radioactivity was determined. After 30 minutes, cardiac arrest was induced

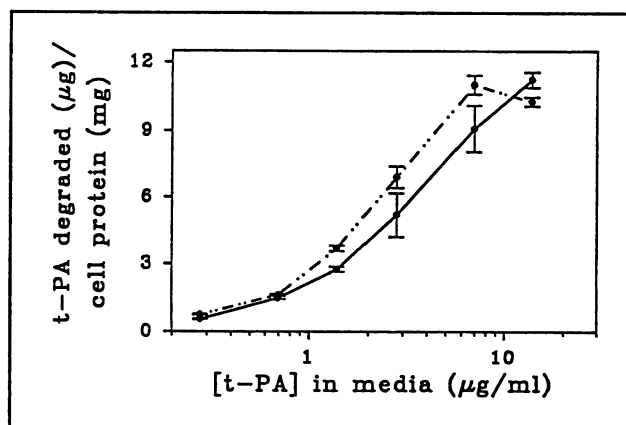


Fig. 4. Degradation of ^{125}I -DLT-t-PA by rabbit alveolar macrophages in the presence (solid line) and absence (dotted line) of mannan (100 $\mu\text{g}/\text{mL}$). Cells were incubated at 35°C for 8 hours. Points are means of three observations; bars are standard error of the mean.

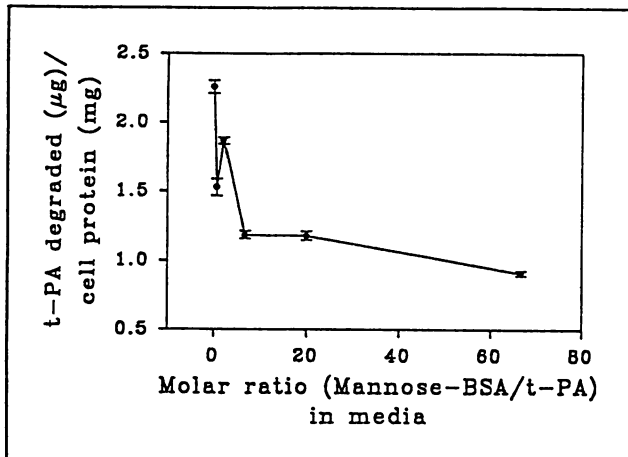


Fig. 5. Inhibition of degradation of 1.5 µg/mL ^{125}I -DLT-t-PA in cultured rabbit alveolar macrophages by mannosylated bovine serum albumin. Cells were incubated at 37°C for 8 hours. Points are means of three observations; bars are standard error of the mean.

with 20 mg xylazine and 10 mL saturated KCl given intravenously.

In separate experiments with ^{125}I -DLT-t-PA and ^{125}I -DLT-PPACK-t-PA, samples of liver were obtained 1, 5, and 30 minutes after administration of the t-PA congener via a 3-cm abdominal incision. In all experiments, liver, spleen, bone marrow, lung, adrenal, and kidney were harvested immediately after cardiac arrest, cut into 4-mm slices, and placed in either 10% (wt/vol) formaldehyde or 4% paraformaldehyde. In all experiments, two to four separate tissue samples (0.1 to 1 g each) were placed in capped tubes, weighed, and assayed for radioactivity in a Packard gamma counter (Downers Grove, IL) with quench and attenuation corrections.

Liver, spleen, heart, kidneys, and adrenal were weighed prior to slicing and corresponded closely to weights determined from morphometric charts [34]. Thus, all whole organ weights were determined based on chart values only. No gross pathology was noted before or after slicing for all organs. The biodistribution studies with each congener were performed in triplicate.

Tissue autoradiography

Fixed tissues were embedded in paraffin, cut into 6-µm sections, and placed on slides, alternating gel-coated slides for autoradiography with polylysine-coated slides for immunocytochemistry. Sections on gel slides were deparaffinized, coated with NTB2 photographic emulsion (Eastman Kodak, Rochester, NY), and stored at 4°C in sealed containers. After selected intervals, emulsions were developed in D-19 developer (Eastman Kodak), fixed, and rinsed in distilled water. Slides were stained with Harris hematoxylin and 1% alcoholic eosin. Slides were examined under bright and darkfield conditions. Two independent, masked observers characterized the patterns of grain distribution in the selected tissues at 40× and 400× magnifications.

Immunocytochemistry

Deparaffinized sections on polylysine slides were stained immunocytochemically with a Biomedex Biostain Kit (Foster City, CA). Sections were rehydrated in automation buffer, incubated in hydrogen peroxide (0.1% in methanol), and washed in automation buffer. Nonspecific protein blocker was added, followed by addition of 1:1000 dilutions of stock RAM11 primary antibody for 1 hour at 37°C. Sections were washed in automation buffer, and secondary biotin-linked antibody was added at 37°C for 30 minutes. 3-Amino, 9-ethyl carbazole peroxidase chromogen was added for 30 minutes, followed by staining of the section with aqueous hematoxylin. Immunohistochemical preparations were compared with corresponding autoradiographic sections to identify labeled cell types.

Results

Binding of tissue-type plasminogen activators

Clots *in vitro*

In previous studies, ^{125}I -DLT-PPACK-t-PA bound with affinity virtually identical to that of radioiodinated PPACK-t-PA to clots *in vitro* [1,2]. ^{125}I -DLT-PPACK high mannose-deficient t-PA binding to clots was assessed as well (Fig. 1). At selected concentrations, ^{125}I -DLT-PPACK high mannose-deficient t-PA bound with affinity similar to that of ^{125}I -DLT-PPACK-t-PA.

Plasminogen activator inhibitor-1 *in vitro*

Following incubation of congeners with PA1-enriched rabbit plasma, SDS PAGE and autoradiography demonstrated no bands at 100 kd or

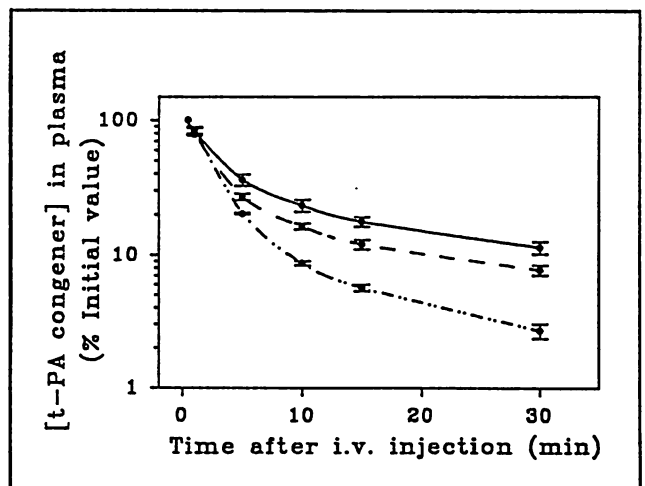


Fig. 6. Clearance of ^{125}I -DLT-PPACK-t-PA (bottom line), ^{125}I -DLT-PPACK-t-PA given concomitantly with a molar excess of 20-fold mannosylated bovine serum albumin (middle line), and ^{125}I -DLT-PPACK high mannose-deficient t-PA (top line) from plasma. Points are means of three observations; bars are standard error of the mean.

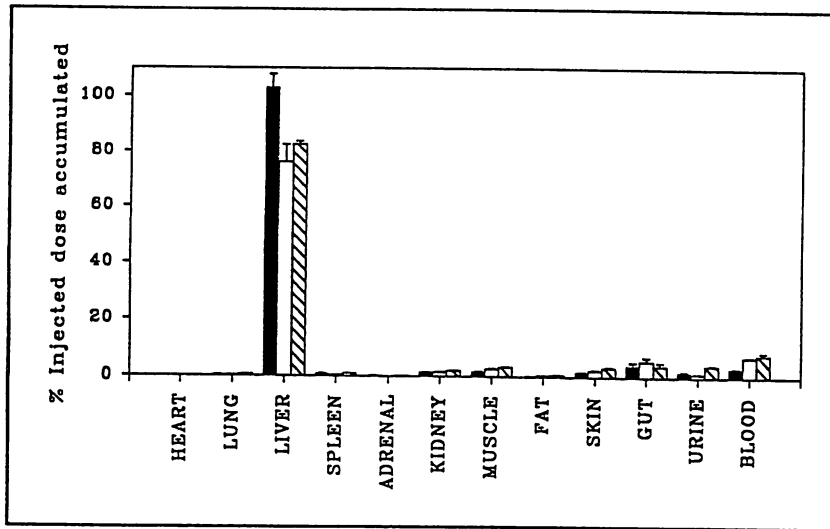


Fig. 7. Organ accumulation of radioactivity 30 minutes after bolus injection of ^{125}I -DLT-PPACK-t-PA (solid bar), ^{125}I -DLT-PPACK high mannose-deficient t-PA (open bar), or ^{125}I -DLT-PPACK-t-PA with mannoseylated bovine serum albumin (crossed bar) expressed as percentages of injected dose accumulated. Values are means of three observations \pm standard error of the mean.

170 kd with ^{125}I -DLT-PPACK-t-PA, but did demonstrate bands at both loci with ^{125}I -DLT-t-PA (Fig. 2). Thus, PAI-1-t-PA and C1-esterase-inhibitor t-PA complexes were not formed with ^{125}I -DLT-PPACK-t-PA. The bands at 110 kd probably represent t-PA dimers, but may also comprise some α_2 -antiplasmin t-PA complexes. Unbound ^{125}I -DLT-t-PA and ^{125}I -DLT-PPACK-t-PA appeared as 55-kd bands.

Binding and degradation of congeners of tissue-type plasminogen activator by rabbit alveolar macrophages

To determine whether t-PA is bound and degraded by macrophages, selected concentrations of radiolabeled congeners of t-PA (specific radioactivity, $0.4 \mu\text{Ci}/\mu\text{g}$) were incubated for 8 hours with cultured rabbit alveolar macrophages in DMEM, with or without cold mannoseylated BSA or mannan. Both ^{125}I -DLT-t-PA and ^{125}I -DLT-PPACK-t-PA were bound and degraded by cultured rabbit alveolar macrophages in a concentration-dependent manner

that was saturable at $10 \mu\text{g}/\text{mL}$ (Figs. 3 and 4). Both mannan and mannoseylated BSA, ligands with high affinity for mannose fucose receptors, competitively inhibited uptake and degradation of ^{125}I -DLT-t-PA by macrophages (Figs. 4 and 5). Uptake and degradation of ^{125}I -DLT-t-PA were diminished by 60% in the presence of a 67-fold molar excess of mannoseylated BSA (Fig. 5).

Pharmacokinetics and biodistribution of radiolabeled congeners of tissue-type plasminogen activator

The results obtained indicated that accumulation of t-PA congeners in bone marrow and other tissue that can interfere with delineation of clots *in vivo* appeared to be mediated by mannose fucose receptors on macrophages. Accordingly, to provide information needed for development of strategies potentially useful in modifying distribution in tissues of t-PA congeners in a fashion favorable for clot imaging, the effects of interactions of t-PA congeners with man-

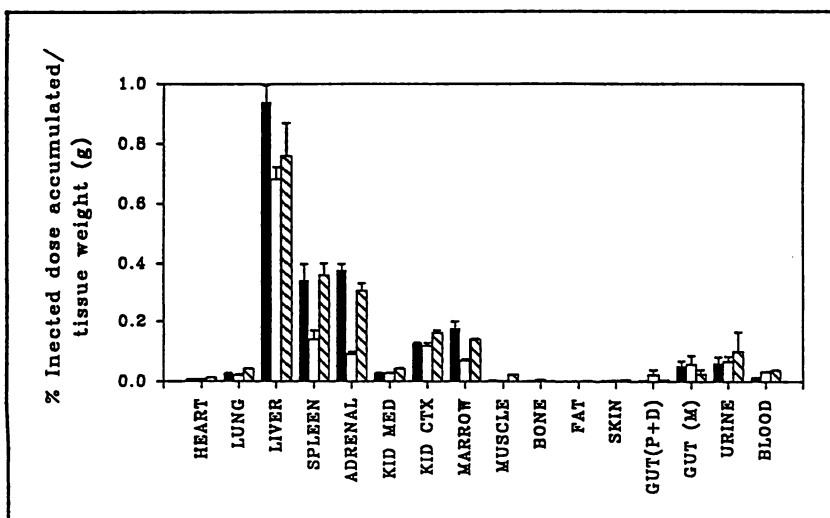


Fig. 8. Tissue accumulation of radioactivity 30 minutes after bolus injection of ^{125}I -DLT-PPACK-t-PA (solid bar), ^{125}I -DLT-PPACK high mannose-deficient t-PA (open bar), or ^{125}I -DLT-PPACK-t-PA with mannoseylated bovine serum albumin (crossed bar) normalized to tissue weight and expressed as percentages of injected dose accumulated/tissue weight (g). Values are means of three observations \pm standard error of the mean. GUT (M)—mid portion of the gut; GUT(P+D)—proximal and distal portions of gut; KID CTX—kidney cortex; KID MED—kidney medulla.

nose fucose receptors were delineated in detail. ^{125}I -DLT-PPACK-t-PA, ^{125}I -DLT-PPACK-t-PA and concomitantly administered mannosylated BSA, or ^{125}I -DLT-PPACK high mannose-deficient t-PA was injected intravenously in rabbits. The t-PA congeners cleared quickly from plasma. ^{125}I -DLT-PPACK-t-PA cleared most rapidly (Fig. 6). Uptake by tissues, expressed as the percentage of injected dose accumulated per organ, was maximal in liver for ^{125}I -DLT-PPACK-t-PA, ^{125}I -DLT-PPACK-t-PA mannosylated BSA, and for ^{125}I -DLT-PPACK high mannose-deficient t-PA (Fig. 7). When normalized for tissue weight, uptake remained maximal in liver, but notable uptake was evident in spleen, adrenal, bone marrow, kidney cortex, lung, and the middle portion of the gut (Fig. 8). Radioactivity of material in urine (Fig. 8) was 97% trichloroacetic acid soluble, indicative of a product of catabolism rather than intact t-PA. Compared with that of ^{125}I -DLT-PPACK-t-PA, accumulation of ^{125}I -DLT-PPACK high mannose-deficient t-PA radioactivity normalized for tissue weight was markedly less in adrenal (only 24% of the amount of the ^{125}I -DLT-PPACK-t-PA), bone marrow (39%), spleen (41%), but was only modestly less in liver (73%), and lung (75%) (Fig. 8). Uptake was the same for both in kidney and middle portion of the gut. Infusion of mannosylated BSA with ^{125}I -DLT-PPACK-t-PA modestly decreased the accumulation of radioactivity in bone marrow (22%), liver (19%), and adrenal (19%), with no change in spleen or kidney and an increase in activity in lung (49%) (Fig. 8).

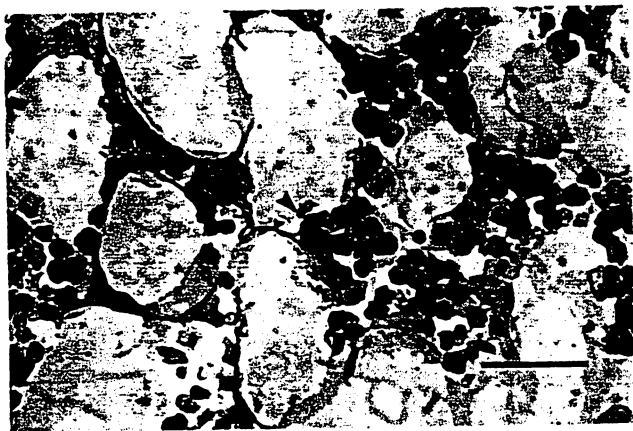


Fig. 9. Immunohistochemical preparation of rabbit bone marrow. Arrowhead points to a large cell with eccentric nucleus and red-staining cytosol, denoting a RAM11-positive macrophage. Bar represents 50 μm .

Macrophages in tissue samples were identified immunohistochemically with the monoclonal antibody, RAM11 [25] (Fig. 9). In alternating sections of liver, spleen, bone marrow, and lung, RAM11 positive cells corresponded to cells with high focal grain densities in autoradiograms of ^{125}I -DLT-PPACK-t-PA-labeled tissues. Neither RAM11 positivity nor

focal grain accumulation was evident in adrenal or kidney samples.

Highly localized grain densities in macrophages in autoradiograms of liver, spleen, bone marrow, and lung were seen after injection of ^{125}I -DLT-PPACK-t-PA (Fig. 10A, 10B). With ^{125}I -DLT-t-PA, no highly localized grain densities were seen in macrophages, but grains were seen predominantly in parenchymal cells in liver and in vascular spaces known to be lined by endothelial cells in liver, spleen, bone marrow, and lung (Fig. 10C, 10D). With ^{125}I -DLT-PPACK high mannose-deficient t-PA, no focal grain densities were seen in macrophages. However, grains were prominent along edges of liver sinusoids (Fig. 10E). These patterns were consistent despite variation of the duration of the interval between injection of the radiolabeled t-PA congeners and harvesting tissues.

These observations indicate that modification of t-PA congeners to preclude recognition by mannose fucose receptors or induction of competition for the receptor with agents given concomitantly diminishes tissue uptake by macrophages in bone marrow, spleen, and elsewhere, and alters the pattern of accumulation in liver with diminution of uptake in macrophages and increased accumulation in sinusoidal and perisinusoidal zones richly endowed with endothelial cells.

Discussion

Our results indicate that recognition of the high mannose moieties at the ASN-117 locus of t-PA mediates extrahepatic uptake of PPACK-t-PA in bone marrow, spleen, lung, and adrenal. Furthermore, attenuation of uptake in these tissues, needed for optimal clot imaging, can be accomplished by induction of competition for the mannose fucose receptors or modification of the t-PA to produce a high mannose-deficient congener.

In contrast to PPACK-t-PA, t-PA with an intact, unmodified active site is taken up in bone marrow, spleen, and lung much less predominantly by macrophage mannose fucose receptors. Such uptake may reflect complexing of t-PA by PAI-1, as well as binding mediated by high- and low-affinity endothelial cell receptors [35-40]. In tissues relatively devoid of macrophages (eg, adrenal and kidney), uptake of PPACK-t-PA was similar to that of t-PA with an unmodified active site. However, uptake of PPACK-high mannose-deficient t-PA by adrenal parenchymal cells was significantly diminished, which is indicative of a nonmacrophage, nonendothelial cell mannose fucose receptor-mediated process.

In contrast to PPACK-t-PA, PPACK high mannose-deficient t-PA distributes along edges of sinusoidal spaces in the liver. This pattern reflects inhibition of mannose fucose receptor-mediated up-

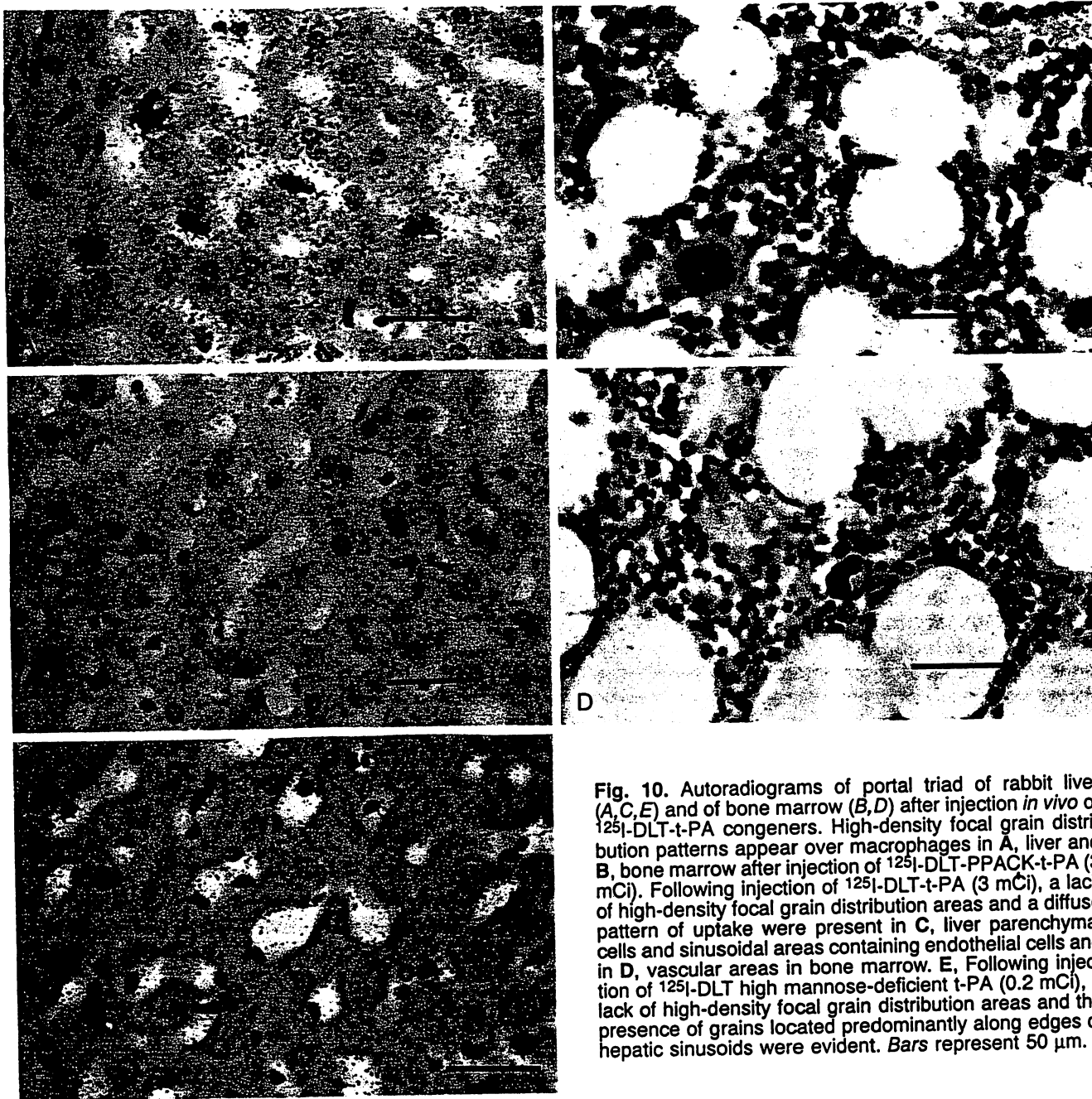


Fig. 10. Autoradiograms of portal triad of rabbit liver (A,C,E) and of bone marrow (B,D) after injection *in vivo* of ^{125}I -DLT-t-PA congeners. High-density focal grain distribution patterns appear over macrophages in A, liver and B, bone marrow after injection of ^{125}I -DLT-PPACK-t-PA (3 mCi). Following injection of ^{125}I -DLT-t-PA (3 mCi), a lack of high-density focal grain distribution areas and a diffuse pattern of uptake were present in C, liver parenchymal cells and sinusoidal areas containing endothelial cells and in D, vascular areas in bone marrow. E, Following injection of ^{125}I -DLT high mannose-deficient t-PA (0.2 mCi), a lack of high-density focal grain distribution areas and the presence of grains located predominantly along edges of hepatic sinusoids were evident. Bars represent 50 μm .

take and possibly the failure of hepatocytes to accumulate aberrant PAI-1-t-PA complexes. Alternatively, binding to low-affinity, PPACK-independent sites on endothelial cells may be responsible [35-40].

Monocytes and macrophages produce PAI-1 [41], which is known to be present in spleen [42]. Thus, complexing of t-PA with PAI-1 may predominate and prevent interactions of t-PA with mannose fucose receptors in extrahepatic tissues richly endowed with macrophages. Conjugation of t-PA with PPACK and inhibition of its active site may interfere, resulting in relatively more uptake by

macrophages. In liver, the density of endothelial cell mannose fucose receptors in close proximity to blood may facilitate binding of t-PA by these cells and further diminish t-PA access and binding by macrophages.

Others have noted a comparative lack of uptake of t-PA by Kupffer cells compared with endothelial and liver parenchymal cells [11,13,18]. We have shown that binding of t-PA and PPACK-t-PA to cultured rabbit alveolar macrophages is concentration-dependent and saturable and that blockade requires high concentrations of selective mannose fucose receptor blocking agents.

Conclusions

The results obtained in the present study indicate that one confounding factor potentially impairing delineation of thrombi *in vivo* with radiolabeled congeners of t-PA, namely uptake in bone marrow, spleen, and other tissues, can be offset by modifying the determinants of tissue distributions of the congeners of t-PA. Competition with mannose fucose receptors induced by concomitant administration of a ligand for the receptor or modification of t-PA to produce a congener deficient in high mannose moieties leads to reduced uptake in bone marrow and spleen favorable for clot imaging. Both maneuvers diminish accumulation of the congener in tissues rich in macrophages that ordinarily accumulate t-PA via mechanisms involving mannose fucose receptors. The clearance of the high mannose-deficient congener used in our study was even more rapid than that of congeners we have shown previously to be suitable for imaging clots. Furthermore, the congener bound avidly to clots at concentrations required for imaging thrombi *in vivo*. Accordingly, our results indicate that enhanced imaging of thrombi with congeners of t-PA can be anticipated by competition for mannose fucose receptors or modification of the imaging agent to diminish its recognition by mannose fucose receptors.

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