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Research Article

Tissue-type plasminogen activator (t-PA) is a serine protease, catalyzing the initial step in the fibrinolytic process. Intravenously administered t-PA is rapidly cleared from the circulation by the liver. Two distinct clearance mechanisms, which are mediated by the low density lipoprotein receptor-related protein (LRP) on liver parenchymal cells and by the mannose receptor on liver endothelial cells, have been described. Using competitors and inhibitors of the receptors, we investigated the role of LRP and carbohydrate receptors in t-PA clearance in vivo. To inhibit LRP, the 39-kD protein, which is a potent inhibitor of LRP activity, was overexpressed in the liver of mice using an adenoviral gene transfer technique. Expression of the 39-kD protein resulted in a sustained plasma concentration and an increase in the plasma half-life of 125I-t-PA from less than 1 min to 4-5 min. Blockade of the mannose receptor by intravenous administration of ovalbumin also prolonged the plasma half-life of 125I-t-PA to 3-4 min. The same degree of inhibition of t-PA clearance was also observed after administration of an inhibitor of the fucose receptor, fucosyl-BSA. However, under the conditions established for the complete blockade of the mannose receptor, no additional inhibition of t-PA clearance was observed using fucosyl-BSA, suggesting little or no role for the fucose receptor in the clearance of t-PA. Furthermore, a dramatic increase of the plasma […]

Two Receptor Systems Are Involved in the Plasma Clearance of Tissue-type Plasminogen Activator (t-PA) In Vivo

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Abstract

Tissue-type plasminogen activator (t-PA) is a serine protease, catalyzing the initial step in the fibrinolytic process. Intravenously administered t-PA is rapidly cleared from the circulation by the liver. Two distinct clearance mechanisms, which are mediated by the low density lipoprotein receptor–related protein (LRP) on liver parenchymal cells and by the mannose receptor on liver endothelial cells, have been described. Using competitors and inhibitors of the receptors, we investigated the role of LRP and carbohydrate receptors in t-PA clearance in vivo. To inhibit LRP, the 39-kD protein, which is a potent inhibitor of LRP activity, was overexpressed in the liver of mice using an adenoviral gene transfer technique. Expression of the 39-kD protein resulted in a sustained plasma concentration and an increase in the plasma half-life of ^{125}I-t-PA from less than 1 min to 4–5 min. Blockade of the mannose receptor by intravenous administration of ovalbumin also prolonged the plasma half-life of ^{125}I-t-PA to 3–4 min. The same degree of inhibition of t-PA clearance was also observed after administration of an inhibitor of the fucose receptor, fucosyl-BSA. However, under the conditions established for the complete blockade of the mannose receptor, no additional inhibition of t-PA clearance was observed using fucosyl-BSA, suggesting little or no role for the fucose receptor in the clearance of t-PA. Furthermore, a dramatic increase of the plasma half-life of ^{125}I-t-PA ($\gg$ 20 min) was observed in mice overexpressing 39-kD protein and administered ovalbumin±fucosyl-BSA. Our results clearly demonstrate that two independent receptor systems, LRP and the mannose receptor, are involved in the hepatic clearance of t-PA. (*J. Clin. Invest.* 1995. 96:1164–1168.) Key words: tissue-type plasminogen activator • 39-kD protein • low density lipoprotein receptor–related protein • adenovirus • mannose receptor

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Introduction

Tissue-type plasminogen activator (t-PA)[1] is an endogenous plasma serine protease that converts the zymogen plasminogen to plasmin. The resultant activated plasmin can proteolytically degrade the fibrin network associated with thrombi. t-PA has been used clinically to dissolve thrombi within coronary arteries associated with acute myocardial infarction (1). A major drawback to the clinical use of t-PA is its rapid plasma clearance, which is 1–2 min in rodents (2), and ~ 5 min in humans (3). Recent studies have suggested that multiple mechanisms exist for t-PA clearance. The low density lipoprotein receptor–related protein (LRP) was shown to be a hepatic receptor for both free t-PA (4) and t-PA complexed with its inhibitor plasminogen activator inhibitor type-1 (5). The mannose receptor, which is expressed mainly on liver endothelial cells, also contributes to the clearance of t-PA (6, 7). Recently, Hajjar and Reynolds have reported an additional mechanism in HepG2 cells in vitro mediated by O-linked fucose, an unusual saccharide found within the EGF domain of t-PA (8). However, the role of O-linked fucose in t-PA clearance in vivo is unknown. To investigate the precise role of LRP and the carbohydrate receptor(s) on t-PA hepatic clearance, we have performed studies in vivo in which each of the receptor systems has been inhibited. Herein, we took advantage of a high affinity inhibitor of LRP, the 39-kD protein that copurifies with and inhibits all ligand interactions with LRP (9). We previously reported that administration of purified recombinant 39-kD protein altered t-PA clearance by inhibiting LRP in vivo (10). In the current study, we used an adenoviral vector to carry the 39-kD cDNA into the liver of mice to inhibit LRP, and we evaluated the role of LRP, mannose, and fucose receptors on the hepatic clearance of t-PA.

Methods

Reagents. Single-chain recombinant human t-PA expressed in Chinese hamster ovary cells was generously supplied by Genentech (lot 9124AX; South San Francisco, CA). Carrier-free sodium [^{125}I]iodide was purchased from DuPont New England Nuclear (Boston, MA). Ovalbumin was purchased from Calbiochem-Novabiochem Corp. (La Jolla, CA). Albumin bovine fucosylamide (fucosyl-BSA) was purchased from Sigma Chemical Co. (St. Louis, MO). IODO-GEN was

1. *Abbreviations used in this paper:* α_2M*, methylamine-activated α_2 macroglobulin; LRP, low density lipoprotein receptor–related protein; t-PA, tissue-type plasminogen activator.

from Pierce Chemical Co. (Rockford, IL). BALB/c mice were obtained from The Jackson Laboratories (Bar Harbor, ME). α_2-Macroglobulin was purified and activated (α_2M*) as described previously (11). Enzymobead was from Bio-Rad Laboratories (Richmond, CA)

Protein iodination. t-PA was iodinated using IODO-GEN as described previously (12). α_2M* was iodinated using Enzymobead according to the manufacturer's instructions. Specific radioactivities were 5–10 μCi/μg of protein.

Adenovirus purification. Recombinant adenovirus containing the full-length rat 39-kD protein cDNA (AdCMV-39-kD) or the *Escherichia coli* β-galactosidase gene (AdCMV-β-Gal) were prepared as described previously (13). Virus particle number was assessed from the OD_{260} of the purified virus preparation (1 OD_{260} = 5×10^{11} virus particles/ml). Virus viability was determined by infecting 293 cells at limiting dilutions and evaluating β-galactosidase production 18 h later. The titer was ~ 100 virus particles per plaque forming unit.

Purification of the 39-kD protein. Purification of the 39-kD protein after expression in *Escherichia coli* was carried out as described previously (10).

Antibody production. Rabbit polyclonal antibodies against the full-length 39-kD protein were prepared as described previously (11).

In vivo viral delivery. Recombinant adenovirus containing either the AdCMV-39-kD or AdCMV-β-Gal was injected intravenously via tail vein into 12–16-wk old BALB/c mice. Plasma 39-kD protein was detected after day 2, peaked at days 4–6, and thereafter declined (see Results). Therefore, all clearance studies were performed on day 5 after virus injection. Various viral particle doses were examined as described in the text. Optimal expression was achieved after administration of 4×10^{11} particles (4×10^9 plaque forming units) of either AdCMV-39-kD or AdCMV-β-Gal.

In vivo plasma clearance. 12–16-wk old BALB/c mice (weighing 20–25 g) were anesthetized with sodium pentobarbital (1 mg/20 g body wt mouse) during the course of the experiment. The indicated radiolabeled protein (6.4 pmol of t-PA, [6.0×10^6 cpm/μg], 13.9 pmol of α_2M*, [1.3×10^7 cpm/μg]) in sterile saline (total vol 100 μl) was injected into a tail vein over 30 s. In studies where glycosylated albumins were administered, these competitors were injected 1 min before administration of the radiolabeled t-PA. At the indicated times, 40–50 μl of blood was collected by periorbital bleeding. ^{125}I-radiolabeled protein in the plasma samples was determined as described previously (10). Generally, at least three mice were independently evaluated for each clearance study, with nearly identical clearance curves within each experimental condition.

Immunoblotting. Plasma from uninjected mice, or from mice after administration of $2–6 \times 10^{11}$ particles of AdCMV-β-Gal or AdCMV-39-kD, was obtained 5 d after virus administration. Samples (3 μl) were electrophoresed on 10% SDS-polyacrylamide gel and transferred electrophoretically to nitrocellulose for immunoblot analysis using polyclonal affinity-purified anti-39-kD IgG.

Pharmacokinetic analysis. Plasma ^{125}I-t-PA concentrations during the 20-min clearance studies were analyzed via the pharmacokinetic clearance formula:

$$C(t) = A_1 e^{(-B_1 t)} + A_2 e^{(-B_2 t)}$$

where the coefficients A and exponential values B were derived via the "cstrip" program using a two-exponential fit (14, 15), herein α phase $t_{1/2} = 0.693/B_2$, β phase $t_{1/2} = 0.693/B_1$.

Results

Expression of adenoviral-mediated 39-kD protein in vivo. To evaluate the efficiency of adenoviral gene transduction in vivo, we performed semiquantitative protein immunoblot analyses. 4×10^{11} viral particles (2×) (~ 4×10^9pfu) of either the AdCMV-39-kD or AdCMV-β-Gal (16) were intravenously injected into 12–16-wk old BALB/c mice. Previous studies have shown expression in a majority of hepatocytes under these conditions (16). 5 d after administration of the recombinant virus, plasma was obtained and subjected to immunoblotting (Fig. 1, *inset*). 39-kD protein was detected in plasma from the AdCMV-39-kD–injected mice (4×10^{11} viral particles injected) (lane *5*), but was undetectable in plasma from uninjected (lanes *1* and *2*) or AdCMV-β-Gal–injected mice (lanes *3* and *4*). Standards containing (10–300 ng) purified recombinant 39-kD protein were also analyzed (lanes *6–9*). Comparison of the intensity of lane *5* to the standards yields a plasma concentration of the 39-kD protein ≥ 200 μg/ml. Similar quantitative analyses were performed on multiple animals 5 d after injection with 2×10^{11} (1×), 4×10^{11} (2×), and 6×10^{11} (3×) viral particles. Plasma concentrations of the 39-kD protein were determined using quantitative immunoblot analysis and were 20–50 μg/ml, 50–400 μg/ml, and 100–600 μg/ml, respectively (data not shown). Upon pathological review, no gross or microscopic morphological differences in the liver (including no evidence of necrosis, fibrosis, or inflammation) were observed 5 d after viral infection. In addition, X-Gal staining (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; Promega Corp., Madison, WI) of liver sections of mice administered AdCMV-β-Gal documented productive infection (data not shown).

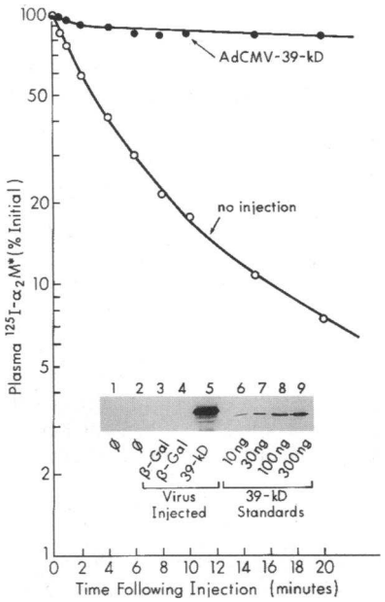

Figure 1. Effect of AdCMV-39-kD on the plasma clearance of ^{125}I-α_2M*. As described in Methods, mice were injected with 13.9 pmol of ^{125}I-α_2M* with (●) or without (○) preinjection of 4×10^{11} particles of AdCMV-39-kD 5 d previously. Blood samples were collected at the indicated times and TCA-insoluble radioactivity was determined. (*Inset*) Immunoblot analysis of 39-kD protein in plasma from uninjected, AdCMV-β-Gal-injected, or AdCMV-39-kD-injected mice. Plasma from mice that were uninjected (lanes *1* and *2*), or infected with AdCMV-β-Gal (lanes *3* and *4*), AdCMV-39-kD (lane *5*) was obtained 5 d after virus administration. Samples (3 μl) were electrophoresed on 10% SDS-polyacrylamide gel and transferred electrophoretically to nitrocellulose. Nitrocellulose was incubated with polyclonal affinity-purified anti-39-kD IgG. Antibody binding was detected by protein A peroxydase. Standards containing 10–300 ng purified recombinant 39-kD protein were analyzed (lanes 6–9). Plasma clearance of ^{125}I-α_2M* was determined for duplicate samples.

Effect of AdCMV-39-kD on the plasma clearance of ^{125}I-α_2M*. To evaluate the functional effect of the 39-kD protein expressed by adenoviral gene transduction, we compared the ability of the mice to clear an LRP-specific ligand, ^{125}I-α_2M*, from the circulation. As shown in Fig. 1, 5 d after injection with AdCMV-39-kD, mice were unable to clear ^{125}I-α_2M* from the circulation, whereas the uninfected animals rapidly cleared ^{125}I-α_2M*. These results demonstrate that mice infected with 4

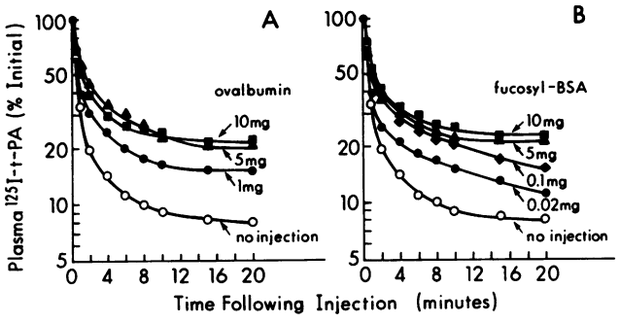

Figure 2. Effect of preadministration of ovalbumin and fucosyl-BSA on the clearance of ^{125}I-t-PA. Various concentrations of ovalbumin (1, 5, and 10 mg)(*A*) or fucosyl-BSA (0.02, 0.1, 5, and 10 mg)(*B*) were preadministered 1 min before the injection of ^{125}I-t-PA. Control mice (no administration of ovalbumin or fucosyl-BSA) are also shown.

Table I. Pharmacokinetic Parameters from ^{125}I-t-PA Clearance Studies

	α phase $t_{1/2}$	β phase $t_{1/2}$
	min	*min*
Control	0.3	11
Ovalbumin (5 mg)	1.1	18
Fucosyl-BSA (5 mg)	0.6	40
AdCMV-βGal	0.6	9.1
AdCMV-39-kD (2×)	0.6	14
AdCMV-39-kD (2×) +ovalbumin (5 mg)	1.2	220
AdCMV-39-kD (2×) +ovalbumin (5 mg) +flucose-BSA (5 mg)	1.2	42

Pharmacokinetic parameters were derived from computer fitting, as described in the text. Doses of competitors are given in parentheses; 2 × = 4 × 10^{11} viral particles. Clearance curves are seen in Figs. 2 and 3.

× 10^{11} particles of the AdCMV-39-kD expressed sufficient 39-kD protein in plasma to inhibit LRP, in agreement with the results of Willnow et al. (13).

^{125}I-t-PA clearance in the presence of various competitors. The plasma clearance of ^{125}I-t-PA after intravenous administration of 6.4 pmol of ^{125}I-t-PA in mice is shown in Fig. 2 (Fig. 2, *A* and *B, no injection*). The initial plasma half-life of ^{125}I-t-PA was 30 s–1 min with < 10% of the administered dose remaining in the circulation at 10 min (α phase $t_{1/2}$ ~ 0.3 min, β phase $t_{1/2}$ ~ 11 min, Table I). This clearance curve is similar to that of the plasma clearance of ^{125}I-t-PA in rats (6).

To confirm that no adverse effect on the clearance of ^{125}I-t-PA was induced by the viral infection, clearance studies of ^{125}I-t-PA were performed in mice after administration of AdCMV-β-Gal. As seen in Fig. 3 *A*, the clearance of ^{125}I-t-PA in AdCMV-β-Gal-infected mice was essentially the same as that of noninfected mice (α phase $t_{1/2}$ ~ 0.6 min, β phase $t_{1/2}$ ~ 9 min, Table I).

To assess the functional effect of the 39-kD protein overexpressed via the adenoviral system, we determined the clearance of ^{125}I-t-PA in AdCMV-39-kD-infected mice. Previously, we have demonstrated that the purified 39-kD protein inhibited ^{125}I-t-PA binding to MH_1C_1 cells (4) and that intravenous administration of the purified 39-kD protein to rats prolonged the plasma half-life of ^{125}I-t-PA in vivo (10). In the present studies, we used an adenoviral vector carrying the 39-kD protein cDNA to generate recombinant protein in vivo. 2 × 10^{11} (1×), or 4 × 10^{11} (2×), or 6 × 10^{11} (3×) particles of AdCMV-39-kD were intravenously administered to mice via tail vein. 5 d after administration, plasma clearance studies of ^{125}I-t-PA were performed. As seen in Fig. 3 *B*, AdCMV-39-kD-infected mice cleared ^{125}I-t-PA more slowly with plasma half-lives of ~ 2 min (1×), 3.5 min (2×), and 4 min (3×) for the respective doses. Although the effect of AdCMV-39-kDa on the clearance of ^{125}I-t-PA was dose dependent, 3× the AdCMV-39-kDa dose (i.e., 6 × 10^{11} particles) resulted in near saturation of t-PA clearance. Since ^{125}I-α_2M* is an LRP-specific ligand, and since ^{125}I-t-PA is cleared not only by LRP but also by other (carbohydrate) receptor(s), the apparent discrepancy in the effect on ligand clearance between ^{125}I-α_2M* and ^{125}I-t-PA is likely explained by the participation of other clearance mechanism(s).

To investigate the possible involvement of carbohydrate receptors in the clearance of ^{125}I-t-PA, we next performed clearance studies of ^{125}I-t-PA using various glycoproteins as competitors for the carbohydrate receptors. As seen in Fig. 2 *A*, preinjection of various amounts of ovalbumin (1, 5, 10 mg), a mannose-terminated glycoprotein, altered the plasma half-life of ^{125}I-t-PA from < 1 min to > 3 min. The α phase $t_{1/2}$ increased from 0.3 min in control mice to 1.1 min in ovalbumin-injected mice, while the β phase $t_{1/2}$ increased from 11 min to ~ 18 min (Table I). These results are in agreement with previous observations (6). The ^{125}I-t-PA clearance profile in mice administered 10 mg of ovalbumin was identical to that seen after the administration of 5 mg of ovalbumin (Fig. 2 *A*). Therefore, administration of 5 mg of ovalbumin saturated the mannose receptor. Recently, using fucosyl-BSA, Hajjar and Reynolds suggested that EGF domain–associated O-linked α-fucose might mediate t-PA binding and degradation by HepG2 cells (8). To investigate a potential role of α-fucose in vivo, we examined the clearance of ^{125}I-t-PA after preadministration of fucosyl-BSA. As seen in Fig. 2 *B*, fucosyl-BSA (0.02–10 mg) altered the clearance of ^{125}I-t-PA similarly to that seen with ovalbumin. Since the mannose receptor recognizes fucose with an affinity similar to that of mannose (17), preadministration of excess fucosyl-BSA should inhibit both the mannose receptor and the fucose receptor. Nevertheless, the observation that fucosyl-BSA inhibited the clearance of ^{125}I-t-PA similarly to that observed with ovalbumin suggests that the fucose receptor plays little or no role in t-PA clearance. To confirm the above results, we simultaneously administered fucosyl-BSA (5 mg) and ovalbumin (5 mg) to mice and evaluated ^{125}I-t-PA clearance in these animals. Identical results were obtained with those shown in Fig. 2, *A* and *B*, for maximal dosage of ovalbumin or fucosyl-BSA when administered independently (data not shown).

To examine whether LRP and the mannose receptor were able to function independently as clearance receptors in vivo, we next combined the administration of the competitors with these two receptor systems. As seen in Fig. 3 *C*, preadministration of ovalbumin (5 mg) to AdCMV-39-kD–infected mice dramatically prolonged the plasma half-life of ^{125}I-t-PA compared with that observed with either competitor alone (Fig. 2 *A* and Fig. 3 *B*). The average half-life of ^{125}I-t-PA in these mice was >> 20 min. The half-life for the α phase ($t_{1/2}$ ~ 1.2 min)

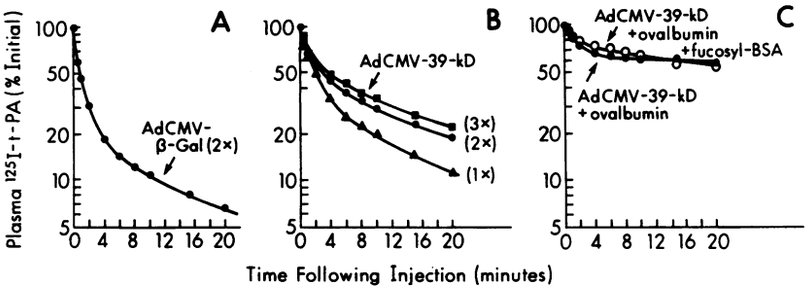

Figure 3. Effects of preadministration of AdCMV-39-kD, ovalbumin, and fucosyl-BSA on the clearance of ^{125}I-t-PA. Either 4×10^{11} particles of AdCMV-β-Gal (*A*), or three different doses of AdCMV-39-kD (2×10^{11} = 1×, 4×10^{11} = 2×, 6×10^{11} = 3× particles) (*B*) were administered intravenously to mice via tail vein. 5 d after virus administration, mice were injected with ^{125}I-t-PA, and plasma radioactivities were determined at the indicated times. 5 mg of ovalbumin with (○) or without (●) 5 mg of fucosyl-BSA were injected into mice infected with 2 × AdCMV-39-kD (*C*).

remained similar to those injected with ovalbumin alone, while the half-life for the β phase ($t_{1/2} > 200$ min) increased dramatically. Furthermore, the addition of fucosyl-BSA did not result in any further effect on ^{125}I-t-PA clearance (Fig. 3 *B*). Although markedly diminished in the presence of LRP and mannose receptor competitors, ^{125}I-t-PA clearance was still detectable. This could be due either to incomplete inhibition of these two receptor systems or to the existence of minor unknown clearance mechanism(s) for t-PA.

Discussion

The present observations demonstrate that (*a*) inactivation of LRP in vivo by gene transfer of a receptor antagonist inhibits hepatic clearance of ^{125}I-α_2M*; (*b*) inactivation of LRP by the 39-kD protein prolongs the plasma half-life of ^{125}I-t-PA; (*c*) blockade of the mannose receptor by administration of ovalbumin also prolongs the plasma half-life of ^{125}I-t-PA; (*d*) under the conditions established for the complete blockade of mannose receptor, no additional inhibition on t-PA clearance is observed when using an inhibitor of the fucose receptor; and (*e*) a dramatic increase of the plasma half-life of t-PA (> 30-fold) is observed in the mice overexpressing the 39-kD protein and coadministered a competitor of the mannose receptor. These results indicate that both LRP and the mannose receptor are responsible for t-PA clearance in vivo.

t-PA plays a central role in the fibrinolytic process by converting plasminogen to plasmin. This role for t-PA has been applied clinically for clot dissolution, especially in patients suffering from acute myocardial infarction (1). However, one major drawback to the clinical use of t-PA is its rapid clearance from the blood in vivo. Exogenously administered t-PA is cleared from the circulation via receptor-mediated endocytosis. So far, two receptor systems have been reported to be responsible for the clearance of t-PA in vivo: (*a*) LRP is a hepatic receptor for both free t-PA and t-PA complexed with plasminogen activator inhibitor type-1 (4, 18, 19); (*b*) the mannose receptor, present primarily on liver endothelial cells, recognizes mannose-rich oligosaccharides (17). Based on subfractionation studies of the liver, parenchymal cells are responsible for > 50% of the interaction of t-PA with the liver (6). Thus, LRP on the parenchymal cells is responsible for the majority of hepatic t-PA clearance. We previously demonstrated that the 39-kD protein, a specific inhibitor of LRP, blocked the binding and uptake of t-PA (4, 18), as well as t-PA: plasminogen activator inhibitor type-1 complexes (19). Recently, we also demonstrated that intravenous administration of purified recombinant 39-kD protein prolonged the plasma half-life of t-PA in vivo (10). In the current study, we demonstrated that the 39-kD protein overexpressed in liver using a gene transfer technique prolonged the plasma half-life of t-PA in vivo. The effects on t-PA clearance of the 39-kD protein after overexpression were similar to those observed after administration of the purified 39-kD protein.

t-PA contains an asparagine-linked (N-linked) mannose-rich oligosaccharide. In general, most glycoproteins are cleared from the circulation via their carbohydrate moieties, which are recognized by various carbohydrate receptors, such as the asialoglycoprotein receptor, fucose-specific receptor on Kupffer cells, mannose receptor of macrophages and hepatic endothelial cells (20). Within the carbohydrate receptor family, the mannose receptor on rat liver endothelial cells has been shown to be responsible in part for the rapid clearance of t-PA (21). The present findings (Fig. 2) are consistent with these earlier observations.

Importantly, we demonstrate a dramatic increase of the plasma half-life of t-PA (from 0.5 to 1 min in control to ≫ 20 min) in mice overexpressing the 39-kD protein and administered ovalbumin, an inhibitor of the mannose receptor (Fig. 3 *C*). These results demonstrate that two independent receptor systems, LRP and the mannose receptor, are involved in the hepatic clearance of t-PA. Consistent with these observations, hepatic cell fractionation studies by Kuiper et al. (6) after administration of ^{125}I-t-PA in vivo have demonstrated that parenchymal cells account for ~ 55% of t-PA uptake, whereas endothelial cells account for an additional ~ 40%. Furthermore, preadministration of ovalbumin virtually eliminated endothelial cell t-PA uptake, without substantial effect on t-PA uptake by parenchymal cells (6). This is consistent with the known localization of the mannose receptor to endothelial cells (6) and LRP to parenchymal cells (22). The physiological significance of the presence of two independent hepatic t-PA clearance mechanisms is not clear at present. However, the differential cellular distribution between the mannose receptor and LRP suggests that LRP may be responsible for t-PA clearance mainly in the liver parenchyma, whereas the mannose receptor on macrophages and endothelial cells may regulate the levels of free t-PA in a more local process, such as inflammation.

In addition to N-glycosylation sites, an O-linked α-fucose moiety has been recently identified on threonine-61 within the EGF domain of t-PA. Fucosylated threonine is also found in rat and mouse t-PA. Similar fucosylation is also observed in prourokinase, a fibrinolytic protein of similar structure and function to t-PA (23). The functional role of fucosylation at threonine-61 is unknown (24). Recently, Hajjar and Reynolds demonstrated that ^{125}I-t-PA binding and degradation by hepatoma

HepG2 cells were mediated by O-linked α-fucose (8). However, our in vivo results demonstrate that α-fucose had no significant role in t-PA clearance from the circulation. Subfractionation studies of liver revealed that liver Kupffer cells, which express a fucose receptor (25), were responsible for $< 5\%$ of the t-PA cleared via the liver (6), in further support of our conclusion.

In the presence of competitors for LRP and the mannose receptor, ^{125}I-t-PA continued to be cleared, but at a very slow rate. This may be due either to incomplete blockade of the two receptor systems or to the existence of a minor unknown clearance mechanism(s) for t-PA. Nonetheless, the present observations cleary demonstrate that LRP and the mannose receptor are responsible for the vast majority of ^{125}I-t-PA clearance in vivo.

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References

1. Lijnen, H. R., and D. Collen. 1991. Strategies for the improvement thrombolytic agents. *Thromb. Haemostasis* 66:88–110.

2. Devries, S. R., K. A. A. Fox, A. Robison, R. U. Rodriguez, and B. E. Sobel. 1987. Determinants of clearance of tissue-type plasminogen activator (t-PA) from the circulation. *Fibrinolysis.* 1:17–22.

3. Bounameaux, H., M. Verstraete, and D. Collen. 1985. Biological and therapeutic properties of new thrombolytic agents. *In* Thrombolysis. D. Collen, H. R. Lijnen, and M. Verstraete, editors. Churchill Livingstone, Edinburgh. 86–91.

4. Bu, G., S. Williams, D. K. Strickland, and A. L. Schwartz. 1992. Low density lipoprotein receptor-related protein/α_2-macroglobulin receptor is an hepatic receptor for tissue-type plasminogen activator. *Proc. Natl. Acad. Sci. USA.* 89:7427–7431.

5. Orth, K., T. Willnow, J. Herz, M. J. Gething, and J. Sambrook. 1994. Low density lipoprotein receptor-related protein is necessary for the internalization of both tissue-type plasminogen activator complexes and free tissue-type plasminogen activator. *J. Biol. Chem.* 269:21117–21122.

6. Kuiper, J., M. Otter, D. C. Rijken, and T. J. C. Van Berkel. 1988. Characterization of the interaction in vivo of tissue-type plasminogen activator with liver cells. *J. Biol. Chem.* 263:18220–18224.

7. Smedsrod, B., and M. Einarsson. 1990. Clearance of tissue-type plasminogen activator by mannose and galactose receptors in the liver. *Thromb. Haemostasis.* 63:60–66.

8. Hajjar, K. A., and C. M. Reynolds. 1993. α-fucose-mediated binding and degradation of tissue-type plasminogen activator by HepG2 cells. *J. Clin. Invest.* 93:703–710.

9. Herz, J., J. L. Goldstein, D. K. Strickland, Y. K. Ho, and M. S. Brown. 1991. 39kDa protein modulates binding of ligands to low density lipoprotein receptor-related protein/α_2-macroglobulin receptor. *J. Biol. Chem.* 266:21232–21238.

10. Warshawsky, I., G. Bu, and A. L. Schwartz. 1993. 39-kD protein inhibits tissue-type plasminogen activator clearance in vivo. *J. Clin. Invest.* 92:937–944.

11. Warshawsky, I., G. Bu, and A. L. Schwartz. 1993. Identifications of domains on the 39-kDa protein that inhibit the binding of ligands to the low density lipoprotein receptor-related protein. *J. Biol. Chem.* 268:22046–22054.

12. Bu, G., P. Morton, and A. L. Schwartz. 1992. Identification and partial characterization by chemical cross-linking of a binding protein for tissue-type plasminogen activator (t-PA) on rat hepatoma cells. *J. Biol. Chem.* 267:15595–15602.

13. Willnow, T. E., Z. Sheng, S. Ishibashi, and J. Herz. 1994. Inhibition of hepatic chylomicron remnant uptake by gene transfer of a receptor antagonist. *Science (Wash. DC).* 264:1471–1474.

14. Boxenbaum, H. G., S. Riegelman, and R. M. Elashoff. 1974. Statistical estimations in pharmacokinetics. *J. Pharmacokinet. Biopharm.* 2:123–148.

15. Yamaoka, K., T. Nakagawa, and T. Uno. 1978. Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. *J. Pharmacokinet. Biopharm.* 6:165–175.

16. Herz, J., and R. D. Gerald. 1993. Adenovirus-mediated low density lipoprotein receptor gene transfer accelerates cholesterol clearance in normal mice. *Proc. Natl. Acad. Sci. USA.* 90:2812–2816.

17. Stahl, P. D. 1990. The macrophage mannose receptor: current status. *Am. J. Respir. Cell Mol. Biol.* 2:317–318.

18. Bu, G., E. A. Maksymovitch, and A. L. Schwartz. 1993. Receptor-mediated endocytosis of tissue-type plasminogen activator (t-PA) by low density lipoprotein receptor-related protein (LRP) on human hepatoma HepG2 cells. *J. Biol. Chem.* 268:13002–13009.

19. Orth, K., E. L. Madison, M. J. Gething, J. F. Sambrook, and J. Herz. 1992. Complexes of tissue-type plasminogen activator and its serpin inhibitor plasminogen activator type 1 are internalized by means of the low density lipoprotein receptor-related protein/α_2-macroglobulin receptor. *Proc. Natl. Acad. Sci. USA.* 89:7422–7426.

20. Drickamer, K. 1991. Clearing up glycoprotein hormones. Minireview. *Cell.* 67:1029–1032.

21. Otter, M., M. M. Barrett-Bergshoeff, and D. C. Rijken. 1991. Binding of tissue-type plasminogen activator by the mannose receptor. *J. Biol. Chem.* 266:13931–13935.

22. Bu, G., I. Warshawsky, and A. L. Schwartz. 1994. Cellular receptors for the plasminogen activators. *Blood.* 83:3427–3436.

23. Harris, R. J., C. K. Leonard, A. W. Guzetta, and M. W. Spellman. 1991. Tissue plasminogen activator has an O-linked fucose attached to threonine-61 in the epidermal growth factor domain. *Biochemistry.* 30:2311–2314.

24. Baenziger, J. U. 1994. Tissue-type plasminogen activator: a role for O-linked fucose. Editorial. *J. Clin. Invest.* 93:459.

25. Haltiwanger, R. S., M. A. Lehrman, A. E. Eckhardt, and R. L. Hill. 1986. The distribution and localization of the fucose-binding lectin in rat tissues and the identification of a high affinity form of the mannose/N-acetylglucosamine-binding lectin in rat liver. *J. Biol. Chem.* 261:7433–7439.