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In Vivo and *in Vitro* Interaction of High and Low Molecular Weight Single-chain Urokinase-type Plasminogen Activator with Rat Liver Cells*

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The plasma clearance and the interaction of high (HMW) and low (LMW) molecular weight single-chain urokinase-type plasminogen activator (scu-PA) with rat liver cells was determined.

^{125}I-Labeled HMW- and LMW-scu-PA were rapidly cleared from plasma with a half-life of 0.45 min and a maximal liver uptake of 55% of the injected dose. Liver uptake of scu-PA was mediated by parenchymal cells. Excess of unlabeled HMW-scu-PA reduced the liver uptake of ^{125}I-HMW-scu-PA strongly. *In vivo* liver degradation of scu-PA was reduced by inhibitors of the lysosomal pathway.

A high affinity binding site (K_d 45 nM, B_{max} 1.7 pmol/mg cell protein) for both HMW- and LMW-scu-PA was determined on isolated parenchymal liver cells. Cross-competition binding studies showed that LMW- and HMW-scu-PA bind to the same site. Tissue-type plasminogen activator, mannose- or galactose-terminated glycoproteins did not affect the scu-PA binding to parenchymal liver cells.

It is concluded that LMW- and HMW-scu-PA are taken up in the liver by a common, newly identified recognition site on parenchymal liver cells and are subsequently degraded in the lysosomes. It is suggested that this site is important for the regulation of the turnover of scu-PA.

Plasminogen activators are proteolytic enzymes that convert plasminogen to plasmin. Plasmin is a broad spectrum protease that degrades fibrin and several proteins of the extracellular matrix. Because of their capacity to degrade fibrin via plasminogen activation, plasminogen activators are considered to be attractive thrombolytic agents. Two types of plasminogen activators (PA)[1] are identified in mammals: tissue-type PA (t-PA) and urokinase-type PA (u-PA).

u-PA is produced as a single chain protein (scu-PA) with a molecular weight of 54,000 by *e.g.* kidney cells (1, 2), many tumor cells (3), and fibroblasts (4). The proenzyme scu-PA is converted to two-chain u-PA (tcu-PA) by plasmin by a proteolytic cleavage between the Lys^{158} and Ile^{159}. A proteolytic derivative of scu-PA (LMW-scu-PA), that lacks the first 143 N-terminal amino acids, has been isolated (2, 5) and might be used for thrombolytic therapy. The HMW-u-PA molecule contains three domains: a growth factor domain, a kringle domain, and a protease domain. These three domains are highly homologous to the comparable domains in the t-PA molecule (6). u-PA has, however, a significantly longer connecting peptide between kringle and protease domain than t-PA.

The use of scu-PA as a thrombolytic agent is, like the use of t-PA, hampered by a short plasma half-life (7-12). The liver and to a lesser extent the kidneys have been identified to play a major role in the organ uptake of scu-PA (both HMW- and LMW-scu-PA) in rabbits and monkeys (7-10). The receptor systems in liver and kidneys, responsible for the clearance of scu-PA from the blood are unidentified, but activation to two-chain u-PA appeared unnecessary (11). A receptor for u-PA has been described on monocytes and tumor cells (see for review, Ref. 13). This u-PA receptor is a heavily glycosylated protein with a M_r of 55,000-60,000 (14-16), which is anchored in the cell membrane by a glycolipid (17). The receptor binds single and two chain HMW-u-PA with a high affinity (K_d 0.5-3 nM), but lacks affinity for LMW-u-PA (18, 19). Binding of HMW-scu-PA to this receptor is not coupled to internalization and degradation (18, 19). Recently, it was shown that interaction of PAI-1 or PAI-2 with receptor bound two-chain u-PA leads to internalization and proteolytic degradation of both u-PA and PAI (20, 21).

In the present study plasma clearance, organ uptake, and mechanism of liver uptake of both high and low molecular weight scu-PA were studied in rats. In rat liver, the main site for scu-PA uptake, a binding site common for HMW-scu-PA and LMW-scu-PA is described on parenchymal liver cells. Binding of both types of scu-PA leads to proteolytic degradation via the lysosomal route.

EXPERIMENTAL PROCEDURES

Materials—HMW- and LMW-scu-PA from human embryonic kidney cell cultures were gifts from Abbott (Abbott Park, IL). Collagenase (type I), fetuin, neuraminidase (agarose bound), and bovine serum albumin (BSA) were from Sigma. Collagenase (type D) and Pronase were from Boehringer Mannheim (Mannheim, Federal Republic of Germany (F. R. G.)). Recombinant t-PA was from Boehringer Ingelheim. Ovalbumin was from Serva Feinbiochemica (Heidelberg, F. R. G.). Culture medium Ham's F-10 was from Gibco (Hoofddorp, The Netherlands). ^{125}I (carrier free) was from Amersham (Buckingham-

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[1] The abbreviations used are: PA, plasminogen activator; u-, urokinase type; t-, tissue type; scu, single-chain urokinase-type; tcu, two-chain urokinase type; HMW, high molecular weight; LMW, low molecular weight; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; BSA, bovine serum albumin; PAI, plasminogen activator inhibitor.

shire, United Kingdom). Nycodenz was from Nycomed A/S (Oslo, Norway). HEPES was from Merck (Darmstadt, F. R. G.). Sephacryl S-200 HR was from Pharmacia LKB Technology AB (Uppsala, Sweden).

Lactosylated bovine serum albumin was made by reduction of the Schiff's base with cyanoborohydride. Asialofetuin was enzymatically prepared as described (22).

Radiolabeling of scu-PA—HMW- and LMW-scu-PA were iodinated as described earlier for tissue-type plasminogen activator (22) by the iodogen method, resulting in a specific radioactivity between 3500 cpm/ng (LMW-scu-PA) and 5000 cpm/ng (HMW-scu-PA).

Purity of labeled scu-PA preparations was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, under reducing and nonreducing conditions. Under both conditions HMW- and LMW-scu-PA were identified as single bands with a M_r of 55,000 and 33,000, respectively. This indicates that after labeling the urokinase-type plasminogen activators have preserved their single-chain form.

Gel filtration of the labeled proteins was performed on a calibrated Sephacryl S-200 HR column using a buffer (0.3 M NaCl, 10 mM Tris-HCl, pH 7.4) containing 0.1% (w/v) BSA as an eluent. The labeled proteins eluted from the column as a single peak. From the retention time of the respective peaks, it could be determined that the labeled proteins eluted under these conditions in their monomeric form.

The latent amidolytic activities of the labeled scu-PA preparations, determined on pyro-Glu-Gly-Arg-*p*-nitroanilide (S-2444) after activation of scu-PA with plasmin (23), were 90% of the unlabeled proteins. This shows that the preparations could still be activated and had intact active sites.

In Vivo Plasma Clearance and Organ Uptake—12-week-old male Wistar rats (225–275 g), used throughout the study, were anesthetized by intraperitoneal injection with 20 mg of pentobarbital. The abdomen was opened, radiolabeled compounds (400 ng/kg rat) were injected via the vena penis and at the indicated times 0.3-ml blood samples were taken with heparinized syringes from the vena cava and liver lobules were tied off. Body temperature of the rats was kept at 36.5–37 °C, using an infrared lamp. Blood samples were centrifuged for 2 min at 10,000 × *g*, and 10% trichloroacetic acid-precipitable and 10% trichloroacetic acid-soluble radioactivity was determined in aliquots of plasma. Excised liver lobules (totally less than 10% of total liver weight) were weighed and radioactivity was counted. Total liver uptake was determined by weighing the total liver at the end of the experiment. Uptake of ^{125}I-scu-PA in other organs was determined by weighing total organs and counting radioactivity. Uptake in the various organs was corrected for the amount of plasma in these organs.

Rats were pretreated with leupeptin (20 mg/kg body weight) by intraperitoneal injection 60 min before ligand injection and with chloroquine (12.5 mg/kg body weight) by intraperitoneal injection 120 and 60 min before ligand injection.

Cell Isolation Procedures—For the determination of the contribution of different liver cell types to total liver uptake, rats were anesthetized and injected with ^{125}I-HMW-scu-PA and ^{125}I-LMW-scu-PA (400 ng/kg rat) via the vena penis. After 10 min the vena porta was cannulated and a liver perfusion at low temperature (<8 °C) was started using Hanks' buffer (supplemented with 10 mM HEPES). Parenchymal liver cells were separated from non-parenchymal liver cells after collagenase (0.05% (w/v) type I) perfusion of the liver at 8 °C. To separate endothelial liver cells from Kupffer cells, the liver residue obtained after collagenase perfusion, was further digested by stirring with Pronase (0.25%, w/v) for 20 min at 4 °C, and separated using counterflow centrifugation, exactly as described earlier (22, 24, 25). The contribution of the various liver cell types to total liver uptake was calculated as described (22, 24–26). As found for a number of substrates (22, 24–26), no loss of cell-bound label and/or formation of acid-soluble radioactivity occurred during the low temperature cell isolation procedure, leading to a quantitative recovery of radioactivity associated with the isolated liver cells as compared to the total liver association. This was checked for each individual liver cell isolation by comparing the calculated liver association (from the relative contribution of the various cell types) and the determined total liver association.

In Vitro Binding Studies—Parenchymal liver cells used for *in vitro* binding studies were isolated after perfusion of the rat liver for 10 min with collagenase (0.05% (w/v), type D) following the method of Seglen (27), modified as described previously (28). Purity of the cells was always more than 99%, while viability (checked by ATP content and trypan-blue exclusion) was more than 95% during the incubations. For binding studies with scu-PA, parenchymal cell suspensions were incubated at a density of 1 mg of cell protein/ml Ham's F-10 medium, containing 2% (w/v) BSA and 25 mM HEPES, pH 7.4. Cell incubations were performed in Kartell plastic tubes at a circulating lab shaker (150 rpm, Adolf Kuhner) at either 4 or 37 °C. At the end of the incubations cells were washed at 4 °C: two times with 10 mM Tris-HCl, 150 mM NaCl, 5 mM $CaCl_2$, pH 7.4, plus 0.2% BSA and once with the same buffer without BSA for 30 s at 50 × *g*. Finally cells were resuspended in this buffer (without BSA) and cell-bound radioactivity was counted in a gamma counter while cell protein was determined according to Lowry with BSA as standard. Dissociation of cell-bound scu-PA was determined to be less than 3% during the time of washing procedure. The nonspecific binding was determined in the binding studies as the residual binding of radiolabeled scu-PA to rat parenchymal liver cells in the presence of a 1000-fold unlabeled scu-PA.

Dissociation constant (K_d) and maximal binding (B_{max}) were determined from displacement curves according to a single site displacement model using a computerized nonlinear fitting program (minimizing the sum of squares via the Simplex-iteration procedure) (Graph-Pad: H. Motulsky, ISI-Software) (29). Plasma clearance curves were analyzed by computerized nonlinear fitting following a biphasic clearance model using the same program.

RESULTS

Plasma Half-life and Organ Uptake of scu-PA—Both ^{125}I-LMW- and ^{125}I-HMW-scu-PA showed after intravenous injection into rats a very similar pattern of plasma clearance and liver uptake (see Fig. 1). For both types of scu-PA, a plasma half-life of 0.45 ± 0.04 min was found for the α-phase, while the β-phase varied from 6.5 ± 0.9 min (HMW-scu-PA) to 8.0 ± 1.2 min (LMW-scu-PA). Maximal liver uptake was at 10 min after injection 56.5 ± 2.5% and 54.4 ± 3.3% for

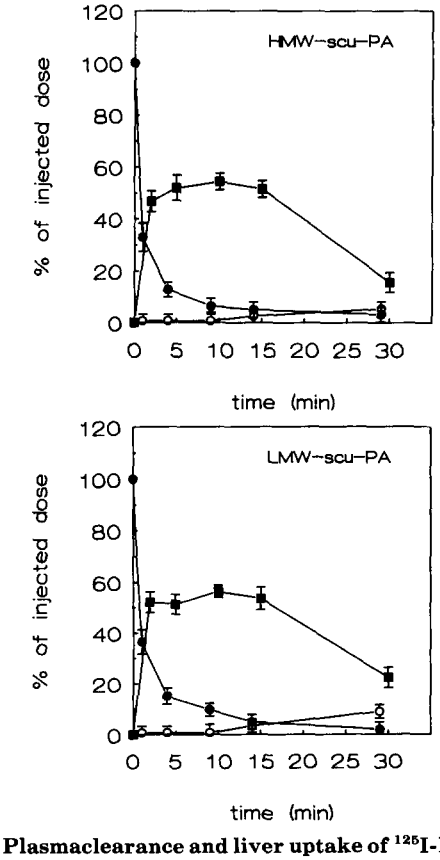

FIG. 1. **Plasmaclearance and liver uptake of ^{125}I-HMW- and ^{125}I-LMW-scu-PA.** At the indicated times after intravenous injection of ^{125}I-HMW-scu-PA (*top*) or ^{125}I-LMW-scu-PA (*bottom*) into male Wistar rats, plasma clearance and liver lobules (■) were determined. In plasma, both 10% trichloroacetic acid-soluble (○) and precipitable (●) radioactivity were measured. Data are mean (n = 4) ± S.D.

LMW- and HMW-scu-PA, respectively. From 15 to 30 min after injection, a decrease in liver-associated radioactivity of scu-PA was observed, while in the same time an increase was found in trichloroacetic acid-soluble radioactivity in plasma. This sequence of events is consistent with liver uptake of ^{125}I-HMW- and ^{125}I-LMW-scu-PA followed by degradation and secretion of degradation products into the circulation.

The pathway of degradation of scu-PA in the liver *in vivo* was further investigated using the lysosomal inhibitors leupeptin and chloroquine (Fig. 2). Both inhibitors had no effect on the plasma clearance of either type of scu-PA (not shown). The initial uptake of scu-PA in the liver was also unaffected, but at 30 and 45 min after injection significantly higher levels of HMW- and LMW-scu-PA were detected in the livers of animals treated with protease inhibitors.

The specificity of the interaction of ^{125}I-HMW- and ^{125}I-LMW-scu-PA with the liver was determined by preinjection of rats with various ligands 1 min prior to injection of scu-PA. Ovalbumin (blocking the mannose-receptor) and asialofetuin (blocking the asialoglycoprotein receptor) (22) did not affect plasma clearance and liver uptake of HMW- and LMW-scu-PA, indicating no involvement of these receptors in the uptake of both types of scu-PA by the liver (not shown). An intravenous injection of 20 mg of unlabeled HMW-scu-PA/kg body weight 1 min prior to injection of ^{125}I-HMW-scu-PA, however, did inhibit the plasma clearance of ^{125}I-HMW-scu-PA (Fig. 3). Concurrently, initial liver uptake of ^{125}I-HMW-scu-PA was reduced at 2 and 5 min after injection to 31.0 and 56.3% of the uptake in control rats, respectively.

It appeared that in addition to the liver other sites in the body must be involved in the plasma clearance of scu-PA. Therefore, we determined the relative contribution of other organs to the plasma clearance of scu-PA (Fig. 4). Besides the liver (51.3 ± 4.3%), kidney (12.9 ± 1.3%), skin (6.0 ± 0.6%), and muscles (10.3 ± 1.0%) contributed to the plasma clearance of ^{125}I-LMW-scu-PA. For ^{125}I-HMW-scu-PA these values were: liver (55.2 ± 2.5%), kidneys (9.5 ± 2.6%), skin (2.3 ± 1.2%), and muscles (3.2 ± 0.4%). The uptake of ^{125}I-LMW-scu-PA by skin and muscles was significantly larger than that of ^{125}I-HMW-scu-PA.

The capacity of organs other than the liver to interact with scu-PA was studied in rats in which, by complete hepatectomy, the liver did not contribute to the plasma clearance. For HMW-scu-PA an increased plasma half-life was found and 30 min after injection of the ligand still 37% of the injected dose was present in the circulation (Fig. 5). The tissue uptake of HMW-scu-PA was performed, in the absence of liver uptake, by kidneys (32.9 ± 2.9%), muscles (5.6 ± 0.2%), and skin (16.9 ± 0.3%). More than 90% of the injected dose was present in the aforementioned tissues plus plasma, and no trichloroacetic acid-soluble radioactivity was found in

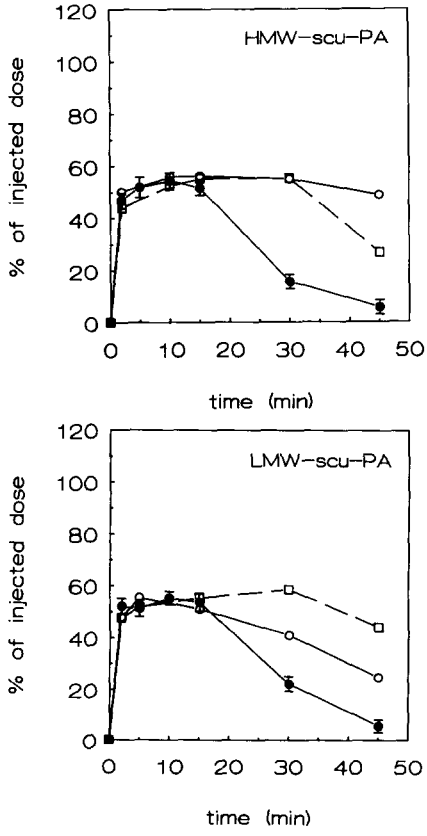

FIG. 2. **Effect of chloroquine and leupeptin on the liver association of ^{125}I-HMW- and ^{125}I-LMW-scu-PA.** Rats were pretreated with chloroquine (○), leupeptin (□), or not (●) as described under "Experimental Procedures." At the indicated times after injection of scu-PA, liver lobules were tied off and radioactivity was counted.

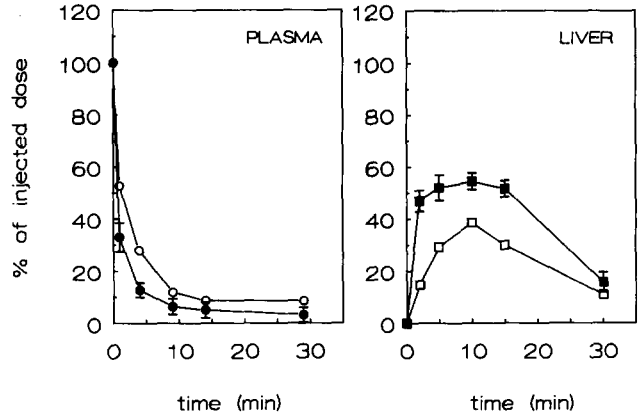

FIG. 3. **Effect of preinjection of unlabeled HMW-scu-PA on the plasma clearance and liver uptake of ^{125}I-HMW-scu-PA.** Rats were injected intravenously with 20 mg of HMW-scu-PA (*open symbols*) per kg of body weight 1 min prior to injection of ^{125}I-HMW-scu-PA or not pre-treated (*closed symbols*). At the indicated times after injection of the radiolabeled ligand, liver uptake (■, □) and plasma clearance (●, ○) were determined. *Bars* represent S.D. of three experiments.

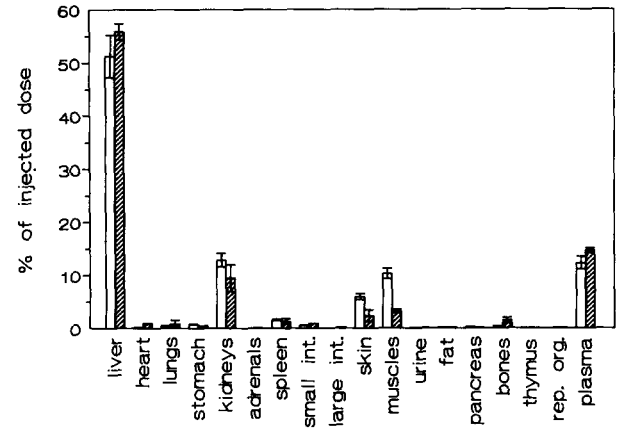

FIG. 4. **Tissue distribution of intravenously injected ^{125}I-HMW- or ^{125}I-LMW-scu-PA.** At 5 min after intravenous injection of ^{125}I-HMW-scu-PA (*hatched bars*) or ^{125}I-LMW-scu-PA (*open bars*) into rats, the amount of radioactivity in the various tissues was determined. Recovery of the injected amount of radioactivity in the tissues shown was 92.1 ± 5.3% for HMW-scu-PA and 97.5 ± 3.5% for LMW-scu-PA (n = 3, mean ± S.D.).

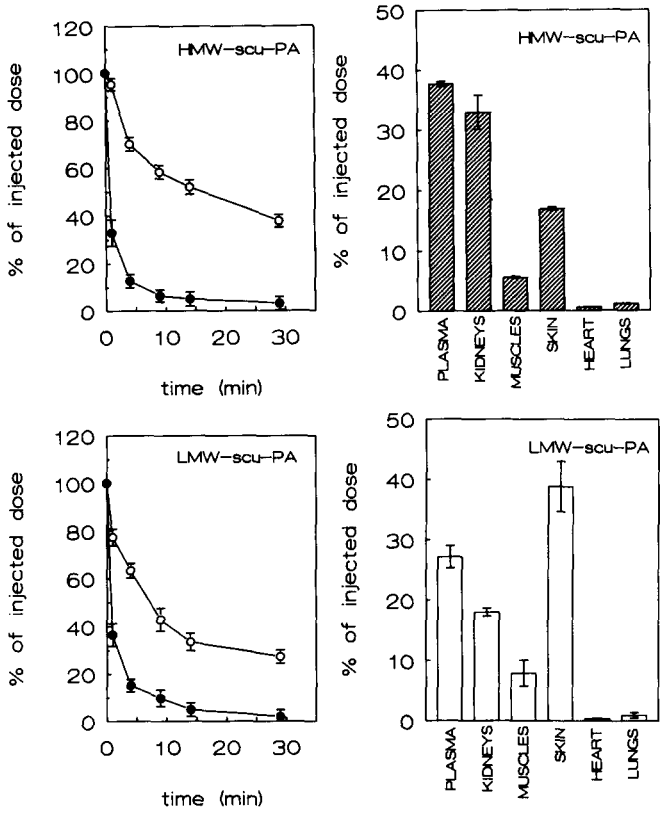

FIG. 5. **Tissue distribution and plasma clearance of intravenously injected ^{125}I-HMW- and ^{125}I-LMW-scu-PA in hepatectomized rats.** Rats were hepatectomized and subsequently injected with radiolabeled HMW-scu-PA (*top*) or LMW-scu-PA (*bottom*). At the indicated times blood samples were taken, and radioactivity (*open symbols*) was counted in aliquots of plasma. Control plasma clearance is shown by the *closed symbols*. At 30 min after injection, hepatectomized rats were sacrificed, and radioactivity was counted in the indicated tissues.

the plasma at 30 min after injection. This indicates that little or no degradation was mediated by extrahepatic organs. Additionally, for ^{125}I-LMW-scu-PA a prolonged plasma half-life was found after hepatectomy, and 30 min after injection 27.2 ± 1.9% of the injected dose was recovered in the plasma. At this time 38.8 ± 4.2% of the injected dose of LMW-scu-PA was recovered in the skin, while kidneys (18.0 ± 0.7%) and muscles (7.9 ± 2.2%) also contributed substantially to the plasma clearance of LMW-scu-PA. Again more than 90% of the radiolabel was present in the tissues shown and no trichloroacetic acid-soluble radioactivity was found in the plasma, indicating that degradation during hepatectomy was absent.

Liver Cell Distribution of scu-PA—Table I shows the contribution of the various liver cells types to the total liver uptake of ^{125}I-scu-PA. 10 min after injection of ^{125}I-scu-PA the various liver cell types were isolated and it was found that parenchymal liver cells were responsible for about 90% to the total liver uptake of both HMW- and LMW-scu-PA. Nonparenchymal liver cell types contributed only about 10% to the total liver uptake of scu-PA.

In Vitro Interaction of scu-PA with Liver Cells: Binding and Competition Studies—Since parenchymal liver cells were responsible for 90% of the liver uptake of HMW- and LMW-scu-PA, the specificity of the interaction of the two types of scu-PA with this liver cell type was studied *in vitro*.

Fig. 6 shows the displacement of ^{125}I-HMW-scu-PA with increasing amounts of unlabeled HMW-scu-PA after a 2-h incubation at 4 °C. After 2 h maximal binding had been

TABLE I

Relative contribution of the different liver cell types to liver uptake of scu-PA

10 min after intravenous injection of ^{125}I-LMW- and ^{125}I-HMW-scu-PA a liver cell isolation at 8 °C was started. Multiplication of the % of the injected dose/mg cell protein (not shown) with the amount of protein that each liver cell type contributes to total liver protein, results in the scu-PA uptake (expressed as % of total liver) by each cell type. Recovery of the injected dose in the different liver cell types was 112.1 ± 11.6% (HMW) and 105.1 ± 4.0% (LMW). Data represent the mean of three experiments ± S.D.

Cell type	^{125}I-LMW-scu-PA	^{125}I-HMW-scu-PA
Parenchymal	87.7 ± 4.6	89.2 ± 4.1
Endothelial	7.2 ± 2.3	6.1 ± 1.6
Kupffer	5.1 ± 2.4	4.9 ± 2.8

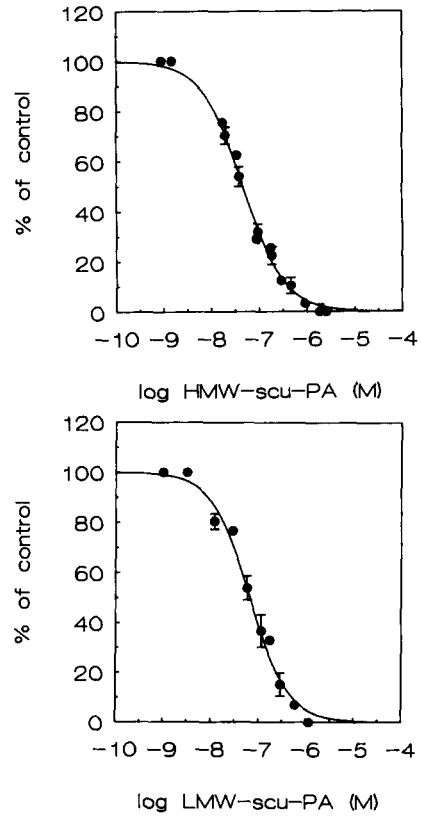

FIG. 6. **Effect of unlabeled scu-PA on the binding of ^{125}I-HMW- and ^{125}I-LMW-scu-PA by rat parenchymal liver cells *in vitro*.** Freshly isolated rat parenchymal liver cells were incubated for 2 h at 4 °C in the presence ^{125}I-HMW-scu-PA (1.49 nM, *top*) or ^{125}I-LMW-scu-PA (24.1 nM, *bottom*) with increasing unlabeled HMW- and LMW-scu-PA, respectively. At the end of the incubation the cell-bound radioactivity was counted, and the amount of cell protein was determined. Data are expressed as % of specific binding. Total binding for HMW-scu-PA was 2.9 ± 0.2 ng/mg cell protein and for LMW-scu-PA 17.8 ± 1.1 ng/mg cell protein; a nonspecific binding was 9.3 ± 0.3 and 9.2 ± 0.3% of the total binding, respectively. Data are mean ± S.D. (n = 3).

reached, and the level of binding was identical to that reached after 20 min of incubation at 37 °C (not shown). From the displacement curves the apparent K_d for the binding of HMW-scu-PA to parenchymal liver cells was 45 nM, while maximally 1.80 ± 0.11 pmol/mg cell protein (or about 800,000 binding sites/cell) was bound. For ^{125}I-LMW-scu-PA a similar inhibition experiment was performed (Fig. 6), which indicated that LMW-scu-PA bound specific to parenchymal liver cells with a K_d of 45 nM and maximal binding of 1.56 ± 0.11 pmol/mg cell protein.

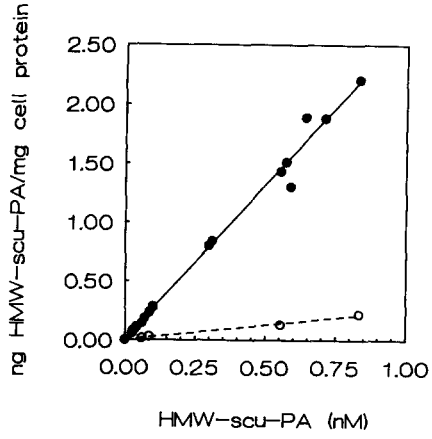

FIG. 7. **Binding of ^{125}I-HMW-scu-PA to parenchymal liver cells as a function of the HMW-scu-PA concentration.** Increasing amounts of ^{125}I-HMW-scu-PA were added to freshly isolated rat parenchymal liver cells in the presence (O) or absence (●) of 1 μM unlabeled HMW-scu-PA. After 2 h of incubation at 4 °C, cells were washed and the amount of radioactivity/mg of cell protein was determined.

Since ^{125}I-HMW-scu-PA was used at a concentration at which the u-PA-receptor on monocytes and tumor cells is reported to be saturated (13), a binding curve was determined at a concentration range from 0 to 1 nM HMW-scu-PA (Fig. 7). It is clear that in this concentration range no saturation of the binding was observed.

The apparent absence of a monocytic type of receptor on rat parenchymal liver cells, which is specific for HMW-scu-PA and does not bind LMW-scu-PA, initiated further studies to the specificity of the binding site on parenchymal liver cells. The coincidence of K_d and maximal number of scu-PA molecules bound/parenchymal cell for HMW- and LMW-scu-PA stimulated us to perform cross-competition experiments. Fig. 8 shows that the binding of ^{125}I-HMW-scu-PA to parenchymal liver cells can be equally well displaced by unlabeled HMW- and LMW-scu-PA, respectively. The binding of ^{125}I-LMW-scu-PA to parenchymal liver cells can also be displaced to the same degree by equal molarities of LMW- or HMW-scu-PA. The specificity of the binding of scu-PA to parenchymal liver cells was further determined by competition experiments with the related protein t-PA, ovalbumin, asialo-orosomucoid, and lactosylated albumin (Fig. 9). t-PA did not influence the binding of ^{125}I-HMW- or ^{125}I-LMW-scu-PA, while unlabeled HMW- or LMW-scu-PA had no effect on the binding of ^{125}I-t-PA by parenchymal liver cells.[2] Lactosylated albumin or asialo-orosomucoid, which block in the applied range the asialoglycoprotein receptor, did not affect the level of binding of ^{125}I-HMW- or ^{125}I-LMW-scu-PA to parenchymal liver cells. The mannose-terminal glycoprotein ovalbumin had also no effect on the binding of ^{125}I-HWM- and ^{125}I-LMW-scu-PA.

DISCUSSION

The presented data show that human LMW- and HMW-scu-PA are cleared from the plasma in rats with very comparable kinetics, a plasma half-life for the α-phase of 0.45 min and for the β-phase of 6–8 min. Both types of scu-PA are mainly taken up by the liver (approximately 55% of the injected dose). Besides the liver, the kidneys, skin, and muscles contributed significantly to the plasma clearance of scu-PA. In rabbits and monkeys (8, 9), like rats, the liver also

[2] J. Kuiper, unpublished results.

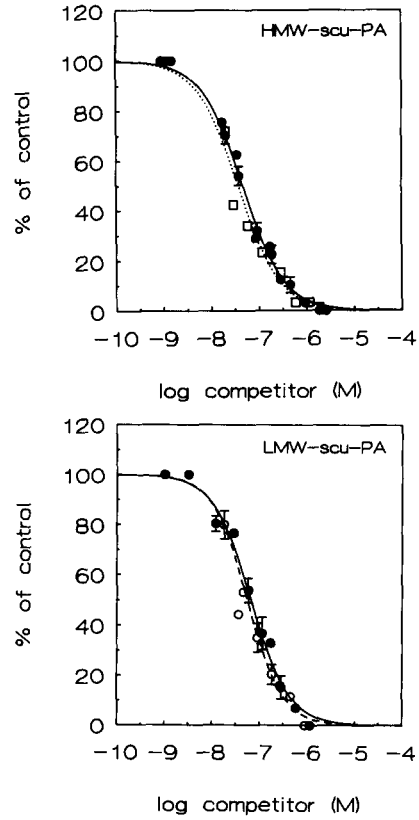

FIG. 8. **Effect of unlabeled scu-PA on the binding of ^{125}I-HMW- and ^{125}I-LMW-scu-PA by rat parenchymal liver cells *in vitro*.** Freshly isolated rat parenchymal liver cells were incubated for 2 h at 4 °C in the presence ^{125}I-HMW-scu-PA (*top*) with increasing amounts of unlabeled HMW-scu-PA (*solid line*) and LMW-scu-PA (*broken line*), or parenchymal cells were incubated in the presence of ^{125}I-LMW-scu-PA (*bottom*) with increasing amounts of unlabeled LMW-scu-PA (*solid line*) and HMW-scu-PA (*broken line*). At the end of the incubation, cell-bound radioactivity was counted and the amount of cell protein was determined. Data are expressed as % of specific binding. Values for total and nonspecific binding are given in the legend of Fig. 6. Data are mean ± S.D. (n = 3).

played a major role in the plasma clearance of human scu-PA. The resemblance between the pharmacokinetic behavior of high and low molecular weight scu-PA in rats is in agreement with the similar pharmacokinetic behavior of LMW- and HMW-scu-PA in rabbits and monkeys (8).

The cellular site of *in vivo* recognition of scu-PA within the liver was determined. For both LMW- and HMW-scu-PA, 90% of the liver-associated radioactivity was recovered in the parenchymal liver cells. *In vivo* and *in vitro* association of ^{125}I-HMW-scu-PA by the liver parenchymal cells could be inhibited by unlabeled HMW-scu-PA. *In vivo* inhibition of liver uptake of ^{125}I-HMW-scu-PA by unlabeled HMW-scu-PA was less efficient than *in vitro* inhibition of ^{125}I-HMW-scu-PA binding to parenchymal liver cells by unlabeled HMW-scu-PA. This may be the consequence of the fact that *in vivo*, in contrast to *in vitro*, unlabeled scu-PA is metabolized and the concentration of competitor is rapidly decreasing. This may also explain *in vivo* the higher percentages of inhibition at the shortest times after injecion. A similar effect has been observed blocking the uptake of ^{125}I-t-PA *in vivo* by unlabeled t-PA (22).

The binding of HMW-scu-PA to isolated parenchymal liver cells showed a K_d of 45 nM, and a maximal binding of 1.8 pmol/mg cell protein was found, which is equivalent to about 800,000 binding sites/cell. For the binding of LMW-scu-PA

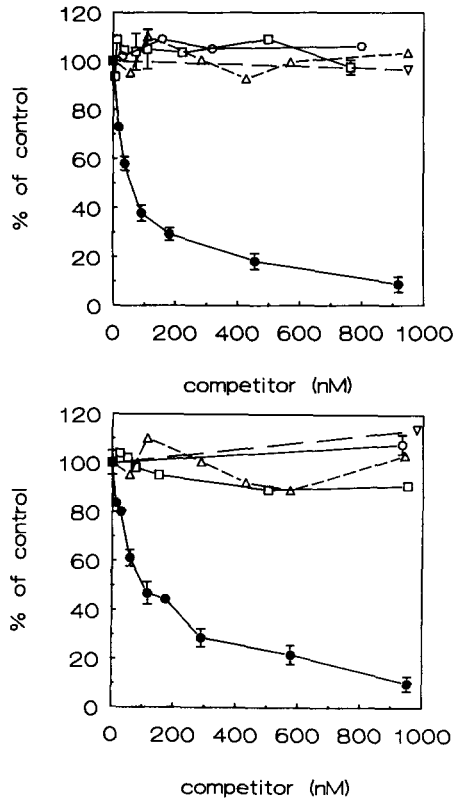

FIG. 9. **Comparison of the ability of unlabeled proteins to compete with the binding of ^{125}I-HMW- and ^{125}I-LMW-scu-PA by rat parenchymal liver cells *in vitro*.** Freshly isolated rat parenchymal liver cells were incubated for 2 h at 4 °C in the presence ^{125}I-HMW-scu-PA (*top*) and ^{125}I-LMW-scu-PA (*bottom*) with increasing amounts of unlabeled HMW-scu-PA (●, *top*) or unlabeled LMW-scu-PA (●, *bottom*), recombinant tissue-type plasminogen activator (□), lactosylated bovine serum albumin (○), ovalbumin (▽), and asialo-orosomucoid (△). At the end of the incubation, the radioactivity was counted and the cell protein was determined. Data are expressed as mean ± S.D. (n = 3).

to parenchymal liver cells, very similar binding characteristics were found. The apparent affinity of the parenchymal liver cell for HMW-scu-PA is a factor 15–150 lower than the apparent affinity of several other cell types (monocytes, tumor cells) for HMW-scu-PA (13), but higher than the affinity reported for rat parenchymal liver cells and highly labeled tcu-PA (K_d > 300 nM) (30). A unique feature of the parenchymal cell recognition site, which contrasts the monocytic u-PA receptor is the fact that the scu-PA receptor on liver cells recognizes both LMW- and HMW-scu-PA with a similar affinity. The monocytic receptor recognizes the growth factor domain in the HMW-scu-PA molecule and therefore shows no affinity for LMW-scu-PA (18, 19). Binding of HMW-u-PA to the monocytic u-PA receptor is reported to be strictly species specific: human u-PA did not bind to mouse monocytes, while murine u-PA did not bind to human monocytes (13). Species specificity for the organ uptake may be less strict, since, despite quantitative differences, in all species studied so far human scu-PA appears to be taken up predominantly by the liver and to a varying extent by the kidneys (7–12). Recently, a common binding site for HMW- and LMW-scu-PA was also suggested by means of ligand blot studies to be present on human endothelial cells (31).

The kinetics of liver association of LMW- and HMW-scu-PA are indicative for uptake coupled to degradation (8, 11). Inhibitors of the lysosomal degradation route (chloroquine and leupeptin) inhibit the disappearance of radioactivity from the liver of both LMW- and HMW-scu-PA, suggesting that degradation of scu-PA inside the liver is executed in the lysosomes. Rat parenchymal liver cells do not produce PAI-1 (32) nor possess m-RNA for PAI-1 or PAI-2 (33) and since *in vitro* HMW- and LMW-scu-PA degradation by parenchymal liver cells was observed,[2] it apparently occurs in the absence of PAI-1 or PAI-2. The degradation of both HMW- and LMW-u-PA in its single-chain form contrasts degradation of HMW-u-PA by monocytes and tumor cells, which only internalize and degrade two-chain HMW-u-PA after binding of PAI-1 or PAI-2 (20, 21).

The specificity of the scu-PA interaction with parenchymal liver cells was indicated by the fact that the related protein t-PA did not affect the binding of HMW- or LMW-scu-PA to parenchymal liver cells. This correlates with the finding that the hepatic uptake of t-PA and tcu-PA is mediated by distinct receptor systems (30). It may also suggest that the amino acids which differ between the rather homologous t-PA and u-PA molecules are involved in the parenchymal liver cell recognition. The binding and uptake of the glycoprotein scu-PA to parenchymal liver cells is not mediated by a galactose- or a mannose-specific receptor, since preinjection of glycoproteins (mannose- or galactose-terminated) does not affect *in vivo* hepatic uptake or *in vitro* parenchymal liver cell binding of LMW- or HMW-scu-PA. This finding correlates well with conclusions, drawn from the use of non-glycosylated recombinant scu-PA in rabbits (8).

The prominent role of the liver in scu-PA clearance and degradation was further subscribed by performing experimental hepatectomy. In hepatectomized rats plasma clearance of LMW- and HMW-scu-PA was significantly reduced. For HMW-scu-PA hepatectomy resulted in a sharp increase in kidney uptake, whereas for LMW-scu-PA hepatectomy resulted in an unexpected high uptake in the skin. In the absence of liver uptake, the kidneys are able to bind high amounts of HMW-scu-PA, but lack apparently the ability to bind LMW-scu-PA. No degradation of both types of scu-PA is found in hepatectomized animals, which may indicate that the specific HMW-scu-PA binding to the kidney does not lead to degradation of HMW-scu-PA. The kidney-binding site shares therefore recognition properties with the u-PA receptor on monocytes and tumor cells (13). Further studies on the metabolism of tcu-PA-PAI by kidneys are, however, needed for further identification of the kidney-binding site. The high interaction of LMW-scu-PA with the skin during hepatectomy may be due to extravascularization as a consequence of its low molecular weight, rather than a specific phenomenon.

It is concluded that rat parenchymal liver cells possess a common receptor for HMW- and LMW-scu-PA. Binding leads to internalization and proteolytic degradation involving a lysosomal route. The relative importance of the newly identified u-PA receptor system in the liver remains to be elucidated, but it may be anticipated to play a role in the regulation of maintaining low plasma levels of scu-PA (34).

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