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Characterization of the interaction both *in vitro* and *in vivo* of tissue-type plasminogen activator (t-PA) with rat liver cells

Effects of monoclonal antibodies to t-PA

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The interaction of ^{125}I-labelled tissue-type plasminogen activator (^{125}I-t-PA) with freshly isolated rat parenchymal and endothelial liver cells was studied. Binding experiments at 4 °C with parenchymal cells and endothelial liver cells indicated the presence of 68000 and 44000 high-affinity t-PA-binding sites, with an apparent K_d of 3.5 and 4 nM respectively. Association of ^{125}I-t-PA with parenchymal cells was Ca^{2+}-dependent and was not influenced by asialofetuin, a known ligand for the galactose receptor. Association of ^{125}I-t-PA with liver endothelial cells was Ca^{2+}-dependent and mannose-specific, since ovalbumin (a mannose-terminated glycoprotein) inhibited the cell association of t-PA. Association of ^{125}I-t-PA with liver endothelial cells was inhibited by anti-(human mannose receptor) antiserum. Anti-(galactose receptor) IgG had no effect on ^{125}I-t-PA association with either cell type. Degradation of ^{125}I-t-PA at 37 °C by both cell types was inhibited by chloroquine or NH_4Cl, indicating that t-PA is degraded lysosomally. *In vitro* experiments with three monoclonal antibodies (MAbs) demonstrated that anti-t-PA MAb 1-3-1 specifically decreased association of ^{125}I-t-PA with the endothelial cells, and anti-t-PA Mab 7-8-4 inhibited association with the parenchymal cells. Results of competition experiments in rats *in vivo* with these antibodies were in agreement with findings *in vitro*. Both antibodies decreased the liver uptake of ^{125}I-t-PA, while a combination of the two antibodies was even more effective in reducing the liver association of ^{125}I-t-PA and increasing its plasma half-life. We conclude from these data that clearance of t-PA by the liver is regulated by at least two pathways, one on parenchymal cells (not galactose/mannose-mediated) and another on liver endothelial cells (mediated by a mannose receptor). Results with the MAbs imply that two distinct sites on the t-PA molecule are involved in binding to parenchymal cells and liver endothelial cells.

INTRODUCTION

Tissue-type plasminogen activator (t-PA) is a serine protease which is synthesized and released by the vascular endothelium. The enzyme plays a key role in the fibrinolytic system. Binding of t-PA to fibrin enhances plasminogen activation and thus the formation of the proteolytically active enzyme plasmin (Collen, 1980; Bachmann & Kruithof, 1987).

In recent years t-PA has been used as a thrombolytic agent. Therapeutic use is impaired by rapid clearance from the circulation after intravenous administration of the drug. *In vivo* and *in vitro* studies have indicated that the hepatocyte plays an important role in this process (Fuchs *et al.*, 1985; Bakhit *et al.*, 1987; Bugelski *et al.*, 1989; Krause *et al.*, 1990; Rijken *et al.*, 1990). In previously described experiments *in vivo*, we have shown that 55% of the liver uptake of ^{125}I-t-PA is mediated by the parenchymal cells and 40% by the liver endothelial cells. The Kupffer cells are of minor importance for the liver uptake of t-PA: only 5% of the liver associated ^{125}I-t-PA is recovered in these cells (Kuiper *et al.*, 1988). These conclusions were confirmed by Einarsson *et al.* (1988), who also reported that both parenchymal cells and liver endothelial cells internalize t-PA. The aim of the present study was to explore the specific characteristics of the cellular uptake mechanisms for t-PA by isolated liver cells, with the use of monoclonal antibodies (MAbs) against t-PA and specific anti-(carbohydrate receptor) antibodies.

MATERIALS AND METHODS

Chemicals

Na^{125}I was obtained from Amersham. Two-chain t-PA was purified from culture media of Bowes melanoma cells (Rijken & Collen, 1981; Kluft *et al.*, 1983) and protein concentrations were based on amino acid analysis. BSA (fraction V), chloroquine, fetuin (type IV) and $GdCl_3$ were from Sigma, St. Louis, MO, U.S.A. Fetuin was neuraminidase-treated to obtain asialofetuin (ASF) (Kuiper *et al.*, 1988). Ovalbumin (5 × crystallized) was from Serva, Heidelberg, Germany. MAbs against t-PA [MAb 1-3-1, MAb 7-8-4 (both IgG1) and MAb 12-5-3 (IgG2a)] were developed by Bos *et al.* (1992). An anti-fibrin antibody [Y22 (IgG1)] was developed by Wasser *et al.* (1989). The antibodies were purified as described earlier (Ey *et al.*, 1978). The goat anti-[human (placenta) mannose receptor] antiserum (Lennartz *et al.*, 1987) was kindly donated by Dr. P. D. Stahl, Washington University School of Medicine, St. Louis, MO, U.S.A. The goat anti-(rat galactose receptor) IgG was a gift from Dr. G. Ashwell (Tanabe *et al.*, 1979). Orosomucoid was purified from human blood as described by Whitehead & Simmons (1966). The orosomucoid was neuraminidase-treated to obtain asialo-orosomucoid (ASOR) as described earlier (Kuiper *et al.*, 1988).

Labelling of t-PA

t-PA was labelled with ^{125}I using the Iodogen method (Fraker

Abbreviations used: MAb, monoclonal antibody; PAI-1, plasminogen activator inhibitor 1; t-PA, tissue-type plasminogen activator; ASF, asialofetuin; ASOR, asialo-orosomucoid.

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& Speck, 1978). To separate the labelled monomers of t-PA from various polymers and free iodide, we performed a gel filtration on a Sephacryl S-300 column (Rijken & Emeis, 1986). The fractions containing monomeric t-PA were pooled. Specific radioactivity was 1.8×10^9 Bq/mg of protein. Radioactivity was monitored on a Minaxi Auto-gamma 5000 series γ-radiation counter from Packard (Downers Grove, IL, U.S.A.). SDS/PAGE was performed according to Laemmli (1970), and autoradiography showed one band with a molecular mass of approx. 70 kDa. The plasminogen activator activity of ^{125}I-t-PA was measured according to Verheijen *et al.* (1982), and was about 70% of that of unlabelled t-PA.

Association and degradation of t-PA

Rat endothelial and parenchymal liver cells were isolated after perfusion of the liver for 20 min with collagenase (Kuiper *et al.*, 1989*a*). Liver parenchymal cells were isolated by differential centrifugation. The purity was greater than 99% and viability was greater than 95%. Endothelial liver cells were isolated by density-gradient centrifugation and centrifugal elutriation. Purity and viability were greater than 99%. The viability of the cells during the experiments exceeded 90%, as assessed by Trypan Blue exclusion. ^{125}I-t-PA (0.15 nM or 1.3 nM) was incubated with 10^6 parenchymal or liver endothelial cells at 4 or 37 °C under constant rotation. The incubation buffer contained Ham's F10 medium supplemented with Hepes (25 mM), BSA (2%, w/v) and Tween 80 (0.01%, v/v), pH 7.4. The total incubation volume was 1 ml. After incubation, the cells were washed twice with cold Tris/HCl (50 mM)/NaCl (0.15 M)/$CaCl_2$ (2.5 mM)/BSA (0.2%)/Tween 80 (0.01%), pH 7.4, and once with buffer without BSA. Association and degradation of ^{125}I-t-PA were expressed as pg of ^{125}I-t-PA per mg of cell protein, measured according to Lowry *et al.* (1951). Degradation products of t-PA were measured as ^{125}I-tyrosine (Otter *et al.*, 1989) and may represent an underestimation of total degradation. Competition curves were fitted by non-linear regression analysis with a Graph-Pad computer programme (ISI Software, Philadelphia, PA, U.S.A.).

Anti-(mannose receptor) and anti-(galactose receptor) antibodies

Association of ^{125}I-t-PA with liver endothelial cells and parenchymal cells in the presence of increasing concentrations of anti-(mannose receptor) antiserum or non-immune goat serum (0.2–5.4%), or of anti-(galactose receptor) IgG antibodies (27–381 μg/ml), was measured after incubation for 10 min at 37 °C.

Mabs against t-PA

Mouse MAbs and ^{125}I-t-PA were pre-incubated at room temperature for 30 min before experiments both *in vitro* and *in vivo*. For the experiments *in vivo* in rats, antibodies were also pre-injected (1 min) prior to the intravenous injection of the t-PA–antibody complex to prevent dissociation of the complex in the circulation. Plasma clearance and liver association studies of ^{125}I-t-PA *in vivo* were performed as described earlier (Kuiper *et al.*, 1988).

RESULTS

Association and degradation of t-PA

Association and degradation of t-PA by parenchymal and endothelial cells were determined at 37 °C for various periods of time. Association of ^{125}I-t-PA with both cell types proceeded rapidly, and after approx. 20 min an apparent equilibrium was reached (Fig. 1*a*). When experiments were performed at 4 °C for 3 h, the binding reached a maximum after 2 h of incubation (results not shown). Association of ^{125}I-t-PA per mg of cell protein was more than 4-fold higher for endothelial cells than for parenchymal cells.

Degradation products (^{125}I-tyrosine) were detected in the medium after a lag phase of 10 min, and subsequently increased progressively with time (Fig. 1*b*). Similar to the higher association of ^{125}I-t-PA with endothelial cells, the degradation of ^{125}I-t-PA by endothelial cells was also about 4-fold higher than that by parenchymal cells. Competition experiments of ^{125}I-t-PA binding to rat parenchymal cells and liver endothelial cells in the presence of increasing amounts of unlabelled t-PA (0–100 nM), measured after incubation at 4 °C for 2 h, showed a marked and concentration-dependent decrease in ^{125}I-t-PA binding to the cells (Figs. 2*a* and 2*b* respectively). Analysis of the competition curves indicated that parenchymal cells possess approx. 68000 binding sites for t-PA with an apparent K_d of 3.5 nM, and liver endothelial cells possess approx. 44000 binding sites with an apparent K_d of 4 nM.

Inhibitors of t-PA degradation

Degradation of ^{125}I-t-PA by parenchymal liver cells, measured after incubation at 37 °C for 3 h, was decreased by inhibitors of the lysosomal pathway, i.e. NH_4Cl (10 mM) and chloroquine

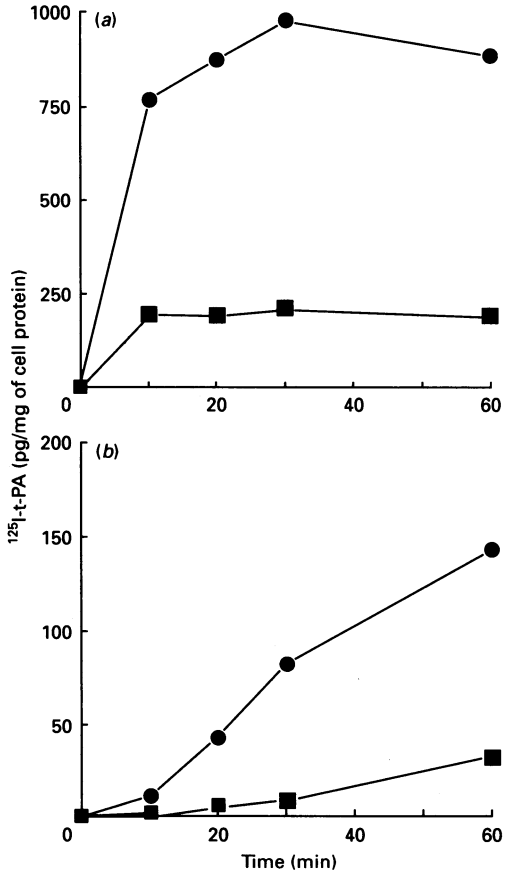

Fig. 1. Cell association and degradation of ^{125}I-t-PA in parenchymal cells and liver endothelial cells

^{125}I-t-PA (0.15 nM) was incubated with the cells for 1 h at 37 °C. Cells were centrifuged for 2 min at 50 *g* and 500 *g* for parenchymal and endothelial cells respectively at the indicated time points and washed with cold buffer, and cell-associated ^{125}I-t-PA was measured. The supernatant was precipitated with trichloroacetic acid and then extracted with chloroform to obtain the water-soluble ^{125}I-tyrosine fraction. Association (*a*) and degradation (*b*) by parenchymal cells (■) and liver endothelial cells (●) are expressed as pg of ^{125}I-t-PA/mg of cell protein.

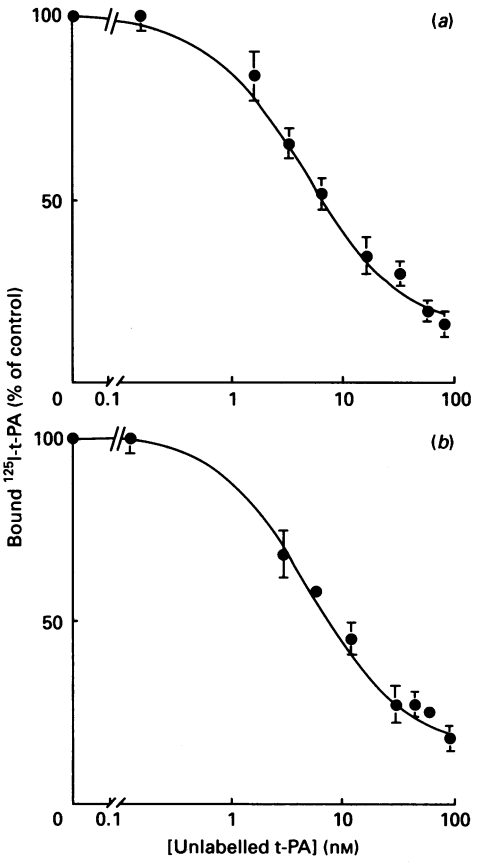

Fig. 2. Competition for ^{125}I-t-PA binding to parenchymal cells and liver endothelial cells

Parenchymal cells (*a*) and liver endothelial cells (*b*) were incubated for 2 h at 4 °C with ^{125}I-t-PA (1.3 nM) and increasing amounts of unlabelled t-PA. Binding is expressed as a percentage of the control, which amounted to 1.2 ng/mg of cell protein for parenchymal cells and 16 ng/mg of cell protein for liver endothelial cells respectively. Curve fitting was obtained by non-linear regression analysis.

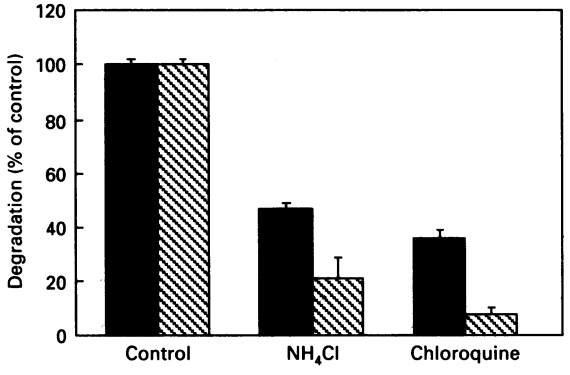

Fig. 3. Inhibitors of degradation of ^{125}I-t-PA by parenchymal and liver endothelial cells

^{125}I-t-PA (0.15 nM) and the inhibitors NH_4Cl (10 mM) or chloroquine (100 μM) were added to parenchymal cells (■) or liver endothelial cells (▨). The cells were incubated for 3 h at 37 °C. Degradation is expressed as a percentage of the control, which amounted to 400 pg of ^{125}I-t-PA/mg of cell protein for liver endothelial cells and 100 pg of ^{125}I-t-PA/mg of cell protein for parenchymal cells.

(100 μM) (Fig. 3). The effects of the two inhibitors were somewhat greater on the degradation of t-PA by liver endothelial cells than by parenchymal cells.

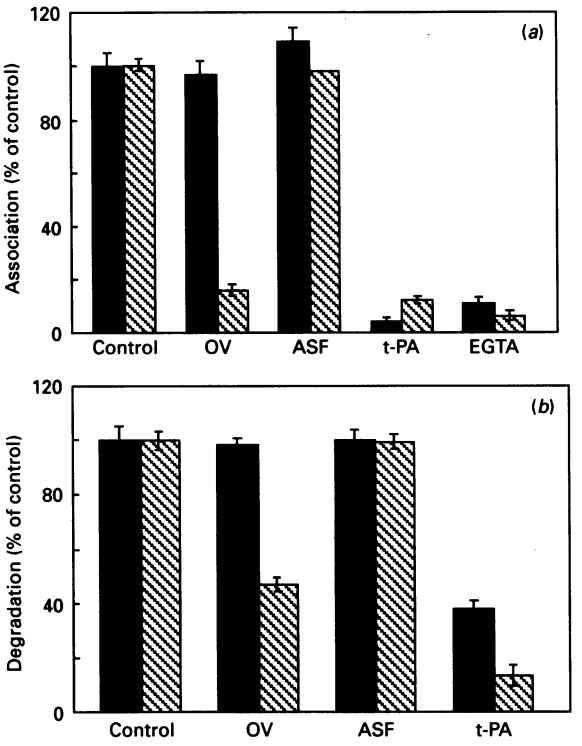

Fig. 4. Competitors for ^{125}I-t-PA association with and degradation by liver endothelial and parenchymal cells

(*a*) Association of ^{125}I-t-PA (0.15 nM) with liver endothelial cells (▨) and parenchymal cells (■) was measured in the presence of ovalbumin (OV, 40 μM), ASF (10 μM), unlabelled t-PA (160 nM) or EGTA (4 mM), for 10 min at 37 °C. Association is expressed as percentage of the control, which amounted for parenchymal cells to 181 ± 23 pg/mg of cell protein and for liver endothelial cells to 1252 ± 294 pg/mg of cell protein. (*b*) Degradation of ^{125}I-t-PA (0.15 nM) by liver endothelial cells (▨) and parenchymal cells (□) was measured in the presence of ovalbumin (OV, 40 μM), ASF (10 μM) or unlabelled t-PA (160 mM) for 3 h at 37 °C. Degradation is expressed as a percentage of the control, which amounted to 400 pg of ^{125}I-t-PA/mg of cell protein for liver endothelial cells and to 100 pg of ^{125}I-t-PA/mg of cell protein for parenchymal cells.

Competitors for cell association and degradation of t-PA

The effect of competitors was measured after incubation at 37 °C for 10 min, because at this time point association was maximal and hardly any degradation had yet occurred. To test the possible involvement of well-known glycoprotein receptors present on parenchymal and endothelial liver cells, we added ASF, ovalbumin or excess unlabelled t-PA (Fig. 4*a*). The association of ^{125}I-t-PA with endothelial cells was decreased by ovalbumin (40 μM) to 15% and by excess unlabelled t-PA (160 nM) to 11%. Binding to parenchymal cells was decreased by excess unlabelled t-PA to 4%, while ASF and ovalbumin were ineffective. These results were confirmed by the degradation experiments: ASF had no effect, ovalbumin was effective only in case of liver endothelial cells and excess unlabelled t-PA had an effect in both cell types (Fig. 4*b*). Association of t-PA with both cell types was Ca^{2+}-dependent: adding EGTA (4 mM) to the cells decreased binding to about 10% (Fig. 4*a*).

Effects of anti-(mannose receptor) and anti-(galactose receptor) antibodies

Association of ^{125}I-t-PA with liver endothelial cells could be inhibited to the level of non-specific cell association by adding anti-(mannose receptor) antiserum to the cells. This effect was

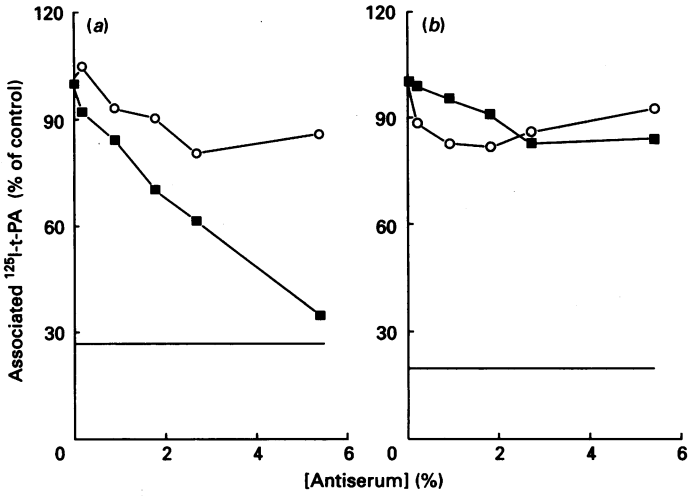

Fig. 5. Effect of anti-(mannose receptor) antiserum on ^{125}I-t-PA association with liver endothelial cells and parenchymal cells

Association of ^{125}I-t-PA (0.15 nM) with liver endothelial cells (*a*) and parenchymal cells (*b*) was measured after an incubation period of 10 min at 37 °C in the presence of increasing amounts of goat anti-(human mannose receptor) antiserum (■) and non-immune goat serum (○). The horizontal lines indicate the level of non-specific binding, obtained at 100 nM unlabelled t-PA.

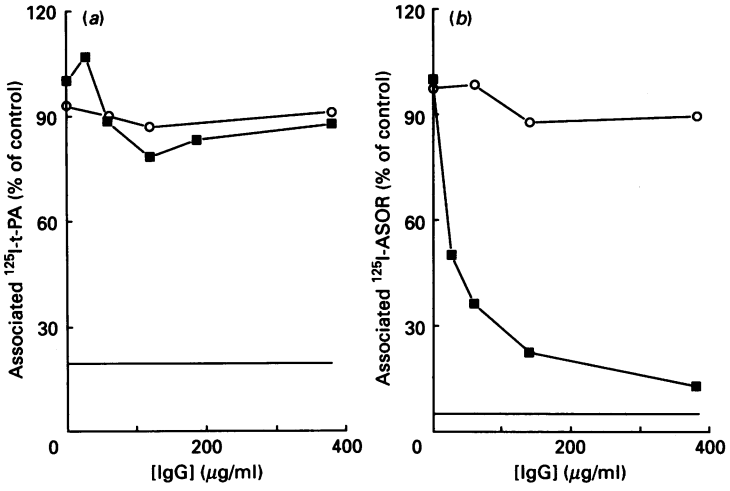

Fig. 6. Effect of anti-(galactose receptor) IgG on association of ^{125}I-t-PA and ^{125}I-ASOR with parenchymal cells

Association of ^{125}I-t-PA (0.15 nM) (*a*) and ^{125}I-ASOR (5 nM) (*b*) with parenchymal cells was measured in the presence of increasing concentrations (27–381 μg/ml) of anti-(rat galactose receptor) antibodies (■) and non-immune goat IgG (○) after an incubation period of 10 min at 37 °C. The horizontal lines indicate the level of non-specific binding, obtained at 100 nM unlabelled t-PA and 250 nM unlabelled ASOR. Association of ^{125}I-t-PA and ^{125}I-ASOR is expressed as percentage of control, which amounted to 181 pg of ^{125}I-t-PA and 12 ng of ^{125}I-ASOR/mg of cell protein respectively.

concentration-dependent (Fig. 5*a*). Control experiments with parenchymal cells showed essentially no decrease in association (Fig. 5*b*). Similar association experiments were performed with anti-(galactose receptor)IgG. These antibodies did not have an inhibitory effect on ^{125}I-t-PA association with either parenchymal

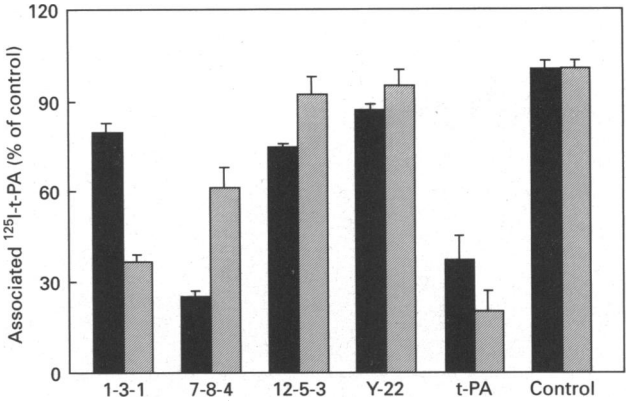

Fig. 7. Effects of MAbs against t-PA on ^{125}I-t-PA association with parenchymal and liver endothelial cells

^{125}I-t-PA (0.15 nM) was pre-incubated with the MAbs (100 nM) or unlabelled t-PA (100 nM) for 30 min at room temperature and then incubated with parenchymal cells (■ bars) or liver endothelial cells (▨) for 10 min at 37 °C. Binding is expressed as a percentage of control, which was 181 pg/mg of cell protein for parenchymal cells and 1252 pg/mg of cell protein for endothelial cells.

cells (Fig. 6*a*) or liver endothelial cells (results not shown). Control experiments with parenchymal cells incubated with ^{125}I-ASOR (a specific ligand for the galactose receptor on parenchymal cells) indicated that the anti-(galactose receptor) antibodies led to a 80 % decrease in the association of this ligand with these cells (Fig. 6*b*).

Effect of MAbs against t-PA on cell association

Three MAbs against t-PA (MAb 1-3-1, MAb 7-8-4 and MAb 12-5-3) were studied for their effects on t-PA association with parenchymal and liver endothelial cells (Fig. 7). Antibodies and ^{125}I-t-PA were pre-incubated at room temperature for 30 min before the mixture was added to the cells. MAb 1-3-1 decreased the association of t-PA with endothelial cells by 63 %, but had little effect on association with parenchymal cells (20 % decrease). Experiments with MAb 7-8-4 indicated the opposite; t-PA association with parenchymal cells was decreased by 75 %, but a significantly smaller effect was observed on t-PA binding to endothelial cells (39 % decrease). Antibody MAb 12-5-3 had little effect on t-PA association with either cell type, nor did the control antibody Y22.

In vivo experiments

An intravenous pre-injection of rats with MAb 1-3-1 or MAb 7-8-4, combined with pre-incubation of ^{125}I-t-PA with the antibodies, inhibited the clearance of ^{125}I-t-PA from the plasma (Fig. 8*a*). Clearance was further delayed when a combination of both antibodies was used. Following injection of ^{125}I-t-PA, 80 % was recovered in the livers of control rats after 7 min (Fig. 8*b*). Liver association was decreased after a pre-injection with either MAb 1-3-1 or MAb 7-8-4. Again, a further enhancement of this effect could be achieved when a combination of the antibodies was used. To investigate whether the Kupffer cells internalize the t-PA–MAb complexes, we blocked the uptake mechanism of the cells by pre-treatment of the rats with $GdCl_3$ (20 μmol/kg) as described by Bouma & Smit (1989). This treatment had no effect on the residual uptake of ^{125}I-t-PA by the liver (results not shown), so we can exclude the possibility that the complex is cleared through the Fc receptor on the Kupffer cells.

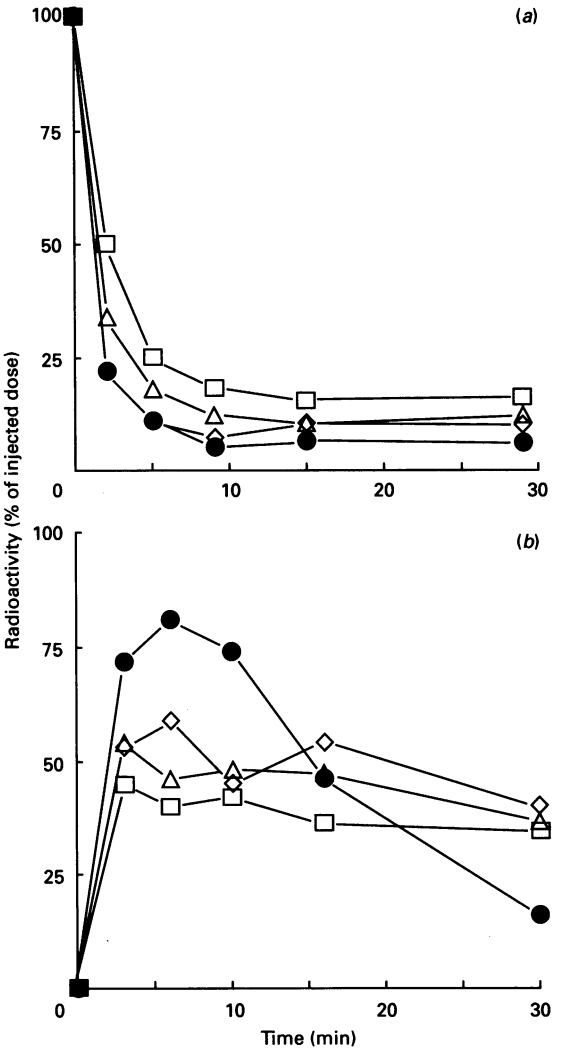

Fig. 8. Plasma clearance of (*a*) and liver association with (*b*) ^{125}I-t-PA *in vivo*

MAbs were pre-injected into rats (100 nM plasma concentration). ^{125}I-t-PA (0.15 nM) was pre-incubated with the MAbs (100 nM) (●, control; △, MAb 1-3-1; ◇, MAb 7-8-4; □, MAb 1-3-1 + MAb 7-8-4) for 30 min at room temperature prior to injection of the complex. At the indicated time points plasma samples were taken and liver lobules were tied off.

DISCUSSION

We previously reported that intravenously administered t-PA is predominantly taken up in the liver by two cell types: parenchymal and endothelial liver cells (Kuiper *et al.*, 1988). These data supported many earlier clearance studies of t-PA *in vivo* which indicated that the liver played a prominent role (Korninger *et al.*, 1981; Emeis *et al.*, 1985; Nilsson *et al.*, 1985; Krause, 1988). In the present study we show *in vitro* that t-PA can bind to, and subsequently be degraded by, freshly isolated parenchymal and liver endothelial cells. The association of ^{125}I-t-PA with both cell types occurred rapidly, and after a lag phase of 10–20 min degradation products began to appear. The kinetics are similar to those for the interaction of ovalbumin with the mannose receptor on rat liver endothelial cells (Magnusson & Berg, 1989) and of asialoglycoproteins with the galactose receptor on parenchymal cells (Tolleshaug *et al.*, 1977; Berg *et al.*, 1983).

The binding of ^{125}I-t-PA to liver endothelial cells in the presence of increasing amounts of unlabelled t-PA indicates that the binding is saturable, with approx. 44000 binding sites per cell and an apparent K_d of 4 nM. Association of ^{125}I-t-PA with liver endothelial cells can be reduced by 90% by ovalbumin, a glycoprotein containing a high-mannose-type carbohydrate group similar to that in t-PA (Pohl *et al.*, 1987). Additionally, the inhibitory effect of the anti-(mannose receptor) antiserum (Lennartz *et al.*, 1987) on the association of ^{125}I-t-PA with liver endothelial cells proves that t-PA is bound to such cells via a mannose receptor. This corresponds well with our recent finding in a purified system that t-PA represents a suitable ligand for the mannose receptor isolated from bovine macrophages (Otter *et al.*, 1991).

Binding of ^{125}I-t-PA to parenchymal cells in the presence of increasing amounts of unlabelled t-PA demonstrates that this binding is also of high affinity (apparent K_d 3.5 nM) and that the parenchymal cells possess approx. 68000 binding sites per cell. Our findings are in agreement with results from Bakhit *et al.* (1987) that ASF and ovalbumin do not interfere with the binding of t-PA to isolated rat hepatocytes. In contrast to Smedsrød *et al.* (1988, 1990), who concluded that t-PA uptake by parenchymal cells was mediated by the asialoglycoprotein receptor, we could find no evidence for a galactose-mediated interaction of t-PA with parenchymal cells as ASF, a well-known ligand for the asialoglycoprotein receptor, did not affect the cell association and degradation of t-PA by parenchymal cells. Moreover, the anti-(galactose receptor) antibodies had no effect on ^{125}I-t-PA association with parenchymal cells. These observations indicate that a role for the asialoglycoprotein receptor in the uptake process of t-PA by parenchymal cells can be excluded.

Degradation of t-PA by both cell types is inhibited by NH_4Cl and chloroquine, indicating that degradation follows a lysosomal pathway. Specific cell association and degradation of t-PA by liver endothelial cells (per mg of cell protein) is at least 4-fold higher than by parenchymal cells. This supports our studies on the cellular distribution on t-PA *in vivo* (Kuiper *et al.*, 1988). Einarsson *et al.* (1988) found a still higher ratio of endothelial cell to parenchymal cell uptake *in vitro*.

For the human hepatoma cell line Hep G2 an extracellular-matrix-independent t-PA-binding mechanism has been described (Otter *et al.*, 1989), which correlates well with our present studies with freshly-isolated parenchymal cells *in vitro*. A different type of binding on Hep G2 cells was described (Owensby *et al.*, 1988, 1989; Morton *et al.*, 1989, 1990; Wing *et al.*, 1991), indicating that plasminogen activator inhibitor 1 (PAI-1) present in the extracellular matrix of the Hep G2 cells plays a crucial role in the binding of t-PA to this human hepatoma cell line (subclone a16). To address the question of whether PAI-1 affects the t-PA binding to isolated rat hepatocytes in suspension, we blocked the active site of ^{125}I-t-PA with D-Phe-Pro-Arg-CH_2Cl. This blockade did not have any effect on the level of t-PA binding to either parenchymal or liver endothelial cells (results not shown). Since freshly isolated rat parenchymal cells do not produce any PAI-1 (Kuiper *et al.*, 1989*b*) and since no mRNA for PAI-1 is detectable in untreated rat parenchymal (Quax *et al.*, 1990), we conclude that PAI-1 is not involved in t-PA binding by rat parenchymal cells. These observations are in line with results from studies *in vivo* (Mohler *et al.*, 1988; Ord *et al.*, 1990), which show that PAI-1 does not play a major role in the clearance of t-PA by the liver.

MAbs to t-PA have mainly been tested with respect to their inhibitory effects on the enzymic properties (van Zonneveld *et al.*, 1987; Wojta *et al.*, 1989). They could, however, also be useful in analysing the mechanisms of t-PA interaction with liver cells. This possibility was examined with the cell line Hep G2 by Reilly *et al.* (1989). The MAbs we developed, MAb 1-3-1 and MAb 7-8-4, had a marked and cell-specific effect on the binding of t-PA

by endothelial and parenchymal cells respectively. Experiments based on epitope competition showed that the antibodies are directed against different epitopes on the t-PA molecule (Bos *et al.*, 1992). This was illustrated by the fact that MAb 7-8-4 affected the enzymic activity of t-PA, whereas MAb 1-3-1 did not (Bos *et al.*, 1992). The differential effects of MAb 1-3-1 and MAb 7-8-4 on t-PA binding by parenchymal and liver endothelial cells indicates that distinct sites on the t-PA molecule are involved in recognition by the two liver cell types. The fact that each MAb decreases t-PA binding to a liver cell type involved in t-PA clearance may explain the additional inhibitory effect on the liver association as well as the clearance of t-PA.

We conclude from our data that t-PA is cleared from the circulation through at least two independently operating pathways localized on the liver endothelial cell (mediated through a mannose receptor) and on the parenchymal cell (mediated through a non-carbohydrate, Ca^{2+} dependent specific uptake mechanism). The t-PA molecule possesses different binding sites for recognition by the parenchymal cell receptors and the liver endothelial cell receptors.

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