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The receptor for tissue plasminogen activator (t-PA) in complex with its inhibitor, PAI-1, on human hepatocytes

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The binding of t-PA-PAI-1 to human hepatocytes at 4°C reached a maximum at 2 h. Scatchard analysis indicated 74000 ± 11000 high-affinity binding sites for complex per human hepatocyte, with a K_d of 0.87 ± 0.09 nM. Almost identical results were achieved with the human hepatoma cell line Hep G2. Binding of [^{125}I]t-PA-PAI-1 complex was unaffected by high concentrations of unlabelled t-PA, PAI-1, u-PA or u-PA-PAI-1 complex; only t-PA-PAI-1 complex competed for binding. Hepatocyte-bound t-PA-PAI-1 was internalized and degraded at 37°C. Thus, hepatocytes have a specific t-PA-PAI-1 receptor that participates in clearance of this complex.

Hepatocyte; Receptor; Endocytosis; t-PA; PAI-1

1. INTRODUCTION

Tissue-type plasminogen activator (t-PA), an important therapeutic thrombolytic agent [1], is cleared rapidly from the circulation. The plasma half-life of t-PA is only 3–5 min [2–4]. The liver is the major site for the clearance of t-PA [2–4] but the mechanism is not yet established. Evidence has emerged recently for at least two receptor systems for t-PA. Hepatic endothelial cells contain a mannose receptor that is involved in the clearance of t-PA [5,6]. Liver parenchymal cells appear to have a specific receptor for t-PA [5,7]; this receptor probably recognizes the finger and growth factor domains of t-PA [8].

We have previously suggested that the high concentration of plasminogen activator inhibitor (PAI-1) in human liver may have a role in the clearance of t-PA [9]. Studies in the human hepatoma cell line, Hep G2, have shown that t-PA uptake occurs after complex formation with PAI-1 in the extracellular matrix of these cells [10,11]. We have established that the uptake of pre-formed t-PA-PAI-1 complex by the isolated perfused rat liver occurs twice as rapidly as that of free t-PA [12]. It was notable that the most significant difference in the clearance of the two proteins was the stronger binding of complex to liver cells, suggesting the presence of a receptor specific for the complex. Here we present evidence for such a receptor on human hepatocytes.

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2. MATERIALS AND METHODS

2.1. *Reagents*

[^{125}I]T-PA was supplied by Amersham (650–800 Ci/mmol). Single-chain recombinant human t-PA was from Genentech. T-PA-PAI-1 complex was purified from HT1080 cells as described previously and contained no detectable free t-PA or PAI-1 [12]. Radiolabelled t-PA-PAI-1 was prepared by incubating [^{125}I]t-PA with Hep G2 extracellular matrix, which contains active PAI-1 [10] for 2 h at 37°C. The [^{125}I]t-PA-PAI-1 complex was analysed by SDS-PAGE and autoradiography and 80–90% of the radiolabel was present as complex. PAI-1 was purified from human endothelial cells and activated by guanidine as described previously [13]. U-PA (two-chain) was from Medac GmbH, Hamburg, and u-PA-PAI-1 complex was prepared by incubation of u-PA with guanidine activated PAI-1 for 1 h at 22°C. Complex formation was assessed by SDS-PAGE, followed by immunoblotting or zymography [12], and was 80% complete.

2.2. *Isolation of human hepatocytes*

Human hepatocytes were isolated after collagenase dispersion of 20–30 g pieces of liver tissue, obtained from renal donors, as described previously [14]. The hepatocytes, 90% viable by Trypan blue exclusion, were washed and resuspended in binding medium (see below) for 15–30 min at 37°C [15] and kept at 4°C.

2.3. *Hep G2 cells*

The human hepatoma cells, Hep G2, were maintained in DMEM supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin under 5% CO_2:95% O_2 atmosphere. For preparation of cell suspension, the cells (90% confluent in 24-well plates) were washed 3 times in 10 mM phosphate buffer pH 7.2 with 0.15 M NaCl (PBS) and incubated with 3 mM EDTA in PBS for 10 min at 37°C. DMEM was added and the cells were dislodged from the culture dish with a plastic pipette. The resulting cell suspension was washed 3× with DMEM and the cells were 90–95% viable as assessed by Trypan blue exclusion.

2.4. *Binding assay*

Washed cells (10^5 hepatocytes or Hep G2), resuspended in binding medium (1 mg/ml BSA, 0.01% Tween 80 in DMEM), were incubated at 4°C with ligands. ^{125}I-t-PA-PAI-1 complex was added and each sample was mixed for 2 h at 4°C. The cells were washed (×3) with PBS at 4°C, lysed with 1 M NaOH and radioactivity counted.

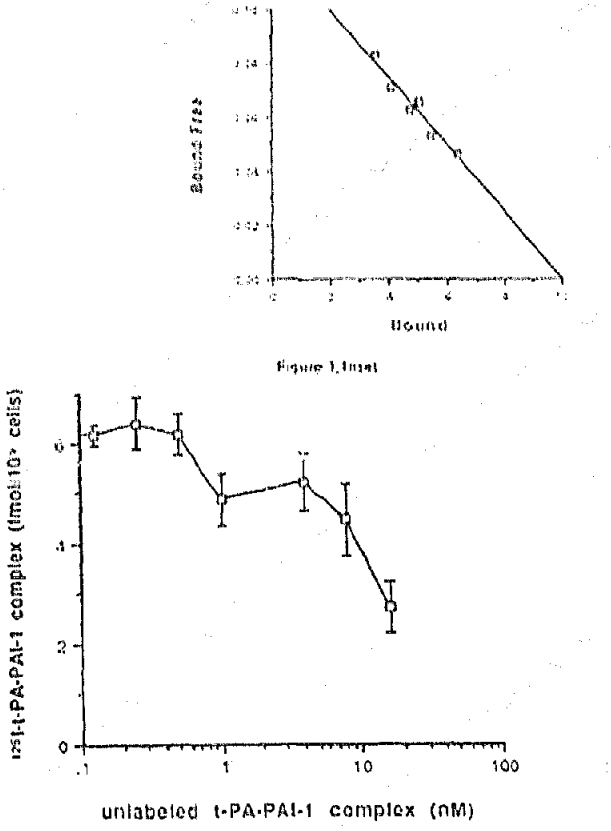

Fig. 1. Specific binding of [^{125}I]t-PA-PAI-1 complex (0.77 nM) to human hepatocytes expressed as fmol bound per 10^5 cells, competed by increasing concentrations (nM) of unlabelled t-PA-PAI-1 complex. Values shown are the mean of duplicate determinations ± SD and are typical of the three such experiments performed. Inset, Scatchard plot of the data.

2.5. *Internalization assay*

Binding was as in Section 2.4 and the cells were washed with PBS at 4°C as above. The total counts of the bound ligand were determined. Binding medium was then added and each sample was incubated at 37°C for up to 2 h. At various time points, the cells were centrifuged (900 × *g* at 4°C). The supernatant and that obtained after treating the cells with 0.02% trypsin in PBS (30 min at 4°C) was counted, as was the cell pellet after lysis with 1 M NaOH.

3. RESULTS

The binding of [^{125}I]t-PA-PAI-1 complex to human hepatocytes, in the presence of different concentrations of unlabelled t-PA-PAI-1 complex, was analysed (Fig. 1). Scatchard analysis indicated specific and saturable binding, with a K_d of 0.87 ± 0.09 nM and 74000 ± 11000 binding sites per cell (Fig. 1, inset). The binding was temperature-dependent and equilibrium was achieved at 4°C after 2 h (data not shown). After binding at 4°C, incubation at 37°C allowed rapid uptake (maximal at 30 min) and degradation of [^{125}I]t-PA-PAI-1 complex (Fig. 2). The uptake was inhibited totally by 0.5 mM chloroquine (data not shown).

Competitive binding assays demonstrated that t-PA,

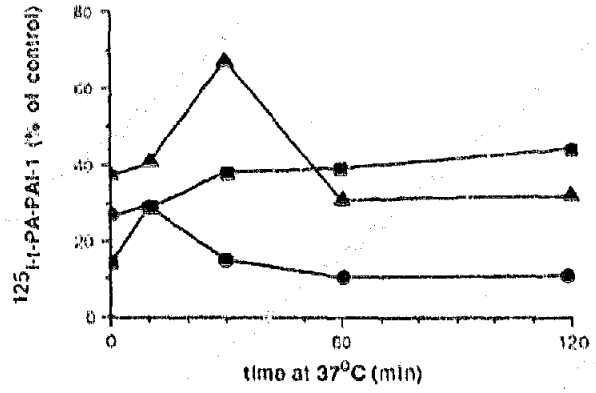

Fig. 2. Internalization of receptor-bound [^{125}I]t-PA-PAI-1 complex. Human hepatocytes were incubated for 2 h at 4°C with [^{125}I]t-PA-PAI-1 complex. The cells were then washed and incubated at 37°C in binding medium for the indicated times. The data are expressed as percentage of the total counts bound to the cells after 2 h at 4°C. (●) Receptor-bound ligand (trypsin-sensitive), (▲) cell-associated ligand (not sensitive to trypsin wash) and (■) released ligand in the supernatant.

u-PA, u-PA-PAI-1 complex and PAI-1, either latent or guanidine-activated, did not compete with [^{125}I]t-PA-PAI-1 binding, even at the relatively high concentrations used (Table I).

Comparable data were achieved with suspensions of Hep G2 cells; the K_d was 0.98 ± 0.06 nM t-PA-PAI-1 and there were 70000 ± 8600 binding sites per cell. Rat hepatocytes also showed comparable affinity for complex (K_d 0.86 ± 0.05 nM) but the number of binding sites per cell was lower (10000–25000). No specific binding of t-PA-PAI-1 to the human lymphoma cell line U937 was detectable (data not shown).

4. DISCUSSION

Evidence is presented for a t-PA-PAI-1 receptor on human hepatocytes. The receptor is saturable and of

Table I

Specificity of [^{125}I]t-PA-PAI-1 complex binding to human hepatocytes

Competitor	(nM)	[^{125}I]t-PA-PAI-1 bound (fmol/10^5 hepatocytes)	(% residual binding)
None		6.51 ± 0.40	100
t-PA-PAI-1	16	2.97 ± 0.40	46
t-PA	700	7.74 ± 0.50	118
u-PA	700	6.59 ± 0.13	101
u-PA-PAI-1	200	6.50 ± 0.01	100
PAI-1	700	5.93 ± 0.16	91
PAI-1 (activated)	700	6.77 ± 0.01	104

Human hepatocytes were incubated with [^{125}I]t-PA-PAI-1 complex (0.77 nM) in the absence or presence of unlabelled competitors. Cell-bound radioactivity was determined as described in Section 2. Results are expressed as the mean ± standard deviation of duplicate determinations of 3 separate experiments.

high affinity. Further, it participates in the uptake and degradation of t-PA-PAI-1. These findings explain and extend our previous data, which showed that the uptake of t-PA-PAI-1 in the isolated perfused rat liver was even more rapid than that of free t-PA [12].

This receptor system appears to be specific for t-PA-PAI-1 complex. Free t-PA or PAI-1 did not compete with binding, even when present in a 1000-fold excess over labelled t-PA-PAI-1. The existence of a receptor for t-PA in complex with its principal inhibitor is in line with the known mechanism of serine protease clearance, which are cleared in complex with their inhibitors [16].

No competition of t-PA-PAI-1 binding was observed when either u-PA or u-PA-PAI-1 complex was present in excess. This suggests that the receptor identified here is distinct from the well-characterized u-PA receptor [17]. This distinction was confirmed by the lack of binding of t-PA-PAI-1 complex to the histiocytic lymphoma cell line U937, a cell line on which the u-PA receptor occurs. A further point of contrast is that the u-PA receptor exhibits species specificity [18], while human t-PA-PAI-1 complex bound with equal affinity to the receptor on human or rat hepatocytes.

This study on primary human hepatocytes indicates that receptor function recovers from the collagenase treatment used in their isolation [15]. The results achieved with hepatocytes could be reproduced using suspensions of the human hepatoma cell line, Hep G2. It has previously been reported that Hep G2 cells bind and take up t-PA [19]. The binding data for t-PA were similar to those reported here; binding required complex formation with the PAI-1 that abounds in the extracellular matrix of these cells [11]. By using suspensions of Hep G2 cells, to avoid interference by matrix PAI-1, and pre-formed t-PA-PAI-1 complex, we have been able to show directly that the complex is the species recognized by this hepatic receptor. We suggest that this receptor represents an important mechanism for the clearance of endogenous t-PA, which occurs in plasma as t-PA-PAI-1 complex [20].

Definition of the relative importance of this receptor and other recently discovered t-PA receptors requires further study.

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REFERENCES

[1] Collen, D., Lijnen, H.R., Todd, P.A. and Goa, K.L. (1989) Drugs 38, 346–388.
[2] Emeis, J.J., Van den Hoogen, C.M. and Jense, D. (1985) Thromb. Haemostas. 54, 661–664.
[3] Korninger, C., Stassen, J.M. and Collen, D. (1981) Thromb. Haemostas. 46, 658–661.
[4] Fuchs, H.E., Berger, Jr H. and Pizzo, S.V. (1985) Blood 65, 539–544.
[5] Rijken, D.C., Otter, M., Kuiper, J. and Van Berkel, T.J.C. (1990) Thromb. Res. Suppl. X, 63–71.
[6] Smedrod, B. and Einarsson, M. (1990) Thromb. Haemostas. 63, 60–66.
[7] Krause, J., Seydel, W., Heinzel, G. and Tanswell, P. (1990) Biochem. J. 267, 647–652.
[8] Larsen, G., Metzger, M., Henson, K., Blue, Y. and Horgan, P. (1989) Blood 73, 1842–1850.
[9] Simpson, A.J., Booth, N.A., Sewell, H., MacGregor, I.R. and Bennett, B. (1989) Thromb. Haemostas. 62, 171 (abstract).
[10] Morton, P.A., Owensby, D.A., Sobel, B.E. and Schwartz, A.L. (1989) J. Biol. Chem. 264, 7728–7735.
[11] Owensby, D.A., Morton, P.A. and Schwartz, A.L. (1989) J. Biol. Chem. 264, 18180–18187.
[12] Wing, L.R., Hawksworth, G.M., Bennett, B. and Booth, N.A., J. Lab. Clin. Med., in press.
[13] Booth, N.A., MacGregor, I.R., Hunter, N.R. and Bennett, B. (1987) Eur. J. Biochem. 165, 595–600.
[14] Grant, M.H., Burke, D., Hawksworth, G.M., Duthie, S.J., Engeset, J. and Petrie, J.C. (1987) Biochem. Pharmacol. 36, 2311–2316.
[15] Rush, G.F. and Alberts, D. (1987) Life Sci. 40, 679–685.
[16] Pratt, C.W., Church, F.C. and Pizzo, S.V. (1988) Arch. Biochem. Biophys. 262, 111–117.
[17] Blasi, F. (1988) Fibrinolysis 2, 73–84.
[18] Blasi, F., Stoppelli, M.P. and Cubellis, M.V. (1986) J. Cell. Biochem. 32, 179–186.
[19] Owensby, D.A., Sobel, B.E. and Schwartz, A.L. (1988) J. Biol. Chem. 263, 10587–10594.
[20] Thorsen, S. and Philips, M. (1984) Biochim. Biophys. Acta 802, 111–118.