© 1991 Elsevier Science Publishers B V (Biomedical Division)
The clinical pharmacology of biotechnology products
M M Reidenberg, editor

Pharmacokinetics of human tissue-type plasminogen activator

P. Tanswell[1], E. Seifried[2] and J. Krause[3]

Departments of Pharmacokinetics[1] and Biochemistry[3], Dr. Karl Thomae GmbH, D-7950 Biberach; Med. Klinik und Poliklinik der Universität Ulm[2], D-7900 Ulm (Federal Republic of Germany)

1. INTRODUCTION

Recombinant human tissue type plasminogen activator (t-PA; *Actilyse*[R]) is the most effective agent currently available for coronary thrombolytic therapy of acute myocardial infarction [1]. Physiological t-PA is an endogenous glycoprotein protease that binds to the fibrin component of intravascular thrombi and specifically activates fibrin-bound plasminogen to plasmin, which subsequently dissolves the clot, Fig. 1. Recombinant t-PA administered at pharmacological doses to lyse pathological thrombi causes only limited generation of plasmin in the circulation, avoiding the systemic lytic state and associated coagulation defect which occur during therapy with the first-generation plasminogen activators streptokinase and urokinase.

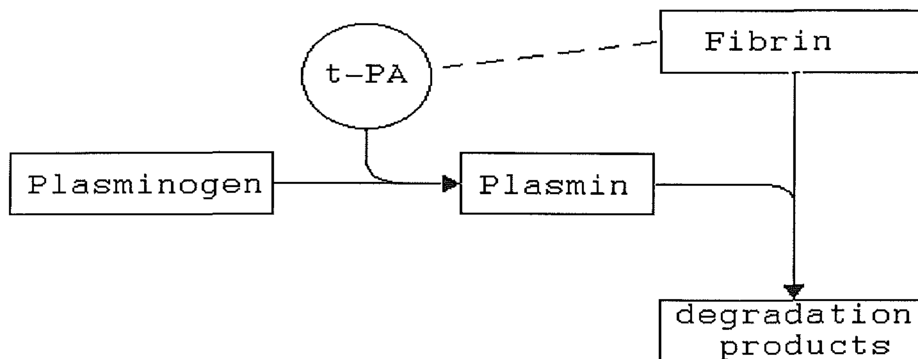

Fig. 1: Mechanism of action of t-PA

The pharmacokinetic properties of t-PA evoked considerable interest early in its development as a thrombolytic agent, when it was discovered that the plasma half-life of the molecule in vivo was only a few minutes due to rapid hepatic elimination [2]. An adequate understanding of t-PA pharmacokinetics is no less important at present, in view of three significant current trends: *(a)* continuing clinical studies aimed at further optimization of dosage regimens [3,21]; *(b)* rapidly advancing knowledge concerning the molecular mechanism of t-PA catabolism [4,22]; and *(c)* substantial ongoing efforts to modify the pharmacological properties of wild type t-PA using site-directed mutagenesis [5 - 7].

2. STRUCTURAL ELEMENTS OF t-PA RELEVANT FOR PHARMACOKINETICS

The t-PA molecule (reviewed in [6]) consists of a single polypeptide chain of 527 amino acids, containing 17 disulfide bridges, Fig. 2. The protease sensitive peptide bond R275-I276 is cleaved by plasmin during fibrinolysis, resulting in a 2-chain form of the molecule in which the amino terminal A-chain remains connected to the carboxy terminal B-chain by a single disulfide bridge. The B-chain comprises a serine protease domain that specifically cleaves the substrate plasminogen.

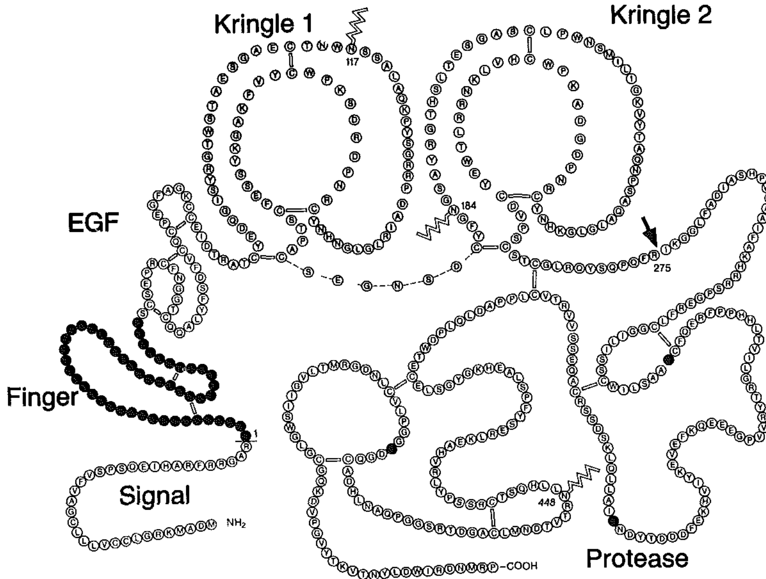

Fig. 2: 2-dimensional structure of human t-PA. N-glycosylation sites are represented by zig-zag lines; the arrow indicates the cleavage site for generation of 2-chain t-PA.

The A-chain mediates other biological functions such as fibrin binding, acceleration of plasminogen activation by fibrin, interaction with the specific inhibitor PAI-1, and binding to cells, including the process of *hepatic clearance*. It contains four autonomous domains which are termed "finger", "EGF" (growth factor), and "kringles 1 and 2", by virtue of their structural homology with other plasma proteins [6]. About 8% of the molecular mass of t-PA (M_r = 65,000) is carbohydrate; recombinant t-PA comprises comparable proportions of the carbohydrate variants termed type I and type II. In type I t-PA, asparagine linked carbohydrate side chains are present at N117 (oligomannose) and 184 and 448 (complex oligosaccharides), whereas in type II t-PA N184 is not glycosylated [8].

3. DETERMINATION OF t-PA CONCENTRATIONS DURING *IN VIVO* STUDIES

The form of circulating t-PA in plasma

After intravenous infusion, t-PA appears to circulate in plasma both in its free form and as complexes with the plasma protein inhibitors PAI-1, α2-antiplasmin, and C_1-esterase inhibitor [9]. Obviously, the interpretation of pharmacokinetic parameters depends on whether inhibitor-bound or unbound t-PA is detected by the analytical method employed. Further, binding sites for t-PA on platelets and monocytes have been reported. However, t-PA binding to blood cells is probably pharmacokinetically insignificant, since only negligible distribution from plasma into the cellular fraction was found on addition of ^{125}I-t-PA at pharmacological concentrations to whole blood in vitro [10].

Analytical methods

t-PA concentrations in plasma and tissues after administration of the drug in vivo have been analyzed using *(a)* ^{125}I-labeled t-PA and determination of radioactivity precipitated by trichloracetic acid (TCA) or bound by specific antibody, *(b)* functional assays based on plasminogen activation, *(c)* enzyme-linked immunosorbent assays (ELISA), or combinations of these methods.

^{125}I-determination is semi-quantitative and yields meaningful results only up to a few minutes after dosing, since ^{125}I is rapidly cleaved from t-PA by intracellular dehalogenases [11], and TCA and antibodies can also precipitate/bind small peptide fragments. There are however few practicable alternatives for estimating t-PA concentrations in cells and tissues.

Functional assays for t-PA activity in plasma have been numerously reported. The most commonly used are the fibrin plate method, and spectrophotometric assays of plasmin via the chromogenic substrate S-2251 after activation of plasminogen by t-PA in the presence of a noncoagulable fibrin analog such as CNBr fragments of fibrinogen [12,13]. Such assays provide a direct measure of plasmingen activation and are inherently sensitive, but suffer the disadvantage of susceptibility to interference by plasma components. In addition, it is difficult to prevent artifacts caused by loss of t-PA activity via complexation with plasma protein inhibitors *in vitro* in the interval between blood sampling and assay.

2-site ELISA methods measure antigenic mass and utilize a solid phase antibody to capture t-PA from the plasma samples, followed by binding of a second, enzyme labeled antibody for photometric detection via a suitable substrate. ELISA techniques are generally the method of choice due to their high sensitivity, specificity and robustness, and a large number of such assays, utilizing both polyclonal and monoclonal antibodies, has been reported in the literature (e.g. [12]). However, the extent to which a particular ELISA distinguishes between free t-PA and t-PA complexed to plasma protein inhibitors depends on the antigenic determinants recognized by the antibodies used and must be investigated in each case.

4. PHARMACOKINETIC MODEL FOR t-PA: CURRENT CONCEPTS

Route of administration

The high molecular mass of t-PA effectively prevents diffusional transport across biological membranes, hence intravenous bolus and/or infusion is at present the only viable method of delivering t-PA for human therapy. Although intramuscular dosing in combination with absorption enhancers yields thrombolytic plasma concentrations in animal models [14], this route of administration has not yet been investigated in man.

General model

The pharmacokinetic model shown in Fig. 3 can be used to fit all intravenous t-PA plasma concentration/time data so far observed [15], and is equally applicable to t-PA mutants. The model incorporates t-PA bolus or infusion into a central (plasma) compartment postulated to include the

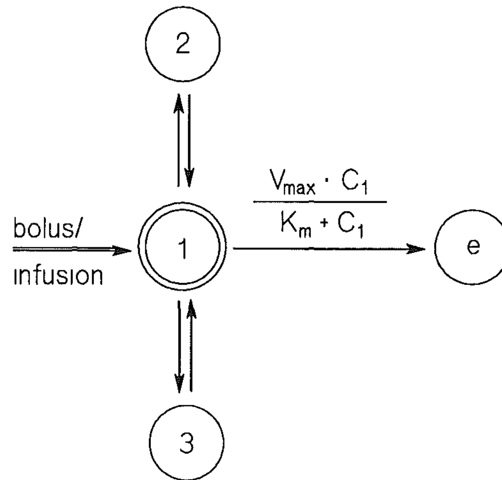

Fig. 3: Pharmacokinetic model for t-PA

liver; first order transfers between the central compartment and 1 or 2 peripheral (tissue) compartments, and capacity-limited (saturable) Michaelis-Menten elimination from the central compartment. The number of compartments and degree of saturation detected in a particular experiment depend on the dosage regimen, the duration of plasma sampling, and the sensitivity of the analytical method.

The elimination rate of t-PA from plasma in this model is given by $-dC_1/dt = V_{max}.C_1/(K_m+C_1)$, where C_1 is the t-PA plasma concentration, V_{max} is the maximum elimination rate and K_m is the Michaelis-Menten constant (value of C_1 for half-maximal elimination rate). Two limiting cases can be distinguished. At very high t-PA plasma concentrations ($C_1>>K_m$), elimination is zero order (i. e. independent of C_1), with $-dC_1/dt = V_{max}$. On the other hand, at low plasma concentrations

($C_1 << K_m$), the more familiar condition of first order t-PA elimination is attained with $-dC_1/dt = k_{1e}.C_1$, where k_{1e} (=V_{max}/K_m) is the elimination rate constant. The effect of nonlinearity (saturation) on the shape of t-PA plasma concentration-time profiles and dose-concentration relationships is illustrated in Fig. 4.

Conditions of nonlinear pharmacokinetics

In a recent study in rats, rabbits and marmosets, using t-PA infusion rates of up to 530 µg/kg/min for 30 min, maximum plasma concentrations (C_{max}) of >110 µg/ml based on ELISA analysis

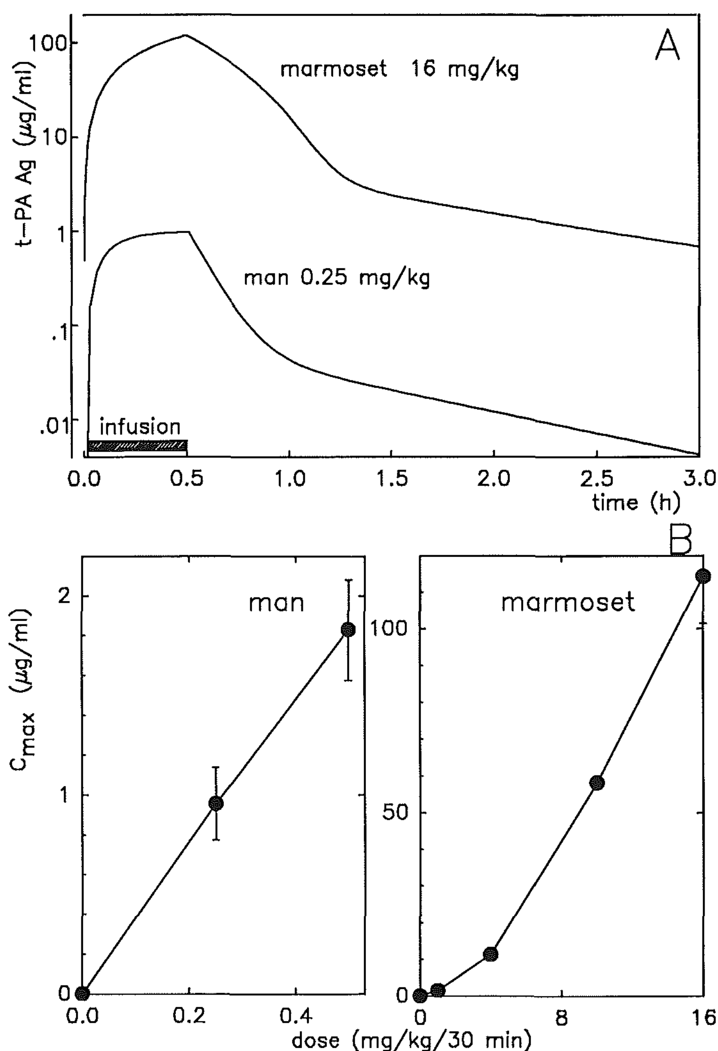

Fig. 4: Effect of nonlinear kinetics (saturation of catabolism) on t-PA plasma concentration profiles (A), and relationship between infusion rate and C_{max} (B). Data based on [13] and [15].

were reached [15]. K_m and V_{max} were computed by simultaneous fitting of multiple plasma concentration-time curves. There was little species variability, with K_m = 12 - 15 µg/ml and V_{max} = 200 - 350 µg/ml/h. Although a zero order component of t-PA disposition was perceptible in the immediate post-infusion phase at the highest infusion rate in the form of a convexity (Fig. 4A), the nonlinearity became apparent at much lower doses on plotting C_{max} or AUC (area under the curve) against infusion rate (Fig. 4B). Further, in *isolated perfused rat liver*, t-PA elimination capacity was considerably reduced compared to the *in vivo* experiments, with K_m = 1.5 µg/ml and V_{max} = 3.7 µg/ml/h [15].

The practical conclusion to be drawn from this study is that kinetic data from single doses of t-PA preparations are of limited value. It must be emphasized that the commonly used pharmacokinetic parameters clearance, volumes of distribution and half lives are *not meaningful* under nonlinear conditions, since they are a function of plasma concentration. In particular, reported differences in "clearance" between mutant molecules and wild-type t-PA in animals may be partially attributable to alterations in K_m and V_{max}, resulting in misleading conclusions regarding the potential clinical utility of such variants.

Conditions of linear pharmacokinetics

If the results of the 3 species study can be generalized [15], wild-type t-PA pharmacokinetics in mammals will be effectively linear as long as average plasma concentrations are less than about 10 - 20 % of K_m, i.e. 1.5 - 3 µg/ml. This is the case in clinical studies reported to date. Under these circumstances, the following pharmacokinetic parameters can be calculated from the model (Fig. 3) using standard formulae: volumes of distribution of the central compartment (V_1) and at steady state (V_{ss}), total plasma clearance ($CL = k_{1e}.V_1$), the half lives $t_{1/2\alpha,\beta,\gamma}$ of the α, β- and γ-phases of disposition, and their relative contributions $AUC_{\alpha,\beta,\gamma}$ to the area under the curve.

Evidence for the hepatic elimination of t-PA

The inference of a capacity-limited process for t-PA elimination based on pharmacokinetic data both *in vivo* and in isolated perfused rat liver, with evaluations of K_m and V_{max} [15], confirmed and quantified many independent earlier observations (e.g. [2] and review in [5]), which present strong evidence that the predominant mechanism of t-PA is via hepatic catabolism. In brief, *(a)* increased plasma concentrations of t-PA occur in liver disease, liver transplantation, experimental ligation of the portal vein, liver bypass and partial or total hepatectomy; *(b)* rapid accumulation of t-PA in the liver is observed in tissue distribution studies and whole body autoradiography [16]; *(c)* t-PA has been shown to undergo avid, saturable binding by a hepatic uptake system, followed by intracellular degradation [17].

5. PHARMACOKINETIC PARAMETERS OF t-PA UNDER LINEAR CONDITIONS

Effect of t-PA preparation

This subject has been reviewed [18]. Briefly, the very first pharmacological studies on t-PA were conducted with material derived from Bowes melanoma cell culture. *Recombinant t-PA* (Genentech, Inc.) for initial clinical trials up to early 1985 was produced by a small scale

methodology and consisted exclusively of the 2-chain molecule. Subsequently, for pivotal clinical trials and marketing, a large scale manufacturing process was used, yielding t-PA that comprised 70 - 80 % of the single chain molecular form (*Activase*[R]*; Actilyse*[R]). Large scale recombinant t-PA exhibited a higher plasma clearance in myocardial infarction patients than small scale t-PA, which necessitated a dosage adjustment [19]. This alteration in clearance was however clearly shown *not* to be connected with the chainedness of the molecule [18].

Pharmacokinetics in animals

Pharmacokinetics of t-PA have been studied in mice, rats, rabbits, dogs and monkeys [5]. Exact quantitative comparisons between published studies are hindered due to differences in the origin of the t-PA preparations administered and/or in the assay methodologies employed to measure t-PA in plasma. However, the following consistent qualitative picture emerges. After intravenous bolus or infusion of t-PA, plasma concentrations decrease rapidly in an initial, α-phase of clearance characterized by a half-life of < 5 min. The α-phase is dominant, since it encompasses more than two-thirds of AUC. A second, β-phase of disposition follows with $t_{1/2}$ >10 min, and often a third, γ-phase with $t_{1/2}$ >1 h. This final phase, when observed, represents the elimination of only a few percent of t-PA dose. In a study in which Actilyse[R] was administered to rats, rabbits and marmoset monkeys under identical experimental conditions [15], total plasma clearance of t-PA antigen at low doses was 16 - 23 ml/min/kg, which is close to the hepatic plasma flow rate in these species. V_1 was 46 - 91 ml/kg, corresponding to the plasma volume, and V_{ss} was 230 - 330 ml/kg, indicating moderate tissue distribution and/or binding. $t_{1/2\alpha}$ was 1.1 - 2.4 min with AUC_α= 65 - 77 %, $t_{1/2\beta}$ was 10 - 40 min with AUC_β = 23 - 25 %, and $t_{1/2\gamma}$ was 1 - 1.7 h with AUC_γ = 7 - 10 %.

Pharmacokinetics in man

Clinical pharmacokinetic studies of t-PA satisfying the criteria of adequate blood sample scheduling and computerized data analysis that have been commonplace for more than a decade in investigations of chemical drugs are unfortunately rare. Surprisingly, many earlier studies appeared to use "pencil and graph paper" methods to determine half-lives from post-infusion data although specialized programs such as "TOPFIT" [13] and "NONLIN" [18], which fit preinfusion and multiple infusion plasma concentration data using nonlinear least-squares algorithms, were readily available.

Healthy volunteers. Large scale process t-PA was pharmacokinetically characterized based on plasma antigen and activity in 2 studies at subclinical doses. In the first [12], 8 subjects were administered 0.25 mg/kg t-PA over 30 min; plasma concentrations were compared using fibrin plate, S2251 chromogenic assay, and ELISA methodologies. Clearance based on antigen was 687 $\pm$ 63 (SD) ml/min (8.3 $\pm$ 0.76 ml/min/kg). The biological assays yielded lower plasma concentrations and thus higher clearances: 1050 $\pm$ 104 ml/min (fibrin plate) and 1235 $\pm$ 170 ml/min (S2251). However, half lives (2 compartment model) were virtually identical in all assays, with $t_{1/2\alpha}$ = 3.3 - 3.5 min and $t_{1/2\beta}$=26 - 34 min; AUC was 82 - 96 %.

In a further study [13], dose linearity was tested and proved by administering 0.25 and 0.5 mg/kg Actilyse[R] in 2 groups of 6 volunteers (see also Fig. 4). Clearance was equivalent at both

doses, the mean value being 620 ± 68 ml/min (8.2 ± 1.3 ml/min/kg) based on t-PA antigen. $t_{1/2\alpha}$ was 4.4 ± 0.3 min, AUC_{α} 87 ± 1% and $t_{1/2\beta}$ 40 ± 2.7 min. Chromogenic activity analysis yielded CL = 924 ± 160 ml/ min. Comparable results based on ELISA were reported in an earlier study, in which 3 different large scale t-PA preparations were administered to groups of 9 volunteers [18]. In common with the animal studies, CL values in healthy volunteers approximated to hepatic plasma flow [15].

Myocardial infarction patients. In the opinion of the authors, only one study adequately describes pharmacokinetics of the marketed large scale t-PA preparation using the recommended dosage regimen (100 mg over 3 hours; 10 mg as an initial bolus, 50 mg infused in the first hour and 20 mg/h over the subsequent 2 hours) [20]. In 12 myocardial infarction patients, 3-compartment kinetics were observed, referenced both to t-PA antigen and activity. Mean data based on antigen were: $t_{1/2\alpha}$=3.6 ± 0.9 min, $t_{1/2\beta}$= 16 ± 5.4 min, $t_{1/2\gamma}$ = 3.7 ± 1.4 h. AUC_{α}, AUC_{β} and AUC_{γ} were 66, 31 and 3 % respectively, CL was 383 ± 74 ml/min, V_1 2.8 ± 0.9 L and V_{ss} 9.3 ± 5 L. Peak plasma concentration after the bolus was 3.3 ± 0.95 µg/ml, and steady state concentrations during the ensuing infusions were 2.21 ± 0.47 and 0.93 ± 0.2 µg/ml respectively. A typical data fit is shown in Fig. 5. Similar kinetic parameters were obtained in a novel study using a single 50 mg bolus injection; C_{max} was 9.8 ± 3.6 µg/ml [21].

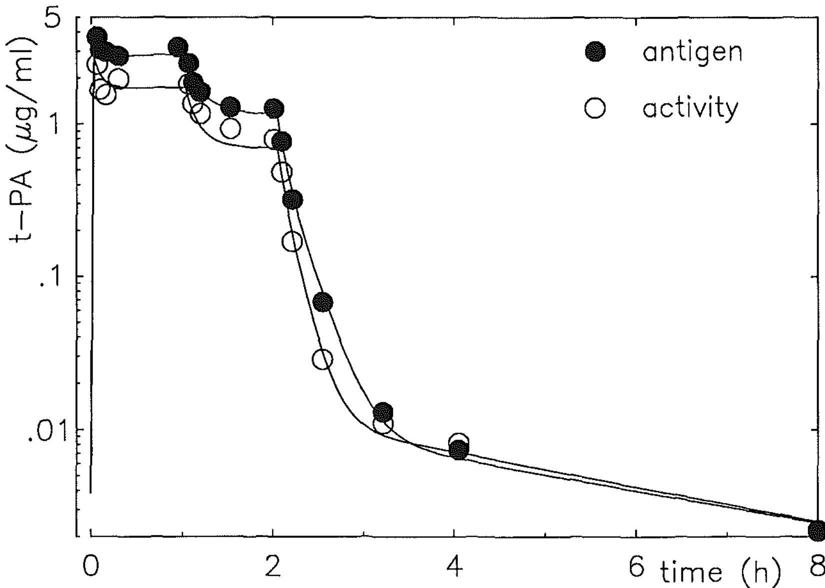

Fig. 5: t-PA plasma concentration-time profile in a myocardial infarction patient (male, 75y); dose regimen given in the text.

6. MECHANISM OF HEPATIC CATABOLISM OF t-PA

Consequent to the pharmacokinetic findings that t-PA is eliminated hepatically, intensive efforts are currently being devoted to elucidate the interaction of t-PA with hepatocytes *in vivo* and *in vitro* using biochemical, cell biological, immunocytochemical and electron microscopy techniques [22, 23]. The present status can be summarized as follows.*(a)* t-PA catabolism is initiated via binding of the molecule to hepatic receptors, followed by internalization in coated pits, degradation by lysosomes, and release of soluble t-PA fragments into the circulation. Urokinase is cleared by a completely different receptor system [4]. Binding of ^{125}I-t-PA to primary rat hepatocytes revealed a high affinity and a low affinity site with K_D values 4 nM and >100 nM respectively, Fig. 6.

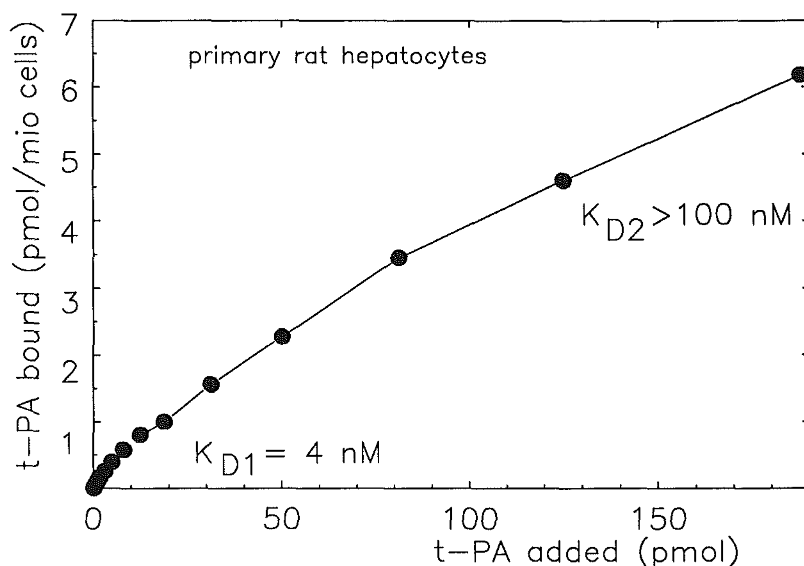

Fig. 6. Saturation binding of ^{125}I-t-PA to primary rat hepatocytes (based on [4]).

(b) Parenchymal, endothelial and Kupffer cell fractions contribute 55 %, 40 % and 6 % to total t-PA uptake in the liver respectively [22]. *(c)* Catabolism in endothelial cells occurs via a mannose receptor recognizing t-PA carbohydrate at N114 (see Fig. 2), and in parenchymal cells via a specific t-PA receptor that recognizes a polypeptide structure localized in the A-chain. Preliminary characterization of the rat hepatic parenchymal receptor indicates that it is located on the cell surface, and is a protein with a molecular mass of about 35000 in SDS gel electrophoresis [24]. The t-PA-specific clearance determinant probably comprises amino acid side chains located in the finger, EGF and kringle 1 domains [25]. The enzymatic active site of t-PA, or other moieties in the B-chain, do not appear to be necessary for recognition by either hepatic receptor system. *(d)* As demonstrated by numerous studies investigating pharmacokinetics of t-PA variants, total plasma

clearance in vivo or in isolated perfused liver is an extremely sensitive indicator of structural modification of t-PA. For example, the single amino acid point mutation R275E, or removal of the high mannose carbohydrate at N114, resulted in a reduction in CL of more than 50 % in both experimental systems [7,26]. Deletion of whole domains reduces the elimination rate of t-PA even more drastically [5]. *(e)* The catabolic fate of t-PA complexes with plasma protein inhibitors, the extent to which these contribute to the overall clearance of t-PA, and the existence (if any) of extra-hepatic clearance pathways for t-PA at pharmacologiocal doses are questions that are as yet unresolved.

7. CONCLUSIONS

The objective of pharmacokinetic studies is to relate doses to pharmacological effects via determination of drug concentrations in plasma and tissues, ultimately enabling prediction of clinical outcome via suitable models. Since the site of action of t-PA is the blood compartment, its therapeutic effect, defined as the rate of thrombolysis, can be expected to correlate directly with t-PA plasma concentrations. Future clinical studies in the setting of acute myocardial infarction should therefore aim at increasing the predictive value of t-PA kinetic parameters, by plasma concentration monitoring and model fit analysis during novel dosage regimens such as front loaded infusions or repeated boluses. In parallel, continuing basic research into the regulation of endogenous t-PA synthesis and catabolism can be expected to yield novel molecular targets for the treatment of chronic thrombotic diseases.

REFERENCES

1. Collen DC, Gold HK (1990) New developments in thrombolytic therapy. Thromb. Res. Suppl. X: 105 - 1312

2. Korninger C, Stassen JM, Collen D (1981) Turnover of human extrinsic (tissue-type) plasminogen activator in rabbits. Thromb. Haemostas. 46: 658 - 661

3. Neuhaus KL, Feuerer W, Jeep-Tebbe S, Niederer W, Vogt A, Tebbe U (1989) Improved thrombolysis with a modified dose regimen of recombinant tissue-type plasminogen activator. J. Am. Coll. Cardiol. 14: 1566 - 1569

4. Krause J, Seydel W, Heinzel G, Tanswell P (1990) Different receptors mediate the hepatic catabolism of tissue-type plasminogen activator and urokinase. Biochem. J. 267: 647 - 652

5. Krause J (1988) Catabolism of tissue-type plasminogen activator (t-PA), its variants, mutants and hybrids. Fibrinolysis 2: 133 - 142

6. Pannekoek H, de Vries C, van Zonnefeld AJ (1988) Mutants of human tissue-type plasminogen activator (t-PA): structural aspects and functional properties. Fibrinolysis 2: 123 - 132

7. Krause J, Tanswell P (1989) Properties of molecular variants of tissue-type plasminogen activator. Drug Res. 39: 632 - 637

8. Spellman M, Asa LJ, Leonard CK, Chakel JA, O'Connor JV, Wilson S, van Halbeek H (1989) Carbohydrate structure of human tissue plasminogen activator expressed in chinese hamster ovary cells. J. Biol. Chem. 264: 14100 - 14111

9. Lucore CL, Sobel BE (1988) Interactions of tissue-type plasminogen activator with plasma inhibitors and their pharmacologic implications. Circulation 77: 660 - 669

10. Seifried E, Tanswell P (1987) Comparison of specific antibody, D-Phe-Pro-Arg-CH_2Cl and aprotinin for prevention of in vitro effects of recombinant tissue-type plasminogen activator on hemostasis parameters. Thromb. Haemostas. 58: 921 - 926

11. Greischel A, Tanswell P, Busch U, Schumacher K (1988) Pharmacokinetics and biodisposition of recombinant human interferon α-2C in rat and marmoset. Drug Res. 38: 1539 - 1543

12. Seifried E, Tanswell P, Rijken DC, Barrett-Bergshoeff MM, Su CAPF, Kluft C (1988) Pharmacokinetics of antigen and activity of recombinant tissue-type plasminogen activator after infusion in healthy volunteers. Drug Res. 38: 418 - 422

13. Tanswell P, Seifried E, Su CAPF, Feuerer W, Rijken DC (1989) Pharmacokinetics and systemic effects of tissue type plasminogen activator in normal subjects. Clin.Pharmacol.Ther. 46:155-162

14. Sobel BE, Sarnoff SJ, Nachowiak DA (1990) Augmented and sustained plasma concentrations after intramuscular injections of molecular variants and deglycosylated forms of tissue-type plasminogen activators. Circulation 81: 1362 - 1373

15. Tanswell P, Heinzel G, Greischel A, Krause J (1990) Nonlinear pharmacokinetics of tissue-type plasminogen activator in three animal species and isolated perfused rat liver. J. Pharm. Exp. Ther. in press

16. Bakhit C, Lewis D, Busch U, Tanswell P, Mohler M (1988) Biodisposition and catabolism of tissue-type plasminogen activator in rats and rabbits. Fibrinolysis 2: 31 - 36

17. Bakhit C, Lewis D, Billings R, Malfroy B (1987) Cellular catabolism of recombinant tissue-type plasminogen activator. J. Biol. Chem. 262: 8716 - 8720

18. Baughman RA (1987) Pharmacokinetics of tissue plasminogen activator. In: Sobel BE, Collen D, Grossbard EB (eds). Tissue plasminogen activator in thrombolytic therapy. Marcel Dekker, New York, pp 41 - 53

19. Garabedian HD, Gold HK, Leinbach RC, Johns JA, Yasufa T, Kanke M, Collen D (1987) Comparative properties of two clinical preparations of recombinant tissue-type plasminogen activator in patients with acute myocardial infarction. J. Am. Coll. Cardiol. 9: 599 - 607

20. Seifried E, Tanswell P, Ellbrück D, Haerer W, Schmidt A (1989) Pharmacokinetics and haemostatic status during consecutive infusions of recombinant tissue-type plasminogen activator in patients with acute myocardial infarction. Thromb. Haemostas. 61: 497 - 501

21. Tebbe U, Tanswell P, Seifried E, Feuerer W, Scholz KH, Herrmann KS (1989) Single bolus injection of recombinant tissue-type plasminogen activator in acute myocardial infarction. Am. J. Cardiol. 64: 448 - 453

22. Rijken DC, Otter M, Kuiper J, van Berkel TJC (1990) Receptor mediated endocytosis of tissue-type plasminogen activator (t-PA) by liver cells. Thromb. Res. Suppl. X: 63 - 71

23. Seydel W, Stang E, Roos N, Krause J (1990) Endocytosis of tissue-type plasminogen activator by hepatic endothelial cells. Drug. Res., in press

24. Seydel W, Hoffmann H, Krause J (1990) Characterization of the receptor mediating the hepatic catabolism of t-PA. Fibrinolysis 4 (Suppl. 3): A225

25. Bennett WF, Paoni NF, Sethy I, Kerr E, Pena L, Keyt B, Nguyen H, Baldini L, Jones A, Meunier A, Wurm F, Johnson A, Zoller MJ, Higgins D, Botstein D (1990) Mapping the functional determinants of tissue plasminogen activator. Fibrinolysis 4 (Suppl. 3): A37

26. Tanswell P, Schlüter M, Krause J (1989) Pharmacokinetics and isolated liver perfusion of carbohydrate modified recombinant tissue-type plasminogen activator. Fibrinolysis 3: 79 - 84

Discussion - PHARMACOKINETICS OF HUMAN TISSUE-TYPE PLASMINOGEN ACTIVATOR

H.J. Röthig

We have seen today that by blocking receptors one can influence certain systems. Have you ever thought of blocking the catabolic system in the liver? Because I understand that t-PA is a very extremely expensive drug, you could reduce the amount of drug needed.

P. Tanswell

This is certainly something which could be done. It has been quite clearly shown that there are two pathways for t-PA catabolism. The mannose receptor on endothelial cells is a universal receptor which is responsible for the elimination of plasma glycoproteins in general. It might be quite difficult to block this because of the very large number of receptors available in the body. But what might be more promising would be to block the parenchymal receptor which only gets the t-PA after it has been processed by the endothelial cells, and I think that is probably an approach which could be quite interesting.

H.R. Lijnen

It is known that t-PA has a relatively high affinity for fibrin. Do you think that in a thrombolytic setting adsorption of t-PA to a fibrin clot plays a significant role in its clearance.

P. Tanswell

It would depend on the size of the fibrin. If you regard fibrin as being a compartment then the size of this compartment is probably extremely small. The binding to fibrin both at the site of the clot and circulating and bound to endothelial cells in the vasculature may be responsible for the multi-compartment kinetics which we have seen, in particular the gamma phase. So I agree there is some binding but I don't think it is important from a pharmacokinetic point of view. From the point of view of pharmacodynamics, it could be very important.

J.A. Galloway

Your mentioned that this material is protein-bound. How tenacious is that binding? Is that in the order of IGF 1 or not so strong?

P. Tanswell

It is very difficult to determine this. t-PA is known to bind to alpha 1 antiplasmin. There is also a specific inhibitor (PAI-1) and t-PA also binds to C1 esterase inhibitor. This binding is probably covalent; so it is probably irreversible. However, it is very difficult to determine the extent of binding while avoiding in vitro artifacts, because if one takes a blood sample this binding can continue in vitro. Further, there are conflicting reports in the literature as to what extent t-PA actually circulates in the form of these complexes in vivo. One report states there is very little, whereas another has described quite considerable complex formation.

J.A. Galloway

Are your pharmacokinetics based on total t-PA in the serum?

P. Tanswell

We use a ELISA method which measures t-PA predominantly in the free form. We obtained similar results using the activity assay.

S. Erill

Non enzymatic glycosylation of many proteins occurs in diabetic patients. Do you know whether in diabetic patients the endothelial pathway is, let us say, saturated or behaves just in the same way as in non-diabetic patients?

P. Tanswell

It would depend on whether the glycosylation is of the mannose type. If the glycosylation were of the mannose type then it would be quite possible that there is increased utilization of endothelial cells. If it were of the galactosamine type, these proteins would be eliminated by the parenchymal cells.