Pharmacokinetics in Chimpanzees of Recombinant Human Tissue-Type Plasminogen Activator Produced in Mouse C127 and Chinese Hamster Ovary Cells

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Pharmacokinetics of recombinant tissue-type plasminogen activator (rt-PA) produced in mouse C127 cells (t-PA(C127)) and Chinese hamster ovary cells (t-PA(CHO)) was investigated in chimpanzees. rt-PA was administered *via* a constant rate i.v. infusion for 60 min, and t-PA concentration and activity in plasma were measured during and after infusion. The noncompartmental parameters were calculated according to the moment analysis method, and a population pharmacokinetic analysis was performed to obtain the mean and interindividual variability of the pharmacokinetic parameters. The mean residence time of t-PA(C127) was significantly longer and the total body clearance was significantly less than that of t-PA(CHO). t-PA(C127) has an α-galactosyl moiety in its carbohydrate chains, whereas such a structure is not found in t-PA(CHO). These results demonstrate that two preparations of rt-PA's with different carbohydrate structures show different pharmacokinetics, and suggest that the carbohydrate structure can affect the efficiency of hepatic uptake of t-PA. A possible mechanism is an interaction of t-PA(C127) with the natural anti-α-galactosyl antibody. The anti-α-galactosyl antibody level in plasma decreased in association with the plasma levels of t-PA(C127) but was unaffected by t-PA(CHO) levels.

Keywords tissue plasminogen activator; recombinant tissue plasminogen activator; pharmacokinetics; chimpanzee; moment analysis; population pharmacokinetics; anti-α-galactosyl antibody

Introduction

Tissue-type plasminogen activator (t-PA) is a glycoprotein that converts plasminogen into plasmin and can thereby induce thrombolysis.[1-4] Advances in recombinant deoxyribonucleic acid technology have made it possible to produce human t-PA[5] in large quantity for the treatment of thrombo-embolic disorders.[6-9] t-PA has a specific affinity for fibrin[10] and its enzymatic activity is markedly increased in the presence of fibrin.[2,11] Therefore, t-PA may be clinically superior to streptokinase and urokinase, which do not have an affinity for fibrin and sometimes cause systemic hyperfibrinogenolysis.[12]

Recently, we found that the recombinant t-PA (rt-PA) expressed in mouse C127 cells had a different carbohydrate structure than that expressed in Chinese hamster ovary cells.[13] No difference was observed in the analysis of amino acid contents.[14,15] The major carbohydrate structural difference is the occurrence of α-galactosyl epitopes. Glycosylation is known to be highly host cell-dependent as shown by the studies of Sindbis virus glycoproteins,[16] and human interferon-β_1.[17] In this paper, we investigated whether this difference in carbohydrate structure can affect the pharmacokinetics of rt-PA in chimpanzees.

Experimental

Materials rt-PA produced in mouse C127 cells, denoted by t-PA(C127), was obtained from Toyobo Co., Ltd. (lot A8803, Osaka, Japan). Recombinant t-PA produced in Chinese hamster ovary cells, denoted by t-PA(CHO), was purchased from Genentech, Inc. (Activase®, lot L9103A, South San Francisco, CA). All other materials were of reagent grade.

Animals Male and female *Pan troglodytes* chimpanzees were used in this study (Primate Research Institute Chimpanzee Colony, New Mexico State University, U.S.A.). A total of 16 animals were used with an average body weight of 37.4±14.0 kg. Prior to the start of the study each animal was given a thorough physical examination by a qualified veterinarian. This examination included routine blood chemistry and hematology evaluations as well as examination for ecto- and endoparasites. Just prior to dose administration and at 24 h post dose, fecal samples were obtained for fecal occult blood determinations. All animals were housed in an environmentally-controlled room. Animals resided in their home cages during the study. Temperature and humidity were recorded at least twice daily. Fluorescent lighting provided illumination for 12 h per day. Food (Wayne 15% Monkey Diet®) and tap water were provided daily. No materials which would interfere with the outcome of the study were expected to be present in the water or diet. No signs of illness or pain were detected in any animal throughout the study. The study was conducted in accordance with Good Laboratory Practice Standards.[18]

Experiments The dose solution was aseptically reconstituted immediately before administration. The lyophilized rt-PA powder was dissolved in sterile water for injection, USP, and added to the same volume of 0.9% sodium chloride for injection, USP, to make the solutions. The dose of t-PA(C127) was 25.0 mg/animal (specific activity, 64.0×10^4 IU/mg protein) and the dose of t-PA(CHO) was 28.6 mg/animal (66.2×10^4 IU/mg protein). 30 ml of the solution was given intravenously over 60 min *via* a catheter placed in left brachial vein at a constant rate using an infusion pump (Harvard apparatus).

A cross-over design was used in these experiments. Animals 1—8 received t-PA(C127) and animals 9—16 received t-PA(CHO) in experiment 1. In experiment 2, the former received t-PA(CHO) and the latter received t-PA(C127). There was a washout period of one week between experiments. Ketamine–HCl was administered *via* intramuscular injection immediately prior to administration of rt-PA and as needed during blood sample collection. Atropine was also administered to control excessive salivation. Blood samples were collected *via* a catheter placed in the femoral vein. 2.5 ml of blood was drawn into sodium citrate vacutainer® tubes containing 0.35 ml sodium citrate solution before dosing and at the following times after start of dose administration: 15, 30, 45, 59, 61, 63, 65, 67, 70, 80, 90, 105, 120, and 180 min. The blood was centrifuged immediately at 4 °C and 1500 *g* for 5 min to obtain plasma. Blood sample tubes were held on ice for no longer than 20 min to promote efficient centrifugation. The plasma was kept frozen at less than −20 °C until assayed. The concentration and activity of t-PA and anti-α-galactosyl antibody titer in the plasma were measured.

Assay Methods The concentration of t-PA in plasma was determined by an enzyme linked immunosorbent assay (Imubind t-PA ELISA Kit, American Diagnostica Inc., Greenwich, CT).[19] The wells of the microtitration plate were filled with 100 μl goat antihuman t-PA IgG (5 μg/ml) diluted in 0.1 M $NaHCO_3$. After incubation for at least 16 h at 4 °C, the plate was emptied and washed three times with Dupont plate wash buffer (phosphate buffer saline (PBS) with 0.05% Tween-20 and 0.1% chloroacetamide). The dilutions of t-PA standard and plasma samples were made

into PBS/Tween buffer (0.01 M sodium phosphate buffer pH 7.4 containing 0.15 M NaCl and 0.05% Tween-20) containing bovine serum albumin (1 mg/ml). A 100 μl volume of standards or samples was added to each well and incubated for 1 h with shaking (50 rpm and 37 °C). The wells were emptied and washed as described above. The wells were then filled with 100 μl of peroxidase-conjugated anti t-PA immunoglobulin G (IgG) solution and the plate incubated for 2 h at 37 °C, 50 rpm. After an additional wash step, bound conjugate was detected by adding 100 μl of citrate-phosphate buffer (pH 5.0) containing 0.42 mg/ml *o*-phenylenediamine and 0.01% H_2O_2 and the plate incubated for 30 min in the dark at room temperature. The enzyme reaction was then stopped by adding 50 μl of 4.5 M H_2SO_4. The absorbance was measured at 490 nm with a reference wavelength of 650 nm using a microtiter plate reader (Molecular Devices, Menlo Park, CA). Sample concentrations were determined by interpolation from the standard curve.

The t-PA activity in plasma was measured using the chromogenic plasmin substrate S2251 (Helena Laboratories, Beaumont, TX). WHO t-PA standards (lot no. 83/517) were prepared at 10 IU/ml in 50 mM Tris–acetate buffer, pH 7.4, 0.01% Tween-80. The chromogenic substrate S2251 was reconstituted with 15.1 ml of H_2O. Fibrin-monomer prepared by digestion of fibrinogen (Helena Laboratories) with cyanogen bromide was diluted by deionized H_2O to 1—1.5 mg/ml. Plasminogen (American Diagnostica) was reconstituted to 1 mg/ml with distilled H_2O. Incubations were carried out in 100 μl volumes in 96-well Falcon Micro Test III flexible assay plates (Becton Dickinson, Oxnard, CA). Incubation mixtures consisted of 10 μl of sample, 10 μl of fibrin-monomer, 20 μl of 3 mM S2251, 10 μl of plasminogen, and 50 μl of 50 mM Tris–acetate buffer, pH 7.4, with 0.01% Tween-80. The assay plates were covered and incubated at 38 °C in a water bath. After incubation for 30 min, the plates were removed from the water bath and 20 μl of acetic acid was added to stop the reaction. The t-PA activity in plasma was determined by the production of *p*-nitroaniline, whose absorbance was read at 405 nm with a reference wavelength of 650 nm using a Molecular Devices plate reader.

Sample activities were calculated using a Televideo computer with a linear regression program which interpolates sample values from the standard curve (0—20 IU/ml).

Anti-α-galactosyl Antibody Titer in Plasma We have reported that there exists a natural antibody with specific binding to t-PA(C127) in nearly all human subjects, including those who have not had prior exposure to t-PA. The antibody recognizes terminal α 1—3 linked galactose residues.[13] Furthermore, this antibody is observed in serum of several primate species (human, apes, Old World monkeys) but is not observed in New World monkeys, lemurs and non-primate mammals.[20,21] Therefore, we measured the anti-α-galactosyl antibody levels to determine their relationship with plasma t-PA levels in the experimental animals.

The antibody titers were determined by ELISA. Microtiter plates were coated with 100 μl of 10 μg/ml t-PA(C127) in 0.1 M $NaHCO_3$ at 25 °C in a moist chamber overnight. The plate was emptied and washed five times with 0.01 M PBS (pH 6.7) containing 0.1% Tween-20. Plasma samples were diluted 100-fold in 0.01 M PBS (pH 6.7) containing 1% casein (Oxoid Ltd., Hampshire, England), 0.05% ethylenediaminetetraacetic acid (EDTA) and 0.1% Tween-20. A sample volume of 100 μl was added to each well and incubated for 2 h at 37 °C in a moist chamber. After washing 5 times, a peroxidase-conjugated anti-human IgG (Organon Teknika-Cappel, Malvern, PA) was added and then incubated for 2 h at 37 °C. After an additional wash step, 100 μl of substrate solution containing 3 mg/ml *o*-phenylenediamine dihydrochloride and 0.02% H_2O_2 in 0.1 M citrate-phosphate buffer (pH 5.7) was added for 15 min at room temperature. The enzyme reaction was stopped by adding 100 μl of 2 N H_2SO_4. Absorbance at 490 nm was measured using the microtiter plate reader.

Data Analysis

Moment Analysis Plasma t-PA concentration or activity *vs.* time curves were analyzed first by a noncompartmental moment analysis. Area under the curve (AUC), the mean residence time (MRT) and the variance of residence time (VRT) were computed by a trapezoidal integration according to conventional equations.[22] Integration over time was performed over the range of zero to 180 min. Extrapolation to infinite time was unnecessary because t-PA levels at 180 min were sufficiently low. Since measured t-PA level included the endogenous t-PA, the t-PA levels corresponding to exogenous t-PA were calculated by subtracting the endogenous t-PA level before dosing (zero point) from the measured t-PA level at each point.

MRT was calculated by subtracting the mean input time from $AUMC/AUC$ as follows[22]:

$$MRT = AUMC/AUC - \text{mean input time} \tag{1}$$

where $AUMC$ is the area under the first moment curve. Since the drug was given as a constant rate infusion for 60 min, the mean input time was 30 min. The total body clearance (CL_t) was calculated from AUC and the dose.

$$CL_t = \text{dose}/AUC \tag{2}$$

The steady-state equivalent volume of distribution was computed as follows[23,24]:

$$Vd_{ss} = CL_t \cdot MRT \tag{3}$$

The comparison between the pharmacokinetic parameters for t-PA(C127) and t-PA(CHO) was performed according to a standard two-stage method, that is, in the first step pharmacokinetic parameters were estimated for each animal, then the parameters obtained were compared by a two-way analysis of variance with respect to type of drug and individual. A $p<0.05$ was taken as being significant.

Population Pharmacokinetic Analysis To obtain a structural model describing the pharmacokinetics of t-PA, a population pharmacokinetic analysis was performed using both plasma t-PA concentration and activity data. The two-compartment open model was used as a basic structural model because the observed time courses showed bi-exponential decline. The basic pharmacokinetic parameters were total body clearance (CL_t), intercompartmental clearance (Q), volume of the central compartment (Vc) and total volume of distribution (Vd_{ss}). Micro-rate constants can be easily derived from these parameters, that is, distribution rate constants k_{12} and k_{21} are obtained as Q/Vc and $Q/(Vd_{ss}-Vc)$, respectively, and elimination rate constant as CL_t/Vc.

The differences in the basic pharmacokinetic parameters between t-PA(C127) and t-PA(CHO) were analyzed using fixed effect parameters as follows[25]:

$$\begin{aligned}
\overline{CL}_t &= \overline{CL}_{t,C127}(1-\theta_1 \cdot DF) \\
\overline{Q} &= \overline{Q}_{C127}(1-\theta_2 \cdot DF) \\
\overline{Vc} &= \overline{Vc}_{C127}(1-\theta_3 \cdot DF) \\
\overline{Vd}_{ss} &= \overline{Vd}_{ss,C127}(1-\theta_4 \cdot DF)
\end{aligned} \tag{4}$$

where $\overline{CL}_t$, $\overline{Q}$, $\overline{Vc}$ and $\overline{Vd}_{ss}$ are population mean parameters, and the subscript C127 denotes the mean parameters for t-PA(C127). DF is a categorical variable of unity if t-PA(CHO) was administered, and otherwise zero. $\theta_1-\theta_4$ indicate the fractional decrease in mean parameters for t-PA(CHO) compared with those for t-PA(C127). A null hypothesis assuming θ equals zero was examined by a log-likelihood test.[25] In this case ($\theta=0$), the parameters for t-PA(C127) and t-PA(CHO) would be identical.

As a random effect with respect to interindividual variability, a log-normal distribution was implemented into the population model.

$$\begin{aligned}
\ln CL_{t,j} &= \ln \overline{CL}_t + \eta_{CL_t,j} \\
\ln Q_j &= \ln \overline{Q} + \eta_{Q,j} \\
\ln Vc_j &= \ln \overline{Vc} + \eta_{Vc,j} \\
\ln Vd_{ss,j} &= \ln \overline{Vd}_{ss} + \eta_{Vd_{ss},j}
\end{aligned} \tag{5}$$

where $CL_{t,j}$, Q_j, Vc_j and $Vd_{ss,j}$ are parameters for individual j, and η is a random variable describing interindividual variability with mean zero and variance ω^2. Thus ω expresses the coefficient of variation (C.V.) of interindividual variability for the pharmacokinetic parameters.

The residual variability including intraindividual variability, measurement errors and model misspecification was modeled in similar manner as follows:

$$\ln Cp_{ij} = \ln \hat{Cp}_{ij} + \varepsilon_{ij} \tag{6}$$

where Cp_{ij} is an observed t-PA level at time t_{ij} for individual j, $\hat{Cp}_{ij}$ is a level predicted using parameters for the individual, and ε_{ij} is a random variable describing residual variability with mean zero and variance σ^2.

The computation was carried out using the NONMEM77 program with PREDPP package[26] on a mainframe computer (FACOM M382, Kyoto University Data Processing Center).

Results

Plasma Concentration Profile of rt-PA Figure 1 shows the plasma t-PA concentration *vs.* time (mean ± S.E. of 16 chimpanzees) for t-PA(C127) and t-PA(CHO). The bi-

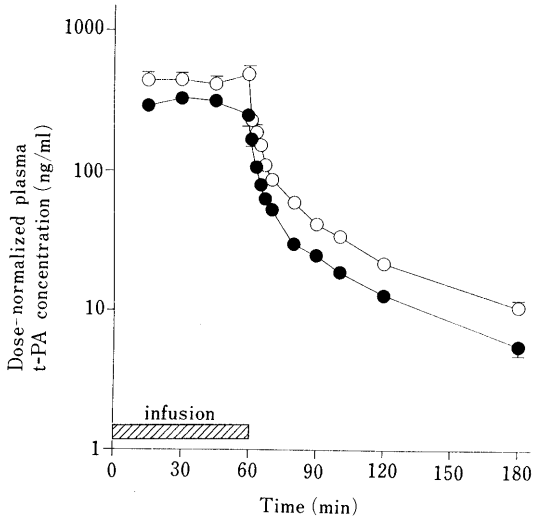

Fig. 1. Plasma Concentration *vs.* Time Curves for t-PA(C127) and t-PA(CHO) during and after Constant Rate i.v. Infusion in Chimpanzees

○, t-PA(C127); ●, t-PA(CHO). Each value represents the mean ± S.E. ($n=16$). Although each animal received the same dose, dose per body weight varies among individuals due to the variability of body weight. To illustrate the two rt-PA profiles, the t-PA concentrations at each point were normalized equivalent to 600 μg/kg dose and plotted.

TABLE I. Noncompartmental Parameters for t-PA Disposition Kinetics Based upon Plasma t-PA Concentration Data

	t-PA(C127)	t-PA(CHO)	ANOVA[a]
AUC (μg·min/ml)	35.2 ± 14.8	27.0 ± 10.6	—[b]
MRT (min)	14.5 ± 3.7	12.1 ± 2.4	$p<0.05$
VRT (min^2)	834 ± 218	732 ± 161	N.S.
CL_t (ml/min/kg)	22.9 ± 6.5	33.1 ± 7.6	$p<0.001$
Vd_{ss} (ml/kg)	345 ± 176	408 ± 157	N.S.

Values are mean ± S.D. of 16 animals. *a*) Two-way layout analysis of variance without replication. *b*) Statistical analysis was not adopted due to the different dose of each drug.

TABLE II. Hypothesis Testing for the Construction of Population Pharmacokinetic Model of t-PA Based upon Plasma Concentration Data

Hypothesis	Twice the log likelihood ratio	p
Random effect model		
$\eta_{CL_t}=0$	25.737	<0.001
$\eta_Q=0$	0.113	N.S.
$\eta_{Vc}=0$	0.362	N.S.
$\eta_{Vd_{ss}}=0$	14.328	<0.001
$\eta_{Vc}=\eta_Q=0$	0.498	N.S.
Fixed effect model[a]		
$\theta_1=0$	68.917	<0.001
$\theta_2=0$	12.246	<0.001
$\theta_3=0$	8.205	<0.01
$\theta_4=0$	6.555	<0.02

a) Hypothesis testing for the fixed effect was performed using the best variance model, that is $\eta_{Vc}=\eta_Q=0$.

phasic decline was observed during the post-infusion period for both rt-PA's in accordance with previous reports.[27-29] It is apparent from the profiles that post-infusion levels of t-PA(C127) are higher than that of t-PA(CHO).

Table I shows non-compartmental parameters obtained by the moment analysis. A significant difference was observed in MRT and CL_t between t-PA(C127) and t-PA(CHO), whereas Vd_{ss} was not significantly different.

TABLE III. Final Estimates for Population Pharmacokinetic Parameters of t-PA Based upon Plasma Concentration Data

	t-PA(C127)	t-PA(CHO)
Mean parameters		
CL_t (ml/min/kg)	19.5 (8)	31.5 (13)
Q (ml/min/kg)	4.42 (15)	6.51 (25)
Vc (ml/kg)	73.8 (12)	107 (24)
Vd_{ss} (ml/kg)	298 (18)	418 (29)
Interindividual variability		
ω_{CL_t} (%)	11.4 (30)	
$\omega_{Vd_{ss}}$ (%)	26.1 (27)	
Residual variability		
σ (%)	35.5 (10)	

Values in parenthesis are standard error of estimate as coefficient of variation (%).

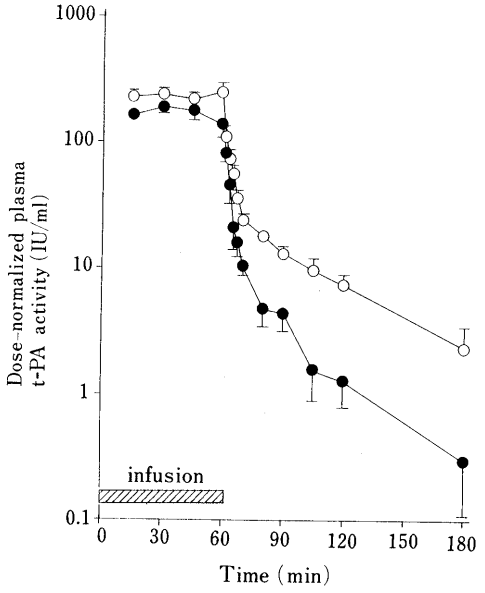

Fig. 2. Plasma t-PA Activity *vs.* Time Curves for t-PA(C127) and t-PA(CHO) during and after Constant Rate i.v. Infusion in Chimpanzees

○, t-PA(C127); ●, t-PA(CHO). Each value represents the mean ± S.E. ($n=16$). Although each animal received the same dose, dose per body weight varies among individuals due to the variability of body weight. To illustrate the two rt-PA profiles, the t-PA concentrations at each point were normalized equivalent to 360000 IU/kg dose and plotted.

Table II shows the results of hypothesis testing for the construction of a population pharmacokinetic model of rt-PA. The interindividual variability for Vc and Q was approximately zero, indicating that random effects can be excluded in a final model. As for fixed effect parameters, all θ's were significantly different from zero, which means that all pharmacokinetic parameters were different between t-PA(C127) and t-PA(CHO). Table III lists the final estimates for population pharmacokinetic parameters of t-PA in chimpanzees based upon plasma concentration data. CL_t, Q, Vc and Vd_{ss} for t-PA(C127) were significantly lower than those for t-PA(CHO). The values of CL_t and Vd_{ss} were similar to the values obtained by moment analysis (Table I), confirming the validity of the analysis. The interindividual variations for CL_t and Vd_{ss} were small, whereas the residual variability was relatively large.

t-PA Activity in Plasma Figure 2 shows the plasma t-PA activity *vs.* time courses (mean ± S.E., $n=16$) during

Table IV. Noncompartmental Parameters for t-PA Disposition Kinetics Based upon Plasma t-PA Activity Data

	t-PA(C127)	t-PA(CHO)	ANOVA[a]
AUC ($\times 10^4$ IU·min/ml)	1.59±0.79	1.29±0.52	—[b]
MRT (min)	10.4±3.5	6.2±3.0	$p<0.0025$
VRT (min^2)	573±304	320±94	$p<0.01$
CL_t (ml/min/kg)	29.6±11.3	39.1±10.0	$p<0.01$
Vd_{ss} (ml/kg)	316±191	233±111	N.S.

Values are mean±S.D. of 16 animals. *a*) Two-way layout analysis of variance without replication. *b*) Statistical analysis was not adopted due to different dose of each drug.

Table V. Hypothesis Testing for the Construction of Population Pharmacokinetic Model of t-PA Based upon Plasma Activity Data

Hypothesis	Twice the log likelihood ratio	p
Random effect model		
$\eta_{CL_t}=0$	19.285	<0.001
$\eta_Q=0$	20.030	<0.001
$\eta_{Vc}=0$	15.429	<0.001
$\eta_{Vd_{ss}}=0$	12.753	<0.001
Fixed effect model[a]		
$\theta_1=0$	22.813	<0.001
$\theta_2=0$	1.830	N.S.
$\theta_3=0$	3.070	N.S.
$\theta_4=0$	3.729	N.S.
$\theta_2=\theta_3=0$	8.958	<0.02
$\theta_2=\theta_4=0$	3.850	N.S.
$\theta_3=\theta_4=0$	18.027	<0.001
$\theta_2=\theta_3=\theta_4=0$	18.957	<0.001

a) Hypothesis testing for the fixed effect was performed using the full variance model.

Table VI. Final Estimates for Population Pharmacokinetic Parameters of t-PA Based upon Plasma Activity Data

	t-PA(C127)	t-PA(CHO)
Mean parameters		
CL_t (ml/min/kg)	24.2 (10)	38.6 (13)
Q (ml/min/kg)	2.89 (16)	2.89 (16)
Vc (ml/kg)	63.1 (18)	97.2 (25)
Vd_{ss} (ml/kg)	247 (18)	247 (18)
Interindividual variability		
ω_{CL_t} (%)	21.5 (33)	
ω_Q (%)	63.3 (59)	
ω_{Vc} (%)	46.4 (68)	
$\omega_{Vd_{ss}}$ (%)	64.1 (71)	
Residual variability		
σ (%)	55.9 (10)	

Values in parenthesis are standard error of estimate as coefficient of variation (%).

and after a constant rate i.v. infusion to chimpanzees. Plasma activity, as well as plasma concentration, declines in a biphasic manner for both rt-PA's. The levels of t-PA(C127) tended to be higher than the levels of t-PA(CHO).

The non-compartmental parameters based upon plasma activities are shown in Table IV. Similar to results in Table I, MRT and CL_t for t-PA(C127) show a significant difference from those for t-PA(CHO). Furthermore, a significant difference was also observed in VRT.

Similar results were also obtained in the population pharmacokinetic analysis (Table V). From the hypothesis testing, θ_2 and θ_4 were not different from zero while θ_1 and θ_3 were estimated as -0.593 and -0.541, respectively. The results indicate that CL_t of t-PA(CHO) was 60% larger than that of t-PA(C127). All interindividual variances had a significant component and were included in the final model. Table VI shows the final estimates for the population pharmacokinetic parameters of rt-PA on the basis of plasma activity. The estimates for CL_t and Vd_{ss} were comparable to those calculated by moment analysis (Table IV). Relatively large variances were obtained for interindividual and residual variabilities.

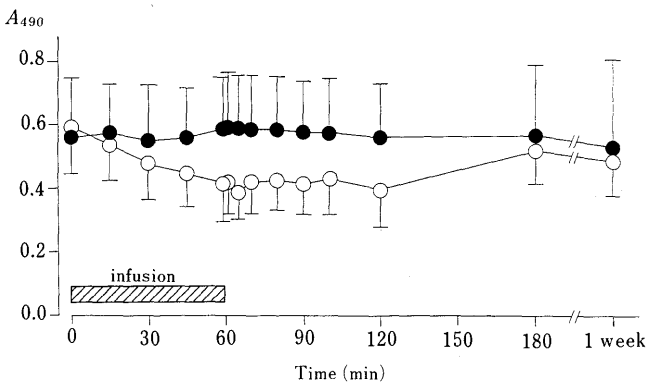

Fig. 3. Natural Anti-α-Galactosyl IgG Antibody Levels during and after i.v. Infusion of rt-PA in Chimpanzees

○, t-PA(C127); ●, t-PA(CHO). Each value represents the mean±S.E. ($n=16$).

Natural Anti-α-galactosyl Antibody Level in Plasma Figure 3 shows the natural anti-α-galactosyl antibody level in plasma during and after i.v. infusion of rt-PA. The antibody levels were almost constant when t-PA(CHO) was administered. In contrast, they decreased gradually when t-PA(C127) was infused, and recovered after the infusion had ended.

Discussion

t-PA is glycosylated either at two or three of the glycosylation sites at Asn-117, Asn-184 and Asn-448. The glycosylation site at Asn-117 carries predominantly high-mannose structures, whereas the Asn-184 and Asn-448 sites contain complex oligosaccharides.[14] Recent studies demonstrated that rt-PA produced in C127 cells has an α1—3 linked galactose moiety in the oligosaccharides at glycosylation sites Asn-184 and Asn-448.[13] In contrast, such a structure was not contained in t-PA(CHO). In this study using chimpanzees, different pharmacokinetics, especially in CL_t, were observed between t-PA(C127) and t-PA(CHO). It is known that t-PA is accumulated rapidly in the liver and cleared predominantly *via* this organ.[30,31] The elimination is thought to be endocytosis of t-PA by liver endothelial cells and parenchymal cells, mediated in part by mannose and galactose receptors, respectively.[32,33] The present results suggest that the difference in glycosylation epitopes may influence the efficiency of receptor-mediated endocytosis for different rt-PAs.

Another mechanism for the different pharmacokinetics of t-PA(C127) and t-PA(CHO) is suggested by these data. It is reported that as much as 1% of the circulating IgG in humans interacts with the Gal α1—3 Gal epitope.[21,34] Also, this antibody was observed in the serum of limited kinds of primates (apes and Old World monkeys).[20] Natural anti-α-

galactosyl antibody levels in the plasma of chimpanzees decreased upon administration of t-PA(C127) while no change was observed upon administration of t-PA(CHO). Therefore, the interaction of t-PA(C127) with the antibody may be an additional mechanism responsible for the decreased clearance, *i.e.* the interaction may obstruct the recognition site in liver and result in the reduction in hepatic uptake rate.

The fibrinolytic activity of t-PA in circulating blood is rapidly neutralized by plasminogen activator inhibitors.[35] This effect may be responsible for the shorter *MRT* of the plasma activity than the *MRT* of t-PA plasma concentration (Tables I and IV). A pronounced difference between t-PA(C127) and t-PA(CHO) plasma activity over time was observed (Fig. 2), suggesting that the interaction with the natural antibody may also influence the neutralization of t-PA by plasminogen activator inhibitors.

Both plasma concentration and activity profiles showed a biphasic decline with rapid distribution and subsequent elimination phases (Figs. 1 and 2). Considering the molecular weight of rt-PA (*ca.* 65 kilodaltons), the first rapid phase might be binding to the receptors located on the surface of the liver, rather than an exchangeable tissue distribution which is usually observed with low molecular weight organic compounds. Therefore, Q may be associated with the affinity of t-PA for the receptors and the volume of peripheral compartment denoted by $Vd_{ss} - Vc$ may correspond to the amount of t-PA which binds to the receptors. Further investigation is necessary to confirm the above mechanism.

The mean parameters for CL_t and Vd_{ss} calculated by the moment analysis were similar to those obtained by the population analysis. However, population analysis was more sensitive in detecting the difference between the t-PA's as a significant difference in Vd_{ss} was observed in Table II but not observed in Table I. This is due to the difference in methodology for evaluation of the interindividual variability. In moment analysis, the standard two-stage method is performed, that is, parameters for individuals are estimated independently in the first step, then the parameter means and variances for the population are obtained in the second step. This two-stage method tends to overestimate the interindividual variability because the parameters obtained in the first step include estimation errors. This disadvantage comes essentially from the two-stage method and is not a shortcoming of the moment analysis itself. A similar problem has been raised for the two-stage method using a conventional curve-fitting technique.[36,37]

On the other hand, our population analysis using the mixed effect model calculates simultaneously the population mean and variance for the pharmacokinetic parameters. Therefore, this method avoids the overestimation of interindividual variability, thus increasing the sensitivity in detecting the difference of the means.

We also attempted a conventional two-stage method based upon the fitting of individual time courses; the individual parameters were computed by fitting the data to a compartment model in the first step, and the obtained parameters were averaged to produce the population mean. However, this approach was problematic in that satisfactory fitting was not always obtained at the first step, that is, some individuals showed an abnormal parameter estimate due to the computation or to misspecification of the model. The latter problem is more severe when one individual of a population shows mono-exponential decline while the others show bi-exponential decline. To average or compare the population mean, data from all individuals must be fitted to the same model even if data from certain individuals are best represented by another model. Given these considerations, the moment analysis and the population analysis are advantageous for analyzing these pharmacokinetic data. The moment analysis is a model-independent method. In the population analysis, the most reasonable model is assigned for the population and does not require that every member of the population be best fit individually. Therefore, the population modeling is more flexible and robust than a conventional two-stage method based upon the fitting of individual time courses and averaging the parameters.

In conclusion, rt-PA's produced in mouse C127 cells and CHO cells, which have different carbohydrate structures, showed distinct pharmacokinetics in chimpanzees. *MRT* of t-PA(C127) was significantly longer and CL_t was conversely less than those of t-PA(CHO). The present results indicate that the carbohydrate structure is a significant determinant of the clinical properties of recombinant glycoproteins and suggests strategies for designing new drugs by modifying a native glycoprotein.

Acknowledgements We thank Dr. K. Watanabe and Dr. S. Mannen for their valuable advice. We thank Ms. J. Groody, Mr. R. Mealy, Mr. T. Olson, Mr. M. Quinn, and Ms. B. Siddall for their expert technical assistance.

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