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Pharmacokinetic studies of Gln117 tissue-type plasminogen activator in rats

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Abstract

Gln117 t-PA is a mutant type of tissue-type plasminogen activator (mt-PA), which is generated by the removal of a high mannose oligosaccharide resulting from the mutation of amino acid #117, from asparagine (Asn) to glutamine (Gln). The plasma concentration, distribution, metabolism and excretion of Gln117 t-PA were investigated after a single intravenous administration of ^{125}I-Gln117 t-PA or Gln117 t-PA, comparing with wild-type t-PA (WT t-PA). The plasma concentration of Gln117 t-PA decreased more slowly than that of WT t-PA, plasma clearance (CL_p), and that of Gln117 t-PA in rats was approximately 2.6 times lower than that of WT t-PA. The highest radioactivity was found in the liver at 5 min after intravenous administration of [^{125}I]Gln117 t-PA to rats, but the radioactivity in the liver was lower than that after intravenous administration of [^{125}I]WT t-PA in our previous paper. Within 288 h after intravenous administration of [^{125}I]Gln117 t-PA to rats, 88.5 and 5.5% of administered radioactivity were excreted into urine and feces, respectively. In a gel-filtration chomatographic (GFC) analysis of plasma, Gln117 t-PA formed complexes with plasma proteins, similarly to WT t-PA. The hepatic clearance (CL_{hep}) of both t-PAs was evaluated by comparing the plasma concentration after a constant intravenous infusion with that after a constant intraportal venous infusion. The CL_{hep} of Gln117 t-PA was 6.5 times lower than that of WT t-PA. These results indicate that the low uptake of Gln117 t-PA to the liver reduces CL_p compared with WT t-PA.

Keywords: Distribution; Excretion; Gln117 t-PA; Metabolism; Plasma concentration; Rat

1. Introduction

Tissue-type plasminogen activator (t-PA) is a 527 amino acid serine protease that cleaves plasminogen into its active form plasmin. The plasmin in turn degrades fibrin [1,2]. The activity of t-PA is markedly stimulated in the presence of fibrin [2]. Human t-PA produced by recombinant DNA technology is presently available for clinical use in patients with acute myocardial infarction [3–5]. Although t-PA is thought to be clinically superior to streptokinase and urokinase because of its spe-

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cific affinity for fibrin, the rapid clearance of t-PA from the circulating blood sometimes requires the administration of high doses to obtain therapeutic blood levels, which can lead to bleeding incidents due to a decrease in plasma fibrinogen level [6,7]. Recently, several types of long biological half-life mutant t-PAs (mt-PAs) were developed [8–11]. However, their activity and fibrin affinity are lower than WT t-PA. t-PA is composed of five domains: finger, EGF, kringle 1 and 2, and serine protease domains. All of them are necessary to act as t-PA. These mt-PAs are changed at the domain, therefore, they are leaded to the loss of activity [12–14]. Therefore, we are trying to develop an mt-PA that is better than the wild type in activity as well as in biological half-life. t-PA is glycosylated at thee glycosylation sites of Asn117, Asn184 and Asn448. That at Asn117 carries a high mannose structure, whereas those at Asn184 and Asn448 carry complex type oligosaccharides. The high mannose oligosaccharide is known to be the most important for hepatic clearance of t-PA, mainly mediated via the mannose receptor [15–18]. Our Gln117 t-PA is lacking the high mannose oligosaccharide at Asn117 because of the mutation of the amino acid from Asn117 to Gln. In this study, we investigated plasma concentration profile, distribution, metabolism and excretion after a single administration of Gln117 t-PA, comparing with WT t-PA, and evaluated the hepatic clearance of both t-PAs by plasma concentration after a constant infusion.

2. Materials and methods

2.1. Chemicals

Recombinant Gln117 t-PA (lot RM9102, GYL003B) and WT t-PA (lot R8803) expressed in mouse C127 cells were obtained from Toyobo Co., Ltd. Gln117 t-PA and WT t-PA were labeled by the iodination reaction consisted of Na[^{125}I] and Iodogen (Pierce) [19]. [^{125}I]Gln117 t-PA and [^{125}I]WT t-PA were then purified on a PD-10 column (Amersham Pharmacia Biotech). The protein concentration in stock solution was determined by the Lowry method [20]. The specific radioactivities of [^{125}I]Gln117 t-PA and [^{125}I]WT t-PA were $1.5 \sim 46.6$ and 17.9 MBq/mg, respectively, and their radiochemical purities, determined by GFC analysis, were 91% or more. GFC condition is shown in Section 2.10. Monoclonal and polyclonal antibodies were obtained from Toyobo Co., Ltd. Fibrinogen and plasminogen were purchased from Nacalai Tesque (Osaka, Japan) and Welfide (Osaka, Japan), respectively. All other chemicals were of reagent grade.

2.2. Animals

Male Sprague–Dawley strain rats of 7–8 weeks old weighing 220–310g (Charles River Japan Inc.) were used.

2.3. Administration of dosing solution

The dosing solution for a single intravenous administration was adjusted to 0.25 mg protein/ml, the radioactivity concentration being 0.40 or 2.68 MBq/ml. It was administered to the caudal vein at a dose of 1 ml/kg. The dosing condition for hepatic clearance is shown in Section 2.6.

2.4. Measurement of plasma concentration

2.4.1. Radioactivity

Radioactivity in each sample was counted for 1 min with a γ-counter (ARC-300, Aloka).

2.4.2. Immunoreactive Gln117 t-PA and WT t-PA [21,22]

The immunoreactivities of Gln117 t-PA and WT t-PA in plasma were determined by ELISA. The wells of a microtitration plate (Costar, Cambridge, MA) were filled with 100 μl of mouse anti human t-PA monoclonal antibody (5 μg/ml). After incubation for 4 h at 37°C, the plate was emptied and washed four times with phosphate-buffered saline containing 0.05% Tween 20. The dilutions of t-PA standard and plasma samples were made into 0.01 M phosphate buffer (pH 7.4) containing 0.14 M NaCl, 0.2% bovine serum albumin, 5% newborn calf serum, 3% normal rabbit serum and 0.05% EDTA disodium salt. A volume

of 50 μl of the standard or sample and 50 μl of the peroxidase-conjugated rabbit anti-human t-PA polyclonal antibody solution were added to each well and incubated for 16 h at 37°C. The wells were emptied and washed as described. Bound conjugate was detected by addting 100 μl of citrate-phosphate buffer (pH 5.0) containing 3 mg/ml of *o*-phenylenediamine and 0.02% H_2O_2. The plate was then incubated for 30 min in the dark at room temperature. The enzyme reaction was then halted by adding 100 μl of 2 N H_2SO_4. The absorbance was measured at 490 nm, with a reference wavelength of 630 nm using a microtiterplate reader (EL-311SL, Biotek, Winooski, VT). Sample concentrations were determined by the spline interpolation from the standard curve.

2.4.3. Fibrin clot lysis time (FCLT) activity [22]

One hundred microliters of plasma sample were treated with 1.9 ml of 0.016% acetic acid and centrifuged (1500 × *g*, 4°C, 10 min). The resulting precipitate was redissolved in 250 μl of Tris–HCl buffer (pH 7.4), mixed with 600 μl of 0.3% bovine fibrinogen in Tris–HCl buffer (pH 8.0) in a polystylene tube (75 × 8 mm i.d.), and then incubated at 37°C for 10 min (solution A). Human plasminogen (10 IU/ml) in saline and human thrombin (40 IU/ml) in saline were mixed at the same volume and incubated at 37°C for 10 min (solution B). Two hundred microliters of solution B were added to solution A. The mixture was incubated at 37°C with stirring. One minute later, a Teflon ball (6 mm i.d.) was put on the top of the clot in the tube. The clot lysis time was determined by timing the interval of the ball to reach the bottom of the tube. There was a good relationship between the clot lysis time and the concentrations of Gln117 t-PA and WT t-PA. Activity was expressed relative to the WHO t-PA standard in international units.

2.5. Plasma concentration

Blood (0.35 ml) was withdrawn periodically from the jugular vein after a single intravenous administration of WT t-PA, Gln117 t-PA or [^{125}I]Gln117 t-PA into an Eppendorf tube containing 20 μl of 3.8% sodium citrate solution and centrifuged (1500 × *g*, 4°C, 10 min). The separated plasma was stored frozen at −80°C until assay.

2.6. Hepatic clearance

The hepatic extraction ratio was assessed by comparing the AUC obtained following intravenous and intraportal venous administration [23]. Male rats were anesthetized with pentobarbital (40 mg/kg, i.p.). The right femoral artery was cannulated with polyethylene tubing (SP31, o.d. 0.80 mm, Natsume, Tokyo, Japan) for drug administration. A volume of 1.3 ml of Gln117 t-PA or WT t-PA was infused into the femoral vein or the hepatoportal vein over 60 min via a catheter placed using an infusion pump (model 975, Harvard Apparatus, South Natick, MA). Blood samples (0.1 ml) were withdrawn via the catheter placed in the femoral artery into an Eppendorf tube containing 10 μl of 3.8% sodium citrate solution and centrifuged (1500 × *g*, 4°C, 10 min). The separated plasma was stored frozen at −80°C until assay.

2.7. Radioactivity concentration in tissue

Male rats were exsanguinated from the abdominal aorta under ether anesthesia after a single intravenous administration of [^{125}I]Gln117 t-PA, and the tissues were excised and weighed. The radioactivity in the plasma was counted. About 50 mg of each tissue were weighed and counted with a γ-counter.

2.8. Radioactivity excreted in urine and feces

Male rats were placed in metabolic cages (KN-646B, Natsume) after a single intravenous administration of [^{125}I]Gln117 t-PA, and excreted urine and feces were collected. The urinary sample was diluted to 50 or 100 ml with water, and the radioactivity in 1 or 2 ml was counted as described above. The fecal sample was homogenized in water and diluted to 200 ml. The radioactivity in each 0.5 ml aliquot of the homogenate was counted as described above.

After collection of the excreta until 288 h, the rats were sacrificed by ether treatment, and then the thyroid gland and liver were collected. Each of them was processed in the same manner as the tissues.

2.9. TCA treatment

Approximately 0.2 g of the male rat tissues (brain, thyroid gland, heart, lung, liver, kidney, spleen, stomach and small intestine) was homogenized with 2 ml of 0.1 M phosphate buffer, pH 7.4, containing 1% bovine serum albumin and 0.15 M NaCl (1% BSA/PBS). Each 1 ml aliquot of the homogenate was mixed with 15% trichloroacetic acid (TCA). The radioactivity was counted in the supernatant and precipitate obtained after centrifugation ($1500 \times g$, 4°C, 10 min). One hundred microliters of plasma were treated with 0.5 ml of 1% BSA/PBS and 1 ml of 15% TCA, and the radioactivity was counted in the supernatant and precipitate according to the method for tissue homogenate.

2.10. GFC analysis

The HPLC system (LC-6AD; Shimadzu) was equipped with a UV detector (SPD-6A, 220 nm), gel filtration column (TSKgel G3000SW, 7.5×600 mm, TOUSOU), and fraction collector (Model FC-80, GILSON). The separation was achieved with 50 mM phosphate buffer (pH 7.0) containing 2 M KSCN and 0.01% Tween 80. The flow rate of the mobile phase was 1 ml/min. Plasma samples (20 μl) were injected onto the column, and 0.5 ml fractions were collected and analyzed with respect to radioactivity. Similarly, the radiochemical purities of [^{125}I]Gln117 t-PA and [^{125}I]WT t-PA (20 μl) were determined by GFC analysis.

2.11. Pharmacokinetic analysis

The observed plasma t-PA concentration curves after a single intravenous administration were fit to a two-compartment open model using the nonlinear least-squares regression program MULTI [24] as follows:

$$C_p(t) = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

where $C_p(t)$ is the concentration of immunoreactive Gln117 t-PA or WT t-PA in the plasma at time t. A and B are the y intercepts of the fast and slow clearance phases, which have slopes of α and β, respectively. D is the dose. k_{10} is the first-order elimination rate constant from the central compartment. k_{12} and k_{21} are the rate constants for drug transfer between the central and the peripheral compartments. The distribution volume of the central compartment (V_c), the ditribution volume at steady state (V_{ss}), the plasma clearance (CL_p) and the mean residence time (MRT) were calculated by the following equations [25]:

$$V_c = D/(A + B) \quad (2)$$

$$V_{ss} = V_c(1 + k_{12}/k_{21}) \quad (3)$$

$$CL_p = k_{10}V_{ss} \quad (4)$$

$$MRT = V_{ss}/CL_p \quad (5)$$

To evaluate the parameters that characterize the hepatic clearance of both t-PAs, the observed plasma t-PA concentration curves during and after a constant infusion were analyzed by noncompartment analysis. The CL_p and extraction ratio in the liver (E_{hep}) were computed according to the following equations [23]:

$$CL_p = D/AUC_{iv} \quad (6)$$

$$E_{hep} = 1 - AUC_{ipv}/AUC_{iv} \quad (7)$$

AUC_{iv} and AUC_{ipv} were calculated by the trapezoidal integration over a period of 0–300 min after the start of a constant infusion into the femoral vein and the portal vein, respectively. Extrapolation to infinite time was unnecessary, because the levels of Gln117 t-PA and WT t-PA were sufficiently low.

The hepatic clearance (CL_{hep}) was estimated by multiplying E_{hep} by the hepatic plasma flow rate (Q) as follows:

$$CL_{hep} = Q\ E_{hep}. \quad (8)$$

The reported hepatic plasma flow rate (47 ml/min/kg) in rats was used in calculation [26].

2.12. Expression of results

The radioactivity concentration and FCLT activity were converted to the equivalent value of Gln117 t-PA or WT t-PA. Results were expressed as mean values and standard errors (S.E.).

3. Results

3.1. Plasma concentration

Fig. 1 shows the plasma concentration after a single intravenous administration of [^{125}I]Gln117 t-PA or Gln117 t-PA to male rats at a dose of 250 μg/kg. The ratio of TCA insoluble radioactivity to total radioactivity in plasma decreased with time. Immunoreactive Gln117 t-PA in plasma was lower than either of them at any time interval after a single i.v. administration. These results were also found in the case of WT t-PA [22]. Fig. 2 shows the immunoreactive Gln117 t-PA or WT t-PA and FCLT activity in plasma after intravenous administration of Gln117 t-PA or WT t-PA to male rats at a dose of 250 μg/kg. The level of FCLT activity was lower than that of the immunoreactive Gln117 t-PA or WT t-PA in plasma. Pharmacokinetic parameters calculated from the immunoreactive Gln117 t-PA or WT t-PA in plasma after intravenous administration of Gln117 t-PA or WT t-PA to male rats were shown in Table 1. The CL_p of Gln117 t-PA was approximately 2.6 times lower than that of WT t-PA. Similarly, MRT of Gln117 t-PA was approximately 4.8 times longer than that of WT t-PA. As to the distribution volume, V_c of Gln117 t-PA was similar to that of WT t-PA, whereas, V_{ss} of Gln117 t-PA was 1.8 times larger than that of WT t-PA.

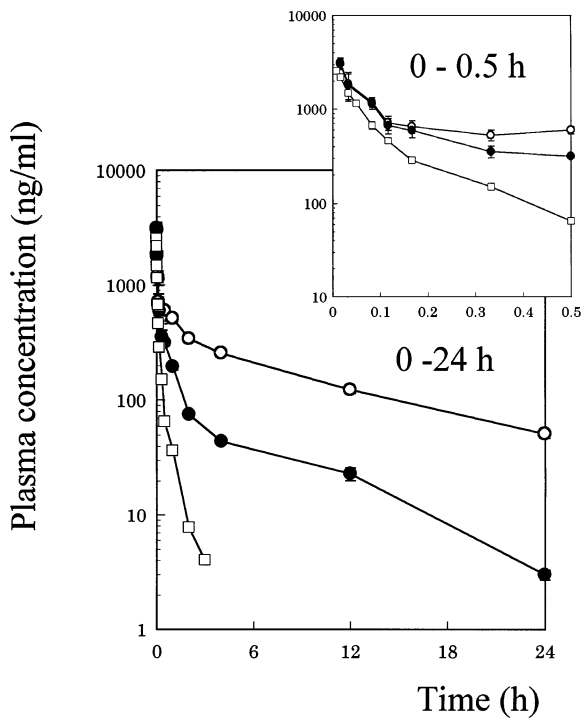

Fig. 1. Total radioactivity (○), TCA insoluble fraction (●) and immunoreactive Gln117 t-PA (□) concentration in plasma after a single intravenous administration of [^{125}I]Gln117 t-PA or Gln117 t-PA to male rats at a dose of 250 μg/kg. Each point represents the mean ± S.E. ($n = 3$).

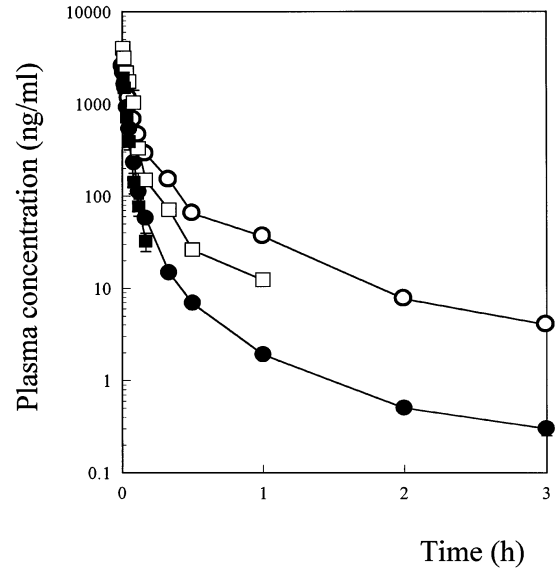

Fig. 2. Immunoreactive Gln117 t-PA or WT t-PA and FCLT activity in plasma after a single intravenous administration of Gln117 t-PA or WT t-PA to male rats at a dose of 250 μg/kg. ○ and □ show the immunoreactive Gln117 t-PA and FCLT activity in plasma after administration of Gln117 t-PA, respectively. ● and ■ show the immunoreactive WT t-PA and FCLT activity in plasma after administration of WT t-PA. Each point represents the mean ± S.E. ($n = 4$).

Table 1
Pharmacokinetic parameters of male rats calculated from the immunoreactive Gln117 t-PA or WT t-PA in plasma after a single intravenous administration of Gln117 t-PA or WT t-PA[a]

Parameter	Gln117 t-PA	WT t-PA
CL_p (ml/min/kg)	17.1 ± 4.1	44.5 ± 11.1
MRT (min)	22.9 ± 0.1	4.8 ± 0.3
V_c (ml/kg)	105.2 ± 17.9	112.8 ± 17.6
V_{ss} (ml/ kg)	393.4 ± 90.7	212.9 ± 41.1

[a] Each parameter represents the mean ± S.E. ($n = 4$).

3.2. Hepatic clearance

Fig. 3 shows the immunoreactive Gln117 t-PA or WT t-PA in plasma during and after a constant infusion of Gln117 t-PA or WT t-PA into the femoral vein (i.v.) or portal vein (i.p.v.) at a dose of 250 μg/kg. A marked difference between the i.v. and i.p.v. infusions for both agents was found, which suggests a significant hepatic elmination during the first circulation through the liver. The pharmacokinetic parameters that characterize the hepatic clearance are summarized in Table 2. Gln117 t-PA showed a CL_{hep} approximately 6.5 times lower than that with WT t-PA.

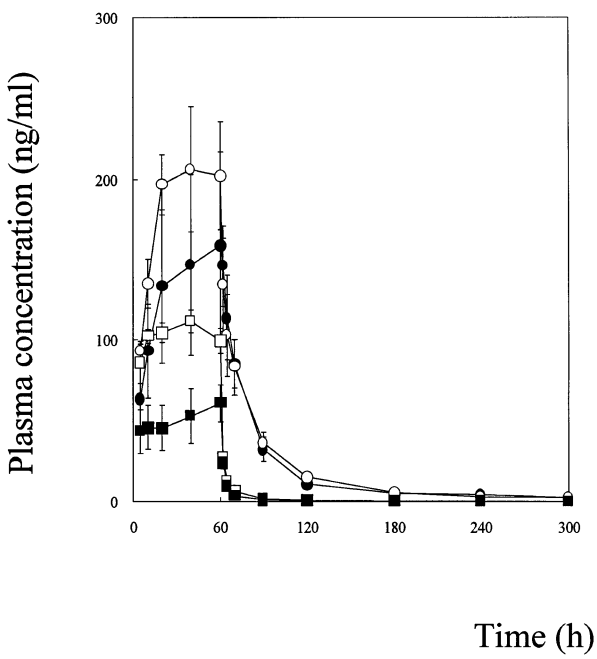

Fig. 3. Immunoreactive Gln117 t-PA or WT t-PA in plasma during and after a constant infusion of Gln117 t-PA or WT t-PA into the femoral vein or portal vein of male rats at a dose of 250 μg/kg. ○ and ● show the immunoreactive Gln117 t-PA in plasma during and after a constant infusion of Gln117 t-PA into the femoral vein and portal vein, respectively. □ and ■ show the immunoreactive WT t-PA in plasma during and after a constant infusion of WT t-PA into the femoral vein and portal vein. Each point represents the mean ± S.E. ($n = 4$).

Table 2
Hepatic clearance of male rats calculated from the immunoreactive Gln117 t-PA or WT t-PA in plasma after a constant infusion of Gln117 t-PA or WT t-PA[a]

Parameter	Gln117 t-PA	WT t-PA
AUC_{iv} (% of dose · min/ml)	18.73 ± 3.27	9.52 ± 1.10
AUC_{ipv} (% of dose · min/ml)	17.27 ± 1.97	5.21 ± 1.40
CL_p (ml/min/kg)	23.66 ± 3.87	44.97 ± 5.90
E_{hep}	0.08	0.45
CL_{hep} (ml/min/kg)	3.66	21.26

[a] Each parameter represents the mean ± S.E. ($n = 4$).

3.3. Radioactivity concentration in tissue

Table 3 shows the percentage distribution of radioactivity in tissues and gastro-intestinal contents at 5, 30 min, 1, 6, 12 and 24 h after intravenous administration of [^{125}I]Gln117 t-PA to male rats. The radioactivity in blood, liver, adrenal gland and spleen reached the highest levels at 5 min, those in testis, prostate gland, epididymus, bladder and stomach peaked at 1 h, that in the seminal vesicle peaked at 6 h, the thyroid gland peaked at 24 h, and those in any other tissues peaked at 30 min. Seventy-one percent of administered radioactivity was found in the liver at 5 min. The distribution in gastro-intestinal contents reached the highest level at 1 h. Although the gastric and intestinal contents were 8.8 and 3.6% of the dose at 1 h, respectively, they decreased to 0.8 and 1.1% at 24 h. At 24 h, the highest level of radioactivities was found in the thyroid gland.

Fig. 4 shows the radioactivity concentration of TCA insoluble fraction in tissue after a single intravenous administration of [^{125}I]Gln117 t-PA

to male rats. The radioactivity concentration of TCA insoluble fraction in the lung, stomach and thyroid gland reached the highest at 30 min, 1 h and 24 h, respectively, whereas those in any other tissues peaked at 5 min.

3.4. Radioactivity excreted in urine and feces

Table 4 shows that 40.0, 58.7, 79.0 and 88.5% of the dose were excreted in urine by 6, 12, 24 and 288 h after a single intravenous administration of [^{125}I]Gln117 t-PA to male rats. Fecal excretion of radioactivity after administration was 1.5 and 5.5% of the dose within 24 and 288 h. The total excretion of radioactivity in urine and feces was 94.0% of the dose within 288 h. The remaining radioactivities in the liver and thyroid gland were 1.1 and 0.04% of the dose at 288 h, respectively.

3.5. GFC analysis of plasma

Fig. 5 shows the radio-GFC chomatograms of plasma at 2, 5, 10 and 30 min after a single intravenous administration of [^{125}I]Gln117 t-PA to male rats at a dose of 250 μg/kg. In each chomatogram, 4 main peaks (I ~ IV) were found. The molecular weights of peaks I, II, III and IV, determined by calibration of the elution times

Table 3
Percentage distribution of radioactivity in tissues and gastro-intestinal contents after a single intravenous administration of [^{125}I]Gln117 t-PA to male rats at a dose of 250 μg/kg[a]

Tissues	Distribution of tissues or gastro-intestinal contents (% of dose)					
	5 min	30 min	1 h	6 h	12 h	24 h
Blood	13.619 ± 0.318	11.638 ± 0.036	9.099 ± 0.561	4.310 ± 0.229	2.558 ± 0.214	0.712 ± 0.099
Brain	0.025 ± 0.002	0.079 ± 0.010	0.062 ± 0.001	0.023 ± 0.003	0.014 ± 0.002	0.007 ± 0.001
Hypophysis	0.002 ± 0.001	0.004 ± 0.000	0.002 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	N.D.
Eyeball	0.006 ± 0.001	0.034 ± 0.003	0.032 ± 0.004	0.017 ± 0.001	0.010 ± 0.001	0.003 ± 0.001
Exolacrimal gland	0.009 ± 0.003	0.050 ± 0.001	0.044 ± 0.001	0.020 ± 0.002	0.013 ± 0.001	0.003 ± 0.001
Submaxillary gland	0.029 ± 0.005	0.139 ± 0.008	0.117 ± 0.009	0.063 ± 0.003	0.038 ± 0.004	0.009 ± 0.001
Thyroid gland	0.025 ± 0.001	0.857 ± 0.181	2.174 ± 0.038	8.380 ± 1.192	9.755 ± 1.007	12.237 ± 0.535
Thymus	0.014 ± 0.001	0.122 ± 0.004	0.093 ± 0.008	0.050 ± 0.003	0.033 ± 0.003	0.004 ± 0.001
Heart	0.096 ± 0.002	0.185 ± 0.009	0.127 ± 0.015	0.062 ± 0.003	0.039 ± 0.003	0.013 ± 0.002
Lung	0.451 ± 0.013	0.854 ± 0.137	0.399 ± 0.048	0.181 ± 0.005	0.095 ± 0.008	0.026 ± 0.005
Liver	70.625 ± 1.072	11.445 ± 0.703	4.055 ± 0.348	1.648 ± 0.045	1.012 ± 0.085	0.575 ± 0.077
Kidney	1.300 ± 0.090	1.533 ± 0.078	0.989 ± 0.048	0.410 ± 0.007	0.266 ± 0.009	0.109 ± 0.006
Adrenal gland	0.099 ± 0.009	0.021 ± 0.002	0.011 ± 0.001	0.003 ± 0.001	0.003 ± 0.001	0.001 ± 0.000
Spleen	0.841 ± 0.053	0.389 ± 0.044	0.202 ± 0.009	0.073 ± 0.009	0.048 ± 0.005	0.014 ± 0.002
Pancreas	0.029 ± 0.002	0.209 ± 0.043	0.139 ± 0.002	0.069 ± 0.009	0.038 ± 0.002	0.010 ± 0.001
Fat	0.177 ± 0.033	0.711 ± 0.095	0.532 ± 0.064	0.244 ± 0.028	0.192 ± 0.031	0.098 ± 0.011
Skeletal muscle	2.297 ± 0.569	11.271 ± 0.263	11.046 ± 1.100	4.250 ± 0.271	2.277 ± 0.277	0.691 ± 0.113
Skin	2.272 ± 0.122	24.273 ± 1.265	21.768 ± 1.530	13.707 ± 0.681	10.689 ± 0.851	3.501 ± 0.439
Testis	0.064 ± 0.003	0.265 ± 0.055	0.340 ± 0.024	0.317 ± 0.004	0.192 ± 0.021	0.040 ± 0.005
Epididymus	0.015 ± 0.001	0.089 ± 0.003	0.095 ± 0.005	0.050 ± 0.001	0.033 ± 0.003	0.007 ± 0.001
Seminal vesicle	0.016 ± 0.002	0.090 ± 0.007	0.089 ± 0.008	0.156 ± 0.074	0.062 ± 0.008	0.028 ± 0.005
Prostate gland	0.008 ± 0.001	0.056 ± 0.009	0.071 ± 0.005	0.038 ± 0.005	0.030 ± 0.003	0.005 ± 0.001
Urinary bladder	0.009 ± 0.001	0.044 ± 0.008	0.104 ± 0.021	0.045 ± 0.011	0.016 ± 0.001	0.006 ± 0.001
Stomach	0.103 ± 0.009	0.896 ± 0.037	1.137 ± 0.032	0.789 ± 0.245	0.354 ± 0.038	0.051 ± 0.016
Small intestine	0.312 ± 0.026	1.266 ± 0.069	0.986 ± 0.082	0.536 ± 0.130	0.566 ± 0.145	0.108 ± 0.029
Caecum	0.031 ± 0.002	0.097 ± 0.012	0.088 ± 0.003	0.052 ± 0.001	0.044 ± 0.005	0.012 ± 0.001
Large intestine	0.052 ± 0.002	0.262 ± 0.009	0.203 ± 0.008	0.117 ± 0.010	0.075 ± 0.013	0.014 ± 0.003
Gastric contents	0.247 ± 0.047	8.096 ± 0.669	8.811 ± 0.344	5.875 ± 1.087	2.793 ± 0.472	0.812 ± 0.209
Intestinal contents	0.268 ± 0.036	3.734 ± 0.381	3.571 ± 0.273	2.701 ± 0.184	3.414 ± 0.389	1.065 ± 0.109

[a] Each point represents the mean ± S.E. ($n = 3$). N.D.: not detected.

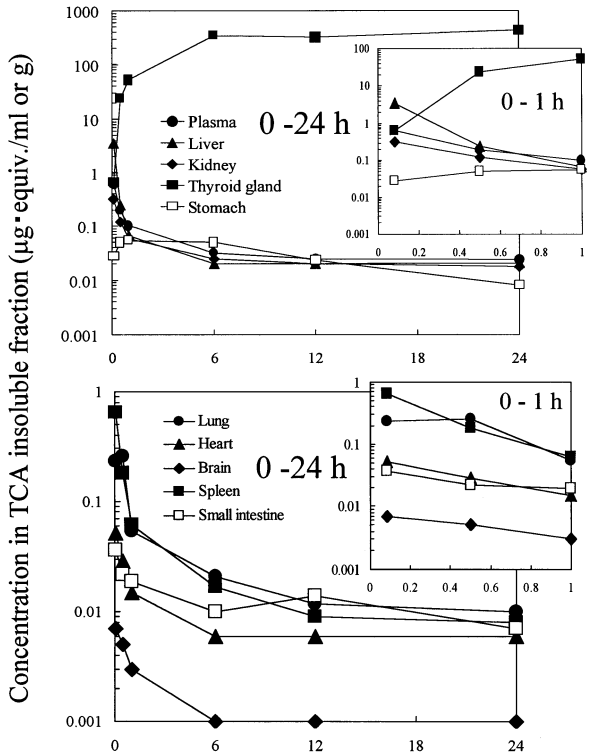

Fig. 4. Radioactivity concentration of the TCA-insoluble fraction in tissues after a single intravenous administration of [^{125}I]Gln117 t-PA to male rats at a dose of 250 μg/kg. Each point represents the mean ($n = 3$).

with standard proteins, were approximately > 600, 130, 65 and < 0.3 kDa. Peak III was thought to be free form, judging from its molecular weight. Peaks I and II had a low level of immunoreactivity, and no activity, whereas neither was found in peak IV (data not shown). The radioactivities of I and II decreased more slowly than III; in contrast, that of IV increased with time. Fig. 6 shows the ratio of total radioactivity in peak I and II after administration of [^{125}I]Gln117 t-PA and [^{125}I]WTt-PA to male rats. No comparative differences between both t-PAs were observed.

4. Discussion

The metabolic fate of Gln117 t-PA was studied in rats, compared with WT t-PA. After intravenous administration of Gln117 t-PA, the levels of immunoreactive Gln117 t-PA and FCLT activity in plasma were higher than those in plasma after inravenous administration of WT t-PA. The CL_p of Gln117 t-PA calculated from the immunoreactive concentration was approximately 1/2.6 as much as that of WT t-PA. Although the V_c of Gln117 t-PA was almost the same as that of WT t-PA, V_{ss} of Gln117 t-PA was 1.8 times larger than that of WT t-PA. Although the CL_{hep} of Gln117 t-PA was 6.5 times lower than that of WT t-PA, the extrahepatic clearance of Gln117 t-PA (20.00 ml/min/kg), which can be assessed by subtracting CL_{hep} from CL_p, was similar to that of WT t-PA (23.71 ml/min/kg). These results indicate that the lack of the high mannose oligosaccharide at Asn117 increases the peripheral distribution volume of t-PA and decreases the hepatic clearance of t-PA but does not affect the extrahepatic clearance of t-PA. In the GFC analysis of plasma, peaks I and II were thought to be the complexes formed with α_2-macroglobulin and α_2-antiplasmin, respectively, judging from their molecular weight. These complexes had some immunoreactivity, but they had no activity in the same manner as WT t-PA [27]. Thus, they might be related to the difference between immunoreac-

Table 4
Cumulative excretion of radioactivity in urine and feces of rats after single intravenous administration of [^{125}I]Gln117 t-PA[a]

Time (h)	Excretion of radioactivity (percentage of dose)		
	Urine	Feces	Total
0–6	40.0 ± 3.0	40.0 ± 3.0	–
0–12	58.7 ± 2.3	0.0 ± 0.0	58.7 ± 2.3
0–24	79.0 ± 1.5	1.5 ± 0.3	80.5 ± 1.3
0–48	83.2 ± 1.4	2.7 ± 0.2	85.9 ± 1.5
0–72	84.9 ± 1.3	3.5 ± 0.2	88.5 ± 1.4
0–96	86.1 ± 1.2	4.2 ± 0.2	90.2 ± 1.2
0–192	87.8 ± 1.1	5.3 ± 0.2	93.1 ± 0.9
0–288	88.5 ± 1.0	5.5 ± 0.5	94.0 ± 0.7

[a] Each parameter represents the mean ± S.E. ($n = 3$). Radioactivity in thyroid gland at 288 h: 1.1 ± 0.2%. Radioactivity in liver at 288 h: 0.04 ± 0.01%.

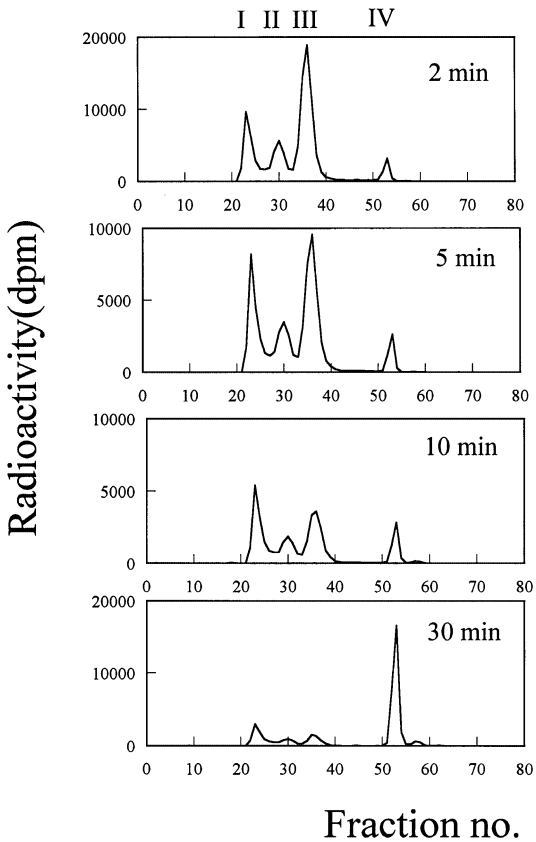

Fig. 5. Radio-GFC chomatograms of plasma at 2, 5, 10 and 30 min after a single intravenous administration of [^{125}I]Gln117 t-PA to male rats at a dose of 250 μg/kg.

tivity and activity in plasma. In contrast, the ratio of total radioactivity in peak IV, which had a low molecular weight (<0.3 kDa), increased with time, so the ratio of TCA insoluble radioactivity to total radioactivity in plasma decreased with time. No comparative differences in the ratio of total radioactivities in these complexes were observed between Gln117 t-PA and WT t-PA. From this result, the high mannose oligosaccharide at Asn117 was not thought to affect the complex forming of t-PA.

Although a high level of radioactivity was detected in the liver soon after a single intravenous administration of [^{125}I]Gln117 t-PA, the radioactivity in the liver was lower than that after administration of [^{125}I]WT t-PA [22]. We assumed that it was caused by the lower uptake of Gln117 t-PA to the liver. However, the increase of radioactivity concentration in thyroid gland with time, most of which was TCA-insoluble, was thought to originate from the uptake of $^{125}I^-$ to the thyroid gland, which generated [^{125}I]thyroglobulin [22]. In addition, the secretion of $^{125}I^-$ in the stomach [28] was supposed to delay the time when the highest distribution of radioactivity was reached in the gastro-intestinal contents.

After intravenous administration of [^{125}I]Gln117 t-PA to rats, 88.5 and 5.5% of the dose were excreted in urine and feces, respectively, within 288 h. The urinary and fecal excretion profile of Gln117 t-PA was similar to that of WT t-PA [22].

Several investigators found that the mannose receptor in the liver recognizes the high mannose oligosaccharide of t-PA [15–18]. These results suggest that the lower CL_p of Gln117 t-PA is due to the decrease of uptake to the mannose receptor in the liver by removal of the high mannose oligosaccharide at Asn117. Recently,

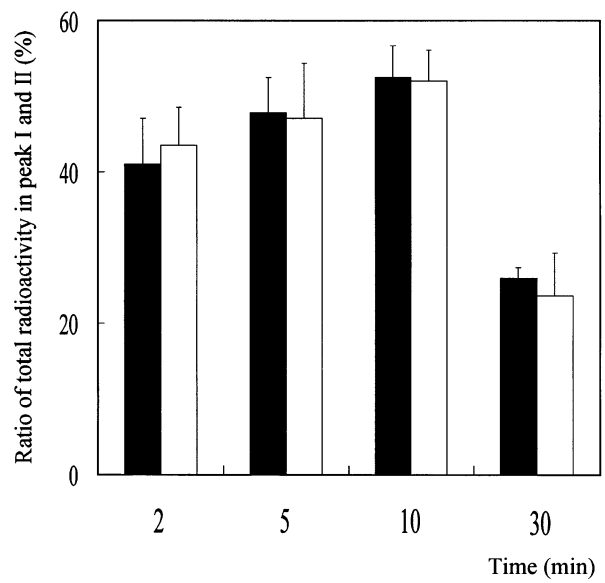

Fig. 6. Ratio of total radioactivity in peak I and II at 2, 5, 10 and 30 min after a single intravenous administration of [^{125}I]Gln117 t-PA (■) and [^{125}I]WT t-PA (□) to male rats at a dose of 250 μg/kg. Each point represents the mean ± S.E. ($n=3$).

Schwartz et al. [29–32] have described two receptor systems, the mannose receptor and the low density lipoprotein-related protein (LRP), which were the most important for hepatic clearance of t-PA. Furthermore, Camani reported that LRP mainly recognizes finger and/or EGF domains of t-PA [33]. Although this suggests that the modification of these domains is necessary to decrease the clearance of t-PA via LRP, some kinds of modification of domains lead to a considerable loss of activity [12–14]. On the contrary, the modification of the sugar chain is not expected to affect the function of each domain. In fact, our Gln117 t-PA is superior to WT t-PA in activity and fibrin affinity [34]. In conclusion, Gln117 t-PA is one of several useful mt-PAs in terms of its high activity and fibrin affinity as well as its long biological half-life.

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References

[1] D. Collen, Thromb. Haemostas. 43 (1980) 77–89.
[2] M. Hoylaerts, D.C. Rijken, H. Lijnen, D. Collen, J. Biol. Chem. 257 (1982) 2912–2919.
[3] F. Van de Werf, S.R. Bergmann, K.A.A. Fox, H. de Geest, C.F. Hoyng, B.E. Sobel, D. Collen, Circulation 69 (1984) 605–610.
[4] D. Collen, E.J. Topol, A.J. Tiefenbrunn, H.K. Gold, M.L. Weisfeldt, B.E. Sobel, R.C. Leinbach, J.A. Brinker, P.A. Ludbrook, I. Yasuda, B.H. Bulkley, A.K. Robison, A.M. Hutter Jr., W.R. Bell, J.J. Spadaro Jr., B.A. Khaw, E.B. Grossbard, Circulation 70 (1984) 1012–1017.
[5] F. Van de Werf, P.A. Ludbrook, S.R. Bergmann, A.J. Tiefenbrunn, K.A.A. Fox, H. deGeest, M. Verstraete, D. Collen, B.E. Sobel, New Engl. J. Med. 310 (1984) 609–613.
[6] The TIMI Study Group, New Engl. J. Med. 312 (1985) 932–936.
[7] A.K. Rao, C. Pratt, A. Berke, A. Jaffe, I. Ockene, T.L. Scheiber, W.R. Bell, G. Knatterud, T.L. Robertson, M.L. Terrin, J. Am. Coll. Cardiol. 11 (1988) 1–11.
[8] S. Suzuki, M. Saito, N. Suzuki, S. Yoshitake, Y. Yui, C. Kawai, Thromb. Haemostas. 65 (1991) 1192.
[9] T. Kawasaki, M. Katoh, S. Kaku, H. Gushima, Y. Yui, C. Kawai, Thromb. Haemostas. 65 (1991) 1337.
[10] N.B. Modi, S. Eppler, J. Breed, E. Braunwald, T.W. Love, Thromb. Haemostas. 79 (1998) 134–139.
[11] W.C. Liao, F.A. Beierle, B.C. Stouffer, R.C. Dockens, Z.A. Abbud, L.K. Tay, Circulation 96 (Suppl. 1) (1997) 260–261.
[12] M. Alibhai, A.M. Chow, N.F. Paoni, Thromb. Haemostas. 75 (1996) 107–112.
[13] R.W. Smalling, Eur. Heart J. 18 (Suppl. F) (1997) F11–16.
[14] L.C. Sehl, H.V. Nguyen, L.T. Berleau, P. Arcila, W.F. Bennett, B.A. Keyt, Prot. Eng. 9 (1996) 283–290.
[15] M. Einarsson, B. Smedsrød, H. Partoft, Thromb. Haemostas. 59 (1988) 474–479.
[16] B. Smedsrød, M. Einarsson, H. Partoft, Thromb. Haemostas. 59 (1988) 480–484.
[17] B. Smedsrød, M. Einarsson, Thromb. Haemostas. 63 (1990) 60–66.
[18] M. Otter, M.M. Barrett-Bergshoeff, D.C. Rijken, J. Biol. Chem. 266 (1991) 13931–13935.
[19] P.J. Fraker, J.C. Speck Jr., Biochem. Biophys. Res. Commun. 80 (1978) 849–857.
[20] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, J. Biol. Chem. 193 (1951) 265–275.
[21] R. Hori, Y.-L. He, T. Shima, K. Inui, S. Aoki, K. Okumura, Y. Tanigawara, Drug Metab. Dispos. 20 (1992) 541–546.
[22] K. Okumura, S. Aoki, T. Ohno, Y. Iba, K. Nishimoto, S. Baba, Prog. Med. 9 (1989) 483–503.
[23] Y. Tanigawara, K. Okumura, Y.-L. He, R. Hori, Chem. Pharm. Bull. 39 (1991) 761–764.
[24] K. Yamaoka, T. Nakagawa, T. Uno, J. Pharmacokin. Biopharm. 6 (1978) 547–558.
[25] J.G. Wagner, Pharmacokinetics for the Pharmaceutical Scientist, Technomic Publishing Co., Lancaster, 1993, pp. 15–102.
[26] G.M. Pollack, K.L.R. Brouwer, K.B. Demby, J.A. Jones, Drug Metab. Dispos. 18 (1990) 197–202.
[27] S. I-da, M. Iwamoto, M. Suzuki, N. Hisanaga, N. Tsuzii, S. Komatsu, Xenobio. Metabol. Dipos. 3 (1988) 309–329.
[28] S. Ulberg, B. Ewaldsson, Acta Radiol. 2 (1964) 24–32.
[29] P.A. Morton, D.A. Owensby, B.E. Sobel, A.L. Schwartz, J. Biol. Chem. 264 (1989) 7228–7235.
[30] P.A. Morton, A.L. Schwartz, J. Biol. Chem. 267 (1992) 15595–15602.
[31] D.A. Owensby, B.E. Sobel, A.L. Schwartz, J. Biol. Chem. 263 (1988) 10587–10594.
[32] D.A. Owensby, P.A. Morton, A.L. Schwartz, J. Biol. Chem. 264 (1989) 18180–18187.
[33] C. Camani, O. Gavin, C. Bertossa, E. Samatani, K. Kruithof, Eur. J. Biochem. 251 (1998) 804D–811D.
[34] S. Aoki, N. Shimizu, M. Shimonishi, M. Kitagawa, K. Okumura, Y. Tanigawara, Biol. Pharm. Bull. 24 (2001) 295–298.