COMPARISON OF DRUG DISPOSITION BETWEEN WILD-TYPE AND NOVEL TISSUE-TYPE PLASMINOGEN ACTIVATOR PAMITEPLASE IN RATS

KEISHI OIKAWA, TAKASHI WATANABE, AND SABUROU HIGUCHI

Drug Metabolism Laboratories, Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan

(Received November 29, 1999; accepted May 18, 2000)

This paper is available online at http://www.dmd.org

ABSTRACT:

The pharmacokinetics of pamiteplase in rats was compared with the pharmacokinetics of recombinant wild-type tissue-type plasminogen activator (rwt-PA). The half-life in the β-phase and total clearance after administration of 125I-labeled pamiteplase (125I-pamiteplase) to rats were 480 and 22% of those of 125I-labeled rwt-PA (125I-rwt-PA), respectively. The amount of radioactivity distributed in the liver after administration of 125I-pamiteplase was lower than that of 125I-rwt-PA; consequently, a possible difference in metabolism between the drugs was assessed by an integration plot and a tissue-sampling single-injection technique. Use of these two methods revealed that the hepatic clearances of both compounds accounted for almost all of the total clearance and also revealed that the hepatic clearance of 125I-pamiteplase was markedly lower than that of 125I-rwt-PA. Therefore, the lower distribution of pamiteplase in the liver compared with rwt-PA is thought to contribute greatly to the higher plasma concentration of pamiteplase. Additionally, the uptake of 125I-pamiteplase in the liver was inhibited by rwt-PA, suggesting that there is a common uptake mechanism for both compounds.

Tissue-type plasminogen activator (t-PA)1 is an endogenous glycoprotein that plays a central role in fibrinolysis. Recently, recombinant wild-type t-PA (rwt-PA) has been widely used as a thrombolytic agent to treat acute myocardial infarction. Many clinical trials have been conducted to evaluate the pharmacologic efficacy of various rwt-PAs, and the results of these studies revealed lower patient mortality compared with the use of streptokinase or urokinase (Verstraete et al., 1985; Kanemoto et al., 1991).

One major disadvantage of rwt-PA is its short plasma half-life due to rapid uptake by the liver (Camani et al., 1994). Two kinds of hepatocytes, parenchymal and endothelial cells, are responsible for the uptake of rwt-PA. To improve the short plasma half-life of rwt-PA, various modified rwt-PAs have been developed. Pamiteplase is a novel recombinant t-PA bearing a deletion in the Kringle-1 domain and a point mutation (Arg275 Glu) in the Kringle-2 domain (Kawauchi et al., 1991). Despite these structural modifications, pamiteplase possesses almost the same in vivo affinity for fibrinous thrombi as rwt-PA (Katoh et al., 1991). Furthermore, plasma concentrations after administration of pamiteplase to rats or dogs are higher than those of rwt-PA (Oikawa et al., 1996a). Pamiteplase at one-fifth the dose of rwt-PA has almost the same thrombolytic activity as rwt-PA in rats or dogs with induced thrombi (Kawasaki et al., 1993a,b,c). Consequently, pamiteplase has the same biological effect as t-PA but much lower clearance compared with rwt-PA.

The clearances of high-molecular weight compounds from plasma are usually due to cellular uptakes by endocytosis, especially in the liver and the kidneys, and/or to irreversible bindings of these compounds to plasma proteins. In the case of pamiteplase and rwt-PA, an unchanged form after administration of either pamiteplase or rwt-PA to rats was not excreted in urine, indicating their eliminations are due to these kinds of metabolic clearance (Iida et al., 1988; Kizen et al., 1988; Komuro et al., 1989; Okumura et al., 1989; Oikawa et al., 1996b). The greatest tissue distribution of both compounds is in the liver, where uptake and degradation occur (Iida et al., 1988; Kizen et al., 1988; Komuro et al., 1989; Okumura et al., 1989; Oikawa et al., 1996b). Therefore, these findings suggest a large difference in hepatic uptake between pamiteplase and rwt-PA.

In this study, the distribution of radioactivity in tissues was examined after single i.v. administration of the same dose of 125I-labeled forms of both drugs. A large difference in hepatic distribution between the drugs was observed, so the uptake of pamiteplase and rwt-PA to the liver in rats was examined using the liver uptake index (LUI) measured by a tissue-sampling single-injection technique. Furthermore, because both drugs formed complexes with plasma protein, the rates of formation of these complexes were examined.

Experimental Procedures

All animal experiments complied with the regulations of Yamanouchi Pharmaceutical’s Animal Experimentation Ethics Committee.

Materials. The pamiteplase gene was constructed by combining a part of the t-PA gene and a synthesized DNA fragment coding the finger and epidermal growth factor (EGF) domains, and was then cloned into a mammalian

1 Abbreviations used are: t-PA, tissue-type plasminogen activator; rwt-PA, recombinant wild-type t-PA; EGF domain, epidermal growth factor domain; CLtot, total clearance; CLhepatic, hepatic clearance; t1/2β, half-life in β phase; AUC0–∞, area under the plasma concentration-time curve from zero to infinity; Vdss, distribution volume at the steady state; MRT, mean residence time; GFC, gel filtration chromatography; LUI, liver uptake index; Eth, extravascular hepatic extraction; CLhepatic (LUI), hepatic clearance in LUI studies; RME, receptor-mediated endocytosis.

Send reprint requests to: Keishi Oikawa, 1-8, Azusawa 1-Chome Itabashi-ku, Tokyo 174-8511, Japan. E-mail: oikawa@yamanouchi.co.jp
expression vector, pVY1, under the control of the SV 40 early promoter (Kawauchi et al., 1991). Plasmid pVY1-pamiteplase was transfected into Chinese hamster ovary cells (Kawauchi et al., 1991). The pamiteplase molecule contains a finger domain, an EGF domain, a Kringle-2 domain, a serine protease domain, and a site mutation at the Kringle-2-serine protease linkage site (Kawauchi et al., 1991). A lyophilized pharmaceutical preparation of pamiteplase was used in this study. rwt-PA (Alteplase) was purchased from Genentech, Inc. (San Francisco, CA). Carrier-free Na125I and 3 H2O were purchased from DuPont NEN (Boston, MA). Other reagents were commercially available and of analytical grade.

Animals. Fischer male rats aged 6 weeks (body weight, 162–201 g) were purchased from Charles River Japan, Inc. (Yokohama, Japan), and were acclimated for more than 1 week before the study. They were housed in an air conditioned room (temperature, 23 ± 2°C; relative humidity, 55 ± 5%) and kept on a light/dark cycle of 12 h/12 h. They had free access to pelleted food (MF: Oriental Yeast Co., Ltd., Tokyo, Japan) and water.

Preparation of 125I-Labeled Pamiteplase (125I-pamiteplase) and 125I-Labeled rwt-PA (125I-rwt-PA). Lyophilized formulations containing either 4 mg of pamiteplase or 50 mg of rwt-PA were dissolved in physiologic saline to make stock solutions of both drugs (1 mg/ml, respectively). 125I-pamiteplase or 125I-rwt-PA was synthesized by the chloramine-T method using carrier-free Na125I and stock solutions (Iida et al., 1988; Kizen et al., 1988; Komuro et al., 1989; Okumura et al., 1989; Oikawa et al., 1996b). The specific activity, concentration, and purity of radioactivity of 125I-pamiteplase were 214.3 MBq/mg, 11.4 MBq/ml, and 95%, respectively; those of 125I-rwt-PA were 177.0 MBq/mg, 9.4 MBq/ml, and >95%, respectively.

Intravenous Administration. Dosing solutions of 125I-pamiteplase or 125I-rwt-PA were prepared from the stock solution, 125I-labeled compound, and vehicle A solutions (0.127 M phosphoric acid solution containing 0.2 M arginine and 0.011% Tween 80) to yield a final concentration of 0.15 mg/ml. These dosing solutions were i.v. administered at 0.3 mg/kg to rats via the tail vein.

To determine tissue distribution, different tissues, including blood, plasma, lungs, heart, liver, kidneys, spleen, adrenal glands, stomach, and small intestine, were harvested at 5 or 120 min after dosing. Three rats were used at each time point. To determine time profiles of concentration in plasma and liver, blood and liver were obtained at 2, 5, 10, 15, 30, 45, 60, 90, 120, 180 min after dosing. Three rats were used at each time point. Blood samples were immediately transferred to polypropylene tubes containing 3.8% sodium citrate (final concentration 10%; Kokusai-Shiyaku Co., Kobe, Japan) and 500 μM PPACK (final concentration, 5 μM; Calbiochem-Novabiochem Co., San Diego, CA), and mixed gently, followed by centrifugation (1,800 rpm for 15 min at 4°C) to separate the plasma. The plasma was stored at −80°C until the gel filtration chromatography (GFC) analysis.

Analysis of GFC. Analysis of GFC was: precolumn: TSK guard column SW-xl (6.0-mm×4 cm; Tosoh Corp., Tosoh, Japan); column: TSK-GEL G-3000 SW XL in a single pass. The E drug was given by:

\[ C_T (t) = C_P (t) \cdot \frac{V_T}{V_D (t)} \] (4)

where the line represents pair of symbols.

Intravenous Administration through the Hepatic Portal Vein. The portal vein injection technique to obtain the LUI was performed according to the methods described by Tsuji et al. (1990). Dosing solutions containing 3 H2O (a freely diffusible reference) were prepared from stock solutions, the 125I-labeled compounds, 125I-H2O, and Ringer HEPES buffer (10 mM HEPES buffer (pH 7.4) containing 141 mM NaCl, 4 mM KCl, 2.8 mM CaCl2, 0.2 M arginine, and 0.01% Tween 80). Three rats were used at each point. Rats were anesthetized by ketamine (235 mg/kg) and xylazine (2.3 mg/kg). After laparotomy, the hepatic artery was ligated, and the portal vein was cannulated with a 27-gauge needle. Two hundred microliters of each plasma was sample-injected into the line represents the area under the plasma-concentration-time curve from time 0 to t, When the efflux (or dissociation) is much smaller than the influx within a short period of time, eq. 3 can be simplified to eq. 4:

\[ V_T \cdot C_T = k_1 \cdot \int _0 ^t C_P \, dt - k_2 \cdot \int _0 ^t C_D \, dt \] (2)

\[ = k_1 \cdot \text{AUC}_{0 \rightarrow t} - k_2 \cdot \int _0 ^t C_D \, dt \] (3)

where AUC0→t represents the area under the plasma-concentration-time curve from time 0 to t. When the efflux (or dissociation) is much smaller than the influx within a short period of time, eq. 3 can be simplified to eq. 4:

\[ V_T \cdot C_T = k_1 \cdot \text{AUC}_{0 \rightarrow t} \] (4)

The plot of \( V_T \cdot C_T(t) \) versus AUC0→t yields a straight line, and the slope of the line represents \( k_1 \).

Determination of LUI and Hepatic Clearance (CLhepatic). The LUI is defined in eq. 5 and experimentally determined using eq. 6.

\[ \text{LUI(%) = } 100 \times \frac{E_{\text{drug}}}{E_{\text{reference}}} \] (5)

\[ \text{LUI(%) = } \left( \frac{125I \text{ dpm}}{3 \times \text{dpm}} \right)_{\text{injected}} \times \left( \frac{125I \text{ dpm}}{3 \times \text{dpm}} \right)_{\text{venous}} \] (6)

where where Edrug and Ereference are the fractional extraction of test and reference compounds on a single pass. The Edrug was given by:

\[ E_{\text{drug}} = \text{LUI} \times \frac{E_{\text{reference}}}{100} \] (7)

The Ereference value of 3 H2O has been previously reported as 84% (Tsuji et al., 1990). The vascular volume of the liver is not negligible. Therefore, the extravascular hepatic extraction (Eextra, the extraction is only due to the cellular uptake) is calculated as follows:

\[ E_{\text{extra}} = \frac{E_{\text{extra}} - E_{\text{in}}} {100 - E_{\text{in}}} \] (8)

where Eextra represents the extraction of albumin for distribution in hepatic vessel, and a value of 13% has been reported (Tsuji et al., 1990). Furthermore, hepatic clearance in LUI studies [CLhepatic (LUI)] was calculated with C in

\[ \text{CLhepatic (LUI)} = \text{plasma flow (58.8 ml/min/kg)} \times E_{\text{extra}} \] (9)
Effect of rwt-PA on the $E_{\text{drug}}$ of Pamiteplase.

To determine the effect of rwt-PA on the $E_{\text{drug}}$ of pamiteplase, a dosing solution was prepared using stock solutions of the drugs, $^{125}$I-pamiteplase, $^{3}$H$_{2}$O, and Ringer-HEPES buffer to yield final concentrations of 10 nM pamiteplase and that of rwt-PA ranging from 5 to 500 nM. The experimental procedure is the same as described above.

Examination of Plasma Clearance.

Ten micrograms per milliliter of incubation solutions were prepared using the stock solutions, $^{125}$I-labeled compounds, and vehicle A solutions. After adding an incubation solution (final concentration 1 mg/ml) to rat plasma preincubated at 37°C for 5 min, these mixtures were incubated at 37°C, and samples were collected at 2, 5, 10, 15, 20, 30, 45, and 60 min after incubation. Unchanged drug concentrations in these samples were analyzed using GFC. As the plasma concentrations of both drugs declined in a monophasic manner, the elimination rate constant ($k_{\text{el}}$) was calculated using a first-order exponential equation:

$$C_{p} = A \cdot e^{-kt}$$  \hspace{1cm} (10)

where $C_{p}$ is unchanged drug concentration in plasma at time $t$ and $A$ is the coefficient, respectively.

Results

Tissue Distribution.

Figures 1 and 2 show concentrations and percentages of the administered dose distributed in tissues after a single i.v. administration of 0.3 mg/kg of $^{125}$I-pamiteplase or $^{125}$I-rwt-PA to rats, respectively. As shown in Fig. 1, the concentration at 5 min after administration of $^{125}$I-pamiteplase was highest in the plasma, followed by the blood, liver, and kidneys. The concentration of $^{125}$I-pamiteplase in the liver was 37% of that in the plasma. In contrast to pamiteplase, the concentration of $^{125}$I-rwt-PA at 5 min after administration was highest in the liver, followed by the plasma, spleen, and kidneys. The liver concentration of $^{125}$I-rwt-PA was 2.9 times higher than that of the plasma. At 120 min postdosing, no
difference was observed in the tissue concentrations of the drugs. As shown in Fig. 2, the percentage distribution of radioactivity in the liver at 5 min after administration of $^{125}$I-rwt-PA was 2.5-fold higher than that after administration of $^{125}$I-pamiteplase.

**Plasma Concentration.** Figure 3 shows a time profile of unchanged drug concentration in plasma after a single i.v. administration of 0.3 mg/kg of $^{125}$I-pamiteplase or $^{125}$I-rwt-PA to rats, and Table 1 shows the pharmacokinetic parameters of both drugs. Plasma concentrations of both drugs declined biexponentially. $t_{1/2a}$ of $^{125}$I-pamiteplase was 5.5 times longer than that of rwt-PA, $t_{1/2b}$ was 4.8 times longer, and MRT was 7 times longer, respectively. CL total of pamiteplase decreased to 22% of that of rwt-PA.

**Concentration of Radioactivity in the Liver.** Figure 4 shows liver concentrations, Fig. 5 percentages of the administered dose distributed in the liver, and Fig. 6 percentages of concentration in the liver relative to that in the plasma after a single i.v. administration of 0.3 mg/kg of $^{125}$I-pamiteplase or $^{125}$I-rwt-PA to rats. As shown in Figs. 4 and 5, the maximum liver concentration after administration of $^{125}$I-rwt-PA was 2.5 times higher than that after administration of $^{125}$I-pamiteplase, and the maximum percentage of radioactivity distribution also was 2.6 times higher. As shown in Fig. 6, the percentage of concentration in the liver relative to that in the plasma after administration of $^{125}$I-pamiteplase leveled out from 10 min after dosing, and ranged from 0.69 to 0.95, whereas that of $^{125}$I-rwt-PA ranged from 1.4 to 14.2.

**CL hepatic Calculated by an Integration Plot.** Figure 7 shows a plot of liver drug concentration versus AUC$_{0\rightarrow t}$ of plasma concentration, and the slope reveals $k_1$ (ml/min/g of tissue) by eq. 4. Table 2 shows CL hepatic calculated using $k_1$, liver weight, and body weight. The CL hepatic of pamiteplase decreased to 19% of that of rwt-PA. The CL hepatic of both drugs was 75 and 86%, and accounted for most of the CL total calculated using unchanged drug concentrations in the plasma.

**Hepatic Extraction Ratio by a Portal Vein Injection Technique.** Figure 8 shows $E_{x,drug}$ and CL hepatic after a single administration of $^{125}$I-pamiteplase or $^{125}$I-rwt-PA (ranged from 0.057–57 μg/ml) into the portal vein of rats. $E_{x,drug}$ ranged from 8.94 to 15.36% for $^{125}$I-pamiteplase and from 30.25 to 33.58% for $^{125}$I-rwt-PA, and it fluctuated very little from these values. $E_{x,drug}$ of $^{125}$I-rwt-PA was 2.2 to 3.7 times higher than that of $^{125}$I-pamiteplase. CL hepatic ranged from 5.26 to 9.03 ml/min/kg for pamiteplase and from 17.8 to 20.0 ml/min/kg for rwt-PA.

Figure 9 shows $E_{x,drug}$ after a single administration of 10 nM $^{125}$I-pamiteplase together with rwt-PA ranging from 5 to 500 nM to the portal vein of rats. The $E_{x,drug}$ of pamiteplase together with rwt-PA decreased dose-dependently.

**Examination of Plasma Clearance In Vitro.** Figure 10 shows a time profile of plasma unchanged drug concentration after incubation...
of $^{125}$I-pamiteplase or $^{125}$I-rwt-PA with rat plasma, and Table 3 shows the kinetic parameters. The unchanged drug concentrations of both drugs declined monoexponentially, and the elimination rate constant ($k_{el}$) calculated by a first-order exponential equation was 0.0172 l/min for pamiteplase and 0.0167 l/min for rwt-PA. $CL_{plasma}$ was calculated using $k_{el}$, and plasma volume was almost comparable for both drugs.

### Discussion

Pamiteplase possesses the same biological activity as rwt-PAs despite its structural modifications (Katoh et al., 1991), although having a longer plasma half-life than that of rwt-PAs (Oikawa et al., 1996a). By prolonging the half-life, a bolus administration of pamiteplase at a lower dose compared with other rwt-PAs exhibited a comparable thrombolytic activity to rwt-PA administered by infusion (Kawasaki et al., 1993a,b,c). In this study, the prolonged half-life of pamiteplase was examined in the terms of the clearance mechanism of both drugs in the body.

$^{125}$I-Labeled materials are usually used in drug metabolism studies of bioactive peptides, such as pamiteplase and rwt-PA, and attention should be paid to the relationship of properties between $^{125}$I-labeled and nonlabeled materials. Before this study, the relationship between pamiteplase and rwt-PA was examined using a GFC analysis of plasma after administration of $^{125}$I-labeled materials to rats.

The percentage of $CL_{plasma}$ relative to $CL_{total}$ was 10.2% for pamiteplase and 2.19% for rwt-PA.
and the marked decrease in CL total of pamiteplase in plasma compared to rwt-PA under identical conditions (dose, animal, researcher, and laboratory) was examined. The prolongations of $t_{1/2}$, $t_{1/2}$, and MRT, and the marked decrease in CL total of pamiteplase in plasma compared with rwt-PA were also confirmed in this study, showing the reason why a bolus pamiteplase administration at a one-fifth dose of rwt-PA exhibits comparable pharmacologic effects. On the other hand, the main site of clearance for both drugs was the liver, from their distributions in tissues. Furthermore, the level of pamiteplase in the liver was markedly lower than that of rwt-PA, suggesting a large difference in hepatic uptake between the drugs. CL hepatic, calculated by an integration plot accounted for most of CL total for both drugs (75% for pamiteplase and 86% for rwt-PA), and was markedly lower in pamiteplase than in rwt-PA. Consequently, the difference in CL hepatic was thought to be the direct cause of differences in pharmacokinetics.

Differences in drug uptakes in the liver were directly compared for both drugs by employing the LUI using a tissue-sampling single-injection technique. This technique is widely used to measure transport rates at cell membranes without damaging tissues (Tsui et al., 1990). This technique has been applied to compare in vivo transport rates of a drug in the blood-brain barrier (BBB), the liver, and kidneys (Tsui et al., 1990). Examples include studies of the BBB transport of acidic drugs (Kang et al., 1990), the carrier-mediated uptake of $\beta$-lactam antibiotics in the kidneys (Tsuji et al., 1990), and the hepatic uptake of asialoglycoprotein (Partridge et al., 1983).

The concentration range (from 0.057–57 $\mu$g/ml) examined in this study covered plasma unchanged drug concentrations after administration of the clinical dose (pamiteplase, 0.3 mg/kg; rwt-PA, 1 mg/kg) of pamiteplase or rwt-PA to rats. The Ex,drug of $^{125}$I-rwt-PA was comparable to previous reports studying the high-capacity hepatic uptake of t-PA (Bakheit et al., 1987; Einarsson et al., 1988; Tanswell et al., 1990). In contrast, the lower Ex,drug of $^{125}$I-pamiteplase compared with rwt-PA was thought to result in increased bioavailability of pamiteplase. Furthermore, the values of CL hepatic (LUI) of both drugs were comparable to those calculated by the integration plot, indicating this technique accurately represents in vivo events.

The concentration-dependent inhibition effect of rwt-PA on $^{125}$I-pamiteplase hepatic uptake suggested that pamiteplase would be eliminated by the same mechanism as rwt-PA but would have a lower affinity than rwt-PA to the specific receptor comprising the RME mechanism. The Asn$_{117}$ binding mannose-rich carbohydrate chain in the Kringle-1 domain and the Tyr$_{67}$ in the EGF domain are reported to be responsible for the hepatic uptake of rwt-PA (Bassel-Duby et al., 1992). Pamiteplase does not possess the Asn$_{117}$ binding mannose-rich carbohydrate chain, and has both the Asn$_{336}$ binding N-glycoside-type carbohydrate chain and the Thr$_{61}$ binding O-glycoside-type carbohydrate chain (Kawauchi et al., 1991). Consequently, the affinity of pamiteplase for the mannose receptor on endothelial cells may be lower than that of rwt-PA because of these modifications. Furthermore, possible structural changes in the EGF and finger domains neighboring the deleted Kringle-1 domain may reduce the affinity of pamiteplase for the low-density lipoprotein receptor-related protein/$\alpha_2$-macroglobulin receptor on parenchymal cells.

Various second generation t-PAs improving the short plasma half-life of rwt-PA, such as reteplase (Kuiper et al., 1995) and monteplase (Mizuo et al., 1996), have been developed. The decreases in CL total

![Graph](image-url)

**Fig. 10. Concentrations of unchanged drug after incubation of $^{125}$I-pamiteplase or $^{125}$I-rwt-PA with rat plasma.**

Each value represents mean and standard error of three experiments.

Concentrations calculated by radioactivity, ELISA, and bioassay were comparable with each other. In addition, the pharmacokinetic parameters after administration of nonlabeled materials were comparable with those following the administration of $^{125}$I-labeled materials. Therefore, the iodinated materials are thought to represent nonradiolabeled drug behavior.

Recently, bioactive peptides, such as granulocyte colony-stimulating factor (Kuwabara et al., 1994), EGF (Yanai et al., 1991), erythropoietin (Kato et al., 1997), and t-PA, have been mass-produced for use as therapeutic agents. Clearance of these bioactive peptides from systemic circulation is mainly due to receptor-mediated endocytosis (RME), especially in the liver and kidneys. The clearance mechanism of rwt-PA is reported to be the RME in two hepatocytes and the low-density lipoprotein receptor-related protein/mannose receptor on endothelial cells and the low-density lipoprotein receptor-related protein/$\alpha_2$-macroglobulin receptor on parenchymal cells (Camani et al., 1994).

The difference in pharmacokinetics between pamiteplase and rwt-PA under identical conditions (dose, animal, researcher, and laboratory) was examined. The prolongations of $t_{1/2}$, $t_{1/2}$, and MRT, and the marked decrease in CL total of pamiteplase in plasma compared with rwt-PA were also confirmed in this study, showing the reason why a bolus pamiteplase administration at a one-fifth dose of rwt-PA exhibits comparable pharmacologic effects. On the other hand, the

### TABLE 3

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc.</th>
<th>$k_{el}$</th>
<th>Plasma Volume</th>
<th>$\text{CL}_{\text{plasma}}$</th>
<th>$\text{CL}_{\text{total}}$</th>
<th>$\text{CL}<em>{\text{plasma}}/\text{CL}</em>{\text{total}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pamiteplase</td>
<td>10</td>
<td>0.0172</td>
<td>44.06</td>
<td>0.7564</td>
<td>7.44</td>
<td>10.2%</td>
</tr>
<tr>
<td>rwt-PA</td>
<td>10</td>
<td>0.0167</td>
<td>44.06</td>
<td>0.7345</td>
<td>33.54</td>
<td>2.19%</td>
</tr>
</tbody>
</table>

*a $k_{el}$ calculated by one-exponential model.

*b Ref. 14.

c $\text{CL}_{\text{plasma}} = k_{el} \times (\text{plasma volume}).$

d $\text{CL}_{\text{total}}$ quoted from Table 1.
and liver distribution of reteplase and monteplase compared with rwt-PA are similar to those of pamiteplase (Kuiper et al., 1995; Mizuo et al., 1996). The distribution of reteplase to parenchymal cells is three times larger than to nonparenchymal cells (Kuiper et al., 1995). The binding of reteplase is inhibited by rwt-PA, but the affinity of reteplase for parenchymal cells is lower than for rwt-PA, suggesting that reteplase has the same mechanism as rwt-PA but lower affinity than rwt-PA to the specific receptors (Kuiper et al., 1995). These results suggest that pamiteplase may have the variation of main distribution hepatocytes and the decrease in affinity to hepatocytes in the same way as reteplase.

The serine active site of both pamiteplase and rwt-PA is thought to bind α2-macroglobulin and α2-plasmin inhibitor irreversibly, resulting in both protease inhibition and plasma clearance (Iida et al., 1988; Kizen et al., 1988; Komuro et al., 1989; Okumura et al., 1989; Oikawa et al., 1996b). In this study, a kinetic analysis of the binding of both drugs with these glycoproteins was conducted in vitro. The unchanged drug $K_{el}$ was almost the same for both compounds, and the contribution of $CL_{plasma}$ of both drugs to $CL_{total}$ was relatively small.

The previously described observations lead to the following conclusions. By the i.v. administration study and a tissue-sampling single-injection technique, the difference in $CL_{total}$ between pamiteplase and rwt-PA was shown to be caused by the difference in $CL_{tissue}$. The same receptors would be responsible for pamiteplase and rwt-PA uptake in the liver. The affinity of pamiteplase for these receptors, however, appeared to be lower than that of rwt-PA, resulting in the decrease in $CL_{total}$.

References


