Pharmacokinetics, Pharmacodynamics, Allometry, and Dose Selection of rPSGL-Ig for Phase I Trial

SOO PEANG KHOR, KYLE MCCARTHY, MICHELLE DUPONT, KRISTIN MURRAY, and GREGG TIMONY
Pharmacokinetic and Pharmacodynamic Sciences (S.P.K., Ky.M., G.T.), Department of Pharmacology (M.D.), Department of Bioanalytical Sciences (Kr.M.), Preclinical Research Development, Genetics Institute, Andover, Massachusetts
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ABSTRACT
rPSGL-Ig is a recombinant, soluble, and chimeric form of P-selectin glycoprotein ligand-1, which is developed as an antagonist to P-selectin. Allometric and pharmacokinetic/pharmacodynamic modeling was used to select doses for human clinical trials. Pharmacokinetic parameters of rPSGL-Ig such as clearance (CL), volume of distribution (Vd), and t1/2 across animal species are well described by power functions with body weight as an independent variable. The power functions for CL, Vd, and t1/2 were CL = 0.37W0.93 ml/h (r² = 0.94), Vd = 45.0W0.064 ml (r² = 0.988), and t1/2 = 190W2.159 h (r² = 0.75), respectively. These functions provide a means to predict pharmacokinetics of rPSGL-Ig in humans. For a 70-kg human, the values of CL, Vd, and t1/2 are predicted to be 19.9 ml/h, 4138 ml, and 15.5 days, respectively. The predicted pharmacokinetics in humans is used in conjunction with pharmacological data to estimate appropriate doses for clinical trials. The doses that may provide potential effects in humans range from 0.13 to 4.7 mg/kg. The predicted doses produce concentrations above those that are associated with efficacy in animal disease models and, maintain concentrations above the EC50 of in vitro binding between rPSGL-Ig and stimulated human platelets. Hence, rPSGL-Ig in clinical trials may provide therapeutic activities for P-selectin-mediated diseases.

The selectin family of adhesion molecules mediates the initial attachment of leukocytes to endothelial cells (Albelda and Buck, 1990). This initial attachment is followed by firm adhesion and diapedesis at the site of tissue injury and inflammation. P-selectin (platelet and endothelial selectin) is one member of the selectin family that also includes E-selectin (endothelial selectin) and L-selectin (leukocyte selectin). P-selectin is constitutively expressed in α-granules of platelets and Weibel-Palade bodies of endothelial cells (Hsu-Lin et al., 1984; McEver et al., 1989). In the presence of inducing agents such as thrombin and inflammatory cytokines, P-selectin is mobilized to the cell surface. P-selectin binds to a group of sialylated, fucosylated oligosaccharides, e.g., sialyl-LewisX, in vitro. Several glycoproteins also interact with P-selectin in vitro; CD24 (Aigner et al., 1995) and P-selectin glycoprotein ligand-1 (PSGL-1, CD162). PSGL-1 is a mucin-like, homodimeric, disulfide-bonded glycoprotein (Moore, 1998). The interaction of P-selectin on activated platelets and endothelial cells with PSGL-1 on neutrophils promotes the rolling of neutrophils on vessel walls and eventually results in diapedesis at the injured or inflamed vascular wall. Abnormal accumulation of neutrophils at vascular site results in the development of several pathologic inflammatory diseases (Albelda et al., 1994). Blocking of neutrophil accumulation through inhibition of binding of P-selectin to PSGL-1 provides a means to treat diseases such as acute myocardial infarction and deep vein thrombosis (DVT).

rPSGL-Ig is a recombinant, soluble, and chimeric form of PSGL-1 that is developed as an antagonist to P-selectin. The protein was engineered by linking a truncated PSGL-1 to the Fc portion of human Ig. rPSGL-Ig binds to platelets and endothelial cell-associated P-selectin. Pharmacological studies have shown that rPSGL-Ig is able to reduce hepatic ischemia/reperfusion injury in rats (Dulkanachinun et al., 1998), accelerate thrombolysis and prevent reocclusion in a porcine model (Kumar et al., 1999), and ameliorate acute traumatic shock in rats (Scalia et al., 1999).

Herein, we present the pharmacokinetics of rPSGL-Ig in different animal species. Particularly, the interspecies allometric scaling of pharmacokinetic parameters of rPSGL-Ig is presented. Also presented are the predictions of pharmacokinetics and concentration time profile of an i.v. dose of rPSGL-Ig in humans. The pharmacokinetic and pharmacodynamic results in animals were used to estimate an appropriate dose range for the phase I clinical trial. Once the doses were predicted, the concentration-time profile of rPSGL-Ig in humans from the predicted doses was generated. These concentrations were then compared with the EC50 for the binding of rPSGL-Ig to thrombin-activated human platelets in vitro. The comparisons helped to determine the potential

ABBREVIATIONS: PSGL-1, P-selectin glycoprotein ligand-1; DVT, deep vein thrombosis; ELISA, enzyme-linked immunosorbent assay; MOPS, 4-morpholinepropanesulfonic acid; CL, clearance; AUC, area under the serum concentration-time curve.
activity and duration of the activity of rPSGL-Ig in clinical trials with the predicted doses.

**Materials and Methods**

**Pharmacokinetics.** Pharmacokinetics of rPSGL-Ig was obtained from four animal species: mouse, rat, monkey, and pig. The studies were conducted at either the Genetics Institute or a contract institute and were approved by an Institutional Animal Care and Use Committee.

Animals were dosed with a single dose of rPSGL-Ig through the i.v. route. The doses (our unpublished data indicate these doses are within a range of linear pharmacokinetics) used in the mouse, rat, monkey, and pig were 0.1, 1.0, 1.0, and 0.25 mg/kg, respectively. Blood samples were collected for serum at specific time points over 2, 5, 6, and 7 weeks in the mouse, rat, monkey, and pig, respectively. A total of 10 to 12 time points was collected for each pharmacokinetic profile. These blood collection schemes allowed the full characterization of the concentration-time profile. In mice, each time point consisted of three observations from different animals. In other species, at least three animals were sequentially sampled per time point. The concentrations of rPSGL-Ig in serum were measured with enzyme-linked immunosorbent assay (ELISA) methodology.

**rPSGL-Ig Total Protein ELISA.** The rPSGL-Ig preclinical ELISA uses an anti-PSGL-Ig monoclonal antibody specific for the PSGL portion of the molecule to capture and an anti-human IgG monoclonal antibody specific for the Ig tail of the molecule. Use of this antibody pair ensures that only intact rPSGL-Ig is detected in plasma. High binding plates (Costar, Cambridge, MA) were coated with 2 μg/ml with 4H10 (mouse anti-PSGL-Ig monoclonal antibody) overnight at 2–8°C. Plates were then washed with Tris high salt buffer plus 200 μlWell Tris high salt buffer plus 4% milk for 1.5 h at ambient room temperature. After washing, rPSGL-Ig standard was diluted to 40 ng/ml in 4% milk/4-morpholinepropanesulfonic acid (MOPS)/TWEEN diluted through seven dilutions with log₂₅ series dilutions and added to plates. Controls were set at 12, 7.5, 2.5, and 0.5 ng/ml in 4% milk/MOPS/TWEEN buffer and were added to plates. Samples were diluted at least 1:25 in 4% milk/MOPS/TWEEN to overcome matrix effects and targeted to fall within the range of the assay 0.5 to 12 ng/ml. After sample addition, plates were sealed and incubated overnight at room temperature on a plate shaker. Plates were then washed with Tris high salt Tween buffer and a mouse anti-human IgG horseradish peroxidase-conjugated antibody (Southern Biotechnology Associates, Birmingham, AL) was added to the plates at a 1:10,000 dilution in MOPS/TWEEN buffer for a 1.5-h incubation at room temperature on a plate shaker. After washing, plates were developed for 10 min with 3,3'9,5,5'9-cyanine diacetate. As a positive control, an aliquot of platelets was labeled with a commercial polyclonal anti-P-selectin antibody (anti-CD26P) tagged with a fluorescent label. All samples were stained with an anti-human IgG fluorescein isothiocyanate. As a positive control, an aliquot of platelets was labeled with a commercial polyclonal anti-P-selectin antibody (anti-CD26P) tagged with a fluorescent label. All samples were stained with an anti-human IgG fluorescein isothiocyanate. As a positive control, an aliquot of platelets was labeled with a commercial polyclonal anti-P-selectin antibody (anti-CD26P) tagged with a fluorescent label. All samples were stained with a phycoerythrin anti-CD41a (IIb/IIIa) platelet antibody to ensure that cells to be analyzed were platelets. Samples were then fixed in 0.5% paraformaldehyde and analyzed for positively stained platelets by two-color flow cytometric analysis with an FACS sorter (Becton Dickinson Immunocytometry Systems, Mountain View, CA).

The ED₅₀ values for the binding of rPSGL-Ig to thrombin-activated platelets were calculated using an algorithm for nonlinear regression (WinNonlin; Pharsight Corp.). A sigmoidal E₅₀ model was used to fit the binding data associated with blood samples obtained from different donors. The value of EC₅₀ was calculated based on the parameters E₀, E₅₀, and Hill coefficient) of the sigmoidal E₅₀ model.

**Dose Estimation.** The purpose of the dose estimation for rPSGL-Ig is to provide a dose range for the first-in-human clinical trial that is used to evaluate the pharmacokinetics and safety of the protein in humans. The goal of the dose estimation is to provide exposure (area under the serum concentration-time curve (AUC₀₋₅₀) or C₅₀PED in humans equivalent to the exposure seen in pharmacological animal models where activities are observed. The disease models in animals used for the analyses were a thrombolyis model in the pig (Kumar et al., 1999), a DVT model in the baboon (Wakefield et al., 2000), a traumatic shock model in the rat (Scalia et al., 1999), and...
a hepatic ischemia model in the rat (Dulkanchainun et al., 1998). The doses of rPSGL-Ig in these models that were associated with beneficial effects were 250, 4000, 500, and 400 µg/kg, respectively. In the hepatic ischemia model a fixed dose of 100 µg was used. A body weight of 250 g was used to calculate the dose in milligrams per kilogram. The dose estimation was accomplished by using the predicted pharmacokinetic parameters of rPSGL-Ig in humans. For example, when a dose of rPSGL-Ig in animal disease model provided beneficial effect, the AUC_0→3, or C_max associated with this effective dose was used as the target exposure for the dose in human. The dose was then calculated by the product of AUC_0→3, and predicted CL or that of C_max, and predicted V_z of rPSGL-Ig in humans.

To evaluate the potential activity of the estimated doses in humans, concentration-time profiles of rPSGL-Ig from the estimated doses were generated as described above. The predicted concentrations were then compared with the value of EC_{50} for the binding of rPSGL-Ig to thrombin-activated human platelets. Concentrations of rPSGL-Ig above the EC_{50} or EC_{90} were considered to provide potential activity.

## Results

The concentration-time profiles of rPSGL-Ig followed a biexponential decline after a single i.v. dosing in all the animal species tested (Fig. 1). When the dose-normalized concentrations were plotted against time, the profiles showed a rank order with respect to the size of animals; smaller animals had higher dose-normalized concentrations (Fig. 2).

The values of pharmacokinetic parameters of rPSGL-Ig in the mouse, rat, monkey, and pig are shown in Table 1. The values of pharmacokinetic parameters for the two groups of monkeys were similar, indicating linear pharmacokinetics over the evaluated dose range. Therefore, only the values of pharmacokinetic parameters from one group of animals (3.7 kg) were used to compare pharmacokinetics between species. However, the values of pharmacokinetic parameters from these two groups of monkeys were used in the analysis of allometry.

![Fig. 1. Serum concentrations of rPSGL-Ig in mouse, rat, monkey, and pig after a single i.v. dose of rPSGL-Ig in each species. Two separate studies were conducted for monkeys with different dose levels (1 and 13.5 mg/kg). The dose for mouse, rat, and pig was 0.1, 1, and 0.25 mg/kg, respectively.](image)

The values of clearance of rPSGL-Ig increased with body weight of animals. The body weight of the animals in our studies was 0.018, 0.21, 3.7, and 15 kg in the mouse, rat, monkey, and pig, respectively. The body weight varied over three orders of magnitude. The values of CL in these animals were 0.0056, 0.21 ± 0.046, 0.96 ± 0.2, and 6.9 ± 1.7 ml/h, respectively. Similarly, the values of V_z increased with body weight of animals (0.49, 13.3 ± 1.9, 173.9 ± 22.2, and 982.5 ± 193.2 ml in the mouse, rat, monkey, and pig, respectively). A similar trend was observed for the value of V_ss with increasing body weight in animal (1.01, 26.5 ± 1.8, 326.0 ± 44.4, and 2447.5 ± 476 ml in the mouse, rat, monkey, and pig, respectively). The corresponding values for t_{1/2} were 121, 100 ± 19, 264 ± 39, and 255 ± 51 h, respectively.

Allometric analyses were performed on the pharmacokinetic parameter CL, V_z, V_ss, V_c, and t_{1/2}. Each of these parameters across the animal species were well correlated with body weight (Fig. 3). The allometric equation relating clearance to body weight (W) across species was CL = 0.37 W^{0.93} ml/h (r^2 = 0.94). Similar analyses resulted in V_z = 45.0 W^{1.06} ml (r^2 = 0.988) and t_{1/2} = 190 W^{0.16} h (r^2 = 0.75). The values of the exponent and coefficient for the allometric equation of V_z and V_ss are shown in Table 2. The exponents of the power functions for the three volume terms were similar and very close to one.

### Prediction of rPSGL-Ig Pharmacokinetics in Humans.

The allometric functions relating pharmacokinetics with body weight allow the prediction of rPSGL-Ig pharmacokinetics in humans. For a 70-kg human, the values of CL, V_z, and t_{1/2} are predicted to be 19.2 ml/h, 4138 ml, and 373 h (15.5 days), respectively (Table 2).

A complex Dedrick plot is shown in Fig. 4. Superimposability of the data points generated from different species was observed for the initial part of the curve, whereas divergence
of points between species occurred at the terminal phase of the profile. Nonetheless, all points were close to the species-independent line (the solid line in Fig. 4).

Predicted concentration-time profiles of rPSGL-Ig from a 0.5-mg/kg dose in a human weighing 70 kg are shown in Fig. 5. The predicted profiles A and B are associated with the semilogarithmic and linear Y-scales, respectively. The concentrations of rPSGL-Ig after an i.v. dose in humans were predicted to decline in a biexponential manner.

**In Vitro Binding of rPSGL-Ig to Human Platelets.** The binding of rPSGL-Ig (at various concentrations) to platelets that were maximally stimulated with thrombin followed a sigmoidal $E_{\text{max}}$ binding isotherm (Fig. 6). Maximum binding was observed at 3 μg/ml rPSGL-Ig and the value of $EC_{50}$ was $0.725 \pm 0.241 \mu g/ml$. The value of $EC_{90}$ was $1.96 \pm 1.53 \mu g/ml$.

**Dose Estimation.** The estimated values of $AUC_{0-\infty}$ associated with a beneficial effect in the animal models of thrombolysis, DVT, traumatic shock, and ischemia were 523, 12,224, 602, and 482 μg/ml, respectively. These values of $AUC_{0-\infty}$ were associated with the doses of 250, 4000, 500, and 400 μg/kg, respectively. With these values of $AUC_{0-\infty}$ as target exposures, the doses required in humans were 0.145, 3.4, 0.17, and 0.13 mg/kg, respectively (Table 3).

With $C_{\text{max}}$ as the target exposure, the estimated values in the four disease models were 3.7, 80, 9.4, and 7.5 μg/ml. The

<table>
<thead>
<tr>
<th>Weight (kg)</th>
<th>Dose (mg/kg)</th>
<th>CL (ml/h)</th>
<th>$V_c$ (ml)</th>
<th>$V_{ss}$ (ml)</th>
<th>$V_z$ (ml)</th>
<th>$t_{1/2}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse $^a$</td>
<td>0.018</td>
<td>0.1</td>
<td>0.0056</td>
<td>0.49</td>
<td>1.01</td>
<td>1.01</td>
</tr>
<tr>
<td>Rat</td>
<td>0.21</td>
<td>1</td>
<td>0.21 ± 0.046</td>
<td>13.3 ± 1.9</td>
<td>26.5 ± 1.8</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>Monkey</td>
<td>3.7</td>
<td>1</td>
<td>0.96 ± 0.2</td>
<td>173.9 ± 22.2</td>
<td>328.6 ± 44.4</td>
<td>354 ± 63</td>
</tr>
<tr>
<td>Monkey</td>
<td>6.3</td>
<td>13.5</td>
<td>1.0 ± 0.2</td>
<td>194.8 ± 5.5</td>
<td>443.9 ± 99.1</td>
<td>446 ± 131</td>
</tr>
<tr>
<td>Pig</td>
<td>15</td>
<td>0.25</td>
<td>6.9 ± 1.7</td>
<td>982.5 ± 193.2</td>
<td>2,447.5 ± 476.2</td>
<td>2456 ± 466</td>
</tr>
</tbody>
</table>

$^a$ Standard deviation was not calculated because of the sampling scheme used in blood collection.

**Fig. 3.** Allometric plots of pharmacokinetic parameters (CL, $V_c$, $V_{ss}$, $V_z$, and $t_{1/2}$) of rPSGL-Ig. Each data point within a plot represents an averaged value of the pharmacokinetic parameter. The five data points in increasing weight represent data from mouse, rat, monkey (3.7 kg), monkey (6.3 kg), and pig, respectively. The solid line represents the line of best fit with a power function to relate the pharmacokinetic parameters with body weight.
doses in human required to provide equivalent values of C_{max} were 0.22, 4.7, 0.56, and 0.44 mg/kg, respectively. Collectively, the dose range predicted to have potential activity in humans was 0.13 to 4.7 mg/kg.

### Potential Activity of rPSGL-Ig

To gauge the potential activity of rPSGL-Ig in humans, the values of C_{max} obtained from the predicted doses (with either AUC 0–2′ or C_{max} as a measure of exposure) were compared with the EC_{50} of binding between rPSGL-Ig and human platelets. The values of C_{max} based on AUC 0–2′ equivalence ranged from 2.2 to 58 mg/ml, whereas the corresponding values based on C_{max} equivalence were 3.7 to 80 mg/ml. These C_{max} values were higher than the EC_{50} of 0.73 mg/ml. The expected values of C_{max} are also higher than EC_{90} (1.95 mg/ml), albeit the difference was smaller compared with that observed for EC_{50}.

The time duration over which the concentrations of rPSGL-Ig from different doses were predicted to remain above either the EC_{50} or EC_{90} are shown in Fig. 7. Concentrations of rPSGL-Ig from different doses were simulated based on the predicted values of rPSGL-Ig pharmacokinetic parameters in humans. The simulated profiles corresponded to a dose range of 0.04 to 7 mg/kg in 70-kg humans. These doses encompassed the previously predicted therapeutic dose range of 0.13 to 4.7 mg/kg. Concentrations from a dose of 2.5 mg were predicted to fall below the EC_{50} immediately after administration, whereas a 10-mg dose would provide concentrations that remain above the EC_{50} for at least a week. Neither of these two doses

### Table 2: Values of coefficient and exponent for power functions relating pharmacokinetic parameters of rPSGL-Ig with body weight across different animal species

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mouse (0.1 mg/kg)</th>
<th>Rat (1 mg/kg)</th>
<th>Monkey (1 mg/kg)</th>
<th>Pig (0.25 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL (ml/h)</td>
<td>0.930</td>
<td>0.368</td>
<td>0.94</td>
<td>19.2</td>
</tr>
<tr>
<td>V_{ss} (ml)</td>
<td>1.064</td>
<td>45.0</td>
<td>0.988</td>
<td>4138</td>
</tr>
<tr>
<td>V_{e} (ml)</td>
<td>1.084</td>
<td>95.7</td>
<td>0.988</td>
<td>9567</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>1.087</td>
<td>100.2</td>
<td>0.985</td>
<td>10143</td>
</tr>
</tbody>
</table>

*\( \phi \) and \( a \) are the parameters for the power function \( Y = aW^\phi \).

*\( r^2 \) value for the regression analysis of pharmacokinetic parameters versus body weight.

* Predicted pharmacokinetic parameters of rPSGL-Ig in humans (70 kg).

#### Fig. 4. A complex Dedrick plot of rPSGL-Ig in different animal species.

Symbols represent observed data from different species, whereas the solid line is the species-independent line generated with the values of the coefficient and the exponent of allometric equations.

#### Fig. 5. Simulations of serum concentrations of rPSGL-Ig in humans assuming 70-kg body weight and a single i.v. dose (0.5 mg/kg) of rPSGL-Ig. A denotes concentrations plotted on semilogarithmic scale. B denotes concentrations plotted on linear scale (right y-axis).

#### Fig. 6. Binding of rPSGL-Ig to maximally thrombin-stimulated human platelets from different donors. Symbols represent averaged value (\( n = 3 \)). The vertical bars above and below the symbols represent 1 S.D. away from the averaged values. Solid line is the line of best fit with a sigmoidal \( E_{max} \) model.

**Potential Activity of rPSGL-Ig.** To gauge the potential activity of rPSGL-Ig in humans, the values of \( C_{max} \) obtained from the predicted doses (with either AUC_{0–\infty} or \( C_{max} \) as a measure of exposure) were compared with the EC_{50} of binding between rPSGL-Ig and human platelets. The values of \( C_{max} \) based on AUC_{0–\infty} equivalence ranged from 2.2 to 58 \( \mu \)g/ml, whereas the corresponding values based on \( C_{max} \) equivalence were 3.7 to 80 \( \mu \)g/ml. These \( C_{max} \) values were higher than the EC_{50} of 0.73 \( \mu \)g/ml. The expected values of \( C_{max} \) are also higher than EC_{90} (1.95 \( \mu \)g/ml), albeit the difference was smaller compared with that observed for EC_{50}.

The time duration over which the concentrations of rPSGL-Ig from different doses were predicted to remain above either the EC_{50} or EC_{90} are shown in Fig. 7. Concentrations of rPSGL-Ig associated with i.v. doses of 2.5 to 500 mg were simulated based on the predicted values of rPSGL-Ig pharmacokinetic parameters in humans. The simulated profiles corresponded to a dose range of 0.04 to 7 mg/kg in 70-kg humans. These doses encompassed the previously predicted therapeutic dose range of 0.13 to 4.7 mg/kg. Concentrations from a dose of 2.5 mg were predicted to fall below the EC_{50} immediately after administration, whereas a 10-mg dose would provide concentrations that remain above the EC_{50} for at least a week. Neither of these two doses...
respectively. A concentration of 2,500 mg would remain above the EC 90 for 2, 4, and 6 weeks, respectively.

Concentrations from doses of 125, 250, and 500 mg would yield concentrations above the EC 50 for as long as 7 weeks. Concentrations from doses of 125, 250, and 500 mg would remain above the EC 90 for 2, 4, and 6 weeks, respectively.

**Discussion**

rPSGL-Ig is a genetically engineered protein that binds P-selectin. The abnormal recruitment of platelets and/or neutrophils to the vascular endothelium is a primary event that initiates a variety of disease states that include inflammation, thrombosis, atherosclerosis, and reperfusion injury. The binding of rPSGL-Ig to P-selectin inhibits the cell-cell adhesions between platelets/neutrophils and neutrophils/endothelial cells. Therefore, rPSGL-Ig is a potential therapeutic entity to prevent P-selectin-mediated diseases. The protein was tested in animal models of thrombosis, reperfusion injury, and DVT. Beneficial activities were seen in these models. The doses in these animals were used as the basis to estimate an appropriate dose range for the phase I clinical trial to evaluate the pharmacokinetics and safety of rPSGL-Ig. The doses used in the phase I trial were chosen such that the exposure in humans based on AUC or $C_{\text{max}}$ were similar to the exposure in animals that produced beneficial effects when treated with rPSGL-Ig. The exposures in humans were estimated based on the predicted pharmacokinetics of rPSGL-Ig in humans, which was derived by allometric scaling of pharmacokinetic parameters across the mouse, rat, monkey, and pig.

Pharmacokinetic parameters of rPSGL-Ig from different animal species were well described by allometric equations. The exponents of the power function for clearance and $V_c$ were 0.93 and 1.1, respectively. These values indicated the increase in both of these parameters with body weight approached a linear relationship. The values of $t_{1/2}$ in the animals we studied (4–11 days) were comparable to the values of other recombinant antibodies of 5.4 to 13.8 days (Lin et al., 1999). We evaluated pharmacokinetics of rPSGL-Ig in the animal species where pharmacological studies were conducted. This pharmacokinetic information in relevant pharmacological animal models provides a better understanding of exposure versus activity in various disease models.

The predicted values of CL, $V_c$, and $t_{1/2}$ of rPSGL-Ig in humans were comparable to corresponding values of other reported antibodies. The predicted pharmacokinetic parameters of a new therapeutic entity are useful for designing clinical trials in several aspects. Knowing the likely pharmacokinetic profile before the start of a first-in-human study allows for the optimal designing of the protocol with respect to dose selection, drug product planning, and duration of study. We used the predicted values of CL and $V_c$ to estimate the exposure parameters of AUC 0–$\infty$ and $C_{\text{max}}$, respectively. These exposure parameters provide an alternative to dosing on the basis of amount of drug per body weight. Because P-selectin is expressed on vascular endothelia and platelets, concentration-related parameters such as $C_{\text{max}}$ or AUC are pertinent exposure indicators for rPSGL-Ig.

A complex Dedrick plot allows the prediction of concentration-time profiles of rPSGL-Ig in humans. The near superimposability of data from different species indicates the predictability of results for human. The predicted concentration profile of rPSGL-Ig on a chronological time scale after an i.v. dose shows a distinct biphasic decline. The profile is similar to that observed for i.v. dosing of purified endogenous IgG (Waldmann and Strober, 1969).

The predicted doses that would have potential activity in humans based on AUC 0–$\infty$ and $C_{\text{max}}$ ranged from 0.13 to 4.7 mg/kg. The wide dose range was due to two major factors. The target exposure levels were obtained from different disease models and each model may require different exposure for activity. Also, in most cases only a single dose level was used in the disease models without a dose-ranging component in the studies. Therefore, the dose used in each model could be much greater than the minimum or optimal effective dose. However, since the completion of the analysis presented herein, additional dose levels were tested in baboon.

### TABLE 3

**Estimation of rPSGL-Ig doses in humans that provide exposure equal to those observed in different animal disease models**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Disease Model</th>
<th>Dose in Animal</th>
<th>Predicted Dose in Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AUC Equivalence</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mg/kg</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Pig</td>
<td>Thrombolysis</td>
<td>0.25</td>
<td>0.145</td>
</tr>
<tr>
<td>Baboon</td>
<td>DVT</td>
<td>4</td>
<td>3.4</td>
</tr>
<tr>
<td>Rat</td>
<td>Traumatic shock</td>
<td>0.5</td>
<td>0.17</td>
</tr>
<tr>
<td>Rat</td>
<td>Hepatic Ischemia</td>
<td>0.4</td>
<td>0.13</td>
</tr>
</tbody>
</table>

* Predicted doses that will provide equal AUC of rPSGL-Ig in human as in animals treated with doses shown in column 3.

* Predicted doses that will provide equal $C_{\text{max}}$ of rPSGL-Ig in human as in animals treated with doses shown in column 3.
DVT and pig thrombolysis models. Preliminary findings indicated that the minimum effective doses were 0.1 and 1 mg/kg in the thrombolysis and baboon models, respectively (R. Schaub, personal communication). These doses were translated to human equivalent doses of 0.06 and 0.85 mg/kg, respectively. The selected dose range (0.13–4.7 mg/kg) encompassed the 0.85-mg/kg dose, whereas the 0.06-mg/kg dose was 2-fold lower than the lowest selected dose (0.13 mg/kg). However, the actual starting dose used in a phase I trial was 0.035 mg/kg. For safety consideration, the chosen dose was a half-log lower than the lowest predicted 0.13-mg/kg dose. The start dose is ~280-fold lower than the no-toxic-effect level in the monkey (P. Bouchard, unpublished data). Therefore, the selected starting dose was within a range considered to be safe for a phase I trial.

The predicted human doses provide $C_{\text{max}}$ values of rPSGL-Ig higher than the values of $EC_{50}$ or $EC_{90}$ for the binding of rPSGL-Ig to stimulated human platelets in vitro. The lowest predicted dose of rPSGL-Ig (0.13 mg/kg) provided a $C_{\text{max}}$ (2.2 μg/ml) 3-fold higher than the $EC_{50}$ (0.73 μg/ml). The predicted doses provide exposure for a long time; concentrations of rPSGL-Ig were predicted to remain above $EC_{50}$ for at least a week for doses $\geq$0.14 mg/kg. In monkeys, rPSGL-Ig remains active in the circulation 4 weeks after dosing. The in vitro extent of bindings of rPSGL-Ig to P-selectin before dosing and after circulating for 4 weeks after dosing in monkeys was equal (data not shown). Hence, the long duration at which concentrations remain above $EC_{50}$ may provide a prolonged activity after dosing.

In conclusion, the pharmacokinetic parameters of rPSGL-Ig from different animal species were well described by allometric relationships. The allometric equations provided a means to predict the pharmacokinetics of rPSGL-Ig in humans. The predicted pharmacokinetic parameters along with pharmacological information from animal disease models enable the estimation of appropriate doses for phase I trials. In addition, the allometric relationships allow the prediction of a concentration-time profile after i.v. dosing.

The potential duration of activity in humans can then be evaluated with the predicted profile and the binding of rPSGL-Ig to stimulated human platelets.

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References


Send reprint requests to: Soo Peang Khor, One Burtt Rd., Andover, MA 01810. E-mail: skhor@ genetics.com