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

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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 (Vc), and t1/2 across animal species are well described by power functions with body weight as an independent variable. The power functions for CL, Vc, and t1/2 were CL = 0.37W0.93 ml/h (r² = 0.94), Vc = 45.0W1.064 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, Vc, 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 therapeutic 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 PSGL-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 (Hu-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 (Dulkanchainun 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 three 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 the ELISA. High binding plates (Costar, Cambridge, MA) were coated at 2 μg/ml with 4H10 (mouse anti-PSGL-Ig monoclonal antibody) overnight at 2–8°C. Plates were then washed with Tris high salt Tween buffer and blocked with 200 μl/well 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 log1.5 series dilutions and added to plates. Controls were 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 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.

In vitro Binding of rPSGL-Ig to Human Platelets. Heparinized human blood samples (0.1 ml) were diluted in a Vacutainer tube containing 0.48 ml of 7.5% potassium ethelenediamine tetraacetic acid with Gly-Pro-Arg-Pro acetate salt in a 1:1 ratio. A 10-μl aliquot of this blood mixture was mixed with thrombin (6.0 I.U./ml) to activate the platelets. Tyrode’s buffer alone was used to prepare the unactivated control. Samples were then incubated in a 37°C water bath for 10 min. Various amounts of rPSGL-Ig were added to samples and tagged with goat (Fab’2)-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) fluorescein isothiocyanate. Negative controls, samples were stained with an inactive form of rPSGL-Ig (UMB4) or with rabbit IgG and tagged with a fluorescent label. All samples were stained with a phycoerytherin 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 FACSSort (Becton Dickinson Immunocytometry Systems, Mountain View, CA).

Dose Selection of rPSGL-Ig

The allometric equation was used to relate pharmacokinetic parameters (Yi) of rPSGL-Ig with animal body weight (W). The subscripts c, v, and h refer to CL, apparent Vc, and t1/2, respectively. The values of α and ϕ were estimated by transforming the allometric expression into

\[ \log Y_i = \log \alpha_i + \phi_i \log W. \]  

The slope and intercept of a linear least-squares analysis of log Y, versus log W gave the values of αi and log αi, respectively.

A complex Dedrick plot for rPSGL-Ig in animals was generated using the estimated parameters of the power functions related to CL, Vc, Va, and Va. The parameters were used in the following equation to yield the desired plot (Boxenbaum and Ronfeld, 1983):

\[ C \cdot D/We = \frac{1}{\alpha \cdot \chi \cdot (\gamma - \phi)} \left[ \left( \chi \cdot \phi \cdot \gamma \right)^2 - \left( \chi \cdot \phi \cdot \gamma - a \cdot \gamma \right) e^{-(\alpha/w)} \right] \]

where \[ \phi = \left( a \cdot \delta - \chi \right)/\left( \delta - \epsilon \right) \] and \[ \gamma = \frac{a}{\delta}. \]

The letters C, D, W, v, and δ denote concentration, dose, weight, exponent of the power function for volume of distribution, and clearance, respectively. The symbols α, χ, δ, and ε denote the coefficients of the power function for CL, Vc, Va, and Va, respectively.

Prediction of rPSGL-Ig Pharmacokinetics in Humans.

Pharmacokinetic parameters (CL, Vc, Va, Va), and t1/2) for humans were estimated with eq. 2. The parameter estimates were calculated with 70 kg as the body weight. Similarly, 70 kg was used in eq. 3 to generate predicted concentration-time profile of an i.v. dose of rPSGL-Ig in humans (Boxenbaum and Ronfeld, 1983).
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 AUC₀⁻→∞ or Cₚₚₙₐₓ 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 AUC₀⁻→∞ and that of Cₚₚₙₐₓ and predicted Vₚ 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 EC₅₀ for the binding of rPSGL-Ig to thrombin-activated human platelets. Concentrations of rPSGL-Ig above the EC₅₀ or EC₉₀ were considered to provide potential activity.

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.

**Results**

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 Vₚ, 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 Vₚ, 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 Vₚ, were 1.01, 29 ± 2, 354 ± 63, 446 ± 131, and 2456 ± 466 ml, respectively. The values of t½ were 121, 100 ± 19, 264 ± 39, and 255 ± 51 h, respectively.

Allometric analyses were performed on the pharmacokinetic parameter CL, Vₚ, Vₚ, Vₚ, and t½. 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 = 0.37W⁰.⁹³ ml/h (r² = 0.94). Similar analyses resulted in Vₚ = 45.0W⁰.⁶₆ ml (r² = 0.988) and t½ = 190-W⁰.¹₆ h (r² = 0.75). The values of the exponent and coefficient for the allometric equation of Vₚ and Vₚ, 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, Vₚ, and t½ 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 ± 0.241 μg/ml. The value of EC$_{90}$ was 1.96 ± 1.53 μg/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·h/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.
doses in human required to provide equivalent values of $C_{\text{max}}$ 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 $C_{\text{max}}$ obtained from the predicted doses (with either AUC 0–2' or $C_{\text{max}}$ as a measure of exposure) were compared with the EC 50 of binding between rPSGL-Ig and human platelets. The values of $C_{\text{max}}$ based on AUC 0–2' equivalence ranged from 2.2 to 58 mg/ml, whereas the corresponding values based on $C_{\text{max}}$ equivalence were 3.7 to 80 mg/ml. These $C_{\text{max}}$ values were higher than the EC 50 of 0.73 mg/ml. The expected values of $C_{\text{max}}$ 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 EC50 or EC90 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 EC50 immediately after administration, whereas a 10-mg dose would provide concentrations that remain above the EC50 for at least a week. Neither of these two doses
respectively. Doses of 125, 250, and 500 mg would remain above the EC 90 for 2, 4, and 6 weeks, 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 \( C_{\text{max}} \) was 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 \( V_e \) were 0.93 and 1.1, respectively. These values indicated the increase in both of these parameters with body weight approximate 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, \( V_e \), 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 \( V_e \) to estimate the exposure parameters of AUC 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 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 models.
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 EC50 or EC90 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 mg/ml) 3-fold higher than the EC50 (0.73 mg/ml). The predicted doses provide exposure for a long time; concentrations of rPSGL-Ig were predicted to remain above EC50 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 EC50 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**


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