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Pharmacokinetics of LJP 993, a tetrameric conjugate of domain 1 of β 2-glycoprotein I for antiphospholipid syndrome

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Abstract

β 2-glycoprotein I is the best-characterized antigenic target for antiphospholipid autoantibodies. We synthesized a tetrameric conjugate of the domain 1 of β 2-glycoprotein I (LJP 993) aimed at developing the conjugate as a Toleragen to suppress antiphospholipid syndrome. The present studies focused on determining the stability, tissue distribution, plasma concentration-time profile and excretion of the LJP 993 in mice. The stability of LJP 993 in mouse plasma was quantitatively evaluated using strong cation-exchange high performance liquid chromatography. ^{125}I -labeled LJP 993 was intravenously injected to mice, and levels of ^{125}I -labeled LJP 993 in plasma, tissues, urine and feces were determined at known intervals. Incubation of LJP 993 with mouse serum at 37°C for 8 h resulted in a decrease by 34% of LJP 993 concentration. No degradation fragment was observed during the incubation. After a single intravenous administration of ^{125}I -LJP 993 (0.5 and 5 mg/kg) to mice, both $C_{\max}$ and area-under-curve values increased in a dose-proportional manner, and blood radioactivity disappeared in a bi-exponential manner with the distribution half-lives equal to 1.7 min, and the elimination half-lives 188 and 281 min, respectively. The ^{125}I -LJP 993 was moderately distributed into organs and tissues with the exception that brain level of ^{125}I -LJP 993 was negligible. The major sites of ^{125}I -LJP 993 uptake were the kidney (at 30 min post dosing), and kidney, lung, liver, heart, spleen, skin, muscle and fat tissues (at 4 h post dosing). Cumulative urinary and fecal radioactivity for 0–48 h post dosing accounted for 44.7% and 4.2% of the administered dose, respectively, with the fast rate of urinal excretion occurring within the first 8 h. In summary, LJP 993 was fairly stable in mouse plasma. After administration to mice, ^{125}I -LJP 993 was taken up mainly by kidney and then distributed extensively to tissues except brain. Both $C_{\max}$ and area-under-curve values increased in a dose-proportional manner. It was predominantly excreted in the urine with an elimination half-life longer than 3 h. Kidney is a major route to excrete the tetrameric conjugate.

Keywords

antiphospholipid syndrome; domain 1 of β 2-glycoprotein I; excretion; pharmaco-kinetics; tissue distribution

Introduction

β 2-glycoprotein I (β 2-GPI), also known as apolipoprotein H, is a 50 kDa phospholipid-binding protein presented in normal human plasma at approximately 200 $\mu\text{g/mL}$,¹ with *in vitro* anticoagulant activity.² It is the most common and best-characterized antigenic target for antiphospholipid autoantibodies. β 2-GPI is composed of five homologous sushi domains

designated by their disk-like shape.³ Using recombinant domain-deleted mutants of human β 2-GPI and wild-type human β 2-GPI in competition assays to inhibit the autoantibodies from binding to immobilized wild-type β 2-GPI, Iverson *et al.* demonstrated that only those domain-deleted mutants containing the first domain inhibited the binding to immobilized wild-type β 2-GPI from all of the patients with antiphospholipid syndrome.^{4,5}

The realization that the epitope(s) was located in domain 1, and that the latter plays an important role in suppressing anticardiolipin antibodies, suggested a therapeutic approach for treatment of antiphospholipid syndrome, and has allowed us to begin building Toleragens, which specifically tolerize, or shut down, the **B** lymphocytes producing the pathogenic antibody.⁵ The synthesized Toleragen was composed of linker-attached domain 1 coupled to a non-immunogenic platform at a valency of four domain 1 molecules per platform to produce a tetrameric Toleragen, LJP 993 (Figure 1). The proposal for building such tetravalent conjugate of domain 1 was based on the following hypotheses: (1) from a chemical perspective, attachment of a glyoxyl group of domain 1 to aminooxy groups on the multivalent platform may facilitate an effective and selective chemical reaction between domain 1 and the multivalent platform and provide a well-defined multivalent domain 1 conjugate; (2) from a bioactivity perspective, a single tetravalent conjugate of domain 1 may have fourfold greater activity than a single domain 1 if the conjugate retains both affinity and specificity of antibody binding of a single domain 1. The hypotheses were then tested in our previous studies,^{6,7} resulting in the following conclusions: (1) the reaction was very selective and predictable, and produced a good yield; and (2) the affinity of LJP 993 bound to purified antibody from patients with elevated anticardiolipin antibodies was roughly fourfold higher in valence than that of a single domain 1. The conjugate seems to be biologically stable, non-toxic, and non-immunogenic.

To achieve these therapeutic objectives, a comprehensive evaluation of the pharmacokinetic profile of LJP 993 is important for further development of the Toleragen. Thus, the present study was designed to use a radiolabeling method to fully evaluate the pharmacokinetic parameters, tissue distribution and excretion route of LJP 993. We also developed a high-performance liquid chromatography (HPLC) method to evaluate the integrity of LJP 993 in mouse plasma.

Materials and methods

Synthesis and formulation of [¹²⁵I] LJP 993

The parent LJP 993 was synthesized according to the methods described previously.^{6,7} The ¹²⁵I-labeled LJP 993 was prepared biosynthetically by incubating LJP 993 with ¹²⁵I sodium salt; iodine was attached to the tyrosine residue. One mole [¹²⁵I] LJP 993 contains one mole [¹²⁵I] tyrosine. Unincorporated ¹²⁵I sodium was separated in a disposable chromatography column. The iodination method resulted in a product with a specific activity of ~307 μ Ci/mg. Protein precipitation by using trichloroacetic acid (10%) showed the radiochemical purity to be greater than 98%. [¹²⁵I] LJP 993 was diluted in sterilized phosphate-buffered saline to 1 mg/mL, which resulted in approximately 6.23×10^8 decays per minute (dpm) per milliliter. The concentration of the [¹²⁵I] LJP 993 solution was further adjusted so that 100 μ L administered to a 20 g mouse was equal to a dose of 0.5 and 5 mg/kg of body weight, in order to evaluate the mass balance and tissue distribution of the radiolabeled molecule at pharmacologically relevant dose.⁸

Dosing and sample collection

Balb/c mice of both sexes with body weight ranging 20–30 g ($n = 32$) were purchased from Jackson Laboratory (Bar Harbor, ME). All animals used in these investigations were handled

in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and the Guidelines of the US Department of Agriculture (Animal Welfare Act; Public Law 99-198). The experimental protocol (No. PK-001) was approved by the Institutional Animal Care and Use Committee of the Company. [¹²⁵I] LJP 993 was administered intravenously (i.v.) via the tail vein. Blood samples were collected through the retroorbital sinus of each mouse at 0.5, 5, 15, 30 min and 1, 2, 4, 8, 24 h after dosing. Each mouse was bled at not more than three time points during the experiment (~60 µL per time point) to minimize the total amounts of mice used ($n = 16$) without compromising the scientific validity of the data. The mice were then euthanized immediately. Blood samples were transferred from heparinized blood collecting tubes (Fisher, Pittsburgh, PA) into 0.5 mL polypropylene Eppendorf microfuge tubes. Blood samples were immediately spun at 5000 g for 10 min. An aliquot of plasma (30 µL) was pipetted into tubes for measuring radioactivity.

Four mice receiving 5 mg/kg of [¹²⁵I] LJP 993 were housed individually in metabolism cages (Nalge, Rochester, NY) for the cumulative collection of urine and feces at 4, 8, 24 and 48 h after dosing. Four mice were sacrificed at each time point of 30 min, 4 h and 24 h following administration of [¹²⁵I] LJP 993 (5 mg/kg, i.v.). Tissues were immediately removed for determination of total radioactivity per gram of tissue.

The radioactivity of plasma, tissues, urine and feces was trapped and counted in a Packard Cobra II γ -counting system, model 5010 (Packard Inc., Meriden, CT). Counting efficiency was determined by using the normalization procedure provided by the manufacturer. The observed radioactivity values were converted to compound radioequivalent concentrations. Radioequivalents were defined as the amount of parent compound, at the specific activity as administered, which would result in the observed dpm. Compound equivalents in biological samples were determined by dividing the dpm in the sample by the specific activity of the compound in dpm per microgram as described previously.⁹ Compound equivalents were expressed in nanogram per gram of tissue, when possible, as a percentage of the administered dose per organ or tissue. The radioactivity in the urine and feces was expressed as a percentage of the administered dose for each time interval and as a cumulative percentage. Samples in which the radioactivity was less than the reading of blank samples were considered to have a value of zero.

Analytical procedure for stability of LJP 993 in plasma

The analytical procedures and data processing are similar to that previously described.¹⁰ Briefly, fresh mouse plasma was pre-incubated at 37°C before adding non-radiolabeled LJP 993 to a final concentration of 50 µg/mL. A volume of 100 µL of LJP 993-spiked plasma was removed at known time intervals and added to 100 µL of 3% trichloroacetic acid for separation of LJP 993 from the interfering plasma components. The cloudy reaction solution was vortexed and then spun at 14,000 g for 10 min to pellet the precipitated plasma proteins. The supernatant (100 µL) was analyzed by a strong cation-exchange HPLC to determine the residual concentrations of intact LJP 993 at various incubation times. The HPLC stability-specific verification included separate analysis of peaks, peak shape and peak absorbance. Blanks with and without plasma were carried out to determine the recovery of LJP 993 in the presence of trichloroacetic acid. Addition of trichloroacetic acid to the plasma spiked with LJP 993 resulted in an overall chromatographic recovery about 80%. As extraction yields were known, the results were calculated assuming 100% recovery at the beginning of the experiment.

Samples were analyzed on a strong cation-exchange HPLC system (Model HP 1100, Hewlett Packard, Palo Alto, CA) consisted of a HP 1100 autosampler. The system was controlled and data were processed using ChemStation software. The HPLC analytical column (150×4.6 mm² I.D.) was packed with polysulfoethyl A (PolyLC, Inc., Columbia, MD) and maintained at 25°C in a column block heater. A guard column was employed to protect the analytical

column. A mobile phase was composed of buffer A (5 mM KH₂PO₄, pH 3) and buffer B (5 mM KH₂PO₄ plus 2 M KCl, pH 3). The gradient solution was 0 to 55% buffer B over 10 min, and then 55% buffer B isocratic for 8 min. Flow rate was adjusted from 1 to 0.4 mL/min at 7.5 min for 12.5 min in order to increase the corresponding peak area. The degradation was measured by quantifying the peak area of intact LJP 993 at a wavelength of 210 nm.

Pharmacokinetic analysis

Pharmacokinetic parameters for the [¹²⁵I] LJP 993 equivalents in plasma were calculated with the nonlinear least square regression by using the WinNonlin program (Professional version 2.1; Pharsight Co., Mountain View, CA) as described previously.¹¹ The plasma concentration-time data of [¹²⁵I] LJP 993 after i.v. administration were fitted to a bi-exponential model based on the goodness-of-fit criteria set by lower condition number, higher precision and the smallest value of Akaike Information Criterion¹² when comparing several models for a given set of data. The WinNonlin uses the trapezoidal rule to calculate the area under the serum concentration-time curves (AUC) up to the last measured serum concentration. All other important parameters were analyzed by the WinNonlin.

Statistical analysis

Unless otherwise noted, all results are presented as group means and standard deviations (mean ± S.D). Only one standard deviation is shown in the figures either above or below the mean for figure clarity. Group means and standard deviations were calculated and subjected to *t*-test analysis to assess whether the means of two dose groups of small samples are statistically different from each other.¹³ A significance level of *p* < 0.05 denoted significance in all cases.

Results

Stability analysis of LJP 993 in plasma

The chromatographic peaks of authentic LJP 993 in plasma were identified by comparing the retention time of LJP 993 in the presence and absence of the plasma. Incubation of LJP 993 with mouse plasma at 37°C for 8 h resulted in a decrease by 32% of LJP 993 concentration (Figure 2). However, there was no appreciable degradation fragment of LJP 993 produced over the incubation period, indicating that LJP 993 is stable in mouse plasma to a certain extent.

Pharmacokinetic analysis

The plasma concentration versus time profiles of [¹²⁵I] LJP 993 are illustrated in Figure 3. The profiles indicated that plasma levels of two doses of [¹²⁵I] LJP 993 declined in a parallel fashion up to 24 h. The rapid decline of plasma concentration of [¹²⁵I] LJP 993 in the early phase after dosing can be attributed to the distribution of [¹²⁵I] LJP 993 from the central compartment to the peripheral compartment (tissues and organs), and renal excretion. Indeed, a concomitant accumulation of [¹²⁵I] LJP 993 in the tissues and urine was found within 4 h after dosing (Table 2 and Figure 4). The pharmacokinetic parameters are provided in Table 1. The C_{max} and AUC values of [¹²⁵I] LJP 993 demonstrated dose proportionality in mice over the i.v. dose range of 0.5 and 5 mg/kg. The differences between 0.5 and 5 mg/kg groups in dose-related pharmacokinetic parameters such as C_{max}, and AUC were very significant (*p* < 0.001).

Tissue distribution

Table 2 shows the levels of [¹²⁵I] LJP 993 found in various tissues at 0.5, 4 and 24 h after a single i.v. injection (5 mg/kg). Kidney levels of [¹²⁵I] LJP 993 at 30 min post dosing were 24.8 ± 8.8 µg per gram of tissue, which were about fivefold higher than the all other tissues including the plasma (7.3 µg/mL at 30 min). The concentrations of [¹²⁵I] LJP 993 in tissues then rapidly reached an equilibration level 4 h post dosing. The highest concentrations were found in the

thyroid, probably because the thyroid preferentially took up the radioactive iodine. The order of tissue levels of [125 I] LJP 993 at 4 h was as follows: kidney, lung, fat, skin, spleen, liver, heart and muscle. The ratios of tissue to plasma level were generally greater than 1 at this time point, except for brain which, due to a constituted blood–brain barrier, usually uptakes a small amount of drugs. Twenty-four hours after dosing, the levels of [125 I] LJP 993 in tissues fell to a level slightly higher than that in brain. An approximate total of 55.6% and 42.0% of a single i.v. dose was present in the tested tissues at 0.5 and 4 h, respectively. The percentage decreased to about 5.6% at 24 h (Table 2).

To determine the distribution ratio of [125 I] LJP 993 between plasma and erythrocytes, whole blood was taken from each mouse 0.5, 5 and 30 min post dosing and the radioactivity of plasma and erythrocytes was counted separately. The results showed that the plasma to erythrocyte concentration ratio of [125 I] LJP 993 was about 6, suggesting that [125 I] LJP 993 preferentially associates with the plasma.

LJP 993 Excretion in urine and feces

Total excretion of [125 I] LJP 993-derived radioactivity in mouse urine and feces was 44.7% and 4.3%, respectively, within 48 h post dosing (Figure 4). Hence, a major portion of the radioactivity dose was eliminated in the urine. The losses of radioactivity in the metabolism cage were estimated at up to 2.5% of the total excretion. Figure 4 shows the cumulative excretion of [125 I] LJP 993-derived radioactivity in urine and feces in the various time periods. From 24–48 h after dose, less than 3.8% of the radioactivity was excreted in urine and feces. The excretion rate of [125 I] LJP 993-derived radioactivity in urine of mice ($n = 4$) at intervals was 570 ± 280 (0–4 h), 919 ± 383 (4–8 h), 69 ± 19 (8–24 h) and 3 ± 2 (24–48 h) $\mu\text{g/mL/hr}$. By comparison, the overall fecal excretion rate was quite slow. For instance, the fecal excretion rate after a single dosing was 17 ± 13 (0–4 h), 19 ± 7 (4–8 h), 2.5 ± 2 (8–24 h) and 0.4 ± 0.3 (24–48 h) $\mu\text{g/g/hr}$.

Discussion

The tetrameric conjugate of human $\beta 2$ -GPI domain 1 (Figure 1) was stable in mouse plasma as it was readily detected after 8 h of incubation (Figure 2). The stability of the conjugate was similar to monomeric domain 1 under the same conditions (unpublished observation), indicating that the platform and the linkers used to conjugate the four monomers together are not vulnerable to enzymatic degradation in vitro. Control studies carried out under the same conditions but without plasma showed a residual LJP 993 concentration of $94 \pm 4\%$ after 8 h incubation (only a negligible loss of LJP 993). The major losses (32%) of LJP 993, when it was incubated in mouse plasma, may be due to proteolysis. Most, if not all, proteins are catabolized by proteolysis.¹⁴ Proteolytic enzymes are not only widespread throughout the body, but they are also ubiquitous in nature, and therefore the potential number of catabolism sites on any protein is very large.¹⁵ For most proteins and peptides, the degradation products are not always observed, presumably due to the multiple reaction pathways available to peptides (resulting in different degradation products at undetectable amounts),¹⁶ or to chromatographic mobility of the degradation fragments that is similar to that of parent peaks, and/or to their rapid proteolysis.¹⁷ Hence, we cannot ensure that no potential losses of a particular degradation product occurred. However, the present HPLC stability studies do provide information on the in vitro stability of the tetrameric domain 1.

Protein biodistribution studies are usually performed with radiolabeled compounds to evaluate blood levels of the protein drugs and assess drug targeting to specific tissues. If the protein contains a suitable amino acid such as tyrosine or lysine, an external label such as ^{125}I can be chemically coupled to the protein. This iodinated protein has the advantage over the internally ^3H - or ^{14}C -labeled protein because of the potential for re-utilization of the

radiolabeled amino acid fragments in the synthesis of endogenous proteins.¹⁸ Two-compartment kinetics was observed when sampling was taken at very early times (i.e. 30 sec). This pattern of kinetics distinguished certain tissue reservoirs from the central compartment. Programming the plasma concentration-time data in WinNonlin further confirmed the two-compartment model, and revealed that the tetrameric domain 1 appears to decay in a bi-exponential manner. The initial rapid decline of blood radioactivity concentrations after dosing of [¹²⁵I] LJP 993 at 0.5 and 5 mg/kg yielded a distribution half-life of about 1.7 min. The elimination half-life was slow, which is a derived parameter that changes as a function of both clearance and volume of distribution at steady-state. Because of the large size of the tetrameric domain 1, its apparent volume of distribution was moderately higher than the total plasma volume (Table 1).

The present study shows that [¹²⁵I] LJP 993 administered intravenously to mice is, to a great extent, initially concentrated in kidney during the first 0.5 h, at a level approximately fivefold higher than in the other organs (Figure 5). The short time interval has the advantage that metabolism and the tissue efflux clearance can presumably be ignored. [¹²⁵I] LJP 993 was then distributed evenly in most of the tissues within 4 h (Figure 5). For these tissues, comparisons were made per gram of tissue and not adjusted for the total organ weights.

Of these tissues, kidney was the most important excretory organ. Physiologically, excretion of [¹²⁵I] LJP 993-derived radioequivalent in the urine putatively involves glomerular filtration, catabolism at the luminal membrane and active re-absorption by the proximal tubulus by endocytosis and hydrolysis within the cell to peptide fragments and amino acids.^{19,20} The filtration of macromolecules across the glomerulus decreases with increasing effective molecular radius, approaching zero at a radius of approximately 3.5 nm.²¹ The glomerular basement membrane may be the principal structure responsible for the size discrimination.²² The glomerulus allows greater penetration of neutral and cationic molecules as compared with anionic molecules of the same size.²¹ Besides the size and charge, other determining factors of renal excretion of peptide drugs include lipophilicity, functional groups, sugar recognition, vulnerability to proteases, aggregation to particles, formation of complexes with opsonization factors, etc.²³ In the present study, the renal clearance of [¹²⁵I] LJP 993-derived radioequivalent was calculated at 3–5 mL/h (Table 1). Nevertheless, at this moment the exact mechanism(s) by which LJP 993 is excreted is unclear, and we assumed that the gradient in concentration of [¹²⁵I] LJP 993 accumulated in kidney could kinetically drive the excretory mechanism(s) to filter [¹²⁵I] LJP 993 through the renal glomeruli, thereafter resulting in a rapid decrease in plasma levels of [¹²⁵I] LJP 993 as shown in Figure 3, although a dynamic distribution from the central compartment to the tissues plays an important role in this respect as well.

Tissue levels were much higher than the plasma level at 4 h, suggesting significant partitioning of LJP 993 into the tissues. The terminal decay phase of [¹²⁵I] LJP 993-derived radioequivalent from the tested tissues was longer than 24 h, indicating that accumulation does occur. This was confirmed by distributional data at the steady-state (Figure 3, Table 2). In this case, [¹²⁵I] LJP 993 concentrations in a slowly equilibrating tissue compartment with plasma were directly related to the effect intensity. Interestingly, the total percentage of a single i.v. dose present in the tested tissues 24 h after dosing fell to 5% (Table 2). The duration approximately equals five elimination half-lives of [¹²⁵I] LJP 993. It is commonly accepted that it takes five elimination half-lives for a drug to be removed by 95% from the body.²⁴ In view of the very consistent decreases in percentage of dose in total organs at 4 h (Table 2), it is somewhat surprising that the percentage of given [¹²⁵I] LJP 993 equivalent in fat tissue, unlike that in other tissues, increased at 4 h. This regional peak of [¹²⁵I] LJP 993 in the fat tissue may be the result of a slower rate of redistribution of the radioequivalent from other tissues and blood to fat tissue. A mathematical 3-compartmental model may regionally be suitable for the fat tissue. Also, in thyroid, an increase in [¹²⁵I] LJP 993 was found at later times, probably because of

preferential uptake of iodine by the thyroid. In our distribution studies, brain showed considerably lower levels of radioactivity than other tissues. This reflects the drug's poor penetration into the blood–brain barrier, due most likely to the hydrophilic properties and the large size of the molecule (MW 29,400).

Conclusions

The present studies reported the biostability and pharmacokinetic profile of the synthesized Toleragen composed of linker-attached domain 1 coupled to a non-immunogenic platform at a valency of four domain 1 molecules LJP 993 in animals. These types of agents, including LJP 394²⁵ and LJP 1082²⁶ have been developed to treat systemic lupus erythematosus and related autoimmune diseases such as antiphospholipid syndrome (or Hughes syndrome). Both LJP 993 and LJP 1082 contain four recombinant human domain 1, which binds to different platforms. In animal pharmacokinetic studies, when ¹²⁵I-LJP 1082 was injected to CD-1 mice and Sprague-Dawley rats at 1 mg/kg, ¹²⁵I-LJP 1082 resulted in the mean elimination half-life ($t_{1/2\beta}$) of 0.37 h in mice and 9.5 h in rats, respectively.²⁷ By comparison, when ¹²⁵I-LJP 993 was administered to the same animal species, i.e. CD-1 mice, at 0.5 and 5 mg/kg, ¹²⁵I-LJP 993 seemed to have a $t_{1/2\beta}$ longer than ¹²⁵I-LJP 1082 (3.1–4.7 h; Table 1), although detail pharmacokinetic parameters of LJP 1082 are not available for making a more comprehensive comparison between the two molecules. Nonetheless, LJP 1082 showed better activity in suppressing pathogenic antibodies of autoimmune diseases than its analogs. As a result, it is being further tested in human trials. Table 3 summarizes and compares these molecular structures, their disease targets and pharmacokinetic information.²⁸ Phase II/III clinical trials for LJP 394 and phase I/II study of LJP 1082 have been conducted at many medical centers in Europe and the USA to evaluate their safety and efficacy.^{29,30} The present study may help the principal investigators better design their clinical trials, understand the human data, and explain the drug candidates' safety and effectiveness from pharmacokinetic perspectives.

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NH-(D1-R) = HN-RTCPKPDDL P FSTVVPLKTF YEPGEEITYS CKPGYVSRGG MRKFICPLTG LWPINTLKCT
PR-CO₂H

Figure 1.

Chemical structure of LJP 993. The D1-R stands for the domain 1 of β 2-glycoprotein 1.

Figure 2.

Kinetics of degradation of LJP 993 by mouse plasma. LJP 993 (50 $\mu\text{g/mL}$) was incubated at 37°C with undiluted plasma. The percentage of LJP 993 remaining intact was calculated using ChemStation to integrate the peak area of LJP 993. Results are expressed as the mean $\pm$ SD ($n = 4$).

Figure 3. Plasma concentration-time curves for [^{125}I] LJP 993 after a single intravenous administration to mice at doses 0.5 and 5 mg/kg. Each point represents the mean $\pm$ SD ($n = 4$).

Figure 4. The cumulative percent dose excreted in the urine and feces of mice ($n = 4$) after a single intravenous administration of [^{125}I] LJP 993 at 5 mg/kg.

Figure 5. Tissue levels of $[^{125}\text{I}]$ LJP 993 in mice after 5 mg/kg were administered intravenously. The total radioequivalent concentrations in various tissues were determined in micrograms per gram of tissues and data are represented as the mean $\pm$ SD ($n = 4$).

Table 1

Pharmacokinetic parameters (mean±S.D., $n = 4$ per time point) of ^{125}I -LJP 993 after i.v. administration to mice

<i>Dose (mg/kg)</i>	<i>0.5</i>	<i>5</i>
$t_{1/2\alpha}$ (min)	1.73±0.10	1.70±0.18
$t_{1/2\beta}$ (min)	188.52±19.02	281.17±47.95
A (µg/mL)	9.21±0.19	27.60±0.95
B (µg/mL)	1.17±0.12	7.80±0.41
$C_{\max}$ (µg/mL)	10.38±0.18	35.40±0.96
CL (mL/hr)	3.48±0.49	1.86±0.26
AUC (µg•hr/mL)	2.87±0.44	53.83±7.56
V _{ss} (mL)	6.44±0.68	12.30±0.64

$t_{1/2\alpha}$, distribution half life; $t_{1/2\beta}$, elimination half life; A and B are coefficients in the biexponential equation; $C_{\max}$, maximum plasma concentration; CL, clearance; AUC, area under the curve; V_{ss}, volume of distribution at steady-state.

Table 2

Percentage of dose in various mouse tissues at intervals following a single intravenous administration of ^{125}I -LJP 993 to mice at 5 mg/kg

<i>Tissue^a</i>	<i>0.5 h</i>	<i>4 h</i>	<i>24 h</i>
Brain	0.17±0.03	0.10±0.06	0.01±0.01
Fat	2.47±0.377	9.69±4.53	1.91±3.06
Heart	0.47±0.16	0.19 ±0.03	0.02±0.03
Kidney	9.26±5.01	1.33±0.32	0.10±0.13
Liver	5.80±1.60	2.16±0.51	0.20±0.24
Lung	0.88±0.16	0.49±0.09	0.04±0.04
Muscle	18.80±1.49	15.02±1.31	1.25±1.44
Skin	8.23±1.32	6.06±2.18	0.83±0.52
Plasma	8.42±1.06	5.34±1.46	0.34±0.52
Spleen	0.41±0.15	0.19±0.05	0.01±0.01
Thyroid	0.72±0.83	1.47±0.83	0.85±0.91
Total (%)	55.64	42.03	5.56

^aPercentages were calculated by assuming that skin=11%, fat=15% and muscle=50% of the body weight.⁹ The data are represented as the mean±SD (*n* = 4 per time point).

Table 3

A comparison of key characteristics between LJP993 and LJP1082

<i>Toleragens</i>	<i>Molecular structures</i>	<i>Target</i>	<i>Clinical uses</i>	<i>Pharmacokinetics</i>	<i>References</i>
LJP993	Four recombinant human domain 1 of β_2 -GPI bound to a tetravalent aminoxy platform	Bind to anti- β_2 -GPI antibodies	No clinical trials	0.5 and 5 mg/kg (i.v.) produced in $t_{1/2\beta}$ 3.1 h and 4.7 h, and C_{max} 10 μ g/mL and 35 μ g/mL, respectively, in mice.	Jones et al. ^{6,7}
LJP1082	Four recombinant human domain 1 of β_2 -GPI bound to a tetravalent polyethylene glycol platform	Bind to anti- β_2 -GPI antibodies	Antiphospholipid syndrome; 20–200 mg, i.v.; phase I/II trials	1 mg/kg (i.v.) produced $t_{1/2\beta}$ 0.37 h and 9.5 h in mice and rats, respectively; 200 mg per person produced C_{max} 20–30 μ g/mL and $t_{1/2\beta}$ 10–12 h.	Merni ²⁶ , Cockerill et al. ^{27,31} , Horizon et al. ²⁸