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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

E. S. D.

Re: Expediente No. 06-021510

Solicitud de patente a nombre de Ortho-Mc-Neil Pharmaceutical, Inc.

Yo, JIMENA ESCOBAR URIBE, identificada como aparece al pie de mi firma, domiciliada en la carrera 11 No. 86-53, piso 6, obrando como apoderada de la sociedad **Ortho-Mc-Neil Pharmaceutical, Inc** domiciliada en Raritan, New Jersey, Estados Unidos de América, me permito informar lo siguiente:

Es deseo del solicitante poner en consideración del señor examinador el artículo "*Pharmacokinetics and Hematological Effects of the PEGylated Thrombopoietin Peptide Mometic GW395058 in Rats and Monkeys alter Intravenous or Subcutaneous Administration*" de Mark de Serres y colaboradores publicada en Stem Cells en el año de 1999.

El ánimo de la presentación de este artículo es poner de manifiesto que la solicitud en estudio posea novedad y nivel inventivo sobre éste documento que se podría considerar como el estado del arte más cercano, toda vez que los compuestos peptídicos de bajo peso molecular de la solicitud en estudio presentan de manera sorprendente fuertes propiedades de unión al TPO-R y permiten potencialmente reducir los efectos laterales cuando se comparan con los agonistas de la TPO conocidos en la técnica anterior.

Señor Superintendente,


JIMENA ESCOBAR URIBE
C.C.39.779.452 de Usaquén
T.P. 68228
Cód. 5376

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Pharmacokinetics and Hematological Effects of the PEGylated Thrombopoietin Peptide Mimetic GW395058 in Rats and Monkeys After Intravenous or Subcutaneous Administration

MARK DE SERRES,^{*} RUSS L. YEAGER,^{*} JOHN E. DILLBERGER,^{*} GUY LALONDE,[†] GUY H. GARDNER,^{*}
CATHLEEN A. RUBENS,^{*} ANITA H. SIMKINS,^{*} JEFFERY M. SAILSTAR,^{*} MICHAEL J. McNULTY,^{*}
JOSEPH L. WOOLLEY^{*}

^{*}Glaxo Wellcome Inc., Research Triangle Park, North Carolina, USA;

[†]Affymax Research Institute, Palo Alto, California, USA

Key Words: Thrombopoietin · Mimetic peptide · GW395058 · Pharmacokinetics · Hematology · Rat · Monkey · PEGylated

ABSTRACT

GW395058, a potent PEGylated peptide human thrombopoietin receptor (HuTPOr) agonist *in vitro*, is being evaluated for the treatment of thrombocytopenia. GW395058 shares no sequence homology with TPO. In this report the pharmacokinetics and hematological effects of GW395058 in rats and monkeys are described. Doses eliciting thrombocytosis in rodents (2 or 10 µg/kg s.c.) produced insufficient plasma concentration data for pharmacokinetic parameter estimate calculations. At higher i.v. doses in rats (500, 1,000 or 2,000 µg/kg) serum *t*_{1/2} (half-life) values were >20 h, and the area under the concentration time curve increased proportionally with dose. In cynomolgus monkeys GW395058 plasma *t*_{1/2} values ranged from 37 to 68 h after s.c. or i.v.

dosing, and similar values were observed in rhesus monkeys following s.c. dosing. Rat platelet counts increased following 2 (1.6-fold) or 10 µg/kg (fourfold) s.c. doses. Cynomolgus and rhesus monkey platelet counts did not change significantly at comparable s.c. doses, but did increase slightly (<twofold) in cynomolgus monkeys following a 25 µg/kg s.c. dose and twofold following a 100 µg/kg s.c. dose. Because the plasma *t*_{1/2} of GW395058 is long in mouse, rat, dog, and monkey, yet the dose required to double platelet levels in monkeys is 50-fold that required in rats, differences in hematological responses may be due to interspecies differences in the interaction of GW395058 with the TPOr. *Stem Cells* 1999;17:316-326

INTRODUCTION

Neutropenia and thrombocytopenia are chemotherapy-induced side effects associated with significant morbidity, including spontaneous bleeding. Thrombocytopenia often limits the maximum chemotherapy dose and the dosing regimen that can be administered. Reducing the magnitude of thrombocytopenia might allow administration of higher doses of chemotherapeutic agents or dose-intensification, which could result in improved disease control. Standard practice is to manage thrombocytopenia by administering platelet transfusions, which carry a risk of secondary infection as well as the eventual production of neutralizing antibodies to platelets. Recombinant interleukin 11 (rIL-11) has

been approved for the treatment of chemotherapy-induced thrombocytopenia, although its use has been associated with notable side effects [1-3].

The lineage-specific cytokine that regulates platelet production, thrombopoietin (TPO), has been described [4]. Two forms of the recombinant human protein have been investigated: full-length glycosylated recombinant human protein (rHuTPO) [5] and PEGylated megakaryocyte growth and development factor (PEG-rHuMGDF) [6]. In mice, dogs, and primates [7-14], rHuTPO and PEG-rHuMGDF promote platelet production and reduce chemotherapy-induced thrombocytopenia. Unfortunately, evidence of TPO-neutralizing antibodies in patients participating in cancer and

Correspondence: Dr. Mark de Serres, Division of Bioanalysis and Drug Metabolism, Department of International Development Support, Glaxo Wellcome Inc., 5 Moore Drive, Research Triangle Park, North Carolina 27709, USA. Accepted for publication October 12, 1999. ©AlphaMed Press 1066-5099/99/35.00/0

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platelet donor clinical trials forced the discontinuation of PEG-rHuMGDF development [15].

Not long after TPO was first reported, a 28-amino acid TPO mimetic peptide was described that was equipotent to TPO in cell-based assays [16]. This peptide dimer, AF13948, shares no sequence homology with TPO. Studies showed that while AF13948 plasma concentrations were short in mice (plasma $t_{1/2}$ [half-life] = 1 h), AF13948 stimulated platelet production following single or multiple regimens of 250 or 1,000 $\mu\text{g/kg}$ (unpublished results).

Subsequent to these studies the sequence of AF13948 was modified with amino acid substitution, and the resulting peptide, AF15705, was PEGylated to produce GW395058 (Fig. 1). Similar to AF13948 and AF15705, GW395058 binds to and activates the human TPO receptor (HuTPOr) with an affinity similar to that of TPO *in vitro* [17]. Unlike AF13948, GW395058 has a long $t_{1/2}$ in mice and elevates peripheral platelet counts following a single s.c. dose of as little as 2 $\mu\text{g/kg}$ [18]. In dogs, when coadministered with G-CSF, GW395058 has a long plasma half-life and accelerates platelet recovery in a dog myelosuppression model (unpublished results, manuscript in preparation). Recent studies have shown that GW395058 is not immunogenic in several animal species, and that if an antibody response to GW395058 were elicited, those antibodies would not likely neutralize endogenous HuTPO [19].

In this report, we describe the pharmacokinetics and hematological effects of GW395058 in rats and monkeys following i.v. or s.c. administration of the compound.

EXPERIMENTAL PROCEDURES

Materials

AF15705 and GW395058 were obtained from the Biotechnology Product Development Division, Glaxo Wellcome Inc. (Research Triangle Park, NC). All other chemicals were reagent grade or better. Dosing solutions of GW395058 were prepared in phosphate-buffered saline (PBS). GW395058 was prepared by derivatizing the two N-termini of the AF15705 linear dimer with 20,000 molecular weight (mw) polyethylene glycol (PEG) (unpublished results, manuscript in preparation). The mw of AF15705 and GW395058 are 3,295 and ~43,300, respectively. All dose calculations are based on peptide mass and are thus directly comparable for PEGylated and unmodified peptides. The amino acid sequences of AF15705 and GW395058 are shown in Figure 1.

Animal Handling, Dosing, and Sample Collection

All animal procedures described in this report were approved by the Institutional Animal Care and Use Committee and conducted in accordance with Federal guidelines.

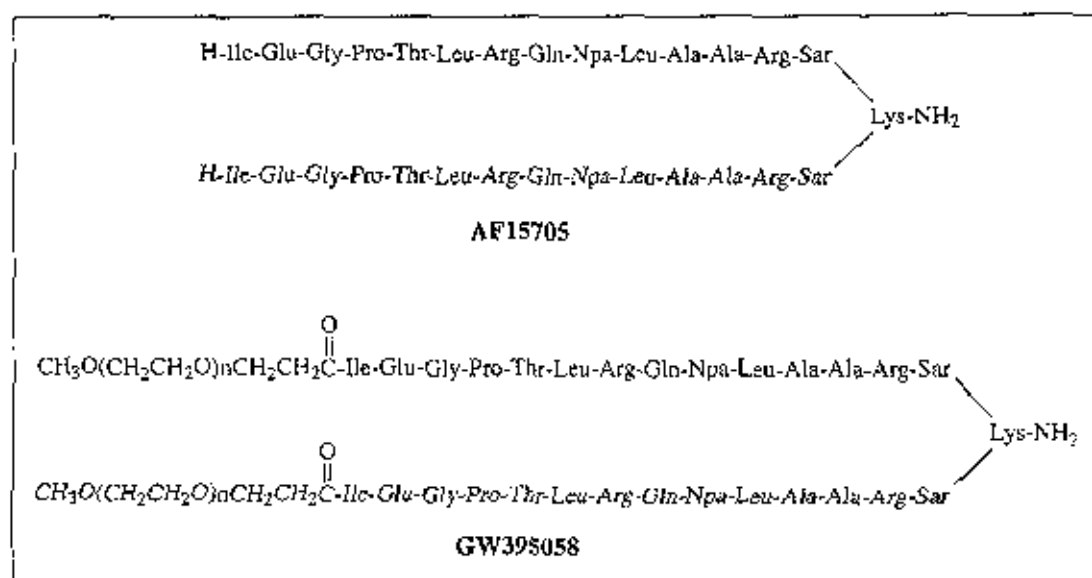


Figure 1. Amino acid sequences of AF15705 and GW395058. AF15705 is a peptide dimer consisting of two identical amino acid chains of 14 residues linked through their carboxyl termini to the α and ϵ amino groups of a lysine residue. The sequence of AF13948 (see text) was modified by the replacement of an alanine and a β -alanine residue with sarcosine (Sar) to remove the chiral centers. Tryptophan residues were replaced with naphthylalanine (Npa) for ease of synthesis. The two resulting peptide amino termini of AF15705 were conjugated to 20,000 mw PEG to produce GW395058.

Studies in Rats

Single-dose and one-month repeat-dose studies were conducted in Han Wistar rats at Glaxo Wellcome. In the single-dose study, male rats (300 to 350 g, three rats/dose) received single s.c. doses of 2 or 10 $\mu\text{g/kg}$. Plasma samples for determination of GW395058 concentrations were obtained from each rat predose and at 3, 4, 7, and 10 days postdose. Plasma samples were stored at -70°C until analysis. Blood samples for hematological analysis were obtained from each rat predose and at 3, 7, 10, 14, 17, 21, and 28 days postdosing and analyzed the same day.

In the one-month repeat-dose study, male and female Han Wistar rats (250 to 300 g; 10 rats/sex at the 500 and 1,000 $\mu\text{g/kg/48 h}$ doses, 18 rats/sex at the control and the 2,000 $\mu\text{g/kg/48 h}$ doses) received single i.v. bolus doses every other day for a month. Upon completion of dosing, 10 rats/sex/dose were euthanized, while remaining control and high-dose rats were allowed to recover for one month and then euthanized to assess the reversibility of treatment-related effects. Doses of GW395058 in this study were chosen to provide exposures well above those necessary for efficacy in rats and those anticipated in clinical trials. Following doses 1 and 11, serum samples for determination of GW395058 concentration were obtained from 3 rats/sex/time point predose and at 0.8, 0.5, 1, 2, 4, 8, 24, and 48 h after dosing. Following dose 11, additional serum samples were taken at 72 and 96 h. Serum samples were stored at -70°C until analysis. Blood samples for hematological analysis were obtained from each rat predose, on day 24 (near the end of the dosing period), and 13 and 28 days after dosing stopped. Hematologic samples were analyzed the same day they were taken.

Single-dose and one-month repeat-dose studies were conducted in monkeys at Covance (Vienna, VA). In the single-dose study, male cynomolgus monkeys (3 to 5 kg) received single s.c. doses at 2, 10, 25, or 100 $\mu\text{g/kg}$ (three monkeys/dose) or a single i.v. dose at 10 $\mu\text{g/kg}$ (two monkeys). Plasma samples for pharmacokinetic analysis were obtained from each monkey predose and at 0.08, 0.25, 0.5, 1, 2, 4, 8, 24, 72, 96, 120, 144, and 168 h postdosing. Plasma samples were stored at -70°C until analysis. Blood samples for hematological analysis were obtained from each monkey at predose (day 1) and 3, 5, 7, 8, 10, 11, 12, 13, 17, 19, and 22 days postdose. Blood samples were stored at room temperature until processed (within 1 h of collection). Because platelet counts increased only slightly at 25 and 100 $\mu\text{g/kg}$ and not at all at $\leq 10 \mu\text{g/kg}$, four rhesus monkeys (3 to 5 kg; two/sex) were added to the study to determine if they responded more strongly to GW395058 than did cynomolgus monkeys. Rhesus monkeys received single s.c. doses of GW395058 at 2 or 10 $\mu\text{g/kg}$. Samples (plasma and blood) were collected as described for cynomolgus monkeys.

In the one-month repeat-dose study, male and female cynomolgus monkeys (3 to 5 kg; three/sex at 500 and 1,000 $\mu\text{g/kg/48 h}$ doses, five/sex at control and at the 2,000 $\mu\text{g/kg/48 h}$ dose) received single i.v. doses every other day for a month. Upon completion of dosing, three monkeys/sex/dose were euthanized, while remaining control and high-dose monkeys were allowed to recover for one month and then euthanized to assess the reversibility of treatment-related effects. Following doses 1 and 14, serum samples were obtained predose and at 0.08, 0.5, 1, 2, 4, 8, and 24 h postdose. Following the first dose, an additional serum sample was collected at 48 h from all animals. Following dose number 14, additional serum samples were taken from animals (two animals/sex) in the vehicle control and 2,000 $\mu\text{g/kg/48 h}$ groups at 48, 72 and 96 h after dosing. Serum samples were stored frozen at or below -20°C until analysis. Blood samples for hematological analysis were obtained from each monkey predose, on days 14 and 26 during the dosing period, and 10 and 24 days after dosing stopped. Hematologic samples were analyzed the same day they were taken.

Determination of GW395058 Plasma Concentrations

Radioimmunoassay (RIA)

Concentrations of GW395058-like immunoreactive material in rat and monkey plasma samples were determined by using a competitive binding RIA. A tritiated analog of AF15705 was prepared by derivatizing the two N-termini of AF15705 with succinimidyl propionate. N-[propionate-2,3- ^3H] obtained from Dupont Company NEN Research Products (Boston, MA). The reaction product was purified by RP-HPLC (2.5 $\times$ 150 mm Symmetry[®] C18 5 μm column, Waters Corp. [Milford, MA]; mobile phase A = 0.1% trifluoroacetic acid (TFA) in water, mobile phase B = 0.1% TFA in acetonitrile) by using gradient chromatographic conditions (0 to 60% mobile phase B for 30 min followed by 60% to 75% mobile phase B for 5 min). The purified radioligand had a specific activity of $\sim 25\text{Ci/nmol}$.

In this RIA diluted rabbit antiserum [19] raised to a bovine thyroglobulin conjugate of an AF15705-analog was incubated overnight at 4°C with radioligand (^3H -AF15705) and aliquots of study samples or spiked (GW395058) plasma calibration standards. The next day bound and free radioligands were separated by incubating for 1 h at 4°C with anti-rabbit immunoprecipitation reagent (Sac-Cel[®]; IDS Ltd.; Belden, Tyne and Wear, UK), followed by the addition of 2 ml of cold PBS (pH 7.2) containing 0.1% bovine serum albumin and 0.1% Tween-20. Assay tubes were centrifuged for 10 min and the supernatant aspirated to waste. The pellets were resuspended in 2 ml of water and decanted into 20 ml scintillation vials containing 5 ml of scintillation fluid. Bound

radioligand was quantified in a liquid scintillation counter, and calibration curves were derived by using the program, Multicalc (Wallac, Turku, Finland), from which control and study sample concentrations were interpolated. All samples were assayed in duplicate. The lower limit of quantitation (LLOQ) was set at 1.6 ng/ml. Because this RIA may also detect metabolic fragments of GW395058, the results here are reported as GW395058 immunoreactivity equivalents.

TPOr Biological Activity Assay

Concentrations of GW395058-like biologically active material in monkey plasma samples following a 25 μ g/kg s.c. dose of GW395058 were determined by using a reporter assay based on a Baf3 [HuTPOr fos/lux cell line. In these cells activation of the HuTPOr results in the production of luciferase that catalyzes the emission of light upon addition of the substrate luciferin. The cells were grown in Dulbecco's modified Eagle's essential medium/F12; 10% fetal bovine serum; 10% Wehi-3 supernatant and antibiotics/antimycotics. Approximately 18 h before the assay, cells were starved by transferring them to the same medium made 0.1% Wehi-3 supernatant. On the day of assay, following a washing step in medium without Wehi-3, 1×10^6 cells/ml were combined with study samples or GW395058-spiked plasma calibration standards. Assay plates were incubated for 2 h at 37°C in a 5% CO₂ atmosphere, then Luciferin reagent (Packard Instrument Co.; Downers Grove, IL) was added to each well. Light emission was measured after a five-minute incubation on a Packard Topcount Luminometer (Packard Instrument Co.). The concentrations of GW395058-like biological activity in the plasma samples were interpolated from the linear portion of the standard curve. The LLOQ was set at 0.1 ng/ml. Because this assay does not distinguish GW395058 from other possible agonists, results with this method were reported as GW395058 biological activity equivalents.

Pharmacokinetic Calculations

Area under the concentration time curve (AUC) values for GW395058 were estimated by using the trapezoidal rule, with back-extrapolation of the GW395058 plasma concentrations to zero time. Single day 1 AUC values were calculated from time zero to infinity ($0-\infty$), with the extrapolated area from the time of the last measurable sample concentration ($C_{p_{last}}$) to infinity time estimate as $C_{p_{last}}/k_e$, where k_e is the terminal phase elimination rate constant. Multidose day last AUC values were calculated from time zero to τ , where τ is the dosing interval. C_{max} values were defined as the highest observed plasma concentration postdose. Mean calculations were performed by using Microsoft® Excel 97 SR-1 (Microsoft Corporation; Redmond, WA). Noncompartmental pharmacokinetic calculations were performed by using WinNonlin, Professional Network Version 1.5 (Scientific

Consulting Inc.; Cary, NC) or PKCAL Version 2.0 (Glaxo Wellcome Inc.).

Hematology

Clinical hematology and coagulation analyses were performed at Glaxo Wellcome (rats) or at Covance, Vienna, Virginia (monkeys). Clinical hematology parameters measured in all studies were platelet count, total leukocyte count, differential leukocyte counts, total erythrocyte count, reticulocyte count, hematocrit, hemoglobin, and RBC indices (mean cell hemoglobin [MCH], mean cell volume [MCV], mean cell hemoglobin concentration [MCHC]). In one-month studies, mean platelet volume (MPV), platelet distribution width, and coagulation parameters (prothrombin time and activated partial thromboplastin time) also were measured.

Statistical Analysis

Mean calculations were performed using Microsoft® Excel 97 SR-1. Statistical analysis (ANOVA) of pharmacokinetic and hematology data was performed by using JMP Statistical Visualization Software Version 3.2.5 (SAS Institute, Inc.; Cary, NC) or SAS System for Windows, Version 6.12, TS Level 0020, (SAS Institute). Comparisons of pharmacokinetic parameter estimates were made between the sexes and days of dosing and among the dose groups by using Tukey's Studentized Range Test. Differences were declared statistically significant when $p < 0.05$.

RESULTS

Comparison of RIA with TPOr Biological Activity Assay

To clarify the relationship between GW395058 immunoreactivity and TPOr biological activity, plasma concentrations of GW395058 were measured by RIA and TPOr biological activity assay in samples collected following a single s.c. dose of GW395058 at 25 μ g/kg to male cynomolgus monkeys (threelgroup). Averaged plasma concentrations of GW395058 determined by RIA or TPOr biological activity assay are shown in Table 1. A comparison of individual GW395058 plasma concentrations measured by RIA or TPOr biological activity assay is shown in Figure 2.

Good general agreement ($r^2 = 0.95$; slope = 0.74) was found between the TPOr biological activity assay and the GW395058 RIA. The results show that the RIA detects primarily biologically active material in plasma, and this assay can be used to accurately determine GW395058 plasma or serum concentrations in monkeys or rats dosed with GW395058. Although the TPOr biological activity assay was the slightly more sensitive assay of the two methods, the RIA had a greater throughput. Consequently, the RIA was used to determine GW395058 plasma or serum concentrations for calculation of pharmacokinetic parameter estimates in study samples.

Table 1. Plasma concentrations of GW395058 determined by RIA or TPOr biological activity assay. Averaged plasma concentrations (GW395058 equivalents ng/ml plasma) determined by RIA or TPOr biological activity assay are shown. Plasma samples were obtained from male cynomolgus monkeys (three/group) receiving a single s.c. dose at 25 µg/kg.

Time (h)	RIA (ng/ml GW395058 equivalents ± SD)	TPOr biological activity assay (ng/ml GW395058 equivalents ± SD)
Pre-dose	NQ	NQ
0.08	NQ	NQ
0.25	1.8 ± 0.6	2.0 ± 1.6
0.5	6.7 ± 1.4	6.9 ± 0.6
1	21 ± 14	20 ± 12
2	42 ± 38	45 ± 24
4	107 ± 45	90 ± 31
8	130 ± 23	107 ± 27
24	135 ± 31	127 ± 16
48	141 ± 52	109 ± 18
72	119 ± 37	87 ± 20
96	86 ± 27	61 ± 15
120	64 ± 28	49 ± 15
144	45 ± 21	33 ± 13
168	29 ± 11	25 ± 12

NQ = not quantifiable, below the lower limit of quantitation (LLOQ) for the assay; LLOQ for RIA = 1.6 ng/ml; LLOQ for TPOr biological activity assay = 0.1 ng/ml.

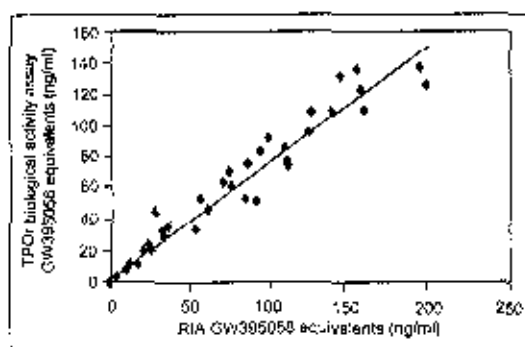


Figure 2. Comparison of GW395058 plasma concentrations determined by RIA or TPOr biological activity assay. Individual concentrations (GW395058 equivalents ng/ml plasma) determined by RIA or TPOr biological activity assay are shown. Plasma samples were obtained from male cynomolgus monkeys (three/group) receiving a single s.c. dose at 25 µg/kg.

Plasma Concentrations and Pharmacokinetic Parameter Estimates for GW395058 in Han Wistar Rats

Following s.c. dosing with GW395058 at 2 or 10 µg/kg, there were insufficient plasma concentration versus time data

available to estimate pharmacokinetic parameters due to the limited amount of blood and plasma that could be collected from each animal over the extended duration of this study. The highest plasma concentrations of GW395058 were observed on day 3 following the 10 µg/kg dose. GW395058 was undetectable in plasma following the 2 µg/kg dose (data not shown).

Pharmacokinetic parameter estimates for GW395058 in male and female Han Wistar rats following i.v. dosing at 500, 1,000, or 2,000 µg/kg/48 h are shown in Table 2.

In general following i.v. dosing at 500, 1,000, or 2,000 µg/kg/48 h for one month, plasma concentrations did not differ between sexes. Following dose 1, there was good proportionality with increasing dose between AUC and C_{max} values. Plasma t_{1/2} values were long at all dose levels. Plasma clearance (CL) and volume of distribution values (V_z) over the fourfold range in i.v. dose were low and generally comparable among dose levels. Dose 1 and dose 11 pharmacokinetic parameter estimates did not differ (data not shown) suggesting that repeated i.v. dosing did not alter GW395058 systemic exposure.

Plasma Concentrations and Pharmacokinetic Parameter Estimates for GW395058 in Cynomolgus Monkeys

Plasma concentrations of GW395058 in cynomolgus monkeys following s.c. dosing at 2, 10, 25, and 100 µg/kg and i.v. at 10 µg/kg are shown in Figure 3. Pharmacokinetic parameter estimates for GW395058 in cynomolgus monkeys following s.c. dosing at 2, 10, 25, and 100 µg/kg and i.v. at 10 µg/kg are shown in Table 3.

In general, AUC and C_{max} values increased proportionality with increasing dose following s.c. dosing at 2, 10, 25, or 100 µg/kg. Plasma t_{1/2} values were long at all dose levels. Plasma clearance (CL/F) values over the 50-fold range in s.c. dose were low and comparable among dose levels. Volume of distribution (V_z/F) values were similar at the 10, 25, and 100 µg/kg dose levels, while the averaged V_z/F value for the 2 µg/kg dose was slightly higher.

Following i.v. dosing with GW395058 at 10 µg/kg, the AUC was slightly lower than the comparable s.c. dose level. The plasma t_{1/2} following i.v. dosing was at least 10 h less than that observed following s.c. dosing. Plasma CL following i.v. dosing was generally low, but slightly higher than that observed following s.c. dosing at the same level. The V_z following i.v. dosing at 10 µg/kg was essentially the same as that observed following s.c. dosing. The volume at steady state (V_{ss}) was 82 ± 12 ml/kg.

Pharmacokinetic parameter estimates for GW395058 in cynomolgus monkeys following single and repeated i.v. doses at 500, 1,000, 2,000 µg/kg are shown in Table 4. In general for monkeys (male and female) dosed at 500, 1,000,

Table 2. Pharmacokinetic parameter estimates in Han Wistar rats after one-month, multiple-dose studies. Average pharmacokinetic parameter estimates following dose 1 are shown for male and female rats (10/group) receiving single i.v. doses of GW395058 at 500, 1,000 or 2,000 $\mu\text{g/kg/48 h}$ for one month.

Average Pharmacokinetic Parameter Estimates							
Sex	Dose ($\mu\text{g/kg/48h}$)	AUC ₀₋₂₄ (h * ng/ml)	C _{max} (ng/ml)	t _{1/2} (h)	CL (ml/h/kg)	V _d (ml/kg)	V _{ss} (ml/kg)
M	500	219,254	10,426	21.6	2.28	71.0	68.7
F	500	350,411	15,145	30.2	1.72	75.1	67.5
M	1,000	565,297	30,061	23.0	1.77	58.7	54.3
F	1,000	618,189	27,017	20.4	1.62	47.6	47.5
M	2,000	932,877	54,047	18.2	2.03	53.6	52.0
F	2,000	1,129,452	58,994	19.4	1.77	49.7	49.3

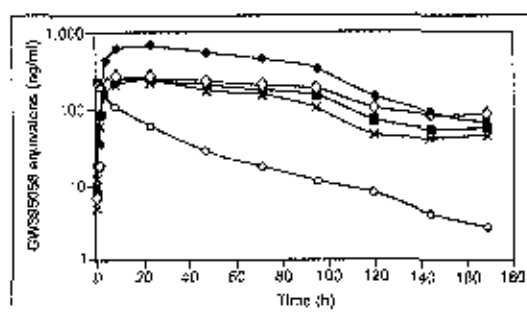


Figure 3. Plasma concentrations in male cynomolgus monkeys dosed with GW395058. Average plasma concentrations (GW395058 equivalents ng/ml plasma) determined by RIA are shown. Plasma samples were obtained from monkeys (three/group) receiving single s.c. doses at 2 ($\square$), 10 ($\blacksquare$), 25 ($\circ$), or 100 ($\bullet$) $\mu\text{g/kg}$ or an i.v. dose at 10 ($\triangle$) $\mu\text{g/kg}$.

or 2,000 $\mu\text{g/kg}$: A) C_{max} and AUC values increased proportionally with dose; B) t_{1/2} values were long, and C) CL values were low and comparable among dose levels.

Following doses 1 and 14, C_{max} and AUC values for GW395058 increased with increasing doses. Increases in C_{max} and AUC values between 500 and 2,000 $\mu\text{g/kg}$ groups were statistically significant. The increases in CL between

500 and 2,000 $\mu\text{g/kg}$ dose groups were small, and only significant in female monkeys. There were no statistically significant differences in t_{1/2} or V_d across the dose groups.

C_{max} values increased from dose 1 to dose 14, but these increases were significant only in the 1,000 and 2,000 $\mu\text{g/kg}$ female and the 500 and 1,000 $\mu\text{g/kg}$ male dose groups. The only significant decrease in CL and increase in AUC values following multiple dosing were observed in female monkeys at the 2,000 $\mu\text{g/kg}$ dose. There were no significant changes in t_{1/2} or V_d with multiple dosing.

C_{max}, AUC, t_{1/2}, and CL values following doses 1 and 14 were not statistically different between male and female monkeys, except at the 2,000 $\mu\text{g/kg}$ dose group, where C_{max}, AUC, and t_{1/2} were greater in males and CL was greater in females. There were no other sex-related trends in any of the other pharmacokinetic parameters examined.

Plasma Concentrations and Pharmacokinetic Parameter Estimates for GW395058 in Rhesus Monkeys

Pharmacokinetic parameter estimates for GW395058 in the rhesus monkey following s.c. dosing at 2 and 10 $\mu\text{g/kg}$ are shown in Table 5. Pharmacokinetic parameter estimates in rhesus monkeys were comparable to those for cynomolgus monkeys following s.c. dosing at 2 and 10 $\mu\text{g/kg}$. AUC values

Table 3. Pharmacokinetic parameter estimates in cynomolgus monkeys after single-dose studies. Average pharmacokinetic parameter estimates are shown for male monkeys (three/group) receiving single s.c. doses at 2, 10, 25, or 100 $\mu\text{g/kg}$ or an i.v. dose (two/group) at 10 $\mu\text{g/kg}$.

Pharmacokinetic Parameter Estimates (Average $\pm$ SD)							
Route	N	Dose ($\mu\text{g/kg}$)	AUC ₀₋₂₄ (h * ng/ml)	C _{max} (ng/ml)	t _{1/2} (h)	CL/F (ml/h/kg)	V _{ss} (ml/kg)
i.v.	2	10	5,154	236	37	2.0	82
s.c.	3	2	1,367	13	68	1.5	145
s.c.	3	10	7,217	71	47	1.4	95
s.c.	3	25	18,054	161	45	1.5	94
s.c.	3	100	65,863	704	45	1.6	104

Table 4. Pharmacokinetic parameter estimates in cynomolgus monkeys after one-month repeat-dose studies. Average pharmacokinetic parameter estimates following doses 1 and 14 are shown for male and female monkeys (three/group) receiving i.v. doses at 500, 1,000, or 2,000 µg/kg/48 h for one month.

Averaged Pharmacokinetic Parameter Estimates Following Dose 1 and 14								
Dose #	Sex	Dose (µg/kg/48h)	AUC ^a (h * ng/ml)	C _{max} (ng/ml)	T _{1/2} (h)	CL (ml/h/kg)	V _z (ml/kg)	V _{ss} (ml/kg)
1	M	500	528,006	18,493	26.1	0.95	35.5	35.3
	F	500	446,043	15,221	25.4	1.17	40.6	39.3
	M	1,000	703,881	32,572	23.4	1.61	49.8	47.1
	F	1,000	776,712	30,441	23.7	1.31	43.9	41.2
	M	2,000	1,808,371	57,915	33.8	1.15	55.0	52.6
	F	2,000	1,004,825	42,009	24.2	1.89	65.4	62.3
14	M	500	635,845	27,093	27.2	0.82	30.9	30.0
	F	500	568,493	21,157	32.7	0.92	41.3	39.4
	M	1,000	1,092,922	44,064	44.0	0.97	53.6	51.6
	F	1,000	923,744	33,589	25.1	1.08	39.1	37.4
	M	2,000	1,540,563	64,364	39.3	1.37	72.1	74.0
	F	2,000	1,468,950	65,525	31.3	1.38	62.6	62.2

^aAUC_{0-∞} for dose 1; AUC₀₋₂₄ for dose 14

Table 5. Pharmacokinetic parameter estimates for GW395058 in rhesus monkeys after single-dose studies. Average pharmacokinetic parameter estimates are shown for monkeys (two/group; one male and one female) receiving single s.c. doses at 10 or 2 µg/kg.

Pharmacokinetic Parameter Estimates (Average ± SD)						
Dose (mg/kg)	AUC _{0-∞} (h * ng/ml)	C _{max} (ng/ml)	T _{max} (h)	T _{1/2} (h)	CL/F (ml/h/kg)	V _z /F (ml/kg)
2	1,032	15	8	39	1.9	98
10	5,710	69	16	45	1.8	113

increased proportionally with increasing dose for rhesus monkeys following s.c. dosing at 2 and 10 µg/kg. Similarly, C_{max} values were comparable with those at the same dose levels for cynomolgus monkeys (Table 3), and there was proportionality with increasing dose. T_{max} values for rhesus monkeys were slightly shorter than those for cynomolgus monkeys. Plasma t_{1/2} values for rhesus monkeys were long following s.c. dosing, although the plasma t_{1/2} value at the 2 µg/kg dose level was approximately half that observed for cynomolgus monkeys. CL/F and V_z/F values were similar for both rhesus and cynomolgus monkeys at the 2 and 10 µg/kg dose levels.

Hematological Effects in Rats After Single-Dose Studies

Single s.c. doses of GW395058 at 2 or 10 µg/kg altered platelet, RBC, and WBC parameters. Platelet counts were increased two- to fourfold within three days postdose and returned to normal within 14 days postdose. The platelet response was dose-dependent, in that increases occurred more rapidly, persisted longer, and were greater in magnitude at

10 µg/kg (Fig. 4). RBC parameters (hematocrit, hemoglobin, RBC count) decreased slightly (10%-15%) at both doses

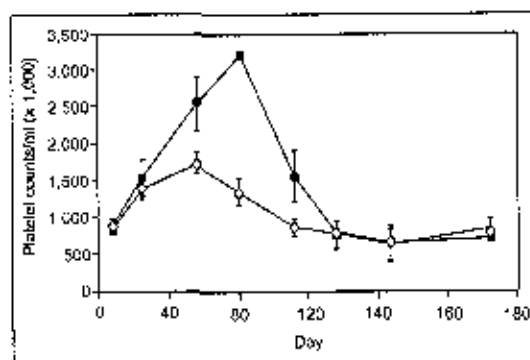


Figure 4. Platelet counts in male Han Wistar rats dosed with GW395058. Average platelet counts in rats are shown. Blood samples were obtained from rats (three/group) receiving single s.c. doses of GW395058 at 2 (○) or 10 (■) µg/kg.

within three days postdose, and did not return to normal until 28 days postdose. Decreases occurred more rapidly and were greater in magnitude at 10 µg/kg. There was a corresponding increase in red cell distribution width, which remained elevated for 28 days postdose, the last time point measured (data not shown).

Total WBC counts increased slightly (<twofold) at both doses within three days postdose and returned to normal within 14 days postdose, reflecting an across-the-board increase in lymphocyte and granulocyte counts. Increases in WBC counts were unrelated to dose (data not shown).

Hematological Effects in Rats After One-Month Repeat-Dose Studies

Repeated i.v. administration of GW395058 at 500, 1,000, or 2,000 µg/kg every other day for a month produced much greater changes in platelet, RBC, and WBC parameters than those observed after single low doses. However, at these elevated dose levels, hematologic effects did not differ among dose groups (Table 6). Platelet counts increased about threefold during the dosing period but increased more (about 10-fold) within two weeks after dosing stopped. Platelet counts had returned to normal by a month postdose. Increases in platelet count were accompanied by an increase in MPV.

RBC parameters (hematocrit, hemoglobin, and RBC count) decreased 15%-25% by the end of the dosing period, and returned to normal within a month postdose. Decreases were accompanied by a reduction in mean corpuscular hemoglobin concentration (5%-10%) and an increased red cell distribution width (15%-30%).

Total WBC counts increased markedly (about eightfold) by the end of the dosing period and returned to normal within a month postdose. WBC count increases reflected across-the-board increases in lymphocyte counts (about fourfold) and granulocyte counts (about 20-fold) observed during this study.

Prothrombin time was somewhat prolonged (30%) at the end of the dosing period, but returned to normal within a month postdose. Activated partial thromboplastin time was unaltered (data not shown).

Hematological Effects in Monkeys After Single-Dose Studies

In cynomolgus or rhesus monkeys, no significant changes in platelet counts were observed following s.c. doses at 2 or 10 µg/kg. In cynomolgus monkeys, a 25 µg/kg s.c. dose produced small but significant increases in platelet counts on days 7 and 11 (1.3 times and 1.5 times, respectively) from day 1 values (data not shown). A 100 µg/kg s.c. dose produced significant increases in platelet counts on days 9, 11, and 13

Table 6. Hematological effects of GW395058 in Han Wistar rats after one-month repeat-dose studies. Changes in average hematological and coagulation parameters at the end-of-dose and postdose at two and four weeks are shown for rats receiving repeated i.v. doses at 500, 1,000, 2,000 µg/kg/48 h for one month.						
Dose (µg/kg/48 h)	Sample time					
	0	End-of-dosing			+ 2 weeks postdose	+ 4 weeks postdose
		500	1,000	2,000	2,000	
Platelet effects						
Platelet count (×10 ⁹ /mm ³)	1,504	2,262	2,343	2,304	6,796	393
MPV (µm ³)	5.1	6.0	6.1	6.1	5.4	5.6
Granulocyte effects						
Neutrophil count (/mm ³)	941	19,802	18,879	27,439	22,330	662
Monocyte count (/mm ³)	160	7,917	6,793	8,246	7,384	215
Eosinophil count (/mm ³)	86	1,857	810	986	1,355	83
Basophil count (/mm ³)	11	653	329	863	290	18
Lymphocyte effects						
Lymphocyte count (/mm ³)	4,210	24,793	24,874	27,439	41,626	5,479
RBC effects						
RBC count (×10 ⁶ /mm ³)	7.05	5.56	5.53	5.52	5.46	7.53
RDW (%)	12.7	18.7	18.7	18.0	19.8	16.3
Coagulation effects						
Prothrombin time (sec)	13.7	16.6	17.2	17.5	n.d.	14.9
n.d. = not determined; RDW = red cell distribution width.						

(Fig. 5). However, platelet counts did not increase proportionally with dose from 25 to 100 $\mu\text{g/kg}$. Additionally, cynomolgus monkeys dosed at 100 $\mu\text{g/kg}$ had significant increases in total WBC counts (1.4-fold) on day 7 (data not shown) and neutrophil counts on day 7 (2.6 times) from day 1 counts (Fig. 6). No significant changes were observed for the other hematological parameters examined.

Hematological Effects in Monkeys After One-Month Repeat-Dose Studies

Repeated i.v. administration of GW395058 to cynomolgus monkeys at 500, 1,000, and 2,000 $\mu\text{g/kg/48 h}$ for a month produced greater increases in platelet counts than were observed in single-dose monkey studies. In addition, RBC and WBC parameters were altered. However, neither the extent nor magnitude of hematological effects differed among dose groups (Table 7). RBC parameters were unaffected

during the dosing period, but a mild transient regenerative anemia occurred postdose. This was characterized by slightly decreased RBC parameters (RBC count, hematocrit, hemoglobin) and increased red cell distribution width. Anemia had not completely resolved a month after dosing stopped, although RBC parameters approached normal values.

Total WBC counts increased slightly (<twofold) by the end of the dosing period and remained slightly increased for a month postdose. WBC count increases reflected an across-the-board increase in lymphocyte counts (about twofold) and granulocyte counts (neutrophils <twofold, monocytes and eosinophils about fourfold) observed in this study.

Platelet counts increased about sevenfold during the dosing period and returned to approximately normal levels within a month postdose. Increases in platelet counts were accompanied by an increased platelet distribution width and a decreased MPV.

Activated partial thromboplastin time was somewhat prolonged (10%) at the end of the dosing period, but returned to normal within a month postdose. Prothrombin time was unaltered (data not shown).

DISCUSSION

This report shows that in rats (i.v.), or cynomolgus (i.v. or s.c.) or rhesus (s.c.) monkeys, administration of GW395058 results in dose-proportional exposures with the peptide exhibiting a low CL/F and Vz/F, and a long plasma $t_{1/2}$. The pharmacokinetic behavior of GW395058 is similar in cynomolgus and rhesus monkeys. While repeated administration of GW395058 for a month at high doses stimulated megakaryocytopoiesis, granulocytopoiesis, and lymphocytopoiesis in both rats and cynomolgus monkeys, the nature and magnitude of the responses differed between species. Following repeated administration of GW395058, platelet counts increased more in monkeys than rats, while WBC counts increased more in rats than monkeys. Platelet count increases were accompanied by a decrease in average platelet size in monkeys but an increase in average platelet size in rats. These differences may reflect species differences in receptor sensitivity or inherent responses to receptor stimulation.

Hematologic effects produced by repeated high doses of GW395058 were reversible when dosing stopped, although recovery was prolonged in some cases. This prolonged recovery phase may reflect GW395058's long $t_{1/2}$, but it also may reflect the inherent rate at which altered hematopoietic cell counts return to homeostatic levels in the species tested.

The observation that hematological effects in repeat-dose studies did not differ with dose suggests that the dose regimen used was beyond that needed to elicit a maximum response. This in turn suggests that maximum responses

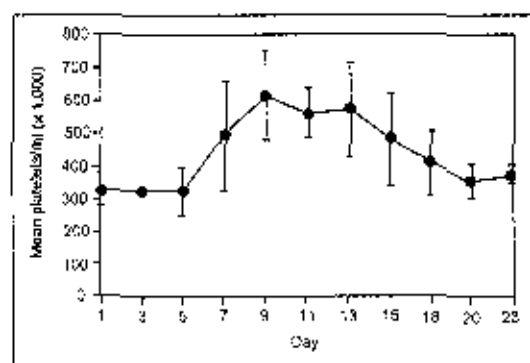


Figure 5. Platelet counts in male cynomolgus monkeys dosed with GW395058. Average platelet counts in monkeys are shown. Blood samples were obtained from monkeys (three/group) receiving a single s.c. dose of GW395058 at 100 $\mu\text{g/kg}$.

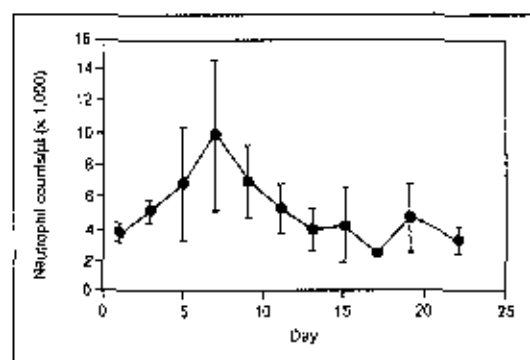


Figure 6. Neutrophil counts in male cynomolgus monkeys dosed with GW395058. Average segmented neutrophil counts in monkeys are shown. Blood samples were obtained from monkeys (three/group) receiving a single s.c. dose of GW395058 at 100 $\mu\text{g/kg}$.

Table 7. Hematological effects of GW395058 in cynomolgus monkeys after one-month repeat-dose studies. Changes in average hematological and coagulation parameters at the end-of-dose and postdose at two and four weeks are shown for monkeys receiving repeated i.v. doses at 500, 1,000, 2,000 $\mu\text{g/kg/48 h}$ for one month

Dose ($\mu\text{g/kg/48 h}$) =	Sample time					
	End-of-dosing				+ 2 weeks postdose	+ 4 weeks postdose
	0	500	1,000	2,000	2,000	
Platelet effects						
Platelet count ($\times 10^9/\text{mm}^3$)	381	1,811	2,823	2,405	2,143	692
PDW (%)	40.6	59.1	56.2	57.0	53.9	40.4
MPV (μm^3)	9.4	6.9	7.1	7.3	8.1	9.4
Granulocyte effects						
Neutrophil count ($/\text{mm}^3$)	4,400	6,900	7,390	5,700	4,400	3,200
Monocyte count ($/\text{mm}^3$)	450	1,150	1,550	1,550	2,000	850
Eosinophil count ($/\text{mm}^3$)	150	450	800	350	400	400
Lymphocyte effects						
Lymphocyte count ($/\text{mm}^3$)	5,900	9,400	8,100	11,300	14,080	10,860
RBC effects						
RBC count ($\times 10^6/\text{mm}^3$)	5.96	5.53	6.16	5.87	5.10	5.56
RDW (%)	14.8	15.4	15.7	15.4	17.5	16.7
Coagulation effects						
APT time (sec)	19.8	21.2	21.7	23.3	n.d.	n.d.

n.d. = not determined; PDW = platelet distribution width; RDW = red cell distribution width.

could be obtained with lower and/or less frequent doses. The dose regimen chosen made it impossible to characterize the dose-response curves for individual hematologic effects, so it is not possible to address the question here of whether the various effects were mediated by a similar molecular mechanism (i.e., action of GW395058 on a single receptor).

GW395058-induced thrombocytosis was not associated with hemorrhage or thrombosis, suggesting that induced platelets function normally, as has been shown with PEG-rHuMGDF [6, 13].

Because GW395058 produces increased numbers not only of circulating platelets that appear functionally normal, but also of circulating granulocytes and lymphocytes, the data presented here suggest that GW395058 has the potential to ameliorate damage or hasten recovery of multiple hematopoietic cell lines from chemotherapy-induced damage, as has been indicated for rHuTPO [20].

Although GW395058 has a long plasma $t_{1/2}$ in mice [18], rats, dogs and monkeys, the single s.c. dose required to double circulating platelet levels in monkeys is 50-fold that required in rats. In previous studies [17] GW395058 and rHuTPO were shown to bind with similar affinity to murine BaF3 cells transfected with the HuTPO α and equipotent in stimulating megakaryocyte colony formation and megakaryocyte maturation in cells cultured from human bone marrow *in vitro*. Additional studies will be required to determine if the observed differences in hematological responses between rodents and monkeys are due to a significantly reduced affinity of the monkey TPO α for GW395058, relative to the human and rodent receptors.

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Pharmacokinetics and Hematological Effects of the PEGylated Thrombopoietin Peptide Mimetic GW395058 in Rats and Monkeys After Intravenous or Subcutaneous Administration

Mark de Serres, Russ L. Yeager, John E. Dillberger, Guy Lalonde, Guy H. Gardner, Cathleen A. Rubens, Anita H. Simkins, Jeffrey M. Sailstad, Michael J. McNulty and Joseph L. Woolley
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