First-in-man Study With Inclacumab, a Human Monoclonal Antibody Against P-selectin

Christophe Schmitt, PharmD, Markus Abt, PhD,* Cornelia Ciorciaro, MD,* Dorothee Kling, PhD,* Candice Jamois, PharmD,* Eginhard Schick, PhD,* Corinne Solier, PhD,* Renée Benghozi, MD,* and Jacques Gaudreault, PhD†*

Abstract: Inclacumab, a novel monoclonal antibody against P-selectin in development for the treatment and prevention of atherosclerotic cardiovascular diseases, was administered in an ascending single-dose study as intravenous infusion to evaluate safety, pharmacokinetics, and pharmacodynamics. Fifty-six healthy subjects were enrolled in this randomized, double-blind placebo-controlled study. Each dose level (0.03–20 mg/kg) was investigated in separate groups of 8 subjects (6 on inclacumab, 2 on placebo). Platelet–leukocyte aggregates, free/total soluble P-selectin concentration ratio, drug concentrations, bleeding time, platelet aggregation, antibody formation, and routine laboratory parameters were measured frequently until 32 weeks. Pharmacokinetic profiles were indicative of target-mediated drug disposition. Platelet–leukocyte aggregate inhibition and soluble P-selectin occupancy showed dose dependency and were strongly correlated to inclacumab plasma concentrations, with IC_{50} of 740 and 4600 ng/mL, respectively. Inclacumab was well tolerated by the majority of subjects and did neither affect bleeding time nor platelet aggregation. These findings allowed the investigation of the potential beneficial therapeutic use of inclacumab in patient study.

Key Words: antiinflammatory, antithrombotic, healthy subject, monoclonal antibody, P-selectin, inclacumab

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INTRODUCTION

P-selectin, a cell adhesion molecule, is a component of the membrane of the α-granules of platelets and Weibel–Palade bodies of endothelial cells. On cellular activation, it can be rapidly translocated to the cell surface. Through interactions with its ligand, P-selectin glycoprotein ligand 1, P-selectin plays a critical role in leukocyte tethering and rolling on the vessel wall and subsequent extravasation. It also promotes platelet rolling and adhesion to the activated vessel wall.[1–5] A truncated soluble form of P-selectin, which is either released by proteolytic cleavage of the cell-expressed P-selectin or by secretion of an alternatively spliced form of P-selectin lacking the cytoplasmic domain, is circulating in blood.[6–8] Soluble P-selectin has been shown to exert prothrombotic and procoagulant activities.[9,10] P-selectin plays therefore a role at the interface of inflammation and thrombosis. In clinics, increased expression of P-selectin on endothelial cells and/or platelets as well as increased plasma concentrations of soluble P-selectin has been reported in a variety of cardiovascular disorders, including peripheral arterial disease,[11] coronary artery diseases,[12,13] diabetes,[14] hypertension,[15] hypercholesterolemia,[16] venous thromboembolism,[17] and atrial fibrillation.[18] In animal models, blockade of P-selectin functions has been shown to inhibit atherosclerosis development,[11,19] thrombus growth and fibrin deposition,[20,21] and ischemia-induced tissue injury.[22,23]

Inclacumab is a recombinant human monoclonal antibody of the immunoglobulin G4 subclass directed against human P-selectin, which is expressed in Chinese hamster ovarian cell line. It has 2 single point mutations (L235E, S228P) introduced into the Fc part to avoid antibody-dependent cell-mediated cytotoxicity and to improve structural stability. The variable regions were derived from transgenic mice that were immunized with soluble P-selectin antigen (technology licensed by Genmab A/S) and the resulting hybridoma. It binds to human P-selectin with high affinity (in nM range) and shows selectivity for P-selectin (>3000-fold) compared with the other members of the selectin family (E-selectin and L-selectin). This selectivity is an essential safety requirement because blockade of P-selectin and E-selectin or of P-selectin and L-selectin results in an immunocompromised phenotype based on evidence from double-selectin knockout mice.[24] Inclacumab inhibits P-selectin–mediated functions and has been shown to exert antiinflammatory and antithrombotic activity. It inhibited the adhesion of leukocytes to endothelial cells in an ex vivo human flow system and reduced the infiltration of leukocytes into a nonhuman primate inflammation model. Furthermore, it attenuated leukocyte adhesion to platelet monolayers ex vivo and formation of platelet–leukocyte aggregates (PLA).[25] Through its inhibitory effects on inflammatory and thrombotic cascades, inclacumab is expected to beneficially interfere with the basic events underlying atherosclerosis. It is therefore being developed for the treatment and prevention of atherosclerotic cardiovascular diseases. First indication of its efficacy in clinics was obtained in a recently completed trial,[26] where it seemed to reduce the myocardial damage after percutaneous coronary intervention in non–ST-segment elevation myocardial infarction patients.

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From the *F. Hoffmann-La Roche AG, Basel, Switzerland; and †JJG Pharma Consulting, GmbH, Basel, Switzerland.
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Reprints: Christophe Schmitt, PharmD, Department of Clinical Pharmacology, F. Hoffmann-La Roche AG, CH-4070 Basel, Switzerland (e-mail: christophe.schmitt@roche.com).

The present single ascending dose study was the first clinical study carried out with inclacumab to assess its safety, tolerability, pharmacokinetics, and pharmacodynamics in healthy subjects. Results of this study provided rationale for the selection of doses in the proof-of-concept trial.

METHODS

Subjects

Healthy male and female subjects aged 18–65 years and of body mass index between 18 and 30 kg/m^2 participated in this study. Female subjects had to be surgically sterile or postmenopausal. Subjects were fully informed of the purpose of the study and risks involved and gave written informed consent before enrollment in the study. Eligible subjects were required to have hematology (including clotting times) and biochemistry panels, platelet aggregation, and bleeding time within normal limits, as well as negative serology (hepatitis A, B, C, and HIV) and drugs of abuse tests (including cannabinoids, amphetamines, opiates, methadone, cocaine, benzodiazepines, and barbiturates). Subjects were excluded if they had inflammatory diseases or infections within 3 months before the start of the study. All subjects were free of other medications for at least 14 days before the study. With the exception of paracetamol and medications to treat adverse events (AEs), no medications were permitted during the study. Antiplatelet drugs and anticoagulants were absolutely prohibited. Consumption of xanthine-containing food and alcohol and the use of tobacco were not permitted while resident in the unit and were limited once discharged [no more than 6 cups of coffee (or equivalent) per day, no more than 21 units of alcohol for males or 14 units for females per week, no more than 5 cigarettes per day] until the end of their participation in the study. Subjects were kept hospitalized for 72 hours after administration of the study drug.

Study Design

This single ascending dose study was designed as a double-blind, randomized, and placebo-controlled study. Seven dose steps were planned (0.03, 0.1, 0.3, 1, 3, 10, and 20 mg/kg). In each dose step, 6 subjects were to receive inclacumab and 2 subjects to receive placebo. The randomization numbers were allocated sequentially in the order in which the subjects were enrolled. Escalation to each new dose step was dependent on the complete review of safety (clinical laboratory tests, vital signs, and ECG), tolerability (AEs), platelet aggregation, and bleeding time data over 72 hours after dose. Dose escalation was stopped if one of the following circumstances occurs in 3 or more subjects on active treatment within the same dose group: severe drug-related AEs of the same character, clinically significant laboratory abnormalities of the same character, clinically significant changes in vital signs or ECGs of the same character, or medically significant infections in 2 or more subjects. The study was approved by the Welwyn Clinical Pharmacology Ethics Committee (Welwyn Garden City, United Kingdom) and was conducted in full conformance with the Declaration of Helsinki, Good Clinical Practice guidelines, and the British law.

Test Compound

Inclacumab (15 mg/mL) and placebo were manufactured by Roche (Basel, Switzerland) and supplied in vials containing 5 mL sterile solution. The infusion bags were prepared at LCG Bioscience (Cambridge, United Kingdom) with normal saline. Study drugs were administered at room temperature by controlled infusion into a forearm vein over a 2-hour period to enable discontinuation of study drug administration in the event of the occurrence of significant AEs.

Safety and Tolerability

Safety and tolerability were assessed for until 16 weeks (doses <3 mg/kg) and 32 weeks (doses ≥ 3 mg/kg) after administration of inclacumab by monitoring of vital signs, ECGs and clinical laboratory tests (hematology, biochemistry, urinalysis), clotting times (prothrombin time and activated partial thromboplastin time), bleeding time, platelet aggregation, and levels of antidrug antibodies (ADAs). Subjects were questioned about any AEs that they might have experienced, and events were also reported by subjects spontaneously. AEs were graded on a 3-point scale according to their severity (mild, moderate, and severe). Additionally, subjects were carefully monitored for signs of infections during the entire study conduct.

Modified Ivy bleeding time test was used to assess potential bleeding risk. After a sphygmomanometer cuff was placed round the subject's arm and inflated to a pressure of 40 mm Hg, 3 punctures were made using a diabetic gun in the flexor aspect of the forearm. The blood was absorbed using the edge of filter paper until all bleeding ceased. Bleeding time was recorded as the mean time from all 3 punctures.[27]

Platelet aggregation assay was performed using the Platelet Function Analyzer instrument (PFA-100; Dade Behring, Marburg, Germany), which aspirated an anticoagulated blood sample under standardized high-shear conditions through a 150-μm-diameter collagen-coated aperture in the presence of adenosine diphosphate or epinephrine.[28] These resulted in platelet activation and aggregation, slowly building a stable platelet plug at the aperture. The assay measured the time required to occlude the aperture (closure time).

Sample Collection and Analysis

Blood samples (5 mL) for pharmacokinetic analyses were collected with a 19-gauge butterfly needle in EDTA containing plastic tube at predose and at 1, 2, 3, 5, 8, 14, 24, 48, 72, and 96 hours and 7, 14, 21, 28, 42, 56, 70, 84, and 98 days after dose. Additional samples were collected on day 112 for subjects in cohorts dosed with less than 3 mg/kg or on days 140, 168, 196, and 224 for subjects in cohorts dosed with 3 mg/kg or more. The blood samples were centrifuged at 1500*g* and 4°C for 10 minutes to collect plasma. Plasma samples were stored below −80°C until analyzed. Plasma concentrations of inclacumab were determined by a validated sandwich enzyme-linked immunosorbent assay (ELISA) at Roche bioanalytical department. The calibration range was 1.64–400 ng/mL. The precision and accuracy of the assay ranged from 8.1% to 11.1% and from 92.3% to 102.7%, respectively. The lower limit of quantification was 1.64 ng/mL in plasma. A

dilution factor of 10 was used for predose and placebo samples and of 10–100,000 for inclacumab-containing samples. The pharmacokinetic parameters of inclacumab were calculated from the plasma concentration–time data using noncompartmental methods[29] with the aid of WinNonlin software (version 5.1; Pharsight, Mountain View, CA).

ADAs were qualitatively detected in human plasma by a validated electrochemiluminescence immunosorbent assay from the same plasma samples as for the pharmacokinetic assessments. The analysis was performed by Roche bioanalytical department at baseline and at follow-up visits (on day 112 for subjects in cohorts dosed with less than 3 mg/kg or on day 224 for subjects in cohorts dosed with 3 mg/kg or more). The electrochemiluminescence immunosorbent assay had a sensitivity of 7 ng/mL and provided a drug tolerance factor of about 50-fold.

The pharmacodynamic effects of inclacumab were assessed by determination of PLA and the measurement of soluble P-selectin plasma concentrations.

Blood samples (3.8 mL with the first 2 mL being discarded) for the measurement of PLA were collected with a 19-gauge butterfly needle in plastic tubes containing 108 nM sodium citrate at predose and at 1, 2, 4, 14, 24, 48, and 96 hours and 14, 28, 56, and 84 days after dose. Additional samples were collected on day 112 for subjects in cohorts dosed with less than 3 mg/kg or on days 140, 168, 196, and 224 for subjects in cohorts dosed with 3 mg/kg or more. Flow cytometric measurements were performed in whole blood within 2 hours of collection using thrombin receptor-activating peptide (TRAP) stimulation as described elsewhere.[25] The analysis was performed by LCG Bioscience (Cambridge, United Kingdom). Intraassay and interassay precision was lower than 11% and 14.7%, respectively.

Plasma concentrations of total and free soluble P-selectin (soluble P-selectin not bound to inclacumab) were determined by ELISA (Human sP-Selectin/CD62P ELISA Kit, R&D Systems, Inc Minneapolis, MN) from the same plasma samples as for the pharmacokinetic assessments. The analysis was performed by Roche biomarker department at the following time points: predose, 3, and 24 hours, and 7, 28, and 84 days after dose. An additional time point was analyzed on day 112 for subjects in cohorts dosed with less than 3 mg/kg or on day 224 for subjects in cohorts dosed with 3 mg/kg or more. The free soluble P-selectin was obtained by immunodepletion on an immunoaffinity column (PROTIA Sigma ProteoPrep Immunoaffinity Albumin & IgG Depletion Kit, Saint-Louis, MO) that depletes albumin and immunoglobulin from plasma samples. The lower limit of quantification of the ELISA for soluble P-selectin was 0.2 ng/mL in human plasma. Interassay precision was lower than 9.9%. Results are presented as ratio of free over total soluble P-selectin concentrations and were expressed as percent.

Statistical Analysis

All pharmacokinetic and pharmacodynamic parameters were subjected to descriptive analysis, including arithmetic mean values, SD, geometric mean values, medians, coefficients of variation, and ranges. Because of the obvious nonlinear pharmacokinetics, no formal statistical analysis of variance for dose proportionality was performed.

Pharmacokinetic/Pharmacodynamic Relationships

The data of all subjects were pooled and analyzed together (naive-pooling). Concentration–effect relationships for the ratio of free/total soluble P-selectin concentration and the PLA were plotted over time to check the absence of a delay in response (hysteresis). Pharmacokinetic/pharmacodynamic relationships were analyzed using WinNonlin software (version 5.1; Pharsight, Mountain View, CA). Simple or sigmoid inhibitory E_{max} model with or without E_{min} were tested for best fit, and selection was made on the basis of the Akaike Information Criterion. Model diagnosis was also performed by visual analysis of the weighted residual plots and by observation of the relative standard error of the estimated parameters. The parameter estimates and associated relative standard error were reported.

Both relationships between inclacumab concentration and PLA and between inclacumab concentration and free/total soluble P-selectin concentration ratio were well described by a sigmoid inhibitory E_{max} model:

$$E = E_0 - \left[(E_0 - E_{min}) \times \frac{C^{\gamma}}{C^{\gamma} + IC_{50}^{\gamma}} \right],$$

where E is the pharmacodynamic effect [PLA (%) or ratio of free/total soluble P-selectin concentration (%)], E_0 is the pharmacodynamic effect at baseline (%), E_{min} is the pharmacodynamic effect at infinite inclacumab concentration (%), C is the inclacumab plasma concentration (ng/mL), IC_{50} is the inclacumab concentration causing half of the maximum effect (ng/mL), and γ is the sigmoid shape factor.

The potential effect of inclacumab on clotting times (prothrombin time and activated partial thromboplastin time) and markers of platelet function (bleeding time and platelet aggregation triggered with adenosine diphosphate or epinephrine) were visually investigated. Data points without inclacumab concentration (below the limit of quantification or from placebo subjects) were assigned to a concentration value of 1 ng/mL to appear on the graphs.

RESULTS

Study Population

A total of 56 subjects were enrolled in the study. Age (mean ± SD) was 44 ± 12 years (range 23–63 years), body weight was 78 ± 12 kg (range 48–103 kg), and body mass index was 26.0 ± 2.8 kg/m^2 (range 19.7–29.8 kg/m^2). Fifty-three subjects were White, 2 Black, and 1 Asian. There were 35 male and 21 female. On average, females were older (50.0 vs. 40.1 years) and lighter (71.9 vs. 81.6 kg) than male subjects. A summary of demographic data by dose groups is given in Table 1. All 56 subjects completed the study per protocol and were included in the analyses of safety, pharmacokinetics, and pharmacodynamics.

Pharmacokinetics

After a 2-hour intravenous infusion, maximum concentrations were reached at the end of the infusion or shortly after the infusion finished. Profiles of mean plasma inclacumab concentrations versus time after intravenous infusion clearly showed nonlinear pharmacokinetics and were indicative of target-mediated drug disposition (TMDD) (Fig. 1), that is, elimination of inclacumab because of binding to its pharmacological target. The elimination was concentration-dependent, that is, more rapid at lower concentrations (doses). Visual inspection of the pharmacokinetic profiles indicated however that for inclacumab concentrations above a certain threshold (approximately 10,000 ng/mL), the elimination appeared linear (Fig. 1). Clearance was faster at the lowest dose (23 $mL \cdot d^{-1} \cdot kg^{-1}$) and decreased with increasing doses to approximately 1.5 $mL \cdot d^{-1} \cdot kg^{-1}$ with the top doses. The volume of distribution (V_{ss}) was limited (~50 mL/kg) and approximated the plasma volume, suggesting that the distribution of the antibody was limited to the vascular compartment. Gender difference was not apparent on pharmacokinetic profiles. The overall variability of the pharmacokinetic parameters of inclacumab was moderate, with intersubject coefficients of variation lower than 20% for AUC_{last} and C_{max}. The pharmacokinetic parameters are summarized in Table 2.

Pharmacodynamics

The time courses of TRAP-activated PLA and free/total soluble P-selectin concentration ratio are displayed in Figures 2A, B, respectively. The percentage of ex vivo -induced PLA at baseline amounted to 45% on average. The changes in percentages of PLA corresponded well to the time course of inclacumab concentrations. Infusion of placebo had no effect on PLA, whereas infusion of inclacumab caused a rapid and dose-dependent inhibition of PLA formation. The maximum effect was reached at the end of the infusion (ie, corresponding to the t_{max} of inclacumab) and attained 4% with the highest doses. The duration of inhibition of PLA formation was also dose-dependent. Almost complete inhibition was maintained for 4, 8, and 20 weeks after intravenous infusion of 1, 3, and 20 mg/kg, respectively. Similar findings were observed with the ratio of free/total soluble P-selectin concentrations. Infusion of placebo had no effect on soluble P-selectin concentration ratio, which oscillated between 50% and 60%. After inclacumab treatment, the ratio declined immediately after the end of infusion to reach a nadir of approximately 5% with 20 mg/kg dose. The extent and duration of effect increased with doses. Only highest doses of inclacumab were able to maintain a maximum effect for 4 weeks with a free/total P-selectin concentration ratio close to 7%.

Pharmacokinetic/Pharmacodynamic Relationship

Plasma concentrations of inclacumab were linked to percentages of TRAP-activated PLA and to free/total soluble P-selectin concentration ratio using a sigmoid inhibitory E_{max} model (Figs. 3A, B). Both parameters were strongly correlated to inclacumab plasma concentrations. The concentrations required to inhibit 50% (IC_{50}) of TRAP-activated PLA and free/total soluble P-selectin concentration ratio were 740 ng/mL and 4600 ng/mL, respectively (Table 3).

Safety and Tolerability

There were overall no relevant differences in the incidence of AEs between placebo and active treatment groups, although the incidence of headache was higher in the 20 mg/kg group (5/6) than in any of the other groups. AEs were reported by 12 of 14 subjects (86%) treated with placebo and by 36 of the 42 subjects (86%) treated with inclacumab. The most commonly reported AEs were headache, cough, oropharyngolaryngeal pain, and upper respiratory tract infection. A majority of AEs were of mild intensity, and only 1 event—a headache in a subject dosed with 20 mg/kg—was of severe intensity.

Subjects were also carefully monitored for signs of infections. Altogether, 23 subjects reported events of infections, the majority of these events being upper respiratory tract infections. All of them had resolved by the end of the study. Five subjects of 14 (36%) dosed with placebo reported an event of infection compared with 18 subjects of 42 (42%) dosed with inclacumab (all doses). No trend of increased rate of infections with dose was discernable.

One serious AE (rhabdomyolysis) was reported 2 weeks after administration of inclacumab 0.1 mg/kg. This event was characterized by an increase in creatine phosphokinase (CPK, 120,000 U/L) and transaminases (< 10-fold the upper limit of normal [ULN]) without associated clinical symptoms and no change in the kidney function. It resolved by itself within 2 weeks without sequels and was considered to be possibly drug-related by the investigator.

TABLE 1. Summary of Demographic Data

	Placebo (n = 14)	0.03 mg/kg (n = 6)	0.1 mg/kg (n = 6)	0.3 mg/kg (n = 6)	1 mg/kg (n = 6)	3 mg/kg (n = 6)	10 mg/kg (n = 6)	20 mg/kg (n = 6)
Gender								
Male	6	3	2	5	4	6	4	5
Female	8	3	4	1	2	0	2	1
Age (yr)	45.9 (9.3)	46.2 (13.5)	43.0 (11.5)	40.3 (15.7)	43.8 (14.4)	35.0 (9.9)	53.0 (7.5)	40.3 (11.3)
Weight (kg)	72.4 (9.7)	70.4 (13.5)	79.8 (9.2)	79.8 (9.5)	77.7 (21.5)	79.3 (13.9)	85.0 (10.6)	86.8 (6.5)
BMI (kg/m^2)	25.3 (2.6)	24.2 (3.4)	27.1 (1.7)	26.0 (3.4)	25.1 (3.5)	25.4 (2.8)	27.9 (1.6)	27.5 (2.1)

No. subjects for gender, and mean (±SD) for all other parameters.
BMI, body mass index.

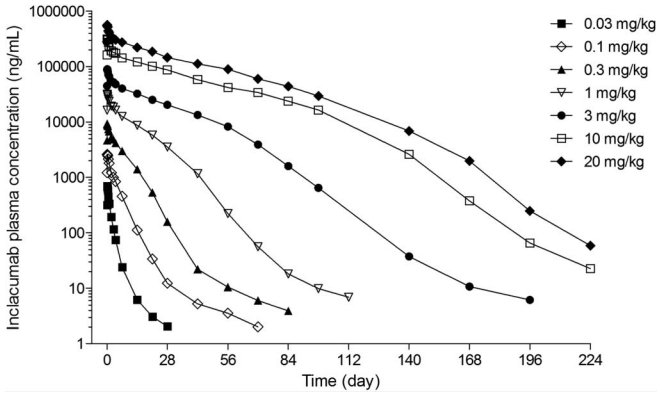

FIGURE 1. Plasma concentration–time profiles of inclacumab after single intravenous infusion (log-linear scale).

No clinically relevant changes in vital signs, laboratory parameters, or ECGs were otherwise reported. The most common laboratory abnormality, reported by 8 subjects, was elevated CPK, which seemed to be evenly distributed across treatment groups including placebo. With the exception of the serious AE detailed above, these increases were limited to 2-fold to 4-fold above the ULN. There were also no changes in coagulation parameters, bleeding time, and platelet aggregation associated with inclacumab (Figs. 4A–E). Two subjects treated with inclacumab (0.1 and 0.3 mg/kg) tested positive for ADA at the follow-up visit.

DISCUSSION

Inclacumab is a recombinant human monoclonal antibody directed against human P-selectin. In this first-in-man study, inclacumab was administered to healthy volunteers as 7, single ascending intravenous doses ranging from 0.03 to 20 mg/kg. The safety, immunogenicity, pharmacokinetics, and pharmacodynamics were investigated.

The nonlinear pharmacokinetics of inclacumab was obvious (Fig. 1). Such profiles were indicative of TMDD,[30] with 2 types of elimination mechanisms: the antigen-mediated saturable clearance and the nonspecific linear clearance by the reticuloendothelial system.[31] At low concentrations, a nonlinear elimination pathway of inclacumab is believed to represent a target-mediated clearance process because of binding to cell-expressed P-selectin and possibly soluble P-selectin. At higher concentrations (above 10,000 ng/mL), this pathway becomes saturated and elimination of inclacumab appears linear; like other antibodies, inclacumab is then believed to be mainly catabolized in tissues throughout the body by the reticuloendothelial system. Total clearance is therefore concentration-dependent, and estimates of elimination half-life from noncompartmental analysis are not very informative. A TMDD modeling, more appropriate in such situation, will be the subject of a dedicated publication. For the highest doses, changes in clearance became small, indicating that the P-selectin-mediated clearance was almost saturated. Total clearance was then mainly determined by the nonspecific linear clearance. Considering high concentrations only, where elimination appeared linear, an apparent "terminal half-life" of approximately 3–4 weeks could be derived. This half-life is similar to that of other human IgG antibodies.[32]

The 2 investigated pharmacodynamic markers, free/total soluble P-selectin concentration ratio and TRAP-activated PLA, demonstrated target engagement and functional P-selectin inhibition, respectively.

Because free soluble P-selectin in this particular setting was defined as soluble P-selectin that was not bound to inclacumab, free/total soluble P-selectin concentration ratio represented soluble P-selectin (receptor) occupancy. Free soluble P-selectin was obtained by immunodepletion of albumin and immunoglobulin from plasma sample. Inclacumab, which is an immunoglobulin, remained retained on the immunoaffinity column, and consequently soluble P-selectin bound to inclacumab as well. Only soluble P-selectin that was not bound to inclacumab, that is, "free," was released. In the absence of inclacumab, a full recovery of soluble P-selectin is to be expected with a ratio of 100%. This was not the case with the analytical method used where unspecific binding occurred. Prestudy method validation demonstrated that the recovery was constant, reaching on average 49.6% across a range of 5.2–180.4 ng/mL of soluble P-selectin concentration. The recovery although incomplete was further showed to be consistent in the study, and ratio amounted to 56% on average in predose and placebo samples.

Inclacumab administration decreased this ratio in a dose-dependent manner, and, with the highest doses, an almost complete soluble P-selectin occupancy was attained.

Elevated circulating PLA have been associated with diverse cardiovascular diseases.[33] Levels of PLA in the circulation are directly associated with platelet expression of P-selectin and consequently has been reported to be relatively low in healthy subjects.[34,35] TRAP activation was therefore

TABLE 2. Pharmacokinetic Parameters of Inclacumab After Single 2-hour Intravenous Infusion of Inclacumab

Parameters*	0.03 mg/kg	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg	10 mg/kg	20 mg/kg
C_{max} (μg/mL)	0.713 (12.3)	2.65 (13.7)	9.35 (12.3)	32.5 (12.2)	90.5 (10.5)	322 (12.0)	566 (10.7)
t_{max} (h)	2.08 (2.0–5.03)	3.00 (2.03–8.03)	2.51 (2.0–3.0)	2.00 (2.0–3.08)	3.03 (2.0–5.0)	2.60 (2.0–5.0)	3.00 (2.25–3.08)
AUC_{last} (μg·d/mL)	1.28 (19.4)	10.4 (14.6)	60.0 (12.8)	335 (8.4)	1519 (16.2)	7089 (7.7)	13,143 (11.2)
CL (mL·d^{-1}·kg^{-1})	23.0 (19.4)	9.56 (14.6)	4.99 (12.8)	2.98 (8.4)	1.97 (16.2)	1.41 (7.7)	1.52 (11.2)
V_{ss} (mL/kg)	79.0 (11.8)	51.2 (11.2)	38.3 (9.4)	39.5 (10.8)	46.8 (9.1)	49.2 (4.2)	54.9 (6.5)

*Median (min–max) for t_{max}, and geometric mean (percentage coefficient of variation) for all other parameters.

AUC, area under the plasma concentration versus time curve; AUC_{last}, AUC from time zero to the last measurable concentration; C_{max}, maximum observed plasma concentration; CL, total clearance; t_{max}, time to C_{max}; V_{ss}, volume of distribution at steady state.

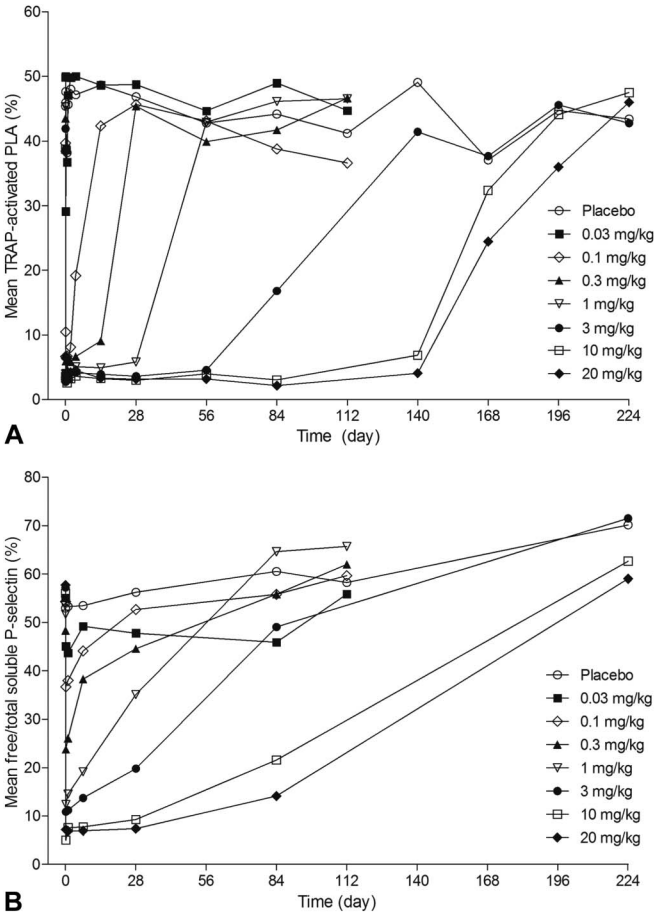

FIGURE 2. Time course of TRAP-activated PLA (A, Reprinted from Kling et al. *Thomb Res* 2013; 131:401–410) and free/total soluble P-selectin ratio (B) after single intravenous infusion of inclacumab or placebo. Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

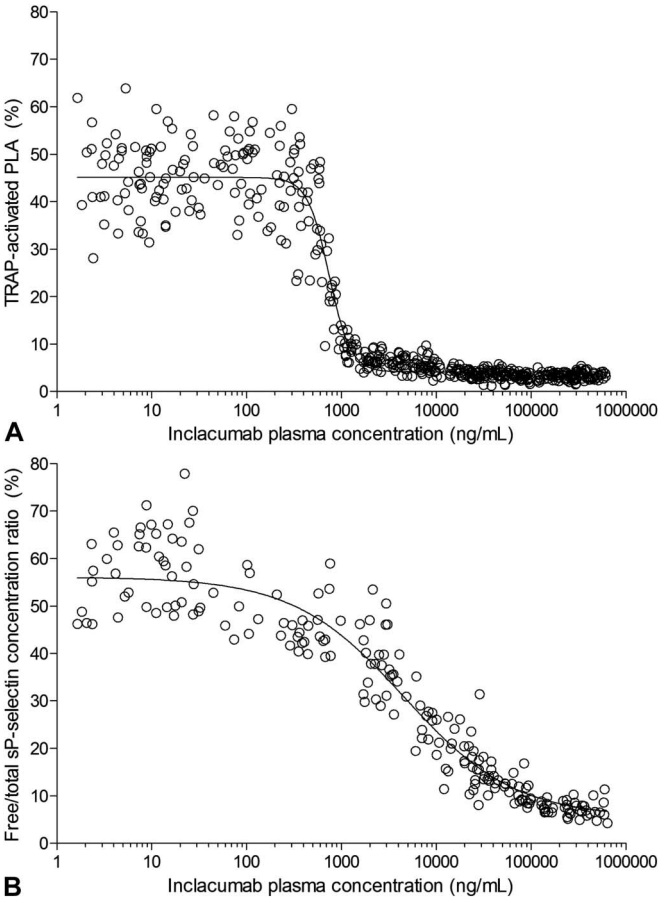

FIGURE 3. Pharmacokinetic/pharmacodynamic relationships. Correlation between inclacumab plasma concentration and PLA (A), and inclacumab plasma concentration and soluble P-selectin ratio (B). ○Observed; ——Model.

used in this study to promote formation of PLA. Activated platelets may then adhere to leukocytes and form mixed aggregates. Approximately 45% of leukocytes were present in the form of aggregates with platelets after TRAP activation in predose and placebo samples. Blockade of P-selectin at the surface of platelets by inclacumab prevented the formation of PLA.

Both pharmacodynamic markers were well correlated with inclacumab plasma concentrations (Fig. 3). The inhibition of PLA formation was more affected by inclacumab (IC_{50} = 740 ng/mL) than the decrease of soluble P-selectin ratio (IC_{50} = 4600 ng/mL). Difference in affinity for these 2 markers is intriguing because both mechanisms involved binding to P-selectin. This could be explained by the different nature of P-selectin involved. PLA formation is mediated through P-selectin present on the surface of platelets predominantly in a dimeric form, whereas soluble P-selectin circulates in the plasma of healthy subjects essentially in a monomeric form.[6,36] In vitro, inclacumab was shown to bind to cell-expressed P-selectin with a higher affinity (assessed with a cell-based ELISA measuring binding of inclacumab to human P-selectin on Chinese hamster ovarian transfectants) than to soluble P-selectin (assessed with a Biacore assay). These 2 PK/PD relationships also showed that maximum inhibition was reached at concentrations greater than 10,000 and 200,000 ng/mL for PLA and soluble P-selectin ratio, respectively.

Doses up to 20 mg/kg of inclacumab were deemed to be safe and well tolerated by the majority of subjects. The most common AEs were headache, cough, sore throat, and upper

TABLE 3. Pharmacokinetic/Pharmacodynamic Parameter Estimates

	PLA		Free/Total P-Selectin Ratio	
Parameters	**Estimates**	**RSE (%)**	**Estimates**	**RSE (%)**
E_0 (%)	45.1	1.00	56.1	3.01
IC_{50} (ng/mL)	740	2.66	4600	13.6
E_{min} (%)	4.1	6.68	5.4	11.2
γ (−)	3.83	8.71	0.75	8.05

E_0, baseline effect; E_{min}, minimum effect; RSE, relative standard error; γ, sigmoid shape factor.

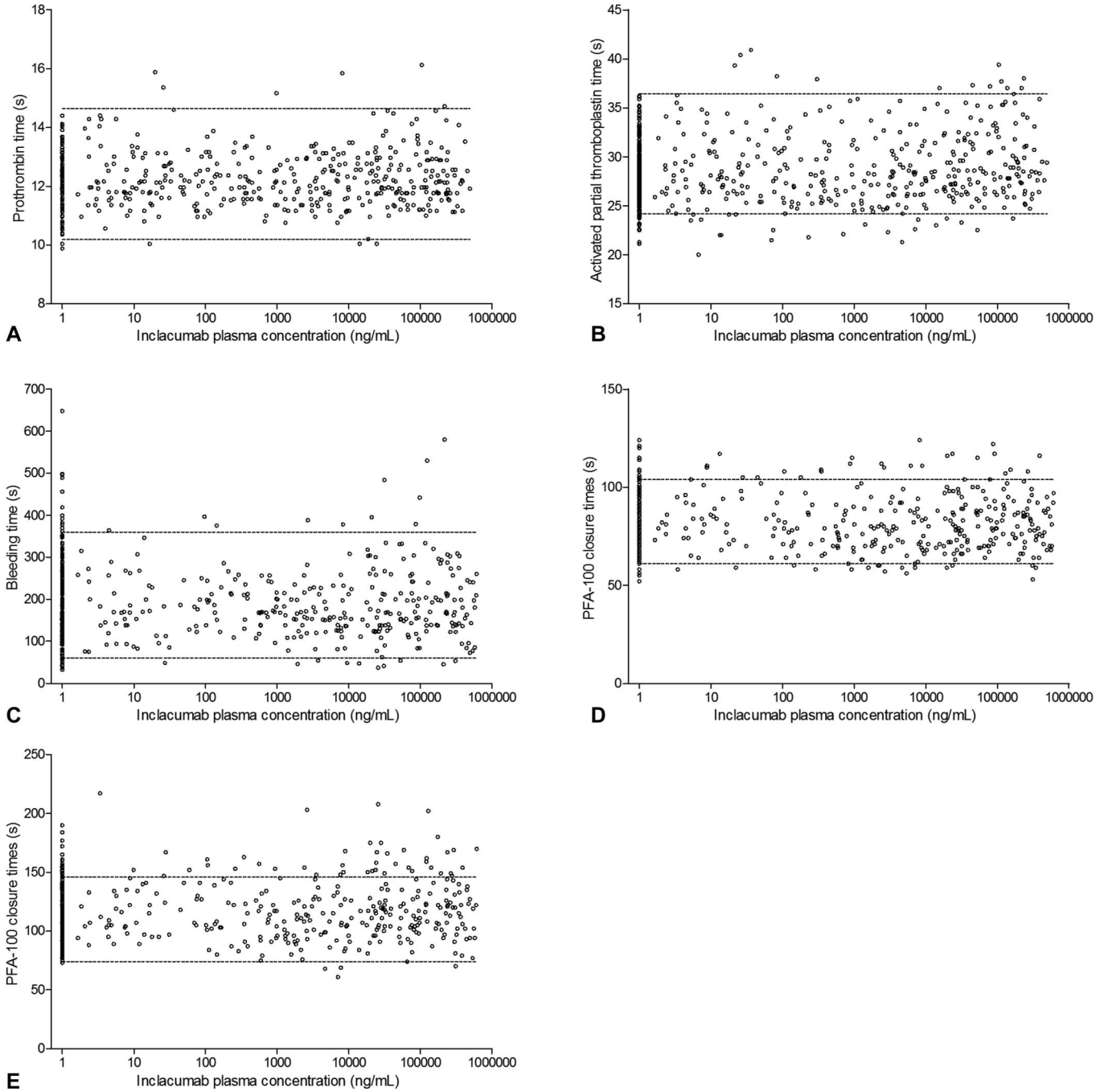

FIGURE 4. Clotting times and markers of platelet function versus inclacumab plasma concentration; prothrombin time (A), activated partial thromboplastin time (B), bleeding time (C), and PFA-100 closure time with collagen/adenosine diphosphate cartridges (D) and with collagen/epinephrine cartridges (E). ○Observed; —Lower and upper limits of normal.

respiratory tract infection, and the majority of AEs were of mild intensity. There were no relevant differences in the incidence of AEs between placebo (86%) and active treatment groups (86%). This relatively high incidence should be looked at in line with the duration of the study of up to 32 weeks after infusion of the drug. Overall, the pattern and nature of AEs were similar in the placebo and the active-treated groups.

Owing to the theoretical risk for an increased rate of infections under P-selectin blockade,[37,38] subjects were carefully monitored for signs and symptoms of infections in this study. A minor imbalance in the proportion of subjects reporting an infection event was noted with inclacumab (42%) compared with placebo (36%), but no trend of increased rate of infections with dose was discernable. Further monitoring in future larger studies is required to allow conclusions about this risk.

One serious AE, an asymptomatic rhabdomyolysis, occurred 2 weeks after infusion of inclacumab 0.1 mg/kg and consisted of marked elevation of CPK. It resolved on its own within 2 weeks. Mainly because of its timing of occurrence and in absence of possible other causes, this event was considered to be possibly drug-related by the investigator. It has been suggested that P-selectin is involved in repair of muscle fibers and actually, P-selectin inhibition has been shown to suppress muscle regeneration after injury.[39] As a matter of fact, the most common laboratory abnormalities observed in this study were elevated CPK. However, these occurred in almost all dose groups including placebo.

The role of P-selectin in hemostasis has been evidenced in P-selectin-deficient mice, where bleeding time after amputation of the tip of the tail was prolonged (+40%) compared with wild type mice,[40] although existing clinical data with P-selectin antagonist did not indicate any prolongation bleeding time.[41] Coagulation tests, including clotting times, bleeding time, and platelet aggregation, were therefore intensively monitored in this study, and none of these tests were affected by inclacumab (Figs. 4A–E).

Two subjects treated with inclacumab tested positive for ADA at the follow-up visit. They did not present allergic reactions, and the presence of ADA had no impact on their safety (platelet count or bleeding time), pharmacokinetic or pharmacodynamic profiles.

CONCLUSIONS

Overall, inclacumab was well tolerated by the majority of subjects after single intravenous infusion and demonstrated pharmacological activity against both cell-expressed P-selectin and soluble P-selectin. Altogether these findings allowed inclacumab to be further tested in a proof-of-concept study in patients.

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