Assessment of Dose Proportionality, Absolute Bioavailability, and Immunogenicity Response of CTLA4Ig (BMS-188667), a Novel Immunosuppressive Agent, Following Subcutaneous and Intravenous Administration to Rats

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Purpose. The objectives of this study were: to delineate the pharmacokinetics of CTLA4Ig in rats after single and multiple intravenous (IV) and subcutaneous (SC) doses; to assess the relationship of the pharmacokinetic parameters of CTLA4Ig vs dose; to calculate the SC absolute bioavailability; and to assess the antibody response of CTLA4Ig.

Methods. A total of 48 (24 male and 24 female) Sprague Dawley rats were divided into eight treatments with 3 rats per gender in each group: a single dose of 10, 80, or 200 mg/kg of CTLA4Ig given either IV or SC and a repeated dose of 10 mg/kg (once every other day for 7 doses over 13 days) given either SC or IV. Serial blood samples were collected up to 43 days after single dose administration and up to 50 days following the administration of the last multiple dose on day 13. The serum concentration of CTLA4Ig and anti-CTLA4Ig antibodies were measured using ELISA assays.

Results. After single IV doses, Cmax and AUCinf increased in a dose proportional manner; CL appeared to be dose independent, while both V1/2 and T1/2 increased as the administered dose increased. Following single SC doses, Cmax and AUCinf increased in a linear manner but not proportionally; mean T1/2 values were prolonged but similar among the three dose levels, while T1/2 increased as the administered dose increased. The absolute SC bioavailability of CTLA4Ig decreased as the dose increased from 10 (62.5%), 80 (55.7%), and 200 mg/kg (41.1%). Comparison of the AUCinf values between the first and last doses suggested an accumulation (3.1–4.7) of CTLA4Ig. However, regardless of the route of dosing, AUCinf after the last dose were comparable to AUCinf values following the single dose. Anti-CTLA4Ig antibodies were detected at the 10 mg/kg dose level after single or multiple doses for both routes of administration. However, regardless of single or multiple doses, antibody titers were relatively greater for the SC compared to the IV administration.

Conclusions. The key findings of this study were: (i) the elimination characteristics of CTLA4Ig were comparable between the SC and IV routes; (ii) the repeated dosing did not alter the pharmacokinetics of CTLA4Ig; (iii) the SC absolute bioavailability tended to decrease as the administered dose increased; and (iv) a greater formation of anti-CTLA4Ig antibodies was observed after SC compared to IV at a single 10 mg/kg dose level; however, after multiple dosing, the formation of antibodies from either of the two routes was relatively slower, and (v) during the study period, no antibodies were observed at either the 80 or 200 mg/kg dose levels regardless of the route of administration.

KEY WORDS: CTLA4Ig; intravenous; subcutaneous; pharmacokinetics; immunogenicity; rats.

INTRODUCTION

CTLA4Ig (BMS-188667) is a recombinant human fusion protein consisting of the extracellular domain of human CTLA-4 and the Fc region (hinge, CH2 and CH3) domains of the human IgG. It has demonstrated immunosuppressive activity and the ability to induce immunogenic tolerance in several in vivo animal models that are associated with T-cell dependent antibody response, i.e., autoimmunity, transplantation, and graft-versus-host-disease (GVHD) (1–6). In a mouse model, suppression of a T-cell dependent antibody response against sheep red blood cells (SRBCs) and keyhole limpet hemocyanin was observed following CTLA4Ig treatment (7). Similar suppression of an antibody response against SRBCs was recently noted in a monkey model following multiple dose treatment with CTLA4Ig (8). The observed suppression of lupus-like disease in NZB/NZW F1 mice, and partial protection against the occurrence of autoimmune glomerulonephritis in rats following CTLA4Ig dosing demonstrates the utility of CTLA4Ig in autoimmune disease models (9–11). Upon treatment of mice in GVHD models with CTLA4Ig, there was a decrease in the lethal effects of the disease (4,12). The attainment of donor-specific immunogenic tolerance has been reported following CTLA4Ig treatment; induction of tolerance to heart allografts in rats (13), marine skin allografts (14) and vascularized cardiac allografts (15). In addition, CTLA4Ig has been shown to promote donor specific tolerance in diabetic mice transplanted with human pancreatic islet cell xenografts (16).

The objectives of the present study were four-fold: (i) to delineate the pharmacokinetics of CTLA4Ig in rats following single and multiple intravenous (IV) and subcutaneous (SC) administration; (ii) to assess the relationship of the pharmacokinetic parameters of CTLA4Ig and the dose administered; (iii) to calculate the SC absolute bioavailability of CTLA4Ig; and (iv) to assess the anti-CTLA4Ig antibody response in rats following SC and IV administration.

EXPERIMENTAL

Study Design

A total of 48 (24 male and 24 female) Sprague-Dawley rats were divided into eight treatment groups with 3 rats per gender in each group: a single dose of 10, 80, or 200 mg/kg of CTLA4Ig was given either IV or SC and a repeated dose of 10 mg/kg (once every other day for 7 doses over 13 days) was given either SC or IV.
Rat Preparation and Handling

Upon receipt, rats were housed individually in stainless steel, wire-bottom cages of appropriate size and type for the duration of the study. Each rat was tagged with a unique identification number (metal ear tag). Each cage was marked with the same unique number as its occupant with appropriate cage labels. Two days prior to the pharmacokinetic sampling, a jugular catheter was placed in the rat. After each bleed, the patency of the cannula was assured by flushing the cannula with 0.2 ml heparinized-saline solution.

Drug Administration

The dosing solutions contained either 40 mg/ml (SC dosing) or 10 mg/ml (IV dosing) of CTLA4Ig. The dosing solution was sterile filtered using a Sterile Acrodisc® filter. An appropriate volume of the dosing solution was injected subcutaneously along the dorsal thorax or intravenously into a tail vein using an appropriate size of needle and syringe.

Sample Collection and Preparation

Blood samples (approximately 0.2 ml) were collected up to 43 days following the administration of single doses: predose, and at 1, 2, 4, 8, 24, 48, 96 h, and 8, 15, 22, 29, 36, and 43 days post-dosing. Two additional blood samples were obtained, at 3 and 30 min after IV administration and at 10 and 12 h after SC administration. In a similar fashion, blood samples were collected up to 50 days following the administration of the last multiple dose on day 13. Serum samples were harvested and kept at or below −70°C until analysis.

Analyses of Serum CTLA4Ig

The serum concentration of CTLA4Ig was quantitated using a validated enzyme immunoassay (17). The standard curve concentrations ranged from 2 to 45 ng/ml in rat serum. A 4-parameter logistic regression model of the form, \( Y = \text{max} + \frac{[\text{min} - \text{max}]}{[1 + (\text{Conc} / \text{ED}_{50})^b]} \), was used to fit the data. Prior to the initiation of the study, three sets of quality control (QC) samples, 9, 24, and 38 ng/ml of CTLA4Ig, were prepared in rat serum. The QC samples were stored and analyzed together with the study samples to verify the accuracy, precision, and reproducibility of the assay.

The \( R^2 \) values of the standard curves were ≥0.994. The mean predicted QC concentrations deviated less than 5.3% of the nominal values. The values for the precision estimates of the QC samples were within 6.7% relative standard deviation. On the basis of the performance of the standard curves and QC samples, the serum assay for CTLA4Ig was accurate, precise and reproducible. The QC data also demonstrated the stability of CTLA4Ig in the rat serum samples during the storage period.

Measurement of Anti-CTLA4Ig Antibodies (Immunogenicity)

Serum titers of antibodies specific for CTLA4Ig were assessed using samples obtained from rats obtained predose and on days 9, 13, 21, 28, 35, 42 for both single and multiple doses. Three additional serum samples obtained on days 49, 56, and 63 following multiple doses were assessed for anti-CTLA4Ig antibody titers. An EIA assay was performed using CTLA4Ig as a capture reagent (plates coated with 2 µg/ml CTLA4Ig) and specific antibody binding was detected using a mixture of commercially available goat anti-rat IgG + IgM specific to heavy and light chains (Jackson Immunoresearch, West Grove, PA) conjugated with alkaline phosphatase as a detection antibody. Prior to detection, serum samples were diluted using a threefold serial dilution scheme starting at an initial dilution of 1:10. Results were represented as end-point titers, where the titer was defined as the reciprocal of the greatest dilution that had an absorbance at least twofold greater than the EIA plate background. An individual rat was judged to have demonstrated a positive immune response against CTLA4Ig when its titer increased by two or more serial dilutions relative to its predose titer.

Pharmacokinetic Analyses

Serum data were subjected to noncompartmental pharmacokinetic analysis (18,19). The following parameters were calculated: peak plasma concentration (\( C_{\text{max}} \)), time to the attainment of \( C_{\text{max}} (T_{\text{max}}) \), area under the serum concentration-time curve from time = 0 to time = infinity (AUC\(_{\text{inf}}\)), area under the serum concentration-time curve in a dosing interval (i.e., 48 h) AUC\(_{\text{int}}\), mean residence time (MRT), terminal serum elimination half-life (\( T_{\text{1/2}} \)), total body clearance (CL) and steady-state volume of distribution (\( V_{\text{ss}} \)). The SC bioavailability (F) was estimated as the quotient of the SC and IV AUC\(_{\text{int}}\) values. The accumulation factor was calculated as the quotient of AUC\(_{\text{int}}\) values obtained after the last dose and the first dose.

Statistical Methods

The pharmacokinetic parameters after single IV doses (\( C_{\text{max}}, \text{AUC}_{\text{inf}}, \text{MRT}, T_{\text{1/2}}, \text{CL}, \text{and} \ V_{\text{ss}} \)) and SC doses (\( C_{\text{max}}, \text{AUC}, \text{MRT}, T_{\text{max}}, \text{and} \ T_{\text{1/2}} \)) were evaluated in the context of an analysis of variance (ANOVA) model. The model included effects for dose, gender, route, gender*route interaction, gender*route interaction, dose*route interaction, gender*dose*route interaction as well as an error term. If statistically significant (p value < 0.05) interactions were found, all subsequent analyses were performed within the effects involved in that interaction for that parameter. Tukey’s unweighted studentized range test was used to make pairwise comparisons among all means for significant model effects as well as to compare the \( T_{\text{1/2}} \) values between single and multiple doses (20).

Weighted linear regression analysis (1/dose) was performed to evaluate the relationship between \( C_{\text{max}} \) versus dose and AUC\(_{\text{int}}\) versus dose. A test for nonlinearity was performed by testing the model for lack of fit (21). In the absence of significant nonlinearity, the parameter was concluded to be dose proportional if the intercept was not statistically significantly different from zero.

RESULTS

The mean serum concentration data following single IV and SC administration of CTLA4Ig are graphically illustrated.