Pharmacokinetics and Leukocyte Responses of Recombinant Human Interleukin-10

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Purpose. To study the pharmacokinetics and ex vivo leukocyte responses of recombinant human IL-10 (rHuIL-10) following single *SC* and *IV* dosing.

Methods. A randomized two-way cross-over study was undertaken in 17 healthy volunteers in which rHuIL-10 was administered as 25 μg/kg *SC* and *IV* doses. Blood samples were collected for 48 hr after dosing to determine serum IL-10 concentrations. Inhibitory activity of IL-10 on *ex vivo* production of inflammatory cytokines (TNF-α and IL-1β) by LPS-treated peripheral blood cells were measured over 96 hr.

Results. A physiologically-relevant modeling approach was developed to determine the pharmacokinetics for two routes of administration (*SC* and *IV*). The *IV* dose showed polyexponential disposition with CL of 65 mL/kg/hr, V_{ss} of 70 mL/kg, and t1/2 of 1.94 hr. Absolute bioavailability averaged 42% for *SC* dosing which produced lower but sustained concentrations. Substantial and prolonged suppression of TNF-α and IL-1β production was achieved during IL-10 treatment. The Hill Function was used to account for the joint concentration-dependent immunosuppressive action of rHuIL-10 after both *IV* and *SC* doses. The IC_{50} values were about 0.03 ng/mL and I_{max} values were about 0.85 for both TNF-α and IL-1β suppression. The degree of change as well as the duration of leukocyte response was greater after *SC* administration than after *IV* administration.

Conclusions. rHuIL-10 shows favorable PK/PD characteristics especially by the *SC* route of administration which produced prolonged suppression of cytokine production (*ex vivo*) which may be applicable in various immune-related disorders.

KEY WORDS: IL-10; cytokines; protein; immunosuppression; pharmacokinetics.

INTRODUCTION

The pleiotropic cytokine, IL-10 originally identified as a product of the T_h2 cell subset (1) has immunomodulatory properties (2,3) that are promising for use in the treatment of various immune disorders. These properties include inhibition of proinflammatory cytokine production such as IL-1, IL-6 and TNF-α by LPS-stimulated macrophages (4) and inhibition of expression and production of polymorphonuclear leukocyte derived chemokines such as IL-8, and MIP-1α (5). It also prevents antigen-specific T cell proliferation (6).

IL-10 provides protection in animal models of LPS-induced (7) as well as enterotoxin B-induced (8) shock in mice and experimental allergic encephalomyelitis in rats (9) by down-regulating excessive cytokine production. IL-10 deficient mice suffer from chronic enterocolitis due to overproduction of cytokines such as TNF-α, IL-1β and IFN-γ (10) signifying a possible role for IL-10 in the treatment of inflammatory bowel diseases. Furthermore, IL-10 was shown to have an immunoregulatory role in rheumatoid arthritis (11). Consistent with these results, recombinant human IL-10 treatment led to the suppression of *ex vivo* LPS-stimulated proinflammatory cytokine production from whole blood in human subjects (12,13,14).

Recombinant human IL-10 is a non-covalently linked homodimeric peptide with a monomeric molecular weight of 18.7 kDa. Data from animals and humans suggest that peptides and proteins including IL-10 have short half-lives (12,15,16). Subcutaneous administration of macromolecules may result in absorption-rate limited disposition causing longer residence times (14,16) due to lymphatic uptake from injection sites (17). The major concern for *SC* or any extravascular dosing is bioavailability as peptides may be degraded locally by proteases.

The present study investigates the *SC* bioavailability and pharmacokinetics of single doses of rHuIL-10 in healthy volunteers with the aid of a general PK model that takes into account the various physiological factors involved in absorption, distribution and elimination of therapeutic macromolecules. The second objective of the study was to assess the immunosuppressive effects of IL-10 with reference to suppression of *ex vivo* LPS-stimulated proinflammatory cytokines, IL-1β and TNF-α, production in whole blood.

METHODS

Experimental

Volunteer Subjects and Dosing Schedule

An institutional review board (IRB) approved the protocol and the volunteers provided informed consent. Seventeen healthy male volunteers were empaneled and completed this

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ABBREVIATIONS: %CV, Coefficient of variation, expressed as a percent; ABEC, Area between baseline and effect curve; AUC, Area under the serum concentration-time curve from time zero to infinity; CL, Systemic clearance; C_{max}, Maximum observed serum concentration; C_0, Theoretical drug concentration in serum at t = 0 after *IV* dosing; E_0, Baseline TNF-α or IL-1β concentration; Bio, Absolute bioavailability; ECL, Electrochemilluminescent assay; ev, Extravascular compartment; Frc, Fraction of IL-10 dose delivered by first-order process; IC_{50}, Drug concentration producing 50% of maximum inhibition; IFN-γ, Interferon-gamma; IL-1β, Interleukin-1beta; IL-10, rHuIL-10, recombinant human interleukin-10; I_{max}, Maximum fraction of inhibition; *IV*, Intravenous; k_{10}, Elimination rate constant from the central compartment; k_{ij}, Distribution rate constant between sites i and j; ka, First-order absorption rate constant from *SC* to L&I sites; ka2, First-order absorption rate constant from L&I to central compartment; L&I, Lymphatic and interstitial compartment; LOQ, Limit of quantitation; LPS, Lipopolysaccharide; *SC*, Subcutaneous; ta, Duration of zero-order input for IL-10; TNF-α, Tumor necrosis factor-alpha; $t_{1/2}$, Terminal phase half-life; T_{lag}, Lag time between zero-order and first-order inputs; T_{max}, Time of maximum observed serum concentration; V_c, Volume of distribution in the central compartment; V_{ss}, Volume of distribution at steady-state.

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randomized, open-label, two-way crossover, single dose study. Each volunteer received two single doses (*IV* and *SC*) of 25 μg rHuIL-10/kg separated by a two-week washout interval. The rHuIL-10 (SCH 52000, nonglycosylated, expressed in *Escherichia coli*) was provided by Schering-Plough Research Institute (Kenilworth, NJ) as a lyophilisate. Volunteers were confined to the study site from approximately 12 hr prior to dosing until approximately 96 hr post-dosing. On the morning following an overnight fast, subjects were administered 25 μg rHuIL-10/kg by either subcutaneous (*SC*) injection in the upper arm or by intravenous (*IV*) bolus injection (over 30 sec). Four mL of blood were drawn for the determination of serum IL-10 concentrations at time 0 (immediately prior to dosing) and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, 20, 24, 36, and 48 hr following *SC* administration. Following *IV* administration, 4 mL of blood were also drawn at times 5, 10, 20, and 45 min. The serum samples were stored at −70°C until assayed.

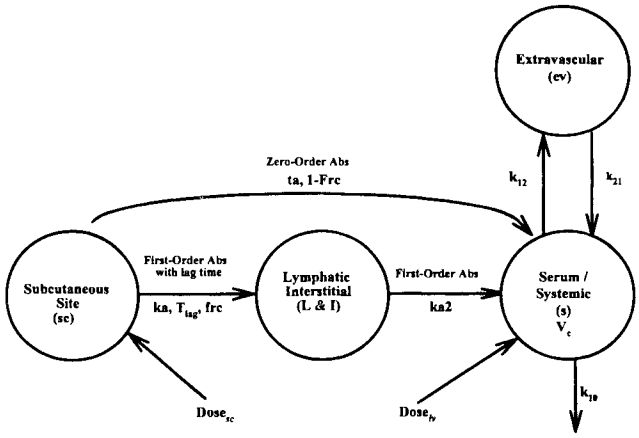

Fig. 1. Proposed pharmacokinetic model for IL-10.

IL-10 Determination

The IL-10 concentrations in serum were determined using a validated ECL method described previously (12). The limit of quantitation (LOQ) was 0.1 ng/mL.

Ex Vivo *Production of Cytokines*

At 0, 0.5, 2, 3, 4, 6, 8, 12, 16, 24, 48, 72 and 96 hr following IL-10 dosing, blood was drawn into heparinized syringes. Blood samples (2.5 mL) were transferred into tubes containing EDTA and 35 ng of *E coli LPS* in 1 mL of RPMI. Samples were incubated at 37°C for 24 hr and the plasma was separated by centrifugation at 1000 g for 20 min at 4°C and then clarified by centrifugation at 13,000 RPM using a microcentrifuge for approximately 5 min. The plasma samples were frozen at −70°C and assayed for TNF-α and IL-1β (12). A differential white blood cell count and CBC were also performed.

Pharmacokinetics

Preliminary analysis of the *IV* data showed that a two-compartment model adequately described the disposition for most subjects, while a three-compartment model better described the profiles for the remaining 3 subjects (subjects 5, 7, 18). The *IV* data from Subject 7 was used as a reference for the three-compartment model, with his inter-compartmental distribution rate constants, k_{12} and k_{13} being fixed for other subjects thereby forcing two peripheral compartments, $k_{12}/k_{21}\cdot V_c$ and $k_{13}/k_{31}\cdot V_c$ to the others. It was assumed for Subjects 5 and 18 that the assay was not sensitive enough to determine the terminal *IV* disposition phase.

IL-10 absorption profiles following *SC* injection (25 μg rHuIL-10/kg) were examined using the Area Function Method which is an application of deconvolution (18,19). The input rate profiles revealed two absorption phases, an initial loading dose followed by more gradual delivery. These were described by a zero-order input over a short duration (ta) reflecting the loading dose phenomenon followed by a first-order input (ka) with an intervening lag time (T_{lag}). The fraction delivered by the two processes were modeled as parameters 1-Frc and Frc.

Finally, an integrated PK model (Figure 1) was used to determine the absorption and disposition characteristics of the drug by fitting simultaneously the *IV* and *SC* data. Parameters obtained directly by simultaneous fitting were k_{12}, k_{13}, k_{21}, k_{31}, k_{10}, V_c, ta, T_{lag}, Frc, ka, ka2, and Bio.

The PK parameters calculated from the proposed model include volume of distribution at steady-state (V_{ss}) and systemic clearance (CL):

$$V_{ss} = (1 + k_{12}/k_{21} + k_{13}/k_{31})\cdot V_c \qquad CL = V_c \cdot k_{10}$$

Linear and stationary pharmacokinetics were assumed for all subjects as established earlier (12). Goodness-of-fit was judged by the convergence of the least-squares regression, absence of systematic deviations of observed versus fitted data points along with the use of the Akaike Information Criterion when comparing alternative models (two- vs. three-compartment).

The differential equations used for the model were:

$$\frac{dC_{s,iv}}{dt} = -(k_{12} + k_{10}) \cdot (C_{s,iv}) + k_{21} \cdot A_{ev,iv}/V_c$$

$$\frac{dC_{s,sc}}{dt} = -(k_{12} + k_{10}) \cdot (C_{s,sc}) + k_{21} \cdot A_{ev,sc}/V_c + ka2 \cdot A_{L\&I}/V_c + Bio \cdot (1 - Frc) \cdot Dose/(ta \cdot V_c)$$

$$\frac{dA_{ev,iv}}{dt} = k_{12} \cdot C_{s,iv} \cdot V_c - k_{21} \cdot A_{ev,iv}$$

$$\frac{dA_{ev,sc}}{dt} = k_{12} \cdot C_{s,sc} \cdot V_c - k_{21} \cdot A_{ev,sc}$$

$$\frac{dA_{L\&I}}{dt} = ka \cdot Bio \cdot Frc \cdot Dose \cdot e^{-ka\cdot(t-ta-t_{lag})} - ka2 \cdot A_{L\&I}$$

where the symbols are defined in the abbreviations.

The zero-order input rate operates from time zero to ta and the first-order absorption occurs at times greater than ta + T_{lag}. The three-compartment model was structured similarly with a second peripheral compartment.

Leukocyte Responses

The inhibition of *ex vivo* production of proinflammatory cytokines (TNF-α and IL-1β) determined from LPS-stimulated blood cells was measured. Data from the first 48 and 72 hr for *IV* and *SC* doses were used to model the concentration-effect relationships. The concentration-effect relationship utilized the

inhibitory I_{max} model assuming the effect as a direct function of serum IL-10 concentration:

$$E = E_0 - \frac{E_0 \cdot I_{max} \cdot C_s}{IC_{50} + C_s}$$

where E and E_0 are the TNF-α and IL-1β concentrations at time t and zero (baseline), C_s is the serum IL-10 concentration at time t corrected by the dilution factor used in the *ex vivo* cytokine production assay, IC_{50} is the drug concentration producing 50% of the maximum inhibition, and I_{max} is the maximum fraction of inhibition observed. Simultaneous fitting for *IV* and *SC* data was done for each subject to enrich the data set. The parameters fitted were E_0 (*IV* dose) and E_0 (*SC* dose), I_{max}, and IC_{50}. The E_0 values were fitted separately due to variability in baseline responses.

$ABEC_{0-48}$ (area between baseline and effect curve) was calculated based on the model predicted effects for individual subjects during both *IV* and *SC* phases by the trapezoidal method using predicted E_0 as the baseline response. The ABEC values were normalized by the respective IL-10 AUC_{0-48}.

Data Analysis

Data analysis was performed with the PCNONLIN regression program (SCI Software Inc., Apex, NC, version 4.0). The Nelder-Mead Simplex and Gauss-Newton with Levenberg's and Hartley's modification algorithms were used to perform nonlinear regressions. Individual data were fitted separately assuming intrinsic differences in the response patterns and the degree of inter-individual variability was determined by the standard deviation of the mean of respective parameters. Weighing of 1/y and 1 were used for the PK and response data. In order to minimize the effects of extreme values on the reported means, median values are also reported. A paired t-test was used to compare the ABEC/AUC ratio for *IV* and *SC* dosing using SAS software (Version 6; SAS Institute, Cary, NC).

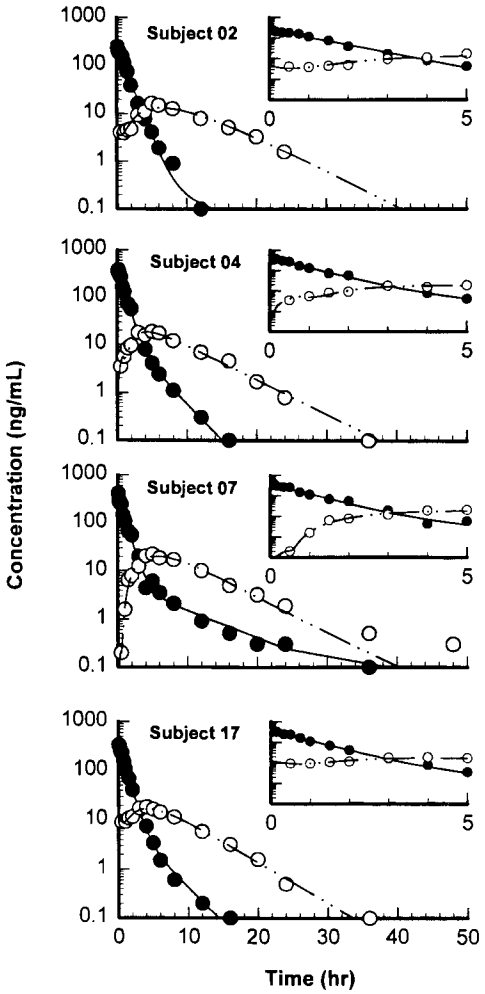

Fig. 2. Time-course of IL-10 concentrations for four subjects following *IV* (●) and *SC* (○) administration.

RESULTS

Pharmacokinetics

Representative serum concentration-time profiles of IL-10 after *IV* and *SC* dosing for four individual subjects are shown in Figure 2. Evident is the sustained-release pattern during *SC* dosing preceded by a rapid absorption phase that can be deemed as a form of loading dose. The *IV* plots show a multi-exponential disposition pattern with a rapid initial decline that later yields a less steep terminal phase with $t_{1/2}$ of 1.94 ± 1.16 hr. The C_{max} after *SC* administration was 13.6 ng/mL versus a C_0 of 471 ng/mL after *IV* administration.

Due to the large molecular size of IL-10, the V_c (55.78 ± 13.65 mL/kg, Table I) approximated the plasma volume implying that the drug remains within the vascular compartment. Also, the V_{ss} (70.26 ± 15.87 mL/kg) remained close to V_c signifying the minimal contribution of any peripheral compartment. The clearance of the drug was 64.56 ± 7.14 mL/kg · hr (Table I). The low CL and low V_{ss} jointly account for a short $t_{1/2}$ ($t_{1/2} \sim 0.7 \cdot V_{ss}/CL$).

The absorption parameters for IL-10 are shown in Table I. As illustrated in Figure 1, upon *SC* administration, IL-10 forms a depot at the injection site from where a small fraction (1 − Frc) was rapidly absorbed (ta = 0.47 ± 0.52 hr) directly into the circulation by an apparent zero-order process. This was followed by a more gradual release of the drug as described by the second fraction (Frc = 0.95 ± 0.04) at a first-order rate (ka = 0.16 ± 0.04 hr^{-1}) whose kinetics is controlled by lymph

Table I. Absorption and Disposition Pharmacokinetics of IL-10

	Mean	SD	Median
Disposition Parameters			
V_c (mL/kg)	55.78	13.65	55.28
V_{ss} (mL/kg)	70.26	15.87	67.11
CL (mL/kg·hr)	64.56	7.14	63.90
$t_{1/2}$(hr)[a]	1.94	1.16	1.81
Absorption Parameters			
ta (hr)	0.47	0.52	0.43
Frc	0.95	0.04	0.96
ka (hr^{-1})	0.16	0.04	0.15
ka2 (hr^{-1})	0.61	0.69	0.35
T_{lag}(hr)	0.23	0.52	0.00
Bioavailability	0.42	0.11	0.43

[a] Patients 5, 7, and 13 were excluded.

flow. A lag time (T_{lag} = 0.23 ± 0.52 hr) between the two absorption phases results from the delay due to slow lymphatic delivery of IL-10. The absorption rate profile (Figure 3) generated by deconvolution depicts the existence of two absorption phases. From the terminal phase of the same plots, it is clear that the input rate governs the terminal phases during *SC* dosing, though one cannot be sure of the true terminal phase due to assay limitations (LOQ for IL-10 is 0.1 ng/mL, while endogenous levels of IL-10 can be less than 1 pg/mL). The absorption rate profile is also identical to the serum IL-10 concentration pattern signifying that the *SC* profile of IL-10 is controlled by its absorption rate. The rate constant ka2 (0.61 hr^{-1}) that describes the absorption from "L & I" to V_c is higher than ka in most cases. However, the difficulty in interpreting ka and ka2 is that they can be interchangeable since the true disposition characteristics from "L & I" are unknown. What is clear is that either ka or ka2 is smaller than the terminal slope of the *IV* profile (data not shown) in most subjects which further strengthens the flip-flop absorption characteristics of IL-10. The drug has moderate bioavailability (42.4%) with low variability (%CV = 25.0).

Figure 4 shows the average and range of the absorption and disposition parameters for all subjects. The overall variability of the absorption parameters is small for Frc (CV = 3.7%), modest for bioavailability (25.0%) and ka (23.9%), while it is large for ta (111%), ka2 (113%) and T_{lag} (222%). The disposition parameters (Table I, Figure 4) showed less variability.

Leukocyte Responses

The concentration as well as the time-dependent suppression of proinflammatory cytokine production (TNF-α and IL-1β) from LPS stimulated whole blood are shown for two subjects (Figure 5). Cytokine concentrations were normalized by simultaneous monocyte counts as IL-10 treatment causes elevation of monocyte counts. IL-10 causes substantial suppression of both TNF-α and IL-1β production. Moreover, the drug produced a high degree of inhibition approaching 100% (I_{max} = 1.0) as shown by the large values of I_{max} (TNF-α: 0.84 ± 0.06, IL-1β: 0.87 ± 0.10, Table II). Parallel responses were seen for TNF-α and IL-1β responses. The *SC* and *IV* data superimposed fairly well in determining the concentration-effect relationship and were fitted simultaneously. The baseline responses (E_0) were, however, estimated separately due to their large differences and variability (Table II). Although the C_{max} for the *SC* route was more than an order of magnitude lower than that observed after *IV* administration, the concentrations of IL-10 after *SC* dosing achieved appreciable immunosuppression and the sustained input prolonged the response in most cases.

The IC_{50} values (TNF-α: 0.029 ± 0.062 ng/mL, IL-1β: 0.029 ± 0.040 ng/mL, Table II) were quite low. These IC_{50} values are markedly below quantifiable concentrations of IL-10.

Comparison of the ABEC/AUC ratios revealed achievement of greater net immunosuppressive efficiency upon *SC* versus *IV* dosing ($p < 0.05$) as shown in Figure 6. The ABEC/

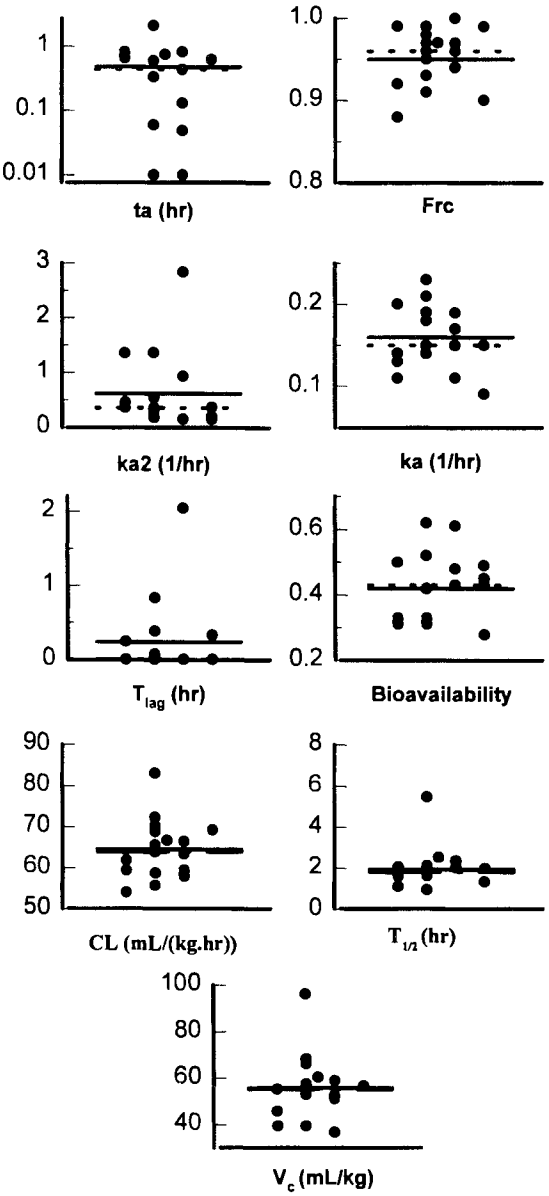

Fig. 4. Range of pharmacokinetic absorption and disposition parameters of IL-10 in 17 healthy subjects.

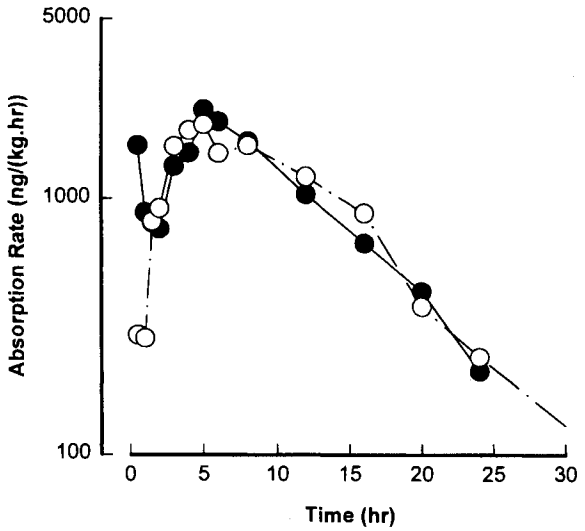

Fig. 3. Absorption rate for IL-10 derived from area function plot for Subject 2 (●) and Subject 18 (○).

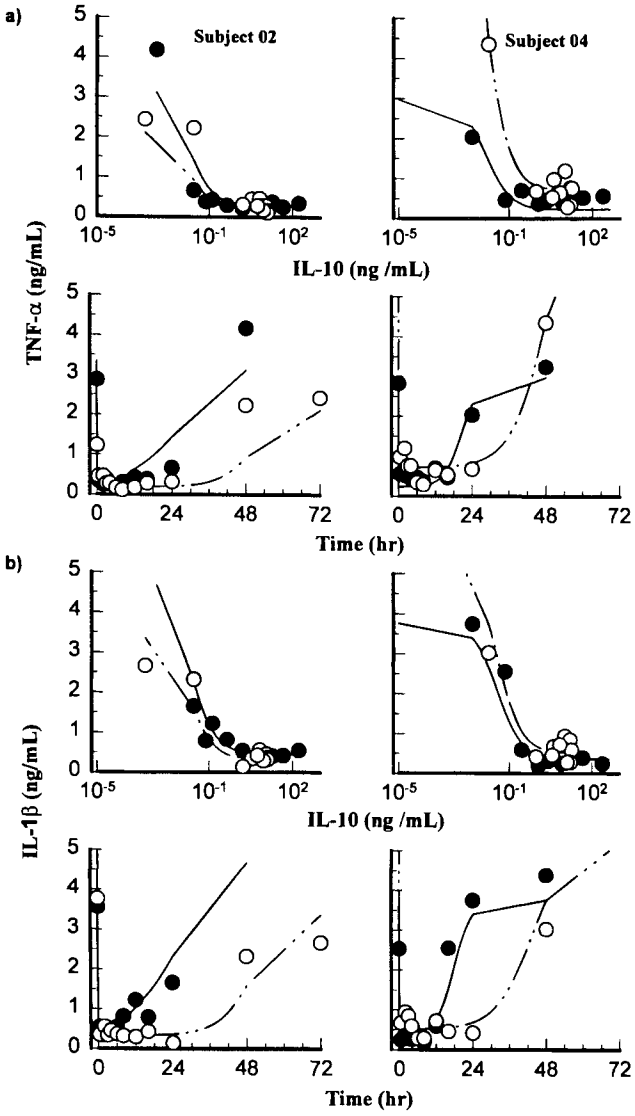

Fig. 5. *Ex vivo* cytokine production (TNF-α and IL-1β) for two subjects following *IV* (●) and *SC* (○) administration of rHuIL-10.

AUC ratios were 0.168 ± 0.157 and 0.583 ± 0.419 for the *IV* and *SC* dosing for TNF-α; the corresponding values for IL-1β production were 0.179 ± 0.143 and 0.788 ± 0.576.

Administration of rHuIL-10 by either the *IV* or *SC* route resulted in complex changes in white blood cell differential counts. There were moderate increases in total WBC count, neutrophil count, and monocyte count in conjunction with a mild decrease in total lymphocyte count. There were mild to moderate decreases in platelet counts. In general, the degree of decrease was greater after *SC* administration than after *IV* administration and were similar to previous findings (14). No clinically significant abnormalities were noted in serum electrolytes, calcium, phosphorous, BUN, creatinine, uric acid, glucose, SGOT, SGPT, alkaline phosphatase or albumin.

DISCUSSION

Pharmacokinetics

Due to its large molecular size, upon *SC* administration, it is hypothesized that most of the drug will be transported to the lymphatic circulation (17). The large gaps between adjacent endothelial cells in the lymphatic capillaries as compared to those in the blood capillaries allow IL-10 to enter the lymphatic circulation (20) from the *SC* injection site before reaching the systemic circulation. However, a fraction of the dose appears in the systemic circulation immediately after *SC* administration without any time delay. Since lymph flow is slow and may

Table II. Summary of Leukocyte Response Parameters of IL-10

Parameters	Mean	SD	Median
TNF-α			
E_0(*IV*, ng/mL)	3.045	1.870	2.356
E_0(*SC*, ng/mL)	2.571	1.958	2.008
I_{max}	0.836	0.059	0.837
IC_{50}(ng/mL)	0.0288	0.0615	0.0099
$ABEC_{iv}/AUC_{iv}$	0.1683	0.1566	0.0911
$ABEC_{sc}/AUC_{sc}$	0.5830	0.4194	0.4408
IL-1β			
E_0(*IV*, ng/mL)	3.419	1.594	3.360
E_0(*SC*, ng/mL)	3.324	2.625	2.524
I_{max}	0.868	0.097	0.904
IC_{50}(ng/mL)	0.0294	0.0404	0.0050
$ABEC_{iv}/AUC_{iv}$	0.1793	0.1425	0.1236
$ABEC_{sc}/AUC_{sc}$	0.7878	0.5762	0.6712

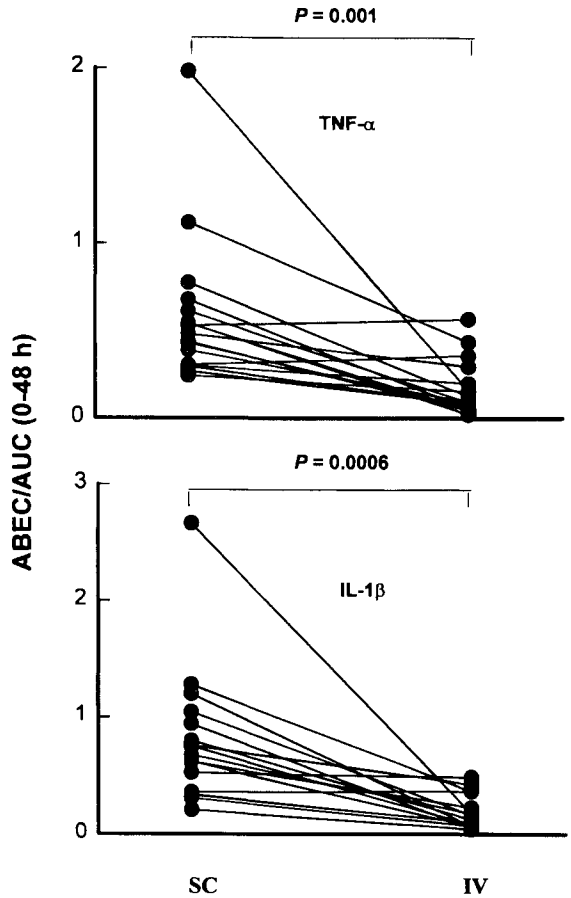

Fig. 6. ABEC/AUC(0–48 hr) ratios for TNF-α and IL-1β for all subjects.

cause a time-lag for the drug to reach the systemic circulation, some leakage of blood vessels may allow entry of a fraction of the injected dose through the interstitial spaces. Thus, the proposed PK model (Figure 1) assumes that *SC* administration forms a depot at the injection site from where a small fraction (1-Frc) is rapidly delivered directly to the circulation by an apparent zero-order process. This event represents an initial loading dose phenomenon. The second phase of absorption (Frc fraction) involves a more gradual uptake of drug where the kinetics are first-order and governed mainly by the lymph flow.

The pharmacokinetic model describes the concentration-time profiles both during *IV* as well as *SC* dosing and could be adapted to other macromolecules administered by *SC* injection. The two input rates well describe the initial time points (inset Figure 2) where there is a quick rise and plateau in drug concentrations following *SC* administration. By virtue of its molecular size, delivery of IL-10 by *SC* dosing is ideal since both immediate as well as prolonged and effective concentrations are achieved. Estimates of V_c approximate the plasma volume while the disposition phase is multiexponential and may be due to some type of binding to nonfunctional sites of IL-10. This phenomenon is common for other macromolecular therapeutic cytokines and monoclonal antibodies (21,22). The rHuIL-10 molecule is nonglycosylated and hence may be catabolized by the kidney. However, there might be other routes of elimination including hepatic metabolism and proteolytic degradation as seen for other peptides (16). The presence of proteolytic enzymes in the *SC* or lymph compartments may partly account for the low bioavailability. Although the C_{max} is considerably lower for the *SC* dose as compared to the *IV* dose, the slow but continuous release profile of the drug enables the achievement of satisfactory delivery rates and bioavailability values. The PK disposition parameters obtained (V_{ss}, CL) agreed well with previous results (12) that used a non-compartmental approach.

Compared to other absorption parameters, the low intersubject variability in estimating Frc values supports the existence of two phases of input for drug absorption from the extravascular *SC* site. This low degree of variability of the disposition parameters and bioavailability facilitated the use of simultaneous fitting for estimating the PK parameters. The variability in the PK profiles arise more due to processes governing the absorption of rHuIL-10 than due to disposition mechanisms (though the sparse data during the initial absorption phase may contribute to some extent for the higher variability). However, some patients (distinctly Subject 7) exhibited a prolonged *IV* terminal disposition phase parallel to *SC* that complicated the fitting to the model. In those cases, flip-flop kinetics do not occur, although, the first-order input following the loading dose still ensures sustained delivery of the drug. This anomaly may be explained by the fact that flip-flop kinetics may not hold true if the true terminal phase is not achieved within the limits of quantitation in most subjects.

Leukocyte Responses

rHuIL-10, a potential immunosuppressive agent, is being investigated for the treatment of patients suffering from immune related disorders. Currently there are data available in patients with Crohn's disease where IL-10 showed encouraging results (23). In the present study, blood samples from healthy male volunteers treated with rHuIL-10 were stimulated with LPS and production of TNF-α and IL-1β was monitored over four days. rHuIL-10 treatment caused prolonged and significant suppression of cytokine synthesis as serum concentrations exceeded IC_{50} values for an extended time frame, especially after *SC* dosing. The information obtained from this study may serve as a therapeutic guideline to determine the dosing pattern for the immunosuppressive action of IL-10 in conditions due to the overproduction of inflammatory cytokines.

IL-10 regulates the TNF-α and IL-1β genes at the transcriptional level as it inhibits their mRNA accumulation when PMNs (polymorphonuclear leukocytes) or PBMCs (peripheral blood mononuclear cells) are stimulated by LPS in the presence of IL-10 (5,24). A direct concentration-effect relationship was utilized assuming that the *ex vivo* measurements were largely a type of bioassay for IL-10 immunosuppressive action. The IC_{50} values obtained for TNF-α and IL-1β suppression (Table II) were very low and I_{max} values of about 0.85 reflect an ability to cause nearly complete inhibition.

The E_0 values (Table II) varied considerably between dosing days in many subjects and contributed to the variability in calculated IC_{50} values. Furthermore, IC_{50} values were very low and occur at concentrations where there is limited PK data (tails of PK profiles). This may also contribute to the uncertainty and variability of the parameter.

ABEC, a summary parameter representing the net effect of the drug was normalized by the respective drug AUC over the same time interval in order to characterize the expected overall efficiency of IL-10 treatment from the two routes of dosing. The ABEC/AUC ratio clearly reflects the prolonged effect during *SC* dosing which is partly accountable by the fact that *IV* dosing "wastes" part of the drug since concentrations greatly exceeded the IC_{50} and attain I_{max} and by the low but sustained and effective serum IL-10 concentrations with *SC* dosing. An extended but effective drug concentration range attains greater efficacy for eliciting any type of pharmacological effect which is easily saturable (25).

There was marked interindividual variability in leukocyte responses to IL-10 treatment. Figure 6 shows the ABEC/AUC ratios for different subjects. Though most of the subjects responded well to IL-10, caution is needed during actual therapy due to inter-individual differences in such responses.

The present studies employed ex vivo whole blood responses as a measure of the inherent potency of IL-10, to assess the intersubject variability in cell responsiveness, and as a means of determining whether the response parameters differ with route of IL-10 administration. The in vivo pharmacodynamics and ultimately patient responses may differ because of the type of inflammatory challenge (e.g., rheumatoid arthritis), multiple mechanism of drug effects, any disease modifications of leukocyte numbers and reactivity, previous and concomitant drug therapy (e.g., steroids, NSAID), disposition of provoked cytokines, and the time-course of drug concentrations (viz. pharmacokinetically controlled rather than static for 24 hours). All of these same considerations apply when comparing in vitro leukocyte responses versus in vivo effects of traditional immunosuppressive agents, yet the former have direct value in anticipating therapeutic efficacy (26). The present results provide useful insights into the potential therapeutic use of *IV* versus *SC* doses of IL-10. The compound exhibits considerable potency in a standard in vitro model and, as an inhibitor of

proinflammatory cytokine production, pharmacodynamic principles for indirect responses argue that improved efficiency will be obtained by maintaining IL-10 concentrations above the IC_{50} for an extended period of time (25).

In summary, *SC* and *IV* administration of single doses of 25 μg rHuIL-10/kg was well tolerated in healthy male volunteers. rHuIL-10 demonstrated potent immunosuppression of *ex vivo* LPS-stimulated cytokine production by whole blood in healthy volunteers. The favorable PK demonstrated by moderate bioavailability and extended release following *SC* administration ensures prolonged effect. The proposed model may help in understanding the pharmacokinetics of IL-10 or other similar macromolecules.

REFERENCES

1. D. F. Fiorentino, M. W. Bond, and T. R. Mosmann. Two types of mouse T helper cell IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. *J. Exp. Med.* **170**:2081–2095 (1989).
2. J. E. de Vries. Immunosuppressive and anti-inflammatory properties of interleukin-10. *Ann. Med.* **27**:537–541 (1995).
3. K. W. Moore, A. O'Garra, R. de W. Malefyt, P. Vieira, and T. R. Mosmann. Interleukin-10. *Annu. Rev. Immunol.* **11**:165–190 (1993).
4. D. F. Fiorentino, A. Zlotnik, T. R. Mosmann, M. Howard, and A. O'Garra, IL-10 inhibits cytokine production by activated macrophages. *J. Immunol.* **147**:3815–3822 (1991).
5. T. Kasama, R. M. Strieter, N. W. Lukacs, M. D. Burdick, and S. L. Kunkel. Regulation of neutrophil-derived chemokine expression by IL-10. *J. Immunol.* **152**:3559–3569 (1994).
6. K. Taga, H. Mostowski, and G. Tosato. Human interleukin-10 can directly inhibit T-cell growth. *Blood.* **81**:2964–2971 (1993).
7. M. Howard, T. Muchamuel, S. Andrade, and S. Menon. Interleukin 10 protects mice from lethal endotoxemia. *J. Exp. Med.* **177**:1205–1208 (1993).
8. A. G. D. Bean, R. A. Freiberg, S. Andrade, S. Menon, and A. Zlotnik. Interleukin 10 protects mice against staphylococcal enterotoxin B-induced lethal shock. *Infect. Immun.* **61**:4937–4939 (1993).
9. O. Rott, B. Fleischer, and E. Cash. Interleukin-10 prevents experimental allergic encephalomyelitis in rats. *Eur. J. Immunol.* **24**:1434–1440 (1994).
10. R. Kuhn, J. Lohler, D. Rennick, K. Rajewsky, and W. Muller. Interleukin-10 deficient mice develop chronic enterocolitis. *Cell* **75**:263–274 (1993).
11. P. D. Katsikis, C-Q. Chu, F. M. Brennan, R. N. Maini, and M. Feldmann. Immunoregulatory role of interleukin 10 in rheumatoid arthritis. *J. Exp. Med.* **179**:1517–1527 (1994).
12. R. D. Huhn, E. Radwanski, S. M. O'Connell, M. G. Sturgill, L. Clarke, R. P. Cody, M. B. Affrime, and D. L. Cutler. Pharmacokinetics and immunomodulatory properties of intravenously administered recombinant human interleukin-10 in healthy volunteers. *Blood* **87**:699–705 (1996).
13. S. Schreiber, T. Heinig, H-G. Theile, and A. Raedler. Immunoregulatory role of interleukin 10 in patients with inflammatory bowel disease. *Gastroenterology* **108**:1434–1444 (1995).
14. R. D. Huhn, E. Radwanski, J. Gallo, M. B. Affrime, R. Sabo, G. Gonyo, A. Monge, and D. L. Cutler. Pharmacodynamics of subcutaneous recombinant human interleukin-10 in healthy volunteers. *Clin. Pharmacol. Ther.* **62**:171–180 (1997).
15. A. Takagi, H. Masuda, Y. Takakura, and M. Hashida. Disposition characteristics of recombinant human interleukin-11 after a bolus intravenous administration in mice. *J. Pharmaco. Exp. Ther.* **275**:537–543 (1995).
16. N. Stute, W. L. Furman, M. Schell, and W. E. Evans. Pharmacokinetics of recombinant human granulocyte-macrophage colony-stimulating factor in children after intravenous and subcutaneous administration. *J. Pharm. Sci.* **84**:824–828 (1995).
17. A. Supersaxo, W. R. Hein, and H. Steffen. Effect of molecular weight on the lymphatic absorption of water-soluble compounds following subcutaneous administration. *Pharm. Res.* **7**:167–169 (1990).
18. H. Cheng and W. J. Jusko. The area function method for assessing the drug absorption rate in linear systems with zero-order input. Pharm. Res. **6**:133–139 (1989).
19. G. M. Ferron, M. Rochi, W. J. Jusko, and J.-M. Schulman. Oral absorption characteristics and pharmacokinetics of colchicine in healthy volunteers after single and multiple doses. *J. Clin. Pharmacol.* **36**:874–883 (1996).
20. T. H. Adair and A. C. Guyton. *Experimental Biology of the Lymphatic Circulation*, Elsevier Science Publishers, 1985, pp. 1–44.
21. C. A. Gloff and R. J. Wills. Pharmacokinetics and Metabolism of Therapeutic Cytokines. In B. L. Ferraiolo *et al.* (eds.), *Protein Pharmacokinetics and Metabolism*, Plenum Press, New York, 1992, pp. 127–150.
22. J. M. Trang. Pharmacokinetics and Metabolism of Therapeutic and Diagnostic Antibodies. In B. L. Ferraiolo BL *et al.* (eds.), *Protein Pharmacokinetics and Metabolism*, Plenum Press, New York, 1992, pp. 223–271.
23. S. J. H. Van Deventer, C. O. Elson, and R. N. Fedorak. Multiple doses of intravenous interleukin 10 in steroid-refractory Crohn's disease. *Gastroenterology* **113**:383–389 (1997).
24. P. Wang, P. Wu, M. I. Siegel, R. W. Egan, and M. M. Billah. IL-10 inhibits transcription of cytokine genes in human peripheral blood mononuclear cells. *J. Immunol.* **153**:811–816 (1994).
25. J. V. S. Gobburu and W. J. Jusko. Role of dosage regimen in controlling indirect pharmacodynamic responses. *Adv. Drug Deliv. Rev.* **33**:221–233 (1998).
26. B. D. Kahan, N. Tejpal, S Gibbons-Stubbers, M. Wang, S. Stepkowski, and T-C Chow. The synergistic interactions in vitro and in vivo of brequinar sodium with cyclosporine or rapamycin alone and its triple combinations. Transplantation **55**:894–900.