Pharmacokinetics of recombinant human interleukin-11 (rhIL-11) in healthy male subjects

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Aims To study the pharmacokinetics of recombinant human interleukin-11 (rhIL-11) in healthy male volunteers following subcutaneous (s.c.) and intravenous (i.v.) administration.

Methods RhIL-11 was infused intravenously at 10–50 $\mu g\,kg^{-1}$ for 1 or 3 h, or administered subcutaneously at 3–50 $\mu g\,kg^{-1}$ to volunteers. RhIL-11 was also administered at 3 $\mu g\,kg^{-1}$ s.c. once daily for 7 days. Plasma and urinary concentrations were measured by enzyme-linked immunosorbent assay (ELISA).

Results RhIL-11 showed linear pharmacokinetics after both intravenous infusion and s.c. administration. Comparison of $t_{1/2}$ and MRT values after i.v. administration with those after s.c. administration indicated that rhIL-11 pharmacokinetics after s.c. administration were absorption rate-limited. Bioavailability after s.c. administration was about 65%. Since RhIL-11 was not detected in urine after a single 50 $\mu g\,kg^{-1}$ s.c. dose, rhIL-11 was considered to be eliminated by metabolism. There was no significant change in the pharmacokinetic profile of rhIL-11 following repeated s.c. administration.

Conclusions RhIL-11 demonstrated linear pharmacokinetics at these dose ranges after single and repeated s.c. administration or constant-rate i.v. infusion in healthy volunteers.

Keywords: pharmacokinetics, recombinant human interleukin-11, ELISA, intravenous, subcutaneous, bioavailability

Introduction

Interleukin-11 (IL-11) was originally identified as a factor produced by IL-1-stimulated PU-34 bone marrow stromal cells which stimulated the proliferation of the IL-6-dependent T1165 plasmacytoma [1]. Native human IL-11 has 178 amino acid residues with proline at its *N*-terminal. In contrast, recombinant human IL-11 (rhIL-11), developed by Genetics Institute (Cambridge, MA, USA), is a *des*-Pro IL-11 which lacks this *N*-terminal proline. However, there is no difference in biological activity between rhIL-11 and native IL-11. RhIL-11 has been shown to act upon the thrombocyte system *in vitro* by enhancing megakaryocyte acetylcholinesterase levels and by increasing megakaryocyte colony size, cellular size and nuclear DNA content in the presence of IL-3 [2, 3]. RhIL-11 has also been shown to increase the number of peripheral platelets in normal and splenectomized mice when administered subcutaneously [4] and further to stimulate post-irradiation thrombocyte-poietic recovery in mice with bone marrow graft, cyclophosphamide-induced bone marrow suppression and irradiation-carboplatin induced myelo-suppression [5]. On the basis of these effects, rhIL-11 is now under investigation for development as a drug for the treatment of thrombocytopaenia.

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In humans, the pharmacodynamic effects of subcutaneously administered rhIL-11 in women with breast cancer have been reported [6, 7]. The pharmacokinetics of rhIL-11, however, have not been well characterized to date. We previously confirmed that rhIL-11 showed linear pharmacokinetics on single s.c. administration at 25–100 $\mu g\,kg^{-1}$ to Sprague-Dawley rats and cynomolgus monkeys, and that bioavailability after s.c. administration was about 30% in rats and 37–55% in monkeys (unpublished data). The aim of the present study was to evaluate the pharmacokinetics of rhIL-11 in healthy male volunteers after i.v. and s.c. administration by measuring the concentration of immunoreactive rhIL-11 in plasma and urine.

Methods

Subjects

Three studies were conducted, a single i.v. infusion study, a single s.c. administration study and a repeated s.c. administration study, involving 9, 12 and 9 healthy male volunteers, respectively. Their mean (±s.d.) ages were 24.3 (±5.7), 27.9 (±2.5) and 22.1 (±1.1) years and mean (±s.d.) body weights were 68.4 (±5.1), 64.8 (±6.3) and 67.5 (±6.5) kg, respectively. Health was confirmed from their medical history and by physical examination, electrocardiography, hematology, blood chemistry and urinalysis. In the single i.v. infusion and single s.c. administration studies, three and

four groups of three subjects each were assigned to each dosage level, respectively. In the repeated s.c. administration study, the nine subjects were randomly allocated to an active drug group ($n=6$) or a placebo group ($n=3$) according to a double-blind design. A placebo group was studied to assess the tolerability of rhIL-11 in humans. All subjects were hospitalized on the evening prior to the day of drug administration and abstained from alcoholic or caffeine-containing beverages and tobacco during the study. In addition, they fasted from the evening meal until dosing. The study protocol was approved by the Institutional Review Board of Kannondai Clinic (Tsukuba, Ibaraki, Japan). The subjects understood the objectives and procedures of the study, anticipated effects and potential adverse reactions of the study, and that they had the right to withdraw at any time. Written informed consent to participate in the study was obtained from all subjects.

Test drug

Lyophilized vial formulations containing 5 mg of rhIL-11 were supplied by Genetics Institute. Dosing solutions were prepared by dissolving the vials in physiological saline to a concentration of 100 $\mu g\,ml^{-1}$ for i.v. study and to 5 $mg\,ml^{-1}$ for s.c. study.

Study design

Single i.v. infusion Three groups of three subjects each were infused i.v. with 10 $\mu g\,kg^{-1}$ of rhIL-11 over 1 h, or 25 or 50 $\mu g\,kg^{-1}$ over 3 h via an infusion pump. Two different infusion durations were used to avoid an excessive increase in plasma rhIL-11 concentration at high dosage levels. It was previously shown that there was little adsorption of rhIL-11 by the equipment used for administration. Blood samples of 1.5 ml were drawn pre-dosing and at 15, 30, 45, 60, 65, 70, 75 and 90 min and 2, 3, 4, 6, 9, 12 and 24 h after the start of infusion in the 10-$\mu g\,kg^{-1}$ group, and pre-dosing and at 15, 45, 90, 135, 180, 185, 190, 195 and 210 min, and 4, 5, 6, 9, 12 and 24 h after the start of infusion in the 25- and 50-$\mu g\,kg^{-1}$ groups. The blood obtained was immediately transferred to polypropylene tubes containing 2.25 mg of ethylenediaminetetraacetic acid disodium (EDTA) and gently mixed, followed by centrifugation at approximately 4° C to separate plasma. The plasma was stored at −80° C until assay.

Single s.c. administration. Four groups of three subjects each received 3, 10, 25 or 50 $\mu g\,kg^{-1}$ of rhIL-11 s.c. Blood samples of 1.5 ml were obtained pre-dosing and at 10, 20 and 30 min and 1, 2, 4, 6, 9, 12, 24 and 48 h post-dosing in the 3- and 10-$\mu g\,kg^{-1}$ groups, or pre-dosing and at 15 and 30 min and 1, 2, 3, 4, 6, 9, 12, 24 and 48 h post-dosing in the 25- and 50-$\mu g\,kg^{-1}$ groups. Plasma was obtained as described above and kept at −80° C until assay. In addition, urine samples spontaneously voided were collected from 15 h before to 48 h after drug administration. A 1-ml portion of each urine sample was immediately transferred to a polypropylene tube containing 9 ml of THST buffer [THS buffer (0.05 M Tris-HCl containing 0.5 M NaCl and 1 mM glycine adjusted to pH 8.0) containing 0.05% (v/v) Tween 20] to avoid adsorption onto the tube and gently mixed. The resulting 10-fold dilution of urine was kept at −80° C until assay.

Repeated s.c. administration. Six subjects received 3 $\mu g\,kg^{-1}$ of rhIL-11 s.c. once daily for 7 consecutive days. Blood samples of 1.5 ml were obtained pre-dosing and at 15 and 30 min and 1, 2, 3, 4, 6, 9 and 12 h post-dosing on Day 1, pre-dosing on Days 2 to 7, and at 15 and 30 min and 1, 2, 3, 4, 6, 9, 12, 24, 32 and 48 h post-dosing on Day 7. Plasma was separated as described above and stored at −80° C until assay.

ELISA for rhIL -11

Preparation of standard solutions, quality control (QC) samples and substrate solution Polypropylene tubes were used for preparation of all samples. All QC samples were prepared on the same day, while standard solutions were prepared on each assay day.

For plasma assay, 100 $\mu g\,ml^{-1}$ of rhIL-11 stock solution was serially diluted with blank human plasma having predetermined endogenous IL-11 levels of $<0.2\,ng\,ml^{-1}$ to concentrations of 0.2–12.8 $ng\,ml^{-1}$. These solutions were further diluted 4-fold with THST buffer to obtain standard solutions at final concentrations of 0.05–3.2 $ng\,ml^{-1}$. QC samples for plasma assay were prepared by diluting rhIL-11 stock solution with blank plasma by a different dilution method from that used for preparation of standard samples to concentrations of 10.0, 2.0 and 0.6 $ng\,ml^{-1}$ and were then stored at −80° C. These QC samples were diluted 4-fold with THST buffer to final concentrations of 2.5, 0.5 and 0.15 $ng\,ml^{-1}$ before use. Unknown plasma samples were also diluted 4-fold with THST buffer and then assayed. In the case of concentrations of unknown plasma samples that were above the upper limit of quantitation, the samples were diluted prior to analysis with blank human plasma.

For urine assay, stock solution was serially diluted with a 10-fold dilution of blank urine in THST buffer to yield standard solutions at concentrations of 0.05–3.2 $ng\,ml^{-1}$ and QC samples at levels of 2.5, 0.5 and 0.15 $ng\,ml^{-1}$ through different dilution routes. These QC samples were stored at −80° C until use.

To prepare substrate solution for ELISA, 8.5 ml of Milli Q water prepared with a portable personal water purifier (ZD20 100Jr; Millipore Japan Ltd., Tokyo, Japan), 1 ml of 65.5 mM K_2HPO_4-16.4 mM citric acid (pH 6.3) prepared in the Milli Q water and filtered through a 0.45-μm filter, 0.5 ml of 3% hydrogen peroxide solution and one *o*-phenylenediamine dihydrochloride 10 mg tablet (Sigma Chemical Company, St Louis, MO, USA) were mixed under protection from light.

ELISA procedure A method developed by Genetics Institute (unpublished findings) was employed with some modification. Fifty microliters of the monoclonal capture antibody solution (11h3/19.6.1, Genetics Institute) diluted with 32 μM anhydrous sodium carbonate and 68 μM sodium hydrogen carbonate (pH 9.6) to a concentration of 2 $\mu g\,ml^{-1}$ was added to each well of 96-well immunoplates (Maxisorp®, Nunc Products, Roskilide, Denmark) and then the plate was

incubated at 4° C overnight (16–24 h). After removal of the solution by aspiration, the plate was washed four times with THST buffer at 300 μl per well. Two hundred microliters of 5% (w/v) gelatin (EIA grade, Bio-Rad Laboratories, Richmond, CA, USA) in THS was added and the plate was incubated at 37° C for 1.5 h. After the solution in the wells was aspirated, the plate was washed four times with 200 μl per well of THST buffer. Fifty microliters of standard solution or sample was added and the plate was incubated at 4° C overnight (16–24 h). All standards and samples were tested in duplicate. The contents of the wells were removed, the plate was washed four times with 200 μl per well of THST buffer, and 50 μl of monoclonal biotinylated detector antibody solution (11h3/15.6.13, Genetics Institute) diluted with THST buffer to a concentration of 1 μg ml^{-1} was added to each well. The plates were then shaken vigorously for 1.5 h at room temperature. After the solution was aspirated, the plate was again washed four times with THST buffer at 200 μl per well, 50 μl of avidin-horseradish peroxidase solution (Vector Laboratories, Burlingame, CA, USA) diluted with THST to a concentration of 1.25 μg ml^{-1} was added and the plate was shaken moderately for 1 h at room temperature. The contents of the wells were aspirated again, the wells were washed four times with 200 μl per well of THST buffer, and 100 μl of substrate solution was added and developed at room temperature for 5 min to permit coloration reaction under protection from light. The reaction was stopped with 100 μl of 2.25 μM sulphuric acid, and the absorbance was read at 490 nm with a Model 3550 Microplate Reader (Bio-Rad).

Using a data analysis program (Microplate Manager; Bio-Rad), drug concentration and absorbance data were processed for log transformation followed by quadratic regression to construct a standard curve. The standard plot was considered acceptable when back-calculated concentrations of the duplicate individual calibration standards were within ±20% of the nominal concentrations. In the case that assay values exceeded these limits, the regression process was resumed with the irrelevant sample excluded, up to a maximum of two samples.

Accuracy and precision The intra-assay accuracy and precision were assessed by performing an analysis of the lower and upper limits of quantitation (0.05 and 3.2 ng ml^{-1}; $n=5$ at each concentration) and QC samples at the three defined concentrations (0.15, 0.50 and 2.5 ng ml^{-1}; $n=5$ at each concentration) in plasma and 10-fold diluted urine against a calibration curve. The inter-assay accuracy and precision were calculated from data obtained during the five-day validation. The procedure was repeated on different days on the same QC samples to determine inter-assay repeatability. All samples were run in duplicate. The accuracy, expressed as percent deviation of measured concentration from prepared concentration, with the relative error (R.E.), was evaluated. The precision was expressed as the coefficient of variation (C.V.) of the measured concentrations.

Pharmacokinetic analysis

An observed value below the lower limit of quantitation was treated as 0 ng ml^{-1}. Pharmacokinetic parameters were calculated by model-independent methods. Peak plasma concentration (C_{max}) and time to C_{max} (t_{max}) were observed values. Elimination half-life ($t_{1/2}$) was calculated from $t_{1/2} = \ln 2/\lambda_z$, where λ_z was the slope obtained by least-squares linear regression of the linear phase of the log plasma concentration–time curve. Area under the plasma concentration–time curve with extrapolation to infinity (AUC) was calculated by adding C_t/λ_z to the $AUC_{(0,t)}$ where C_t was the last detectable plasma concentration at time t and AUC(0,t) was the area up to time t postdosing, calculated by the log linear trapezoidal rule. The mean residence time (MRT) was calculated from MRT = AUMC/AUC using statistical moment analysis [8], where AUMC is the total area under the first moment curve. MRT for the i.v. bolus dose (MRT_{iv}) was calculated from $MRT_{iv} = MRT - [\text{mean input time}]$, where this MRT was the value after i.v. infusion and [mean input time], was [infusion time]/2 [9]. Total body clearance (CL) and volume of distribution as steady state (V_{ss}) were calculated from CL = Dose/AUC and $V_{ss} = MRT \cdot CL$, respectively.

Statistical analysis

To assess any change in the pharmacokinetics of rhIL-11 on repeated s.c. dosing, pharmacokinetic parameters between Day 1 and Day 7 were analyzed by the paired t-test. Differences were considered significant at $P<0.05$.

Results

ELISA for rhIL-11

A typical standard curve obtained from human plasma by the ELISA method is shown in Figure 1. The assay data showed a satisfactory fit to the quadratic regression curve within a concentration range of 0.05–3.2 ng ml^{-1}, corresponding to actual concentrations of 0.2–12.8 ng ml^{-1} in

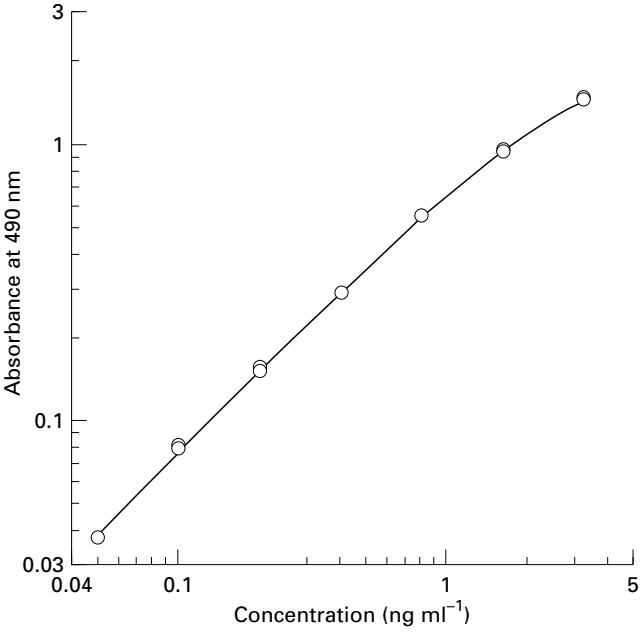

Figure 1 A standard curve (0.05–3.2 ng ml^{-1}) obtained from blank human plasma spiked with rhIL-11.

plasma and 0.5–32 ng ml^{-1} in urine because of the dilution factors of 4 and 10, respectively.

In human plasma assays for rhIL-11, intra-assay accuracy (R.E.) ranged from −7.4% to 2.9% and precision (C.V.) from 2.4% to 10.8%. The inter-assay R.E. and C.V. ranged from −2.8% to −0.7% and from 5.0% to 6.5%, respectively. In human urine assays, intra-assay R.E. and C.V. ranged from −5.6% to 13.4% and from 1.4% to 2.7%, and inter-assay R.E. and C.V. from 6.7% to 7.8% and from 3.3% to 6.8%, respectively (Table 1).

Single i.v. infusion

Figure 2 shows mean plasma concentration profiles of rhIL-11 after i.v. infusion at 10 μg kg^{-1} for 1 h and at 25 and 50 μg kg^{-1} for 3 h, and Table 2 shows pharmacokinetic parameters. In one subject after 10 μg kg^{-1} dosing, a slightly high and sustained plasma concentration profile was seen, including values in the pre-dosing plasma level. This was considered to be due to endogenous IL-11. However, it was considered that the values of this subject had little influence on pharmacokinetic analysis as the level of endogenous IL-11 was very low. We did not subtract from the measured post-dose concentrations the pre-dose concentration, as it was not known whether the level of endogenous IL-11 was constant or not. At all dose levels, the plasma concentration rose rapidly and reached a near-steady state by 30 (10-μg kg^{-1} group) or 45 (25- and 50-μg kg^{-1} groups) min after the start of infusion, and diminished rapidly after the end of infusion. Mean $t_{1/2,z}$ was virtually constant irrespective of dose, ranging from 1.8 to 2.4 h. Mean AUC was 71, 158 and 374 ng ml^{-1} h at these doses, respectively, and thus increased in a dose-dependent manner. Mean V_{ss} was 112–152 ml kg^{-1} and mean CL was 2.2–2.7 ml min^{-1} kg^{-1}; both parameters were constant irrespective of dose.

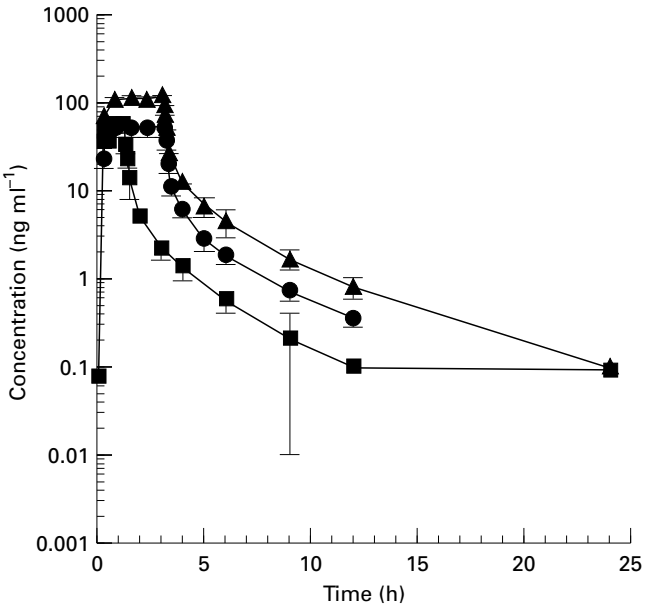

Figure 2 Plasma concentrations of rhIL-11 following constant-rate intravenous infusion for 1 h at a dose of 10 μg kg^{-1} or for 3 h at doses of 25 and 50 μg kg^{-1} to subjects. Values are mean ± s.d. of three subjects. ■, 10 μg kg^{-1} h^{-1}; ●, 25 μg kg 3 h^{-1} (8 μg kg^{-1} h^{-1}); ▲, 50 μg kg^{-1} 3 h^{-1} (17 μg kg^{-1} h^{-1}).

Single s.c. administration

Mean profiles of plasma rhIL-11 concentration after s.c. administration are shown in Figure 3, and pharmacokinetic parameters in Table 2. t_{max} ranged from 2.0 to 3.0 h at doses of 3, 10, 25 and 50 μg kg^{-1}, being almost constant irrespective of dose. Mean C_{max} values were 0.9, 3.5, 8.1 and 19.0 ng ml^{-1}, and those of AUC were 15.0, 48.7, 115 and 242 ng ml^{-1} h, with both parameters increasing in a

Table 1 Accuracy and precision of ELISA method for rhIL-11 in human plasma and urine.

Matrix		*Prepared concentration (ng ml^{-1})*	*Measured★ concentration (ng ml^{-1})*	*R.E.†* *(%)*	*C.V.‡* *(%)*
Plasma	Intra-assay	0.05	0.05 ± 0.00	−7.4	5.0
	(*n* = 5)	0.15	0.15 ± 0.01	2.9	4.0
		0.50	0.51 ± 0.03	1.0	4.9
		2.5	2.3 ± 0.3	−6.5	10.8
		3.2	3.2 ± 0.1	0.5	2.4
	Inter-assay	0.15	0.15 ± 0.01	−0.7	6.0
	(*n* = 25)	0.50	0.49 ± 0.03	−2.0	5.0
		2.5	2.4 ± 0.2	−2.8	6.5
Urine	Intra-assay	0.05	0.05 ± 0.00	−4.0	2.7
	(*n* = 5)	0.15	0.17 ± 0.00	10.8	2.3
		0.50	0.57 ± 0.01	13.4	1.4
		2.5	2.7 ± 0.1	7.2	2.0
		3.2	3.0 ± 0.1	−5.6	1.7
	Inter-assay	0.15	0.16 ± 0.01	7.0	5.4
	(*n* = 25)	0.50	0.54 ± 0.04	7.8	6.8
		2.5	2.7 ± 0.1	6.7	3.3

★Mean ± s.d.

†R.E.: (Measured concentration–Prepared concentration)/Prepared concentration × 100.

‡C.V.: coefficient of variation.

Table 2 Pharmacokinetic parameters of rhIL-11 following constant-rate intravenous infusion for 1 h at a dose of 10 μg kg^{-1} or for 3 h at doses of 25 and 50 μg kg^{-1} and following subcutaneous administration at doses of 3, 10, 25 and 50 μg kg^{-1} to subjects.

Route	*Dose* (μg kg^{-1})	C_{end}★ (ng ml^{-1})	C_{max} (ng ml^{-1})	*AUC* (ng ml^{-1} h)	t_{max} (h)	$t_{1/2,z}$ (h)	V_{ss} (ml kg^{-1})	*CL* (ml min^{-1} kg^{-1})	*MRT* (h)	F† (%)
i.v.	10	53.8±4.3	—	71.0±5.6	—	1.8±0.5	152±98	2.4±0.2	1.1±0.8	—
	25	49.7±4.4	—	158±11	—	2.4±0.1	125±9	2.7±0.2	0.8±0.1	—
	50	120±4	—	374±11	—	2.3±0.1	112±27	2.2±0.1	0.8±0.2	—
s.c.	3	—	0.9±0.2	15.0±8.2	2.0±0.0	8.1±2.0	—	—	14.2±6.6	67
	10	—	3.5±0.9	48.7±6.8	2.7±1.2	6.9±2.1	—	—	11.4±4.8	65
	25	—	8.1±1.3	115±10	3.0±1.0	8.0±2.1	—	—	11.5±2.4	62
	50	—	19.0±3.2	242±19	2.7±1.2	8.1±0.5	—	—	10.9±1.3	65

Values are mean±s.d. of three subjects.

★C_{end}: Plasma concentration at the end of infusion.

†Calculated by using AUC value from intravenous dose study at 50 μg kg^{-1}.

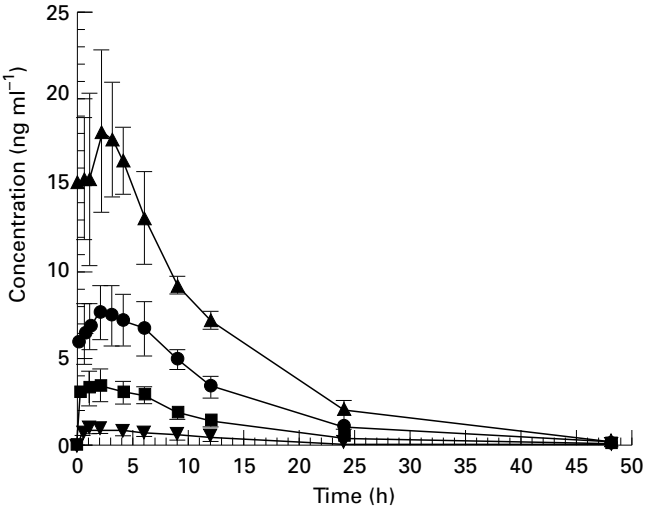

Figure 3 Plasma concentrations of rhIL-11 following subcutaneous administration at doses of 3, 10, 25 and 50 μg kg^{-1} to subjects. Values are mean±s.d. of three subjects. ▼, 3 μg kg^{-1}; ■, 10 μg kg^{-1}; ●, 25 μg kg^{-1}; ▲, 50 μg kg^{-1}.

dose-dependent manner. After t_{max}, plasma concentration declined gradually at all dose levels, with $t_{1/2,z}$ and MRT virtually constant irrespective of dose, ranging from 6.9 to 8.1 h and from 10.9 to 14.2 h, respectively.

In addition, concentrations of rhIL-11 in urine samples obtained at the maximum dose, 50 μg kg^{-1}, were below the lower limit of quantitation (0.50 ng ml^{-1}) at all doses. Urine assay at 3–25 μg kg^{-1} was not performed because the plasma concentration increased dose-dependently at the range of 3–50 μg kg^{-1}, as described above.

Repeated s.c. administration

Plasma concentration curves on Day 1 and 7 after daily repeated s.c. administration are given in Figure 4. Five of the six subjects exhibited similar plasma concentration profiles on both days. In contrast, one subject, subject No. 0904, showed an apparently high and sustained plasma concentration profile, with levels about 2 ng ml^{-1} higher than those of the other subjects throughout the study period, including the pre-dosing plasma level on Day 1. This result was considered to be due to higher plasma levels of endogenous IL-11. Another subject, No. 0908, showed a slightly sustained plasma concentration profile, which was also considered to be due to endogenous IL-11. The values of this subject were not excluded when pharmacokinetic analysis was conducted as the level of his endogenous IL-11 was very low. Daily pre-dosing plasma levels were comparable with those on Day 1. These levels, which were mean values of five subjects excluding those of subject No. 0904, were within the range of 0.06–0.17 ng ml^{-1}. Furthermore, comparison of mean pharmacokinetic parameters excluding those of subject No. 0904 showed no significant difference between Day 1 and 7 in t_{max} (1.5 *vs* 2.0 h), C_{max} (2.0 *vs* 1.9 ng ml^{-1}), AUC(0,24h) (19.3 *vs* 17.5 ng ml^{-1} h), $t_{1/2,z}$ (6.4 *vs* 6.1 h) or MRT (9.9 *vs* 10.9 h), ($P>0.05$; paired *t*-test) (Table 3).

Discussion

Prior to initiation of this clinical study, we modified and then validated an ELISA method developed by Genetics Institute. Standard curves prepared for human plasma and urine with the modified method were all satisfactory within the range of 0.05 to 3.2 ng ml^{-1} (corresponding to actual concentrations of 0.2 to 12.8 ng ml^{-1} in plasma and of 0.5 to 32 ng ml^{-1} in urine due to 4- and 10-fold dilution, respectively). The method was assessed for accuracy and precision at the lower and higher limits of quantitation and also for intra- and inter-assay accuracy and precision at three QC sample concentrations. The resultant R.E. and C.V. were within ±15% at all concentrations determined, indicating that the method described herein satisfies the proposed requirements for analysis in pharmacokinetic studies [10]. Moreover, the lower limit of quantitation (0.05 ng ml^{-1}) indicates that this method is sensitive enough to quantify the drug at low dose levels. These results indicate that this ELISA method is suitable for pharmacokinetic analysis of rhIL-11 in humans.

Following single i.v. infusion at 10, 25 and 50 μg kg^{-1}, the mean plasma concentration of rhIL-11 at the end of infusion was 53.8, 49.7 and 120 ng ml^{-1} (Table 2), which corresponded to 76%, 95% and 96% of plasma concentration at steady state as estimated from infusion rate/CL (70.6, 52.4 and 125 ng ml^{-1}), respectively. In this i.v. infusion study, two different infusion durations were used to prevent an excessive increase in plasma concentration of rhIL-11.

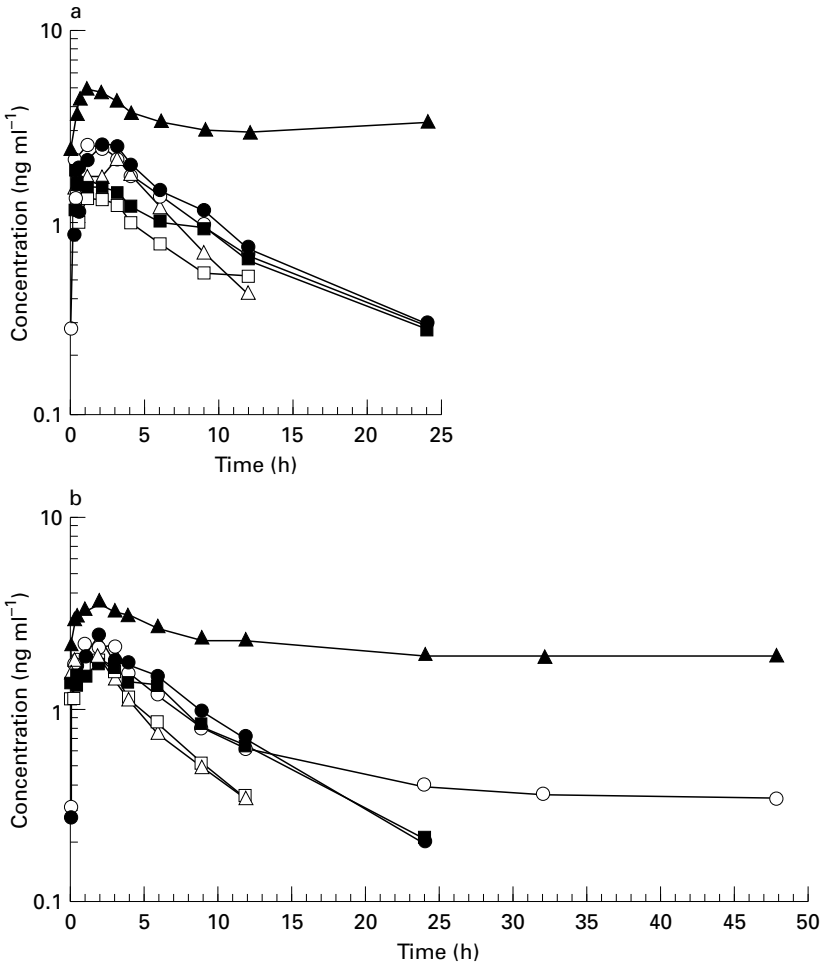

Figure 4 Plasma concentrations of rhIL-11 on (A) Day 1 and (B) Day 7 during single daily repeated subcutaneous administration for 7 days at a dose of 3 μg kg^{-1} to subjects. Each symbol represents a single subject.

Table 3 Pharmacokinetic parameters of rhIL-11 on Day 1 and Day 7 during single daily subcutaneous administration for 7 days at a dose of 3 μg kg^{-1} to subjects.

Subject	*Day*	t_{max} *(h)*	C_{max} *(ng ml^{-1})*	*AUC(0,24h) (ng ml^{-1} h)*	*AUC (ng ml^{-1} h)*	$t_{1/2,z}$ *(h)*	*MRT (h)*
0901	1	0.3	1.7	18.6	—	$9.5_{[6-24h]}$†	13.2
	7	2.0	1.7	19.0	21.3	$7.3_{[6-24h]}$	10.6
0903	1	2.0	2.5	24.5	—	$7.8_{[6-24h]}$	10.9
	7	2.0	2.3	21.6	23.5	$6.5_{[6-24h]}$	9.6
0904	1	1.0	4.8	80.3	—	$16.7_{[4-9h]}$	29.7
	7	2.0	3.5	57.1	131.3	$11.6_{[4-9h]}$	31.6
0905	1	1.0	1.3	13.2	—	$5.1_{[3-9h]}$	8.9
	7	2.0	1.7	12.7	13.0	$4.7_{[4-12h]}$	7.0
0908	1	1.0	2.5	23.2	—	$5.7_{[4-12h]}$	9.8
	7	2.0	2.1	21.5	33.5	$7.3_{[6-12h]}$	20.4
0909	1	3.0	2.1	17.2	—	$4.0_{[4-12h]}$	6.5
	7	2.0	1.7	12.8	13.2	$4.9_{[4-12h]}$	7.1
Mean*	1	1.5	2.0	19.3	—	6.4	9.9
(s.d.)		(1.1)	(0.5)	(4.6)	(—)	(2.2)	(2.5)
	7	2.0	1.9	17.5	20.9	6.1	10.9
		(0.0)	(0.3)	(4.5)	(8.5)	(1.3)	(5.5)

*Calculated by excluding values of subject No. 0904.
†Time range for calculating half-life.

We were concerned that this use of two different infusion periods might have interfered with the ability to observe concentration-dependent pharmacokinetics over the five-fold range of doses. However, we conclude that the results indicated with sufficient veracity that rhIL-11 showed linear pharmacokinetics in humans when administered intravenously within the range of 10–50 μg kg^{-1}.

The results also demonstrated that rhIL-11 shows linear pharmacokinetics after single s.c. administration within the range of 3–50 μg kg^{-1}. When calculated on the basis of AUC after an i.v. dose of 50 μg kg^{-1}, the bioavailability of rhIL-11 in humans following s.c. administration at 3, 10, 25 and 50 μg kg^{-1} was 67%, 65%, 62% and 65%, respectively (Table 2). Supersaxo *et al.* [11] showed that, on s.c.

administration, much protein passes directly into the lymphatics rather than into the blood capillaries on route to the systemic circulation. It was thought that rhIL-11 bound to target cells in the lymph nodes, and that any remaining unbound rhIL-11 then entered the circulation. Another possibility is that rhIL-11 administered subcutaneously was partly degraded at the administration site or in regional lymph nodes, because proteases existing in these sites are thought to contribute to the decomposition of recombinant proteins after s.c. administration [12, 13]. Half-life and MRT after s.c. injection were about 3–5 times and 10–18 times greater than those after i.v. injection, respectively. These findings indicate that the pharmacokinetics of subcutaneously injected rhIL-11 is absorption rate-dependent.

In the repeated s.c. administration study, one subject showed an apparently high and sustained plasma concentration profile. This phenomenon was considered to be caused by assay interference due to endogenous IL-11. However, another possibility, namely assay interference, was also considered. Levinson has shown that heterophilic antibodies, that is, endogenous antibodies with a broad spectrum of reactivities, interfered with immunoassay [14]. The possibility was, therefore, considered that this phenomenon was caused by assay interference due to heterophilic antibodies. In any case, as plasma concentration profile of this subject did not show the true profile of rhIL-11, pharmacokinetic parameters were calculated with these values excluded. There was no significant difference in any pharmacokinetic parameter after repeated s.c. administration between Day 1 and 7 ($P > 0.05$), indicating that the pharmacokinetic profile of rhIL-11 shows little change during repeated s.c. dosing at 3 $\mu g\,kg^{-1}$ once daily for 7 days. In addition, pre-dosing plasma levels of rhIL-11 on consecutive dosing days were comparable with those on Day 1 in all subjects, suggesting that no accumulation of the drug occurs following repeated administration. Moreover, rhIL-11 is tolerated well and shows no evidence of serious irreversible toxicity on subcutaneous dosing.

No immunoreactive rhIL-11 was detected in the urine of human volunteers dosed subcutaneously with rhIL-11. In general, glomerular filtration is considered to be the rate-limiting step in the total body clearance of proteins having a molecular weight of around 20 kD [15]. The renal elimination of GM-CSF (MW 15.5–19.5 kD) has been characterized by glomerular filtration with subsequent tubular reabsorption and enzymatic degradation [16]. The kidneys also play an important role in the disposition of IL-2 (MW 15 kD), the renal clearance of which accounts for about 75% of elimination from the systemic circulation [17]. In addition, it appeared that proteolysis in the proximal tubules contributed to most of the renal elimination of IL-2, as reported by Ohnishi *et al.* [18, 19]. In the present study, total body clearance of rhIL-11 after i.v. administration to healthy adult male volunteers was 2.2–2.7 $ml\,min^{-1}\,kg^{-1}$, which is similar to the value for creatinine clearance in humans (generally 1.7–2.0 $ml\,min^{-1}\,kg^{-1}$). In a biodistribution study conducted by administration of [^{125}I]-rhIL-11 in rats, concentration of radioactivity was about six times higher in kidney than in plasma (unpublished data). Therefore, considering that rhIL-11 has a molecular weight of 19kD, the kidneys probably play an important role in the total body clearance of rhIL-11. Takagi *et al.* [20] have shown that urinary excretion of intact rhIL-11 accounts for as little as about 1% of the dose in mice dosed intravenously with ^{111}In-rhIL-11. They have also shown that rhIL-11 following i.v. administration to mice undergoes glomerular filtration with reabsorption from the proximal tubules. From these findings, rhIL-11 is considered to be metabolized or degraded in humans, as it also is in laboratory animals, and to undergo little excretion in urine as the intact form.

In conclusion, the pharmacokinetics of rhIL-11 in humans can be summarized as follows: 1. rhIL-11 shows linear pharmacokinetics after both i.v. infusion at 10–50 $\mu g\,kg^{-1}$ and s.c. administration at 3–50 $\mu g\,kg^{-1}$. 2. This drug shows absorption rate-dependent pharmacokinetics and bioavailability of about 65% after s.c. administration. 3. No significant pharmacokinetic change and no accumulation in plasma occur during repeated s.c. administration. 4. rhIL-11 is eliminated via a metabolic process.

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