

# Plasma and Lymph Pharmacokinetics of Recombinant Human Interleukin-2 and Polyethylene Glycol-Modified Interleukin-2 in Pigs<sup>1</sup>

SHARON A. CHEN,<sup>2</sup> RONALD J. SAWCHUK, RICHARD C. BRUNDAGE, CHRISTOPHER HORVATH,<sup>3</sup>  
H. VINCENT MENDENHALL, ROBERT A. GUNTHER, and RENE A. BRAECKMAN<sup>4</sup>

Department of Pharmacokinetics and Pharmacodynamics, Chiron Corporation, Emeryville, California (S.A.C., R.A.B.); Bioanalytic and Pharmacokinetic Services, Department of Pharmaceutics, University of Minnesota, Minneapolis, Minnesota (R.J.S., R.C.B.); Department of Experimental Medicine and Surgery, Primedia Corporation, Worcester, Massachusetts (C.H., H.V.M.); and Department of Surgery, University of California, Davis, Davis, California (R.A.G.)

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## ABSTRACT

Modification of recombinant human interleukin-2 (IL-2) with polyethylene glycol (PEG-IL-2) decreases clearance and might favor absorption into the lymphatics, due to its increased molecular weight. In the present study, we compared the plasma and lymph concentrations of IL-2 and PEG-IL-2 in Yorkshire pigs. The IL-2 regimens were i.v. bolus ( $0.1\text{--}1.6 \times 10^6$  I.U., MIU/kg), 15-min i.v. infusion (0.1 MIU/kg), or s.c. bolus (0.1–3.0 MIU/kg). The PEG-IL-2 doses were 15-min i.v. infusion (0.01 MIU/kg) or s.c. bolus (0.01–0.10 MIU/kg). Lymph and plasma data were analyzed using noncompartmental methods and NONMEM. Bioavailability of IL-2 was route- and dose-dependent. Bioavailability of i.v. bolus doses of  $\geq 0.16$  MIU/kg was complete but only 39% at 0.1 MIU/kg. For the infusion and s.c. doses, bioavailability was 28 and 42%, respectively. Noncompartmental and NONMEM estimates of clearance and volume

of distribution at steady state agreed: 300 ml/h/kg and 570 ml/kg, respectively, for IL-2. The ratio of the area under the curve in lymph and plasma increased from 0.67 to 3.4 when comparing i.v. and s.c. routes, and the s.c. delivery advantage (ratio of dose-normalized ratio of the area under the curve in lymph after s.c. and i.v. administration) was 6.6 to 16. For PEG-IL-2, bioavailability was 100%, clearance was 5.9 ml/h/kg, and volume of distribution at steady state was 370 ml/kg. The ratio of the area under the curve in lymph and plasma increased from 0.33 (i.v.) to 1.2 (s.c.), and the s.c. delivery advantage was 3.8. Subcutaneous dosing would be favored over i.v. dosing, and IL-2 would be favored over PEG-IL-2 to maximize lymph and minimize plasma exposure. Because IL-2 efficacy may be related to lymph concentrations, dosing regimens can now be designed to test this hypothesis.

Proleukin (aldesleukin) recombinant human interleukin-2 (IL-2) has antitumor activity in patients, with significant responses occurring most frequently in renal cell carcinoma and malignant melanoma (West et al., 1987; Rosenberg et al.,

1989). The IL-2 receptor-positive T-lymphocytes are thought to be primarily, but not exclusively, associated with efficacy and reside largely in the lymphoid organs (Stites et al., 1994). On the other hand, after IL-2 exposure, natural killer cells and neutrophils in plasma produce cytokines, reactive oxygen intermediates, and proteases, all of which have been shown to be necessary, but not sufficient, to produce the full spectrum of IL-2 toxicities (Caligiuri, 1993). Therefore, adverse in vivo activity of IL-2 may be related to the plasma concentrations, but beneficial activity may be related to lymph concentrations.

Absorption of macromolecules like IL-2 can be targeted to

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<sup>2</sup> Present address: Microcide Pharmaceuticals, Inc., 850 Maude Ave., Mountain View, CA 94043.

<sup>3</sup> Present address: LeukoSite, Inc., 215 First St., Cambridge, MA 02142.

<sup>4</sup> Present address: Ceptyr, 22215 26th Ave. SE, Bothell, WA 98021.

**ABBREVIATIONS:** IL-2, interleukin-2; MIU,  $\times 10^6$  I.U.; PEG-IL-2, polyethylene glycol-modified IL-2; PEG, polyethylene glycol; CL and CL/F, clearance and apparent clearance;  $V_{ss}$  and  $V_{ss}/F$ , volume of distribution at steady state and apparent volume of distribution at steady state; MRT, mean residence time;  $k_a$ ,  $k_{a,central}$ , and  $k_{a,lymph}$ , absorption rate constant and absorption rate constant into the central and lymph compartments, respectively;  $F$  and  $F_{bol}$ ,  $F_{inf}$ ,  $F_{i.v.}$ , and  $F_{s.c.}$ , bioavailability and bioavailability after dosing by bolus and infusion and after i.v. and s.c. administration, respectively;  $F_L$ , fraction of the s.c. dose that initially is absorbed via the lymphatic system; lo, med, and hi, low, middle, and high dose ranges; AUC, AUC<sub>inf</sub>, AUC<sub>p</sub>, AUC<sub>L</sub>, AUC<sub>Li.v.}</sub>, and AUC<sub>LS.c.}</sub>, area under the curve and after dosing by infusion, area under the plasma curve and lymph curves, and area under the lymph curve after i.v. and s.c. administration, respectively;  $Ds_A$ , distribution advantage;  $Dv_A$ , delivery advantage; ELISA, enzyme-linked immunosorbent assay.

the lymphatic system by s.c. rather than i.v. administration, because the plasma concentrations of macromolecules are dependent on capillary and lymphatic absorption processes after s.c. dosing (Bocci et al., 1986; Supersaxo et al., 1988, 1990). Macromolecules like IL-2 diffuse through the interstitium and enter both blood and lymph capillaries (Guyton, 1981a). Proteins circulate within the lymph and are gradually returned to the blood (Guyton, 1981b). Because the primary and secondary lymphoid organs may be the site of action of IL-2, the intensity of a pharmacological response may depend on both the systemic exposure (as measured by the plasma concentrations) and the route of administration. By simultaneously measuring the plasma and lymph concentrations, the rate of absorption directly from the injection site into the blood and lymph and the transfer rate from the lymph to the blood can be measured.

As a small protein (<50,000), IL-2 is rapidly cleared from the body by glomerular filtration, peritubular extraction (Gibbons et al., 1995), and, in humans, an inducible receptor-mediated mechanism (Piscitelli et al., 1996). Therefore, frequent dosing is required for efficacy. To decrease IL-2 clearance, its molecular weight was increased by the addition of monomethoxy polyethylene glycol (PEG) molecules to form PEG-IL-2 (Katre et al., 1987; Knauf et al., 1988). PEG-IL-2 has an apparent molecular weight of 95,000 to 250,000, whereas IL-2 has a molecular weight of 15,000.

There is a direct relationship between molecular weight up to 19,000 and the proportion of a dose transported lymphatically after the s.c. administration of a neutral, water-soluble compound (dextran; Supersaxo, 1990), and a diverse set of proteins between molecular weights 7,500 and 75,000 (Xie and Hale, 1996). Xie and Hale (1996) also found that positively charged proteins had decreased lymphatic absorption relative to negatively charged proteins with similar molecular weight. The addition of PEG to IL-2 increased the molecular weight and resulted in an overall decrease in positive charge of IL-2 after PEG attachment to lysines (Knauf et al., 1988). It was expected that PEG-IL-2 would be preferentially absorbed via the lymphatics compared with IL-2. However, the most profound consequence of changing IL-2 to PEG-IL-2 was the increased water solubility (Katre et al., 1987). Therefore, the extent of absorption of PEG-IL-2 compared with IL-2 into the lymphatic system is not predictable from previously conducted studies.

The purpose of this study was 2-fold: first, to characterize the pharmacokinetics of IL-2 and PEG-IL-2 in Yorkshire pigs after i.v. and s.c. administration using relevant clinical regimens and doses; second, to determine the distributional advantage of IL-2 and PEG-IL-2 into lymph after i.v. and s.c. administration. The results of this study will help determine whether extravascular routes of administration preferentially favor lymphatic exposure of IL-2 and PEG-IL-2. If there is a difference in biodistribution between the i.v. and s.c. administration of IL-2, the toxicity of IL-2 may be reduced by modifying the route and schedule of administration (Whittington and Faulds, 1993; Anderson and Sorenson, 1994). Furthermore, equivalent dosing regimens of IL-2 and PEG-IL-2 may be designed that minimize toxicity based on the plasma pharmacokinetics and result in similar in vivo activity based on the lymphatic pharmacokinetics.

## Experimental Procedures

### Materials

*Escherichia coli*-derived IL-2 (Kato et al., 1985), a 132-amino acid, nonglycosylated protein, was supplied in vials as a sterile lyophilized powder. After reconstitution with sterile water, the solution contained 1.1 mg/ml ( $18 \times 10^6$  I.U., MIU/ml) IL-2 protein. The specific activity of IL-2 has been assigned based on the in vitro activity of native IL-2 in a cell proliferation bioassay and was 16 MIU/mg.

A chemical modification of IL-2 (PEG-IL-2) was prepared by the addition of three or four polyethylene glycol molecules (molecular mass, approximately 7000 Da) as previously described (Katre et al., 1987; Knauf et al., 1988). PEG-IL-2 was also supplied in vials as a sterile lyophilized powder. After reconstitution with sterile water, the solution contained 0.45 mg/ml (1.8 MIU/ml) PEG-IL-2 protein. PEG-IL-2 recognizes and interacts with both the high- and intermediate-affinity IL-2 receptor complexes; however, the biological specific activity of PEG-IL-2 show 4- to 6-fold less activity compared with IL-2. The specific activity of PEG-IL-2 used in these studies was 4 MIU/mg. To compensate for the reduced activity, both materials were dosed based on activity.

### Experimental Design

The study was conducted in two parts using Yorkshire pigs. Part I was a crossover study conducted at the University of California at Davis (Davis, CA) that evaluated the plasma pharmacokinetics of IL-2 after the i.v. and s.c. bolus administration of 0.16 and 1.6 MIU/kg (Table 1). Three animals received all four treatments sequentially, and an additional animal received two s.c. doses of 0.16 MIU/kg. There was a 1- to 3-day washout period between dosing. The IL-2 was administered undiluted, and the injections were made as a bolus into a femoral vein catheter or under the skin of an area of the thigh tented by the restraining thumb and forefinger for i.v. or s.c. administration, respectively.

Part II was a single-dose study conducted at Primedica Corporation (Worcester, MA), which evaluated both the plasma and lymph pharmacokinetics of IL-2 and PEG-IL-2. The animals (three or four per group) received a single injection of either IL-2 or PEG-IL-2 as a 15-min i.v. infusion or s.c. bolus injection; one group received IL-2 as an i.v. bolus injection (Table 1). The IL-2 and PEG-IL-2 were administered undiluted or diluted with 5% dextrose in water to keep

TABLE 1  
Groups, doses, and animal weights  
Values are mean  $\pm$  S.D.

Group	n	Dose	
		MIU/kg	Weight kg
IL-2			
Part I: Crossover design <sup>a,b</sup>			
i.v. bolus	3	0.16	40.5 $\pm$ 4.7
i.v. bolus	3	1.6	40.5 $\pm$ 4.7
s.c. bolus	4 <sup>c</sup>	0.16	38.8 $\pm$ 5.1
s.c. bolus	3	1.6	40.5 $\pm$ 4.7
Part II: Single-dose design <sup>d</sup>			
i.v. bolus	3	0.1	24.1 $\pm$ 1.3
15-min i.v. infusion	3	0.1	22.9 $\pm$ 1.9
s.c. bolus	4	0.1	23.2 $\pm$ 1.3
s.c. bolus	4	1.0	21.6 $\pm$ 2.9
s.c. bolus	4	3.0	23.8 $\pm$ 2.9
PEG-IL-2			
15-min i.v. infusion	3	0.01	21.6 $\pm$ 1.1
s.c. bolus	4	0.01	23.7 $\pm$ 1.5
s.c. bolus	4	0.03	22.1 $\pm$ 3.7
s.c. bolus	3	0.10	25.6 $\pm$ 4.5

<sup>a</sup> Male animals were used in part I.

<sup>b</sup> This study was designed for three animals that received all four treatments sequentially.

<sup>c</sup> A fourth animal was dosed twice with this dose and regimen; therefore, a total of five determinations was made.

<sup>d</sup> Female animals were used in part II.

injection volumes similar. Injections were made through a catheter in a marginal ear vein or as a bolus under the skin of an area of the thigh tented by the restraining thumb and forefinger for i.v. or s.c. administration, respectively. The left side was chosen because the right side was involved in the surgical procedures.

## Animals

Yorkshire pigs were obtained from local farmers near Davis, CA (part I), or from Earle Parsons and Sons, Inc. (Hadley, MA; part II). During a quarantine period of at least 7 days, the pigs were handled daily to acclimate them to close human contact. In part II, the animals were also acclimated to wearing an aluminum protective jacket during the quarantine period.

## Cannulations

Before the study and while the animals were under surgical anesthesia, catheters were placed in the jugular vein for blood sampling and in the femoral vein for i.v. dosing (part I). In part II, surgery to insert a cannula in both the external jugular vein for blood collection and thoracic duct for lymph collection was performed 1 to 3 days before dosing and was based on a previously published method (Jensen et al., 1990).

Briefly, the surgical method to insert the jugular vein and thoracic duct catheters was as follows: the external jugular vein was ligated cranially, and a catheter was inserted and advanced caudally approximately 10 cm so that the tip would be positioned in the superior vena cava, close to the right atrium. The catheter was tunneled s.c. to an area midway between the scapulae.

A right lateral thoracotomy was then performed through the fifth to seventh intercostal space, and for most of the animals, a rib was removed. The thoracic duct was identified subpleurally between the aorta and the vertebral bodies. The thoracic duct was ligated cranial to the insertion point. An appropriately sized Silastic catheter or Hydrocath was inserted into the duct and advanced approximately 1 to 2 cm caudally, so that the tip was positioned at approximately the level of the 10th thoracic vertebral body. The catheter was filled with heparin (5000 I.U./ml) to prevent coagulation. The catheter was passed through the right 7th intercostal space to a site 15 to 20 cm caudal to the site of exit of the jugular catheter.

The thoracic duct catheter was attached to the jugular vein catheter by a single three-way stopcock. The thoracotomy and exit sites were closed in a standard fashion. An aluminum jacket was placed on the animals, which were allowed to recover from anesthesia. The animals wore this jacket continuously throughout the study period; as the animals grew, the jacket was replaced or adjusted as necessary.

The jugular and thoracic duct catheters were flushed twice daily by a retrograde infusion of heparin into the lymph catheter and an

antegrade infusion of heparin into the venous catheter to maintain patency.

## Sample Collection Procedures

At each time of collection, blood (2 ml each) was obtained from the cannula in the jugular vein and placed in labeled tubes containing heparin (part I) or EDTA (part II). The collection times were pre-treatment and 11 to 15 times for up to 48 h after dosing. After centrifugation, plasma samples were placed into sample storage tubes and frozen at  $-70^{\circ}\text{C}$  until assayed.

In addition to blood samples, lymph samples (2 ml each) were collected from the externalized thoracic duct cannula and placed in labeled tubes containing EDTA (part II). The collection times were the same as the blood samples. The lymph samples were stored frozen at  $-70^{\circ}\text{C}$  until assayed.

## Assays

In part I, concentrations of IL-2 in plasma were measured in a bioassay adapted from Gillis et al. (1978). Cell proliferation was quantified by the incorporation of [ $^3\text{H}$ ]thymidine into an IL-2-dependent cell line, HT2A5E, which was a subclone of murine lymphocytes (Watson, 1979). The plasma and lymph samples collected from IL-2-treated pigs in part II were assayed using a commercially available double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) specific for IL-2 (Amersham, Arlington Heights, IL). The quantification of PEG-IL-2 in plasma and lymph samples used an ELISA specific for IL-2 that was developed in-house. A murine monoclonal antibody was used for capture, and a horseradish peroxidase-conjugated affinity-purified rat anti-human IL-2 polyclonal antibody was used for detection. For the bioassay, standards were spiked into pig plasma, but the controls were made in buffer. For each ELISA, both the standards and controls, either IL-2 or PEG-IL-2 was spiked in pig plasma and lymph; the performance of each assay is summarized in Table 2.

## Pharmacokinetic Analysis

**Noncompartmental Analysis.** The plasma and lymph concentration-time data were analyzed separately using Pharm-NCA (Non-compartmental Pharmacokinetic Data Analysis Software, Simed S.A., Creteil, France). The area under the plasma and lymph concentration-time curves ( $\text{AUC}_P$  and  $\text{AUC}_L$ , respectively) were calculated using linear-log trapezoidal rules, with extrapolation to time infinity using the estimated terminal elimination half-life ( $t_{1/2}$ ).

The clearance and apparent clearance (CL and CL/F, respectively), the volume of distribution at steady state and apparent volume of distribution at steady state ( $V_{ss}$  and  $V_{ss}/F$ , respectively), and the mean residence time and mean residence time of drug in the body (MRT) were calculated by standard techniques (Gibaldi and Perrier, 1982). The possible existence of nonlinearities in IL-2 and PEG-IL-2

TABLE 2  
Assay specifications

Type	Matrix	Analyte	Standard Curve Range	Average Recovery	Average Precision	
					Intra-Assay	Interassay
			<i>ng/ml</i>		<i>%</i>	
Bioassay <sup>a</sup>	Plasma	IL-2	0.31–1	N.A. <sup>b</sup>	N.A.	24
ELISA <sup>c</sup>	Plasma	IL-2	0.031–2	100	4.5	8.5
ELISA <sup>c</sup>	Lymph	IL-2	0.031–2	103	5.1	7.6
ELISA <sup>d</sup>	Plasma	PEG-IL-2	1.56–25	108	4.2	17.3
ELISA <sup>d</sup>	Lymph	PEG-IL-2	1.56–25	82	7.0	12.9

<sup>a</sup> A cell proliferation assay with IL-2-dependent murine lymphocytes (Gillis et al., 1978; Watson, 1979). Samples were assayed undiluted or with assay medium so that concentrations fall within the standard curve range.

<sup>b</sup> N.A., not available.

<sup>c</sup> A commercially available ELISA (Amersham). Samples were assayed undiluted or with 50% fetal bovine serum so that concentrations fall within the standard curve range.

<sup>d</sup> This in-house assay used a murine monoclonal antibody for capture and a horseradish peroxidase-conjugated affinity-purified rat anti-human IL-2 polyclonal antibody for detection. The assay required a 1:1 dilution of the plasma with 100% pig plasma containing 4% SDS and a 1:1 dilution of the lymph with fetal bovine serum containing 4% SDS before assay.

absorption, distribution, or elimination kinetics was investigated with one-way ANOVA (JMP Statistical Software for the Macintosh, Version 3.1.6, 1996; SAS Institute Inc., Cary, NC) of the CL/F and  $V_{ss}/F$  values as a function of administration route and dosing level. For any parameter that showed significant differences in the ANOVA, Tukey-Kramer HSD (honest significantly difference) tests were applied to determine which pairs of mean values were significantly different. Significance was set at the .05 level.

**Compartmental Analysis.** A population-based analysis of the pharmacokinetics data was performed using the computer program NONMEM version IV level 1.0 with PREDPP version III level 1.0 and NMTRAN version II level 3.0 (Beal and Sheiner, 1992). All of the available data were analyzed simultaneously for each test article to provide estimates of the average population values of the pharmacokinetic parameters (fixed effects) as well as the degree of variation between and within individuals (random effects). In all, 506 observations in 22 animals constituted the database for IL-2, and 298 observations in 14 animals constituted the database for PEG-IL-2. The observation that the relative concentrations of IL-2 and PEG-IL-2 in plasma and lymph were different depending on the route of administration was justification for assigning them to separate compartments.

For both IL-2 and PEG-IL-2, a linear multicompartment model with observations in a central (plasma) and a peripheral (lymph) compartment was used. The final models adopted for both compounds based on the objective function and diagnostic plots are depicted in Fig. 1.

The final model for IL-2 also included two additional noneliminating peripheral compartments, reversibly connected to the sampling (plasma and lymph) compartments, respectively, to account for the significant distributive phases observed (Fig. 1). Bioavailability fractions,  $F$ , were assigned separately to the i.v. infusion and bolus doses, as well as to the s.c. doses. The model was parameterized with a systemic or an elimination clearance, a clearance from the central to the lymph compartment, and an intercompartmental clearance between each pair of sampled and unsampled peripheral compartments. Four volume terms were also in the model. A dosing compartment for the s.c. dose was also included.

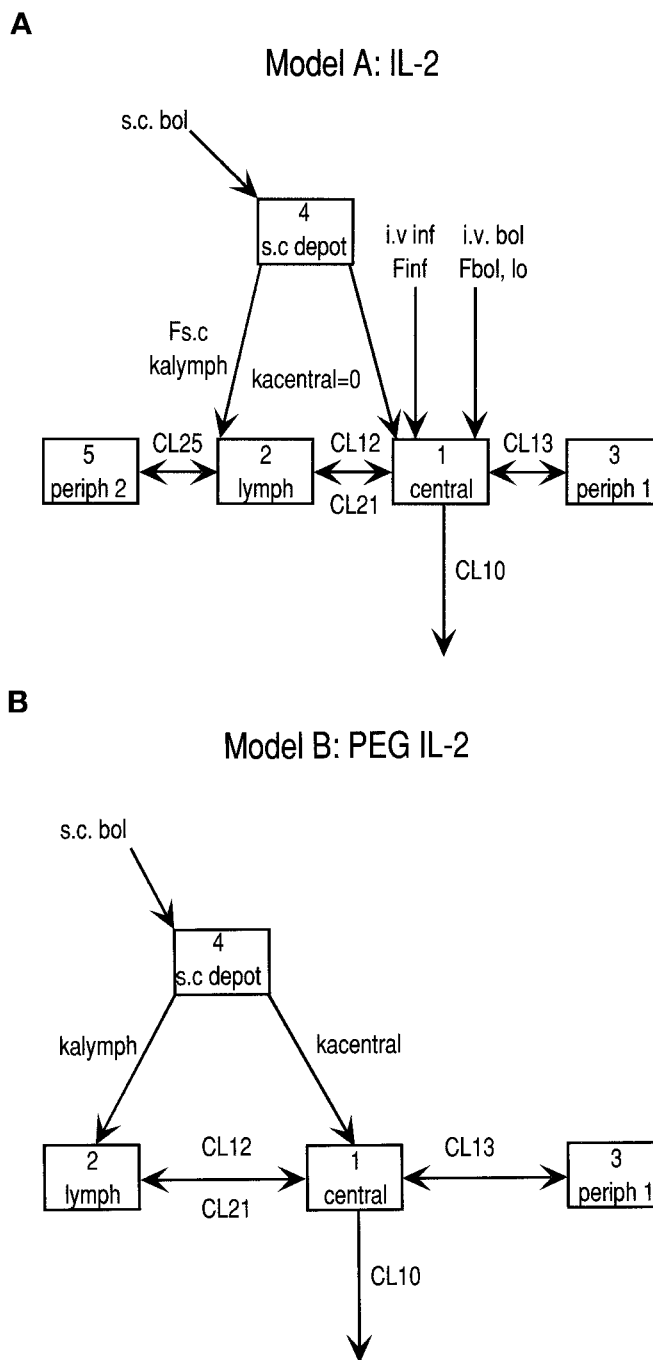
The plasma data from the three pigs that were administered IL-2 both i.v. and s.c. (part I) enabled  $F$  to be calculated in the NONMEM analysis. Bioavailability of IL-2 after i.v. bolus dosing at the intermediate- and high-dose levels (0.16 and 1.6 MIU/kg) was assumed to be complete ( $F = 1$ ). IL-2 bioavailabilities at the lowest i.v. bolus dose level [ $F_{bol, lo}$  (0.10 MIU/kg)], after i.v. infusion [ $F_{inf}$  (0.10 MIU/kg)], and of all the s.c. doses ( $F_{s.c.}$ ) were parameterized between 0 and 1.

First-order absorption from the s.c. compartment into the lymph was assumed. The absorption rate constant for the three highest s.c. dose levels (1.0, 1.6, and 3.0 MIU/kg) was allowed to be some factor of that for the two lowest dose levels (0.10 and 0.16 MIU/kg).

The model for PEG-IL-2 was parameterized similarly; however, this model required an additional first order rate constant for absorption of PEG-IL-2 from the s.c. dosing compartment to the central compartment ( $ka_{central}$ ; Fig. 1). The final model for PEG-IL-2 included only one noneliminating peripheral compartment, reversibly connected to the plasma compartment; therefore, only three volume terms were needed, and one intercompartmental clearance between the plasma-sampled and -unsampled peripheral compartments was included. The bioavailability was assumed to be 1 for the i.v. infusion dose and all the s.c. doses.

In the models for IL-2 and PEG-IL-2, a proportional error model was used for plasma concentrations, and an error model that smoothly interpolated between an additive and proportional error was used for the lymph concentrations.

The distributional clearances between the central and another reversibly connected, noneliminating, sampled compartment were related through the ratio of the AUCs when drug administration occurs directly into the central compartment. Because the ratio of these clearances can be determined by noncompartmental analysis,



**Fig. 1.** Models used in NONMEM analysis of plasma and lymph data. Model parameters were volume of the central compartment,  $V_c$ ; volume of the lymph compartment,  $V_l$ ; volume of the peripheral compartment,  $V_t$ ; clearance from the central to the lymph compartment,  $CL_{12}$ ; systemic or elimination clearance,  $CL_{10}$ ; and intercompartmental clearances between sampling and peripheral compartments,  $CL_{13}$ ,  $CL_{25}$ . For s.c. dosing, an s.c. dosing compartment, the absorption rate constant from the dosing site into lymph is indicated by  $ka_{lymph}$ , and the absorption rate constant from the dosing site into the central compartment is indicated by  $ka_{central}$ .

this was done before compartmental analysis of the data to reduce the number of model parameters by 1.

An advantage for distribution into the lymph is defined [distribution advantage ( $DsA_L$ )] as follows and simplified when equivalent doses are administered via both routes ( $AUC_{Ps.c.} = AUC_{Pi.v.}$ ):

$$DsA_L = \frac{\left[ \frac{AUC_{Ls.c.}}{AUC_{Ps.c.}} \right]}{\left[ \frac{AUC_{Li.v.}}{AUC_{Pi.v.}} \right]} = \frac{AUC_{Ls.c.}}{AUC_{Li.v.}} = 1 + \frac{CL10}{CL12} \quad (1)$$

where CL10 and CL12 are the systemic and distributional clearances, respectively. Where identical doses are administered via both routes but there are differences in the extent of absorption of the s.c. dose ( $F_{s.c.}$ ) and the i.v. dose ( $F_{i.v.}$ ), the ratio of  $AUC_L$  values is defined as a delivery advantage ( $DvA_L$ ):

$$DvA_L = \frac{AUC_{Ls.c.}}{AUC_{Li.v.}} = \left( 1 + \frac{CL10}{CL12} \right) \frac{F_{s.c.}}{F_{i.v.}} \quad (2)$$

The  $DvA_L$  is a more meaningful term because it considers relative bioavailability as well as plasma-to-lymph distribution. As is the case for PEG-IL-2, where bioavailabilities from the s.c. and i.v. routes are equivalent,  $DvA_L$  is equal to  $DsA_L$ . Where  $F_{s.c.}$  is less than  $F_{i.v.}$ , the delivery advantage is less than the distribution advantage.

Estimates of  $DvA_L$  were obtained in two ways. First, it was estimated by noncompartmental analysis, from the ratio of the mean  $AUC_L$  values defined in eq. 2 (middle term). In addition,  $DvA_L$  was calculated for IL-2 (eq. 2, right-hand term) from the clearance ratio and the values of  $F$  for both routes of administration at a given dose level obtained in the population pharmacokinetics analysis.

This analysis assumes that all of the bioavailable s.c. dose enters the lymphatic compartment (compartment 2) directly. Where s.c. dosing (e.g., after PEG-IL-2 dosing) results in a portion of the dose entering the central compartment with the remainder entering the lymph compartment, both the distribution advantage and the delivery advantage would be reduced, with its magnitude dependent on the fraction of the s.c. dose that initially is taken up by the lymph compartment ( $F_L$ ). Where absorption is first-order,  $F_L$  is the ratio of  $ka_{lymph}$  to the sum of  $ka_{lymph}$  and  $ka_{central}$  (model B; Fig. 1). Here, it can be shown that the  $DvA_L$  is equal to the following:

$$DvA_L = AUC_{Ls.c.}/AUC_{Li.v.} = 1 + F_L \cdot CL10/CL12 \quad (3)$$

## Results

### Noncompartmental Analysis

Initially, the pharmacokinetic parameters for the IL-2 plasma data from the animals in parts I and II were evaluated separately (Table 3). In part I, there was no significant difference by dose in  $CL$  or  $V_{ss}$ , but both parameters differed when the route of administration was compared using ANOVA. This indicated that the bioavailability of the s.c. dose was less than complete. Surprisingly, there were no significant differences by dose or route of administration for  $CL$  or  $V_{ss}$  for IL-2 in part II. The pharmacokinetic parameters from the two parts were combined to determine whether the ANOVA results were indicative of the fundamental differences in methodology between the two studies (i.e., plasma assay method and surgical implantation of a lymph catheter).

When the data from the two studies were combined, the noncompartmental estimates of  $CL/F$  evaluated by ANOVA showed differences that were statistically significant when grouped according to treatment.  $CL/F$  was found to be similar (average, 250–300 ml/h/kg) when IL-2 was administered as an i.v. bolus at the two higher dose levels (0.16 and 1.6 MIU/kg) but was approximately 2-fold faster (680 ml/h/kg) at the lowest bolus dose level (0.10 MIU/kg). This parameter was an additional 2-fold faster (1600 ml/h/kg) when IL-2 was administered by infusion at a corresponding low dose (0.10 MIU/kg). There were no statistically significant differences

TABLE 3  
Summary of noncompartmental analysis of plasma and lymph data in pigs receiving IL-2

Part	Regimen	Dose	Plasma Data			Lymph Data			$AUC_L/AUC_P^a$
			$CL/F^{b,c}$	$V_{ss}/F^{b,c}$	MRT <sup>d</sup>	$t_{1/2}^d$	$AUC_L^e$	$t_{1/2}^d$	
II	i.v. 15-min infusion	0.1	1629 ± 633 (39)	1467 ± 360 (25)	0.91	0.71	2.71 ± 1.46 (54)	43	0.65 ± 0.16 (25)
II	i.v. bolus	0.1	678 ± 93 (14)	640 ± 117 (18)	0.94	1.36	6.56 ± 1.32 (20)	105	0.70 ± 0.09 (13)
I	i.v. bolus	0.16	246 ± 58 (24)*	340 ± 41 (12)	1.37	1.64	N.A. <sup>f</sup>	N.A.	N.A.
I	i.v. bolus	1.6	299 ± 113 (38)*	720 ± 387 (54)	2.29	2.82	N.A.	N.A.	N.A.
II	s.c. bolus	0.1	553 ± 235 (43)	1459 ± 958 (66)	4.99	3.07	43.5 ± 3.1 (7)	696	3.50 ± 1.66 (47)
I	s.c. bolus	0.16	1238 ± 335 (27)	2409 ± 1393 (58)	4.44	2.38	N.A.	N.A.	N.A.
II	s.c. bolus	1.0	702 ± 250 (36)	1468 ± 533 (36)	4.69	5.65	233 ± 73 (31)	3728	2.54 ± 0.32 (13)
I	s.c. bolus	1.6	892 ± 314 (35)	2673 ± 605 (23)	5.69	3.12	N.A.	N.A.	N.A.
II	s.c. bolus	3.0	1250 ± 1011 (81)	5810 ± 7270 (125)	5.86	4.05	873 ± 480 (55)	13968	4.10 ± 0.68 (17)

<sup>a</sup> Data are mean ± S.D.; numbers in parentheses represent coefficient of variation as a percentage.

<sup>b</sup>  $CL/F$  significantly different when stratified by route and dose level ( $P = .01$ ).

<sup>c</sup>  $V_{ss}/F$  not significantly different when stratified by route and dose level.

<sup>d</sup> Harmonic mean is reported for MRT and half-life.

<sup>e</sup> A specific activity of 16 MIU/mg was used to convert the IL-2 data from mass units (mg) to activity units (I.U.).

<sup>f</sup> N.A., not available.

\* Comparisons of all pairs using Tukey-Kramer HSD showed that mean values for i.v. bolus, dose levels 0.16 and 1.6 MIU/kg, were significantly different from all other mean values.

among all pairs of mean CL/F values (550–1250 ml/h/kg) for the five s.c. dose levels (0.10–3.0 MIU/kg). These s.c. results suggested that there was no significant difference between the methodologies used in the two studies. Instead, the significant differences in CL/F were attributed to changes in bioavailability related to route of administration and dose, suggesting that a nonlinear model may be required to characterize the pharmacokinetics of IL-2. This assumption was the basis of the compartmental model adopted in the population pharmacokinetics analysis.

Noncompartmental estimates of the volume of distribution ( $V_{ss}/F$ ) were not different when examined by treatment. Although there were large differences in the mean values of the treatment groups, the intragroup variance, especially in the s.c. dosed animals, was extremely large, with coefficient of variations ranging up to 125%. The lack of a significantly different  $V_{ss}/F$  in the face of significantly different CL/F values for the two highest bolus doses is not explained.

The corresponding results for PEG-IL-2 are given in Table 4. Estimates of CL/F and  $V_{ss}/F$  for PEG-IL-2 obtained by noncompartmental analysis were significantly different when grouped by route of administration and s.c. dose level. The post hoc test showed the CL/F value at the highest dose (0.10 MIU/kg) was different from that at the lowest dose (0.01 MIU/kg) after s.c. administration. However, it is noted that the estimates of CL for the i.v. dose of PEG-IL-2 are comparable to those seen when the lowest and middle doses are administered s.c. This suggests that bioavailability of the s.c. doses may be essentially complete.

The post hoc test showed the  $V_{ss}/F$  value of PEG-IL-2 at the highest s.c. dose was different from that after i.v. infusion but not different from that after the lowest s.c. dose. Because the estimates of  $V_{ss}/F$  for the i.v. dose of PEG-IL-2 are also comparable to those seen when the lowest and middle doses are administered s.c., this further suggests that bioavailability of the s.c. doses may be essentially complete at low doses. It is possible, of course, that the extent of systemic absorption may be reduced as the dose is increased; this would be consistent with the observed increase in the apparent CL and  $V_{ss}$  values of PEG-IL-2.

Significant increases in the AUC in lymph were seen when IL-2 was administered s.c. over that noted when the same dose was administered i.v. (Table 3). For example, the AUC in the lymph (mean  $\pm$  S.D.) was  $43.5 \pm 3.1$  ng·h/ml for s.c. dosing, whereas the lymph AUC was only  $2.71 \pm 1.46$  and  $6.56 \pm 1.32$  ng·h/ml when the same dose was administered by infusion and bolus, respectively. The  $AUC_L/AUC_P$  ratios are also presented in Table 3. It is clear that s.c. dosing provides significantly higher lymph levels than i.v. dosing. For example, the  $AUC_L/AUC_P$  ratio increases from 0.65 to 0.70 for i.v. infusion-bolus dosing to 3.5 when the same dose (0.1 MIU/kg) was administered s.c. Thus, the distribution advantage ( $DsA_L$ ) is the ratio of these ratios, or approximately 5.

Table 4 contains the lymph PEG-IL-2 data. The  $AUC_L/AUC_P$  ratio was found to increase from 0.33 to 1.3 when comparing i.v. and s.c. doses. The corresponding estimate of  $DsA_L$  was 3.8.

### Compartmental Analysis

There were significant differences in the structure of the final NONMEM models that described the plasma and lymph concentrations of IL-2 and PEG-IL-2. A noneliminating pe-

TABLE 4  
Summary of noncompartmental analysis of plasma and lymph data in pigs receiving PEG-IL-2<sup>a</sup>

Regimen	Dose	Plasma Data				Lymph Data					
		$AUC_P^a$	$I.U. \cdot h/ml^f$	CL/F <sup>b,c</sup>	$V_{ss}/F^{w,c}$	MRT <sup>d</sup>	$t_{1/2}^d$	$AUC_L^a$	$I.U. \cdot h/ml^f$	$t_{1/2}^d$	$AUC_L/AUC_P^a$
i.v. 15-min infusion	MIU/kg	ng · h/ml	I.U. · h/ml <sup>f</sup>	ml/h/kg	ml/kg	h	ng · h/ml	I.U. · h/ml <sup>f</sup>	h		
	0.01	249 $\pm$ 24 (10)	996	10.1 $\pm$ 1.0 (10)	82.4 $\pm$ 8.4 (10)	8.25	82.4 $\pm$ 10.7 (13)	330	7.19	0.33 $\pm$ 0.07 (22)	
s.c. bolus	0.01	403 $\pm$ 148 (37)	1612	7.07 $\pm$ 3.29 (46)	240 $\pm$ 66 (27)	32.4	467 $\pm$ 424 (91)	1868	12.5	1.20 $\pm$ 0.99 (83)	
s.c. bolus	0.03	932 $\pm$ 431 (46)	3728	9.09 $\pm$ 3.39 (37)	301 $\pm$ 77 (26)	23.7	962 $\pm$ 69 (7)	3848	10.2	1.18 $\pm$ 0.49 (41)	
s.c. bolus	0.1	1494 $\pm$ 671 (45)	5976	19.7 $\pm$ 9.1 (46)*	415 $\pm$ 212 (51)*	22.8	1856 $\pm$ 504 (27)	7424	10.7	1.36 $\pm$ 0.42 (31)	

<sup>a</sup> Data are mean  $\pm$  S.D.; numbers in parentheses represent coefficient of variation as a percentage.

<sup>b</sup> CL/F significantly different when stratified by route and dose level ( $P = .04$ ).

<sup>c</sup>  $V_{ss}/F$  was significantly different when stratified by route and dose level ( $P = .04$ ).

<sup>d</sup> Harmonic mean is reported for MRT and half-life.

<sup>e</sup> A specific activity of 4 MIU/mg was used to convert the PEG-IL-2 data from mass units (mg) to activity units (I.U.).

<sup>f</sup> Comparisons of all pairs using Tukey-Kramer HSD showed that mean values for s.c. bolus, dose level 0.1 MIU/kg, was significantly different from the 0.01 MIU/kg for CL/F. For  $V_{ss}/F$ , the mean for s.c. bolus, dose level 0.1 MIU/kg, was significantly different from the i.v. 15-min infusion dose group.

ripheral compartment reversibly connected to the lymph compartment was added to the model for IL-2, indicating that the distribution of PEG-IL-2 from the lymph was restricted. The NONMEM population estimates for the pharmacokinetic parameters for IL-2 and PEG-IL-2 are in Tables 5 and 6, respectively.

**IL-2.** In examining the i.v. 0.1 MIU/kg (lowest dose level) data shown in Fig. 2 (A and B, bolus; C and D, infusion), it appears that in general, the plasma and lymph concentration data fall off more rapidly than the model-predicted terminal decline. For the i.v. bolus data, some underprediction of the initial time course is also noted. This may be related to an underestimate of bioavailability (NONMEM estimate of  $F_{bol}$ ,  $lo = 0.39$ ) in this dosing group (Table 5). At the intermediate i.v. bolus dose level (Fig. 3A), the correspondence between data and model-predicted function in the plasma concentration in the terminal phase is excellent, whereas at the highest i.v. bolus dose level, there appears to be slight underprediction of the terminal phase plasma concentration (Fig. 3A). No lymph data were available in the crossover experiments portrayed in Fig. 3. Although some misspecification was noted, the model reasonably described the plasma and lymph concentration-time data.

The NONMEM model adopted in this analysis is nonlinear for the i.v. dosing route because it assumes that bioavailability depends on dose and whether dosing occurs by infusion or bolus. Although bioavailability was assumed to be dose-independent for s.c. administration ( $F_{s.c.}$ ), the model is still nonlinear for this route of dosing, because the absorption rate constant was assumed to be different at the three highest dose levels (1.0, 1.6, and 3.0 MIU/kg) from that at dose levels 0.10 and 0.16 MIU/kg. This distinction in fractional rate of absorption was invoked in view of the marked difference in the plasma and lymph concentration profiles as s.c. dose increased (Figs. 3B and 4). Indeed, the parameter  $ka_{lymph, med, hi}$  was found to be more than five orders of magnitude larger than that for the lower dose levels. Thus, s.c. doses of 1.0 MIU/kg or higher were considered to be instantaneously absorbed into the lymph compartment (Table 5).

**PEG-IL-2.** Graphs of the plasma and lymph concentration-time data and of the corresponding NONMEM-predicted

functions are given by sampling site for the i.v. infusion treatment group (Fig. 5, A and B), and s.c. dosing groups (Fig. 6, A–F) for PEG-IL-2. Some model misspecification can be observed in plots of predicted versus observed plasma concentrations in the case of PEG-IL-2. In contrast to IL-2, higher plasma concentrations tended to be overpredicted, and lower values were underpredicted, which is consistent with the nonlinearity in the data observed when subjected to noncompartmental analysis.

Table 6 contains the corresponding compartmental parameters estimated for the model describing the absorption and disposition of PEG-IL-2. The ratio of the absorption constants for PEG-IL-2 into plasma and lymph was 0.4, indicating that absorption via the central compartment was significant for PEG-IL-2 after s.c. administration. The systemic clearance of PEG-IL-2 was found to be about two orders of magnitude slower than that of IL-2. The NONMEM model provides estimates of the clearance close to those obtained at the lower doses in the noncompartmental analysis. The differences in clearance estimates obtained by both methods of analysis are probably due in large part to the use of a linear pharmacokinetics model in the NONMEM analysis.

#### Lymph Delivery Advantage Afforded by s.c. Dosing

The lymph delivery advantage ( $DvA_L$ ) for IL-2 was estimated noncompartmentally and compartmentally (Table 7). At a dose of 0.1 MIU/kg, the calculated  $DvA_L$  is greater for s.c. dosing when defined with reference to infused IL-2 instead of a bolus dose because the bioavailability of infused IL-2 is less than that of a bolus ( $[F_{s.c.}/F_{inf}] > [F_{s.c.}/F_{bol}]$ ; see eq. 2). For instance, the values for  $AUC_L$  for s.c. and i.v. bolus dosing at a dose level of 0.1 MIU/kg are calculated to be 33.39 and 5.243 ng·h/ml, respectively (Table 3). Their ratio ( $DvA_L$ ) is 6.37 (Table 7). This is identical with the value of the lymph delivery advantage ( $DvA_L = 6.4$ ; Table 7) calculated from the right-hand term where NONMEM clearance and bioavailability parameters are used.

Values of  $DvA_L$  for IL-2 could not be estimated noncompartmentally for doses of 0.16 MIU/kg or larger because no lymph data were available after i.v. dosing in this range. However, compartmental estimates are reported here, using

TABLE 5  
Summary of results of NONMEM analysis of IL-2 data after i.v. and s.c. administration to pigs

Parameter <sup>a</sup>	Unit	Estimate	S.E.	% RSE	LCI 95%	UCI 95%
CL10	ml/h/kg	299	46.5	15.6	207.9	390.1
CL12	ml/h/kg	61.2	8.50	13.9	44.5	77.9
CL21 <sup>b</sup>	ml/h/kg	0.67	Fixed			
CL13	ml/h/kg	281	142	50.5	2.7	559.3
CL25	ml/h/kg	58.5	19.4	33.2	20.5	96.5
Vc	ml/kg	201	67.2	33.4	69.3	332.7
Vly	ml/kg	20.6	5.20	25.2	10.4	30.8
V3	ml/kg	243	49.8	20.5	145.4	340.6
V5	ml/kg	162	26.3	16.2	110.5	213.5
$ka_{lymph, lo}$	$h^{-1}$	0.385	0.0941	24.4	0.201	0.569
$ka_{lymph, med, hi}$	$h^{-1}$	$234,000 \times ka_{ly, lo}$	$135,000 \times ka_{ly, lo}$	57.7	191,961	-11,781
$ka_{central}$	$h^{-1}$	0.00	Fixed			
Fs.c.		0.422	0.0343	8.1	0.355	0.489
Finf		0.278	0.0686	24.7	0.144	0.412
Fbol, lo		0.390	0.0654	16.8	0.262	0.518
Fbol, med, hi		1.00	Fixed			
Error plasma		2.80	1.11	39.6		
Error lymph		36.0	4.38	12.2		

RSE, relative standard error; LCI 95%, lower value of the 95% confidence interval; UCI 95%, upper value of the 95% confidence interval.

<sup>a</sup> See Fig. 1 for definition of parameters and structural model (model A).

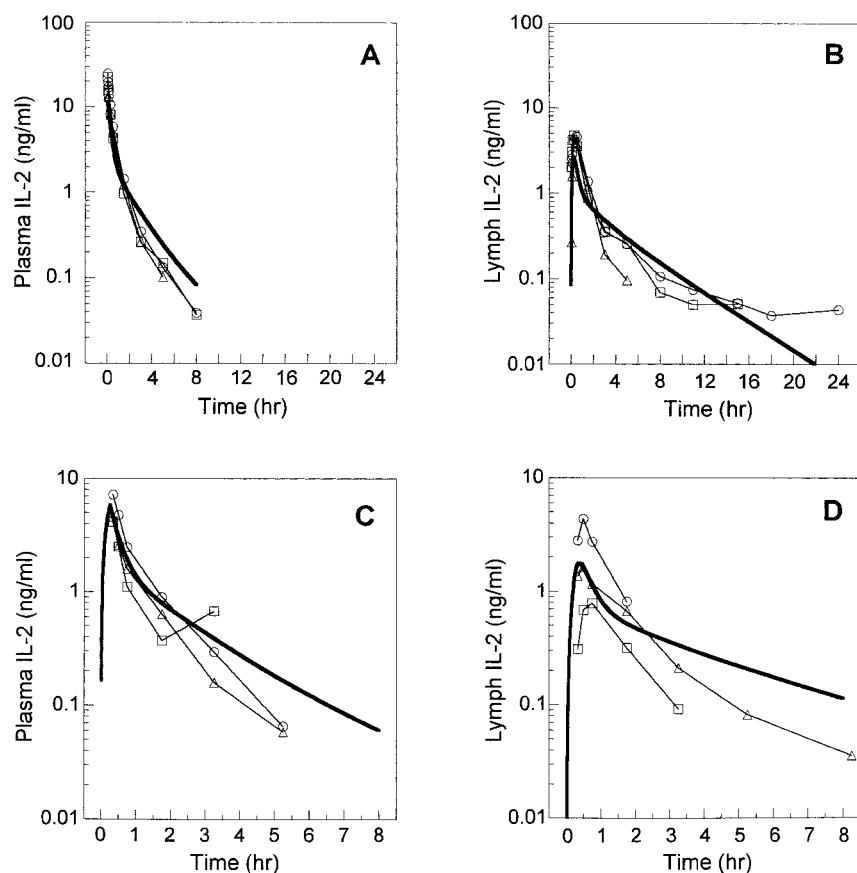
<sup>b</sup> CL21 fixed as CL12/0.67 from a noncompartmental analysis of  $AUC_L/AUC_P$  after i.v. dosing ( $n = 6$ ).

TABLE 6

Summary of results of NONMEM analysis of PEG-IL-2 data after i.v. and s.c. administration to pigs

Parameter <sup>a</sup>	Unit	Estimate	S.E.	% RSE	LCI 95%	UCI 95%
CL10	ml/h/kg	5.87	0.897	15.3	7.63	4.11
CL12	ml/h/kg	1.25	0.437	35.0	2.11	0.39
CL21 <sup>b</sup>	ml/h/kg	0.33	Fixed			
CL13	ml/h/kg	8.40	0.968	11.5	10.30	6.50
Vc	ml/kg	123	17.2	14.0	156.7	89.3
Vly	ml/kg	20.1	8.14	40.5	36.05	4.15
V3	ml/kg	229	68.3	29.8	362.9	95.1
ka <sub>lymph</sub>	h <sup>-1</sup>	3.89	1.38	35.5	6.59	1.19
ka <sub>central</sub>	h <sup>-1</sup>	9.63	1.80	18.7	13.16	6.10
F		1.00	Fixed			
Error		0.155	0.0627	40.5	0.28	0.03

RSE, relative standard error; LCI 95%, lower value of the 95% confidence interval; UCI 95%, upper value of the 95% confidence interval.

<sup>a</sup> See Fig. 1 for definition of parameters and structural model (model B).<sup>b</sup> CL21 fixed as CL12/0.33 from a noncompartmental analysis of AUC<sub>I</sub>/AUC<sub>P</sub> after i.v. dosing ( $n = 3$ ).

**Fig. 2.** Observed (symbols) and predicted (line) plasma and lymph concentrations after i.v. bolus (A and B) and 15-min i.v. infusion administration (C and D) of 0.1 MIU/kg IL-2 in part II. Observed data are from individual animals using the same symbols for the plasma and lymph concentrations.

the clearance values estimated in NONMEM and the values of  $F$  estimated or, in the case of high bolus doses, assumed. Clearly, studies that permit lymph as well as plasma sampling for the assay of IL-2 in the higher dosage range would provide more reliable estimates of the lymph delivery advantage.

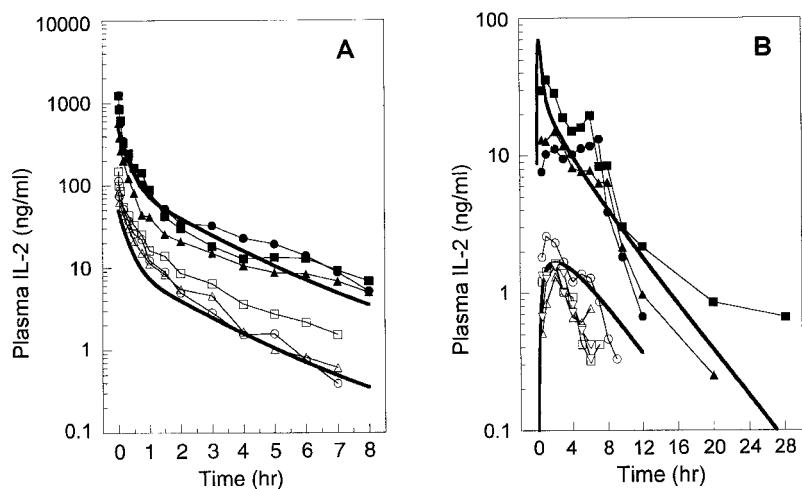
For PEG-IL-2, noncompartmental estimates of  $AUC_I/AUC_P$  increase from 0.33 to approximately 1.2 when comparing i.v. and s.c. doses (Table 4). The corresponding noncompartmental estimate of  $DvA_L$  is 3.8 (Table 7). The NONMEM estimate of  $DvA_L$  is substantially lower than the value estimated noncompartmentally for PEG-IL-2 (Table 7). This may be due to the assumption of linearity, which seems not to hold for PEG-IL-2 at the highest dose as demonstrated by the results of the ANOVA. The difference may also stem from

uncertainty in the estimates of  $ka_{lymph}$  and  $ka_{central}$ , which determine  $F_L$  (eq. 3).

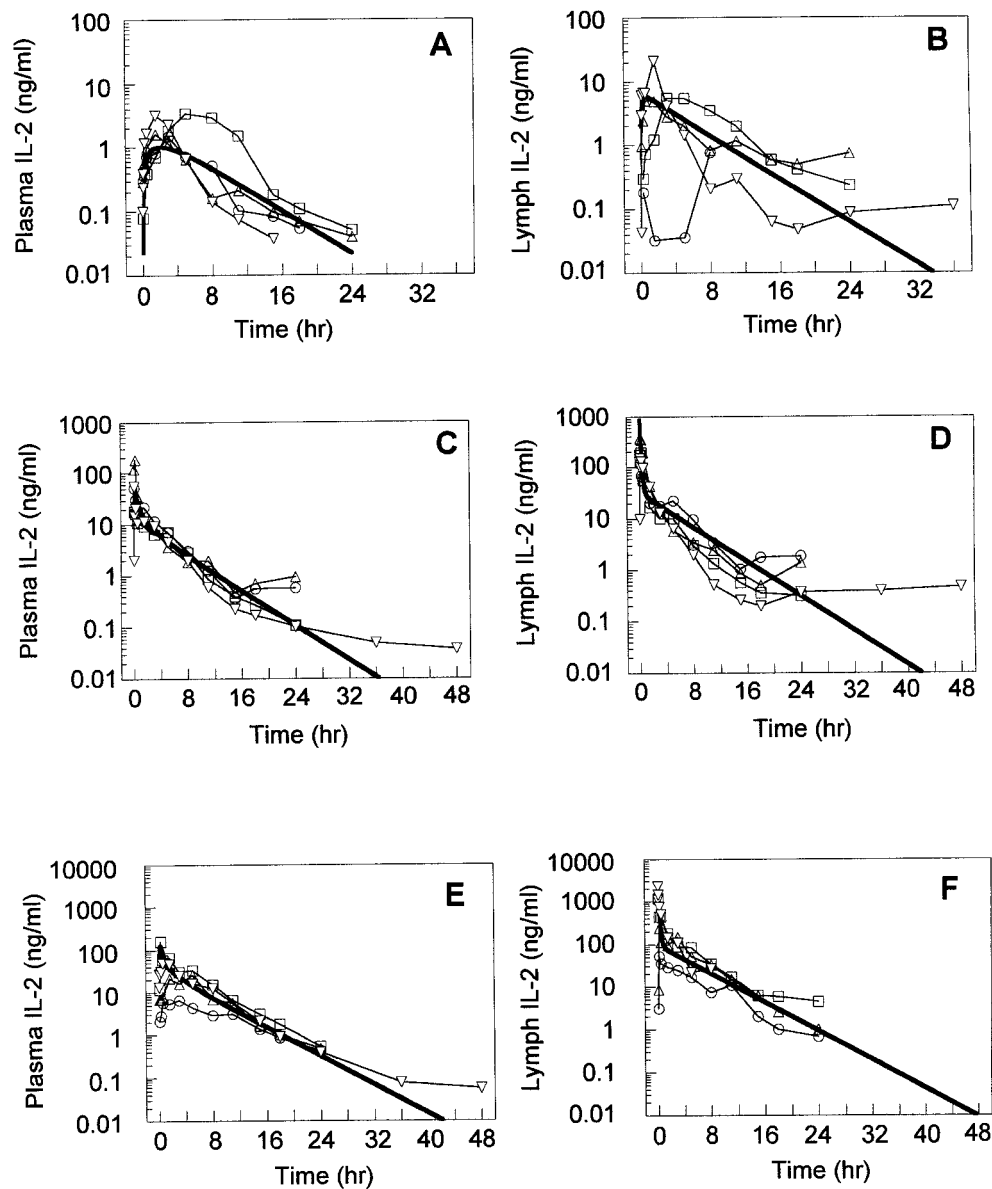
## Discussion

**Animal Model Selection.** The main consideration in selecting the appropriate species to study lymphatic absorption was the ability to predict human data. Before using pigs, the pharmacokinetics of IL-2 and PEG-IL-2 had been characterized in mice, rats, rabbits, sheep, cynomolgus macaques, and cancer patients after i.v. administration. Male/female differences in the pharmacokinetics of IL-2 and PEG-IL-2 have never been reported. Allometric relationships between clearance and body weight could be established, and the exponents were 0.7, which are typical for compounds where in-

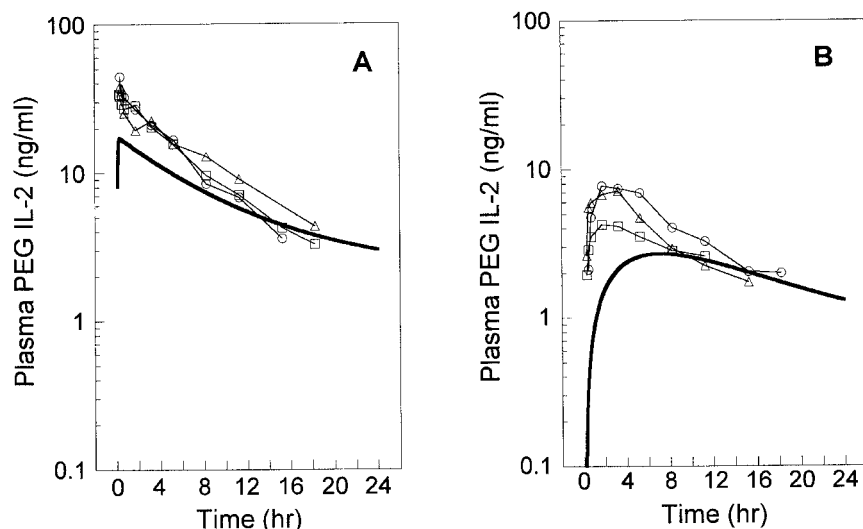




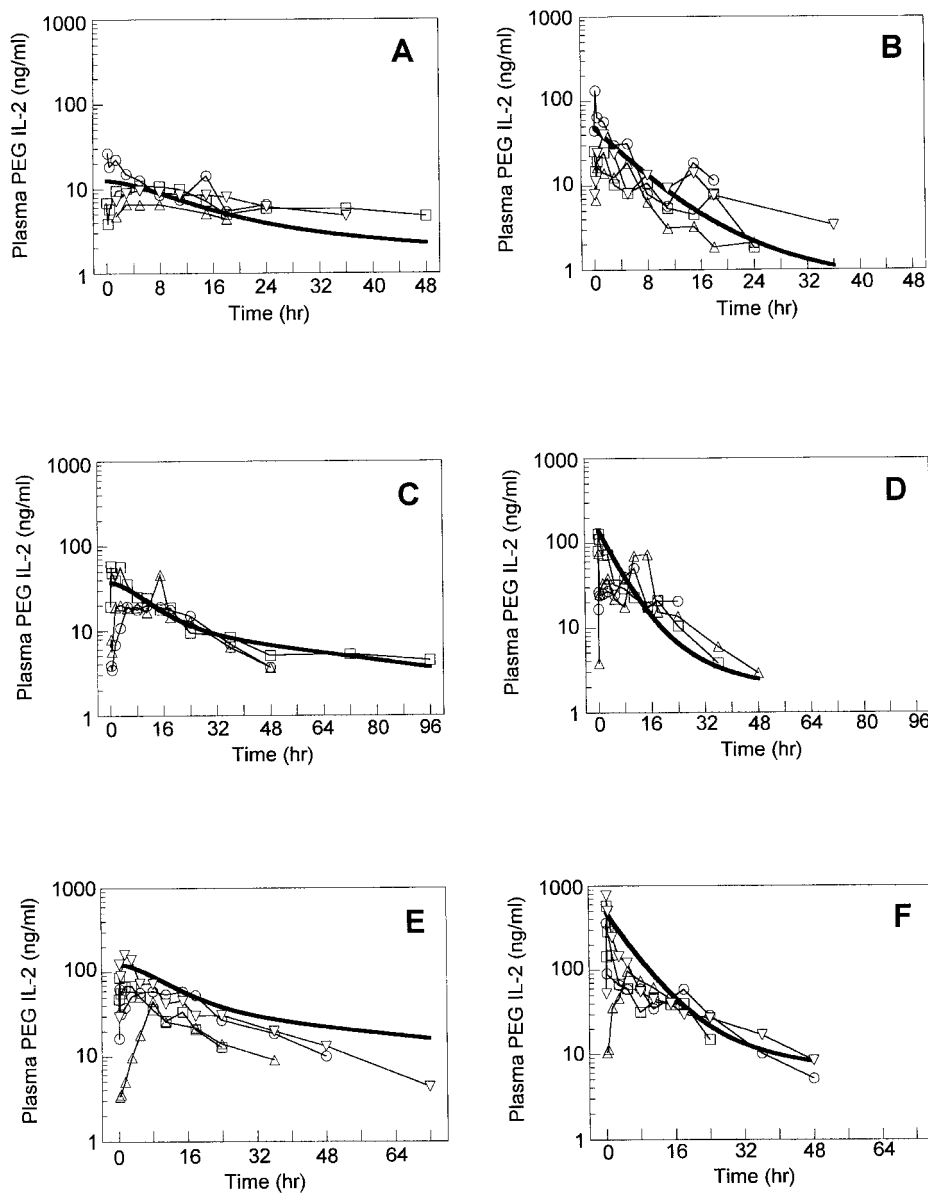
**Fig. 3.** Observed (symbols) and predicted (line) plasma concentrations after i.v. bolus (A) and s.c. administration (B) of 0.16 MIU/kg (open symbols) and 1.6 MIU/kg (filled symbols) IL-2 in part I. Observed data are from individual animals using the same symbols for each animal.



**Fig. 4.** Observed (symbols) and predicted (line) plasma and lymph concentrations after s.c. administration of 0.1 MIU/kg (A and B), 1.0 MIU/kg (C and D), and 3.0 MIU/kg (E and F) IL-2 in part II. Observed data are from individual animals using the same symbols for the plasma and lymph concentrations.



**Fig. 5.** Observed (symbols) and predicted (line) plasma and lymph concentrations after 15-min i.v. infusion of 0.01 MIU/kg PEG-IL-2 (A and B). Observed data are from individual animals using the same symbols for the plasma and lymph concentrations.



**Fig. 6.** Observed (symbols) and predicted (line) plasma and lymph concentrations after s.c. administration of 0.01 MIU/kg (A and B), 0.03 MIU/kg (C and D), and 0.1 MIU/kg (E and F) PEG-IL-2. Observed data are from individual animals using the same symbols for the plasma and lymph concentrations.

TABLE 7

DvA<sub>L</sub> afforded by s.c. dosing as estimated by noncompartmental and compartmental (NONMEM) analysis

Dose	Dosing Route	Noncompartmental	Compartmental
<i>MIU/kg</i>			
IL-2			
0.1	s.c./i.v. 15-min infusion	16	8.9
0.1	s.c./i.v. bolus	6.6	6.4
0.16, 1.0, 1.6, and 3.0	s.c./i.v. bolus	N.A. <sup>a</sup>	2.5
PEG-IL-2			
0.01, 0.03, and 0.1	s.c./15-min i.v. infusion	3.8	2.4

<sup>a</sup> N.A., not available.

terspecies scaling has been applied (Chappell and Mordenti, 1991; Mordenti et al., 1991).

However, allometric relationships between clearance and body weight could not be established when either IL-2 or PEG-IL-2 was administered s.c. Bioavailability was much lower in fur-bearing species. For IL-2, the bioavailability ranged between 21 and 41% in mice and 4.3 to 24% in sheep. Therefore, the pig model was selected to study IL-2 and PEG-IL-2 because skin layers and supporting structures are similar to those in humans. Pigs also have similar physiological, anatomical, nutritional, and metabolic parameters (Dodds, 1982) and are of a similar size as humans. In pigs, the bioavailability of s.c. IL-2 was 42.2% (Table 5), which is comparable to the s.c. bioavailability of IL-2 in cancer patients (35–47%) and in patients infected with human immunodeficiency virus, in whom s.c. bioavailability was approximately 62% (Piscitelli et al., 1996).

After the s.c. administration of PEG-IL-2 to mice, rats, rabbits, and cynomolgus macaques, bioavailability ranged between 22 and 72%; rabbits had the lowest value. In cancer patients, the bioavailability of s.c. PEG-IL-2 was 83%. Similarly, the bioavailability of PEG-IL-2 is essentially complete in pigs after s.c. administration. Therefore, the distribution of IL-2 and PEG-IL-2 to lymph in pigs may predict the lymphatic distribution in patients.

**Comparison between IL-2 and PEG-IL-2.** The addition of PEG molecules decreased the clearance by increasing the hydrodynamic radius of IL-2, which led to a decrease in renal elimination (Knauf et al., 1988). The addition of PEG molecules to IL-2 not only slowed the elimination clearance but also all of the other pharmacokinetic parameters differed between IL-2 and PEG-IL-2. The clearance from central to lymph compartments was about 130-fold less for the higher molecular weight PEG-IL-2, and the clearance from lymph to plasma was approximately 65-fold less (Tables 5 and 6). Thus, AUC<sub>L</sub> relative to AUC<sub>P</sub> was about twice as great (0.67, Table 3) for IL-2 than it was for PEG-IL-2 (0.33, Table 4).

The transfer of proteins into the lymphatic system after i.v. administration has not been consistently observed by other investigators. Significant amounts of recombinant human tumor necrosis factor were found in central lymph after i.v. administration to rats (Kojima et al., 1988); however, recombinant human interferon- $\alpha$ , which is predominantly absorbed via the lymphatic system after s.c. administration, did not distribute to lymph in sheep receiving an i.v. injection (Supersaxo et al., 1988). After i.v. administration, there was significant transfer between plasma and lymph of both IL-2

and PEG-IL-2. However, consistent with the smaller volumes of distribution and slower clearances, the plasma PEG-IL-2 concentrations were always higher than the lymph concentrations.

After s.c. dosing, preferential lymphatic absorption for PEG-IL-2 compared with IL-2 was expected from the data of Supersaxo (1990) and Xie and Hale (1996) based on molecular weight considerations and the overall decrease in positive charge of IL-2 (Knauf et al., 1988). A possible reason for the opposite finding in absorption mechanisms between IL-2 and PEG-IL-2 is a consideration of the differences in lypophilicity of the two molecules. IL-2 requires SDS as a solubilization agent. The addition of hydrophilic PEG polymers to IL-2 results in a significant improvement in solubility (Katre et al., 1987). Lipophilic macromolecules like IL-2 may preferentially be absorbed via the lymphatic system, and the decrease in absorption of PEG-IL-2 via that route may be more due to the decrease in lypophilicity than to the increase in molecular weight.

The estimation of DvA<sub>L</sub> allows for a comparison of the lymph and plasma exposure after i.v. and extravascular administration. However, to compare the systemic exposure of both forms of IL-2, the decreased specific activity of PEG-IL-2 must be considered. Therefore, the average AUC<sub>P</sub> and AUC<sub>L</sub> were expressed in I.U.·h/ml using the appropriate specific activities of 16 and 4 I.U./ng for IL-2 and PEG-IL-2, respectively (Tables 3 and 4, respectively). An s.c. dose of 0.01 MIU/kg PEG-IL-2 resulted in approximately the same AUC<sub>P</sub> as an s.c. dose of 1 MIU/kg IL-2. When AUC<sub>L</sub> is considered, a PEG-IL-2 s.c. dose of 0.03 MIU/kg was equivalent to an IL-2 s.c. dose of 1 MIU/kg. Therefore, to obtain equivalent exposures in lymph of the two IL-2 forms, the exposure of PEG-IL-2 in plasma would be approximately 3 times higher than that of IL-2 if both compounds were dosed s.c.

In conclusion, this report describes the differences in pharmacokinetics between IL-2 and PEG-IL-2 in pigs after i.v. and s.c. administration. Even though there was some model misspecification observed in the NONMEM compartmental analyses, the compartmental model characterizes the general time course of both compounds in plasma and lymph reasonably well, and there is general agreement between the noncompartmental and NONMEM analyses. Therefore, pharmacokinetic parameters from the NONMEM models such as distribution clearance that is not obtainable from the noncompartmental analysis can be used to predict values in patients. Both IL-2 and PEG-IL-2 distribute to lymph after i.v. administration, although distribution to lymph is favored by IL-2. The predominant absorption pathway for IL-2 after s.c. administration is via the lymphatics. At higher s.c. doses of IL-2, only absorption via the lymphatic compartment was observed. It was expected that PEG-IL-2 would also be predominantly absorbed via the lymphatics after s.c. dosing, but absorption via the central compartment was significant for PEG-IL-2 after s.c. administration. IL-2 s.c. bioavailability is approximately 30% in patients; therefore, the systemic exposure is approximately 3-fold lower after s.c. than after i.v. administration. Although the serum concentrations are lower, it is expected, based on extrapolations from results in pigs, that the lymph concentrations would be similar after administration via either route. Although there have been no reports that link the lymphatic concentrations of IL-2 with efficacy, it is speculated that the preferential absorption in

the lymph compartment after s.c. dosing possibly reduces IL-2-related toxicities that are related to the exposure of natural killer cells, neutrophils, and monocytes to high concentrations of IL-2 in the serum while maintaining the exposure of lymphocytes in lymph to IL-2 levels similar to those seen with i.v. dosing. Finally, IL-2 would be favored over PEG-IL-2. Now it is possible to design dosing regimens of IL-2 (i.v. versus s.c.) to produce similar lymphatic concentrations but different plasma concentrations to link the appropriate biophase with efficacy and toxicity.

#### Acknowledgments

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**Send reprint requests to:** Sharon A. Chen, Microcide Pharmaceuticals, Inc., 850 Maude Ave., Mountain View, CA 94043. E-mail: schen@microcide.com

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