Pharmacokinetic Model to Describe the Lymphatic Absorption of r-metHu-Leptin after Subcutaneous Injection to Sheep

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Purpose. The purpose of this work was to develop a pharmacokinetic model to describe the contribution of the lymphatics to the absorption and bioavailability of r-metHu-Leptin administered by subcutaneous (SC) injection to sheep.

Methods. r-metHu-Leptin was administered either by bolus intravenous injection (0.1 mg/kg) into the jugular vein or by SC injection (0.15 mg/kg) into the interdigital space of the hind leg. The SC groups included a non-cannulated control group and a lymph-cannulated group, in which peripheral lymph was continuously collected from a cannula in the efferent popliteal lymph duct. Serum and lymph concentrations were determined by enzyme-linked immunosorbent assay and profiles were modeled using compartmental pharmacokinetic methods. The fraction of the dose reaching the systemic circulation (F_{sys}) and the proportions of the absorbed dose taken up via the blood (F_{blood}) and lymph (F_{lymph}) were determined.

Results. Serum and lymph concentration vs. time profiles were well described by a two compartment model with parallel first order absorption into blood and lymph. F_{sys} for the SC control group was 60.4 ± 8.4%. In the lymph-cannulated group, 21.7 ± 6.4% of the dose was recovered in serum and 34.4 ± 9.7% was recovered in peripheral lymph giving a total fraction absorbed (F_{abs}) of 56.0 ± 10.3%. F_{sys} for the SC control group was not significantly different to F_{abs} in the lymph-cannulated group.

Conclusion. This study has shown that the lymph represents the predominant pathway for absorption of r-metHu-Leptin after SC administration.

KEY WORDS: leptin; lymph; protein delivery; subcutaneous; pharmacokinetic.

INTRODUCTION

Leptin is an endogenous adipocyte-derived protein encoded by the ob gene and contributes to the regulation of body weight. Studies conducted in leptin-deficient obese mice have shown that leptin administration significantly reduced food intake and body weight (1,2) demonstrating its potential as a treatment of obesity.

Recombinant methionyl human leptin (r-metHu-Leptin) is produced in *Escherichia coli* and is a moderately sized protein consisting of 147 amino acids with a calculated molecular weight of 16.2 kDa. Pharmacokinetic studies of r-metHu-Leptin in several species have shown biphasic disposition after intravenous (IV) administration with a terminal half-life ranging from 1 to 3.5 h (3). After subcutaneous (SC) administration in humans, peak concentrations are observed at 3 to 4 h after dosing (4), the terminal half-life averages 3 to 4 h, and the bioavailability is approximately 90% (3,4). Dose-linear pharmacokinetics has been demonstrated in several species (4). r-metHu-Leptin is primarily eliminated by the kidneys via a non-saturable process, most likely glomerular filtration (5). Previous studies have shown that allometric scaling adequately predicts human values for clearance and volume of distribution after both IV and SC administration (3).

SC administered proteins can be absorbed into the systemic circulation via direct uptake into the blood capillaries or indirectly via the lymphatic capillaries. The molecular weight or size of a molecule is thought to be the primary factor governing the absorption route (6). Blood capillaries are permeable to small molecules, but the continuous endothelial basement membrane and tight junctions of blood capillaries hinder and restrict the passage of macromolecules. Proteins larger than approximately 16 kDa are generally considered to undergo preferential absorption into the lymphatics via intercellular junctions within the loosely defined capillary structure (6,7).

Absorption into the peripheral lymphatics has been previously demonstrated for insulin (8), cytochrome *c* (6), interferon α-2a (9), and growth hormone (7) using a cannulated sheep model. A direct correlation between the proportion of the dose absorbed by the lymphatics and the molecular weight has been described for this limited number of compounds. Although molecular weight, or molecular size, is thought to be the predominant determinant of absorption into the lymphatics, it is not known if this is a general correlation or if the extent of lymphatic absorption is influenced by other protein specific factors, such as surface charge or hydrophobicity.

The objective of this study was to assess the contribution of the lymphatics to the absorption and systemic bioavailability of r-metHu-Leptin administered by subcutaneous injection using a lymph-cannulated sheep model, and to develop a pharmacokinetic model to describe the absorption process.

MATERIALS AND METHODS

Materials

r-metHu-Leptin was provided by Amgen Inc. for use in the described studies. Intravenous 16-gauge catheters (133 mm, Angiocath™, Becton Dickinson, Australia) were used for the administration of IV doses and for blood sampling. Sterilized medical-grade polyvinyl cannulae of a 0.58 mm internal diameter and 0.96 mm external diameter (W.F Scientific, Victoria, Australia) were used for lymphatic duct cannulations. All blood samples were collected into 5 ml glass serum collection tubes (Vacutainer®, Becton Dickinson) and peripheral lymph was collected into 10 mL glass collection

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tubes containing no anticoagulant (Vacutainer®, Becton Dickinson).

Study Design

The animal studies were conducted in accordance with the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985) and were approved by The University of Melbourne Animal Experimentation Ethics Sub-Committee. A parallel study design involving three treatment groups was used. A single dose of recombinant human r-metHu-Leptin was administered to each sheep. The first group was an IV control group (n = 4) in which the dose was administered by bolus IV injection. The other two groups, both of which received a bolus SC dose of r-metHu-Leptin, included a non-lymph cannulated control group (n = 4) and a peripheral lymph-cannulated group (n = 4). Blood was sampled from all animals and peripheral lymph was continuously collected from the lymph-cannulated group. During the experimental period, sheep were housed in metabolism cages, and food and water were available *ad libitum*.

Surgical Procedures

Adult male merino wether sheep weighing between 30–60 kg were used in the studies. Intravenous catheters were inserted into the jugular vein of all sheep immediately before dose administration to allow blood sampling.

For the peripheral lymph-cannulation group, the efferent popliteal lymph duct was cannulated using a previously described procedure (10). Cannulation of the popliteal lymph duct enabled the continuous and quantitative collection of peripheral lymph draining the injection site. Antibiotics were administered intramuscularly (600 mg procaine penicillin) to sheep immediately after surgery to reduce the likelihood of post-operative infection. Following an initial recovery period, sheep were transferred to metabolism cages and were allowed to recover for a period of approximately 36–42 h before dosing to ensure normal movement of the cannulated leg. Peripheral lymph flow rates were monitored during recovery, dosing and sampling periods. A consistent flow rate of greater than 3 mL/h was required for lymph-cannulated sheep to be included in dosing studies.

Administration of r-metHu-Leptin and Sample Collection

The r-metHu-Leptin formulation was a sterile, clear, colorless solution. Bolus IV injections of r-metHu-Leptin (0.1 mg/kg) were administered to sheep in the IV control group via an intravenous catheter inserted into the opposite jugular vein to that used for blood sampling. Dosing catheters were flushed with saline before dosing and immediately following administration of the bolus dose and then removed. SC doses of 0.15 mg/kg were administered by bolus injection into the interdigital space of the hind leg. In the lymph-cannulated animals, the dose was administered into the cannulated leg.

Blood samples from the IV group were withdrawn via the in-dwelling jugular vein catheter before dosing and at 1, 3, 6, 10, 15 min, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h post-dosing. Blood samples for the SC control and lymph-cannulated groups were withdrawn before-dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, and 12 h postdosing. One milliliter of blood was initially withdrawn and discarded to flush the catheter and to ensure the collection of circulating blood. Five milliliters of blood was subsequently withdrawn and transferred to serum collection tubes. The jugular vein catheter was flushed immediately following blood withdrawal with heparinized saline (10 U/mL) to ensure patency. Blood samples were allowed to clot for 60 min at ambient temperature and then centrifuged at 2600 × g for 10 min. Serum aliquots were taken and frozen at −80°C until analysis.

In the lymph-cannulated group, peripheral lymph was continuously collected into pre-weighed collection tubes. Peripheral lymph was collected for 30–60 min before dosing and collection tubes were changed at hourly intervals for the duration of 12 h postdosing. Lymph volume was determined gravimetrically after which the lymph samples were allowed to clot at ambient temperature for 60 min. Clotted samples were centrifuged at 2600 × g for 10 min and the supernatant was collected and frozen at −80°C until analysis.

Assay Methodology

Serum and lymph concentrations of r-metHu-Leptin were determined using an in-house solid-phase sandwich enzyme-linked immunosorbent assay. A monoclonal antibody specific for r-metHu-Leptin was precoated onto microtiter plates. Calibration standards, quality controls (QCs), and samples were then added to the wells and the antibody immobilized the r-metHu-Leptin present. After washing away any unbound components, horseradish peroxidase linked monoclonal antibody specific for r-metHu-Leptin was added to the wells. Following a wash to remove unbound antibody-enzyme reagent, a tetramethylbenzidine solution was added to the wells. Color developed in proportion to the amount of r-metHu-Leptin bound in the initial step. Color development was stopped and the intensity measured by UV absorption. The absorbance of the unknown samples and quality control samples was converted to concentration by comparison to a concurrently analyzed calibration curve that was regressed according to a log-log algorithm.

Calibration standards and QCs were prepared in sheep serum. If necessary, serum and lymph samples were diluted with sheep serum to fall within the analytical range. Studies were conducted prior to sample analysis to demonstrate the validity of diluting lymph samples with sheep serum by comparing spike recoveries of lymph samples to serum calibration curves (data not shown). All samples were assayed in duplicate. The limit of quantification (LOQ) for the assay was defined as the lowest calibration standard which met 20% accuracy and precision criteria and was at least twice the background absorbance.

Noncompartmental Analysis

Noncompartmental analysis was performed using WinNonlin (v. 3.2, Pharsight Corporation, Mountain View, CA, USA) for individual serum profiles. The maximum concentration (C_{max}) and time to reach the maximum (T_{max}) were taken directly from experimental data for SC doses. The area under the serum concentration-time curve from zero to infinity ($AUC_{0-\infty}$) was calculated by the log-linear trapezoidal rule. The terminal half-life (t½) was calculated from the terminal rate constant obtained by exponential regression of at least the last three time points that were above the LOQ. The

mean residence time (*MRT*), serum clearance (*CL*), and volume of distribution at steady state (V_{ss}) were determined from the IV data. The mean absorption time (MAT) for the SC groups was calculated by subtracting the individual SC mean residence time (MRT) from the mean IV MRT. The fraction of the dose reaching the systemic circulation (F_{sys}) for the SC control and lymph-cannulated groups was calculated using the equation:

$$F_{sys} = \frac{AUC_{SC} \times Dose_{IV}}{AUC_{IV} \times Dose_{SC}} * 100$$

where AUC_{SC} and $Dose_{SC}$ represent the area to infinity after SC administration and the SC dose, respectively, for each animal, and AUC_{IV} and $Dose_{IV}$ represent the mean values for the IV control group.

In the lymph-cannulated group, the mass of r-metHu-Leptin collected in each lymph sampling interval was calculated from the product of the measured concentration and the volume of lymph. The lymph recovery was calculated by expressing the cumulative mass collected in lymph as a percentage of the administered dose. The total fraction absorbed (F_{abs}) was calculated as the sum of the lymph recovery and F_{sys} in the lymph-cannulated group. The fraction of the absorbed dose taken up via the lymph (F_{lymph}) was calculated from the ratio of the lymph recovery to F_{abs}. The fraction of the absorbed dose taken up via the blood pathway (F_{blood}) was calculated from the ratio of F_{sys} to F_{abs} in the lymph-cannlated group.

Compartmental Analysis and Modeling

Compartmental analysis of the three study groups for r-metHu-Leptin was performed using WinNonlin (v. 3.2, Pharsight Corporation, Mountain View, CA, USA). The models were fit to individual profiles using the reciprocal of the predicted squared weighting factor. Goodness-of-fit for each of the models was assessed by the convergence of the least-squares regression, weighted residual sum of squares (WRSS) and the precision of parameter estimates. Serum data for individual IV doses were fit to a standard two compartment pharmacokinetic model (Eqs. [1] and [4]). Parameter estimates in each animal were generated for the rate constants (k_{12}, k_{21}, and k_{10}) and volume of distribution for the central compartment (V_c). A two-compartment model with first-order absorption and elimination was used to fit the serum concentrations for the SC control animals by setting the mean IV parameter estimates for k_{12}, k_{21}, k_{10}, and V_c as constants (Eqs. [2], [4], [5]). The SC model generated estimates for F_{sys} and the absorption rate constant (k_a).

A model was constructed to simultaneously fit the serum concentration data and the cumulative amount of r-metHu-Leptin in peripheral lymph for the lymph-cannulated animals (Fig. 1). In this group of animals, the absorption into blood and lymph was modeled by separate differential equations (Eqs. [3]–[6]). The mean IV parameter estimates for k_{12}, k_{21}, k_{10}, and V_c were fixed as constants. The SC lymph-cannulated model enabled estimation of k_a, F_{lymph}, and F_{abs}. F_{blood} and F_{sys} were also estimated as secondary parameters.

The complete set of differential equations for the three treatment groups were as follows:

$$\frac{dC_{s(IV)}}{dt} = (k_{21} * A_p/V_c) - (k_{12} + k_{10}) * C_s \quad [1]$$

$$\frac{dC_{s(SC)}}{dt} = (k_{21} * A_p/V_c) - (k_{12} + k_{10}) * C_s + (k_a * F_{sys} * A_{sc}/V_c) \quad [2]$$

$$\frac{dC_{s(SC\_cann)}}{dt} = (k_{21} * A_p/V_c) - (k_{12} + k_{10}) * C_s + (k_a * F_{abs} * A_{sc} * (1 - F_{lymph})/V_c) \quad [3]$$

$$\frac{dA_p}{dt} = (k_{12} * C_s * V_c) - (k_{21} * A_p) \quad [4]$$

$$\frac{dA_{sc(SC,SC\_cann)}}{dt} = -k_a * A_{sc} \qquad \text{where: } A_{sc}(0) = \text{Dose} \quad [5]$$

$$\frac{dA_{l(SC\_cann)}}{dt} = k_a * F_{abs} * F_{lymph} * A_{sc} \quad [6]$$

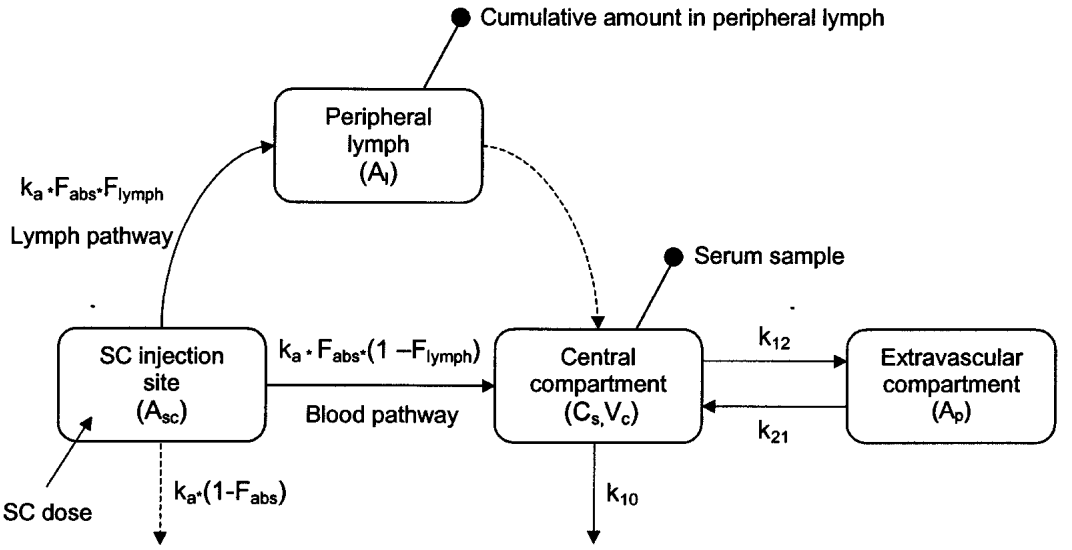

Fig. 1. Proposed pharmacokinetic model for r-metHu-Leptin administered as a SC dose to lymph-cannulated sheep. In this group of animals, peripheral lymph was continuously collected and therefore, the normal transfer of lymph from the peripheral to the central compartment is depicted as a dotted line.

where C_s is the concentration in the systemic circulation, V_c is the volume of distribution for the central compartment, A_p is the amount in the peripheral compartment, A_{sc} is the amount at the injection site, and A_l is the amount absorbed into lymph.

Statistical Analysis

Statistical analysis was performed using SigmaStat (v. 2.03, SPSS Science, Chicago, IL, USA). Noncompartmental and compartmental parameter estimates for the SC control and lymph-cannulated groups were statistically compared using Student's t tests testing for significance at $\alpha = 0.05$.

RESULTS

Assay Validation

The analytical range for r-metHu-Leptin analysis was 1.5 to 98 ng/mL and the LOQ was 1.5 ng/mL. QCs across the assay period were consistently within ±15% of the nominal concentration demonstrating the accuracy of the method. Endogenous sheep Leptin which may have been present in blank serum used to prepare standards and QCs was unquantifiable suggesting either low cross-reactivity between human and sheep Leptin, or endogenous levels in sheep which were below the limit of quantification. Similarly, endogenous Leptin was undetectable in predose serum and lymph samples.

Pharmacokinetics of r-metHu-Leptin after IV Administration

After IV administration, serum levels of r-metHu-Leptin declined in a biexponential manner demonstrating an initial distribution phase and subsequent elimination phase (Fig. 2). The terminal t½ was 1.9 ± 0.2 h, which is similar to that previously reported for sheep (3). Clearance in sheep was 71.7 ± 11.8 ml/h/kg (Table I). r-metHu-Leptin did not distribute extensively outside the serum with a volume of distribution at steady state of 86.3 ± 13.7 ml/kg. The MRT (1.2 ± 0.2 h) was consistent with the previously reported value (3).

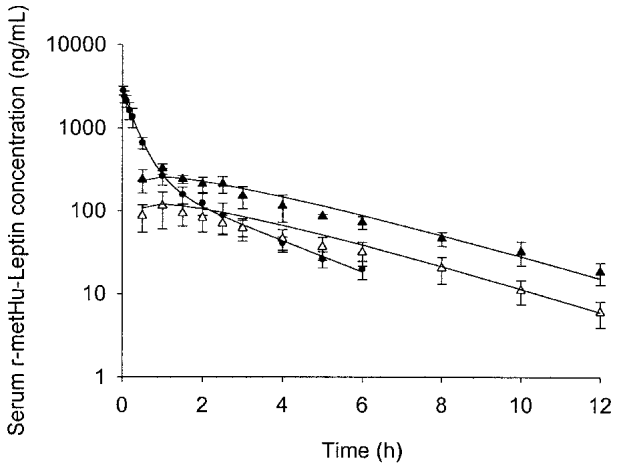

Fig. 2. Serum r-metHu-Leptin concentration vs. time profiles after IV administration of 0.1 mg/kg (●) and SC administration of 0.15 mg/kg to non-cannulated control (▲) and lymph-cannulated (△) sheep (mean ± SD, n = 4) with model estimated fits (solid lines).

Pharmacokinetics of r-metHu-Leptin after SC Administration

Mean serum concentration vs. time profiles for control and lymph-cannulated sheep are shown in Fig. 2. After absorption to a maximum concentration, serum concentrations declined in an apparent monophasic fashion. Collection of lymph in the lymph-cannulated group resulted in a significant reduction in the C_{max} compared to the non-cannulated control animals (Table I). Peak serum levels for both groups occurred at approximately 1 h, which is earlier than that in humans (3,4). r-metHu-Leptin was absorbed over extended periods as demonstrated by the MAT of 3.3 ± 0.7 h for the control group and 3.0 ± 0.1 h for the lymph-cannulated group. The terminal t½ was 3.1 ± 0.6 for the SC control group and 2.3 ± 0.2 h for the lymph-cannulated group, both of which are consistent with the terminal t½ of approximately 3–4 h in humans after SC injection (3,4).

The fraction of the dose absorbed into the systemic circulation (F_{sys}) after SC administration to the control group was 60.4 ± 8.4% which was reduced to 21.7 ± 6.4% in the lymph-cannulated group. The significantly lower F_{sys} in the lymph-cannulated group is consistent with the lymphatics significantly contributing to the systemic availability of r-metHu-Leptin. The cumulative recovery of r-metHu-Leptin in peripheral lymph was approximately 34.4 ± 9.7% of the dose (Table I.). The total fraction of the dose absorbed in the lymph-cannulated group (F_{abs}) was 56.0 ± 10.3%, which is not statistically different from F_{sys} for the non-cannulated group (60.4 ± 8.4%). The fraction of the absorbed dose taken up via the lymph pathway (F_{lymph}) was 0.61 compared to 0.39 for the fraction of the absorbed dose taken up via the blood pathway (F_{blood}).

Modeling of r-metHu-Leptin Pharmacokinetics after IV and SC Administration

A two-compartment model was the most appropriate model to describe the disposition of r-metHu-Leptin after IV administration. Mean volume of distribution (V_c), and rate constants (k_{12}, k_{21}, and k_{10}) were determined for the IV group and were set as constants in the SC control and lymph-cannulated models (Table II).

Despite the apparent monophasic serum decline after SC administration, a two compartment model was adopted based on the biexponential decline observed after IV dosing. Serum profiles for the SC control sheep were successfully fitted to a two compartment model with first order input and elimination. The absorption rate constant (k_a) in SC control sheep was 0.35 ± 0.05 h^{-1} (Table II). The estimates for F_{sys} calculated by compartmental and non-compartmental methods were similar reinforcing the validity of the compartmental estimates.

Serum and peripheral lymph profiles for individual lymph-cannulated sheep were simultaneously modeled using first order differential equations to describe absorption into the blood and peripheral lymph (Fig. 3). The estimate for k_a was 0.34 ± 0.01 h^{-1} (Table II), which is not statistically different to k_a determined for the non-cannulated control sheep. The fraction of the absorbed dose taken up via the lymph (F_{lymph}) was estimated at 0.61, which demonstrates that a large proportion of the bioavailable dose is absorbed via the lymph as opposed to the blood (F_{blood} was 0.39).

Table I. Noncompartmental Pharmacokinetic Parameter Estimates for r-metHu-Leptin after Intravenous (IV) and Subcutaneous (SC) Administration to Sheep[a]

Parameter	IV bolus (0.10 mg/kg) Mean ± SD	SC bolus to non-cannulated control sheep (0.15 mg/kg) Mean ± SD	SC bolus to lymph-cannulated sheep (0.15 mg/kg) Mean ± SD
C_{max} (ng/mL)	–	324.2 ± 47.6	115.5 ± 54.7
T_{max} (h)[b]	–	1.0 [1.0]	1.0 [0.5–1.0]
Terminal t½ (h)	1.9 ± 0.2	3.1 ± 0.6	2.3 ± 0.2
AUC_{inf} (ng · h/mL)	1380.6 ± 231.0	1284.3 ± 154.0	482.7 ± 155.8
V_{ss} (mL/kg)	86.3 ± 13.7	–	–
CL (mL/h/kg)	71.7 ± 11.8	–	–
MRT (h)	1.2 ± 0.2	4.5 ± 0.7	4.2 ± 0.1
MAT (h)	–	3.3 ± 0.7	3.0 ± 0.1
F_{sys} (% of dose)[c]	–	60.4 ± 8.4	21.7 ± 6.4
Lymph recovery (% of dose)	–	–	34.4 ± 9.7
F_{sys} (% of dose)[d]	–	–	56.0 ± 10.3
F_{lymph}[e]	–	–	0.61 ± 0.12
F_{blood}[f]	–	–	0.39 ± 0.12

[a] Values represent the mean ± SD for n = 4.
[b] Values represent median [range] for n = 4.
[c] F_{sys} fraction of the dose reaching the systemic circulation in either the control or lymph-cannulated animals.
[d] $F_{abs} = F_{sys}$ + lymph recovery.
[e] F_{lymph} (proportion of absorbed dose taken up via the lymph) = lymph recovery/F_{abs}.
[f] F_{blood} (proportion of absorbed dose taken up via the blood) = F_{sys}/F_{abs}.

DISCUSSION

Large molecules that have restricted permeability across the endothelial layer of blood capillaries can be absorbed via the lymphatics into the systemic circulation. Intercellular junctions within the endothelial layer of lymphatic capillaries enable large macromolecules such as proteins to readily access the lymphatics (11). Macromolecules larger than about 16 kDa are generally thought to undergo preferential absorption into the systemic circulation via this pathway (6,7). Results from previous studies have reported a correlation between molecular weight and the proportion of the dose absorbed into the peripheral lymph for a limited number of compounds (6–9). Given this limited database, it is not known whether this relationship is a general trend that can be extrapolated to other proteins or whether various protein specific factors may also influence extent of lymphatic absorption.

The systemic availability of r-metHu-Leptin after SC administration was incomplete relative to the IV group with the fraction of the dose absorbed in the control group being 60.4 ± 8.4%. The collection and complete removal of peripheral lymph draining the injection site in the lymph-cannulated group resulted in a significant reduction in the serum concentrations such that only 21.7 ± 6.4% of the administered dose was subsequently accounted for in the systemic circulation of these sheep. The contribution of the lymphatics to the overall systemic availability was independently verified by the cumulative recovery of 34.4 ± 9.7% of the r-metHu-Leptin dose in peripheral lymph.

Through summation of F_{sys} and the lymph recovery in the lymph-cannulated group, the total fraction absorbed

Table II. Compartmental Pharmacokinetic Parameter Estimates for r-metHu-Leptin after Intravenous (IV) and Subcutaneous (SC) Administration to Sheep[a]

Parameter	IV bolus (0.10 mg/kg) Mean ± SD	SC bolus to non-cannulated control sheep (0.15 mg/kg) Mean ± SD	SC bolus to lymph-cannulated sheep (0.15 mg/kg) Mean ± SD
V_c (mL/kg)	35.0 ± 7.2	Fixed	Fixed
k_{12} (h^{-1})	1.0 ± 0.5	Fixed	Fixed
k_{21} (h^{-1})	0.7 ± 0.2	Fixed	Fixed
k_{10} (h^{-1})	2.1 ± 0.4	Fixed	Fixed
k_a (h^{-1})	–	0.35 ± 0.05	0.34 ± 0.01
F_{sys}	–	0.67 ± 0.07	0.23 ± 0.07[b]
F_{abs}	–	–	0.61 ± 0.14
F_{lymph}	–	–	0.61 ± 0.13
F_{blood}	–	–	0.39 ± 0.13
Lymph recovery[c]	–	–	0.38 ± 0.13

[a] Values represent the mean ± SD for n = 4 sheep.
[b] $F_{sys} = F_{abs} * (1 - F_{lymph})$.
[c] Lymph recovery = $F_{abs} * F_{lymph}$.

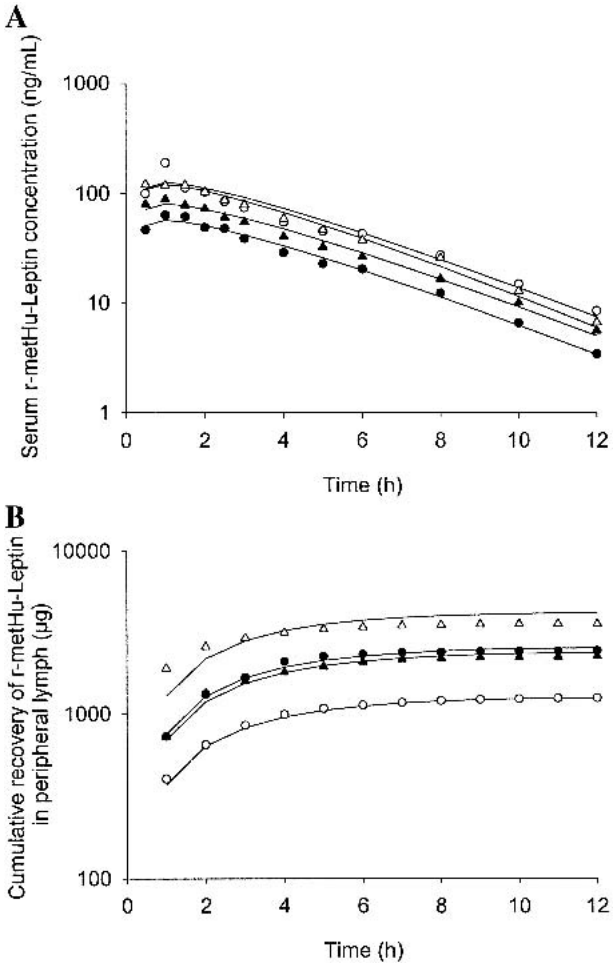

Fig. 3. Individual simultaneous fits for r-metHu-Leptin in serum (A) and peripheral lymph (B) following SC administration to lymph-cannulated sheep. Symbols represent the individual data for sheep A (●), sheep B (▲), sheep C (○), and sheep D (△). The percentage of the administered dose recovered in the peripheral lymph was 36.8% for sheep A, 34.0% for sheep B, 21.6% for sheep C, and 45.1% for sheep D.

(F_{abs}) was determined to be 56.0 ± 10.3% which is not statistically different to F_{sys} for SC control sheep (60.4 ± 8.4%). Comparable F_{sys} in the SC control group and F_{abs} in the lymph-cannulated groups illustrates mass balance and validates the applicability of the lymph cannulated model. Furthermore, the similarity between F_{sys} and F_{abs} suggests that the incomplete bioavailability of r-metHu-Leptin after SC injection is unlikely to be the result of clearance or degradation within the lymphatics. This is in contrast to studies previously reported for human growth hormone, which demonstrated significant loss during lymphatic transport after SC administration (7). The reduced bioavailability of r-metHu-Leptin is most likely attributable to degradation or loss at the injection site.

Collection of the lymph draining the injection site and simultaneous blood sampling allowed estimation of the absorption rate constant (k_a), and the fraction of the absorbed dose taken up via the lymph (F_{lymph}) and blood (F_{blood}) pathways. Estimates for k_a in the SC control and lymph-cannulated animals were not statistically different suggesting internal consistency of the model. The estimated F_{lymph} for r-metHu-Leptin (0.61 ± 0.13) reinforces that the lymphatics are an important absorption route for proteins with a molecular weight of approximately 16 kDa. The consistency of compartmental and non-compartmental estimates for F_{sys}, F_{abs}, F_{lymph}, and F_{blood} supports the proposed pharmacokinetic model.

Absorption into both blood and lymph were successfully modeled as first order processes in this study. In contrast, Radwanski *et al* modeled the absorption of interleukin-10 (IL-10) in patients after SC administration as a rapid zero-order absorption into the blood and a slower first order absorption into the lymph (12). The estimated bioavailability of IL-10 was low (42%) and the fraction of the absorbed dose taken up by the lymph was 0.95 compared with 0.05 taken up by the blood. This value for F_{lymph} for IL-10 is considerably larger than that for r-metHu-Leptin (0.61) despite the similarity in molecular weights for the two proteins (IL-10 18.7 kDa and r-metHu-Leptin 16.2 kDa). These differences could be attributed to a number of different factors including species differences in bioavailability and lymphatic uptake, model differences (i.e., different injection sites, the availability of measured lymph data), or protein specific differences in lymphatic uptake.

According to the previously described correlation between the proportion of the dose absorbed into the peripheral lymphatics and molecular weight, macromolecules greater than 16 kDa are assumed to be absorbed preferentially (i.e., >50%) via the lymphatics (6,7). As shown graphically in Fig. 4, the recovery of 34% of the r-metHu-Leptin dose in peripheral lymph deviates marginally from this correlation. However, if the proportion of the dose absorbed via the lymphatic pathway is expressed relative to the absorbed dose, the lymphatic pathway contributes approximately 61% to the overall r-metHu-Leptin absorption providing better agreement with the previous molecular weight trend. It is therefore apparent that expression of the lymphatic uptake as a proportion of the injected dose may lead to a substantial underestimation of the

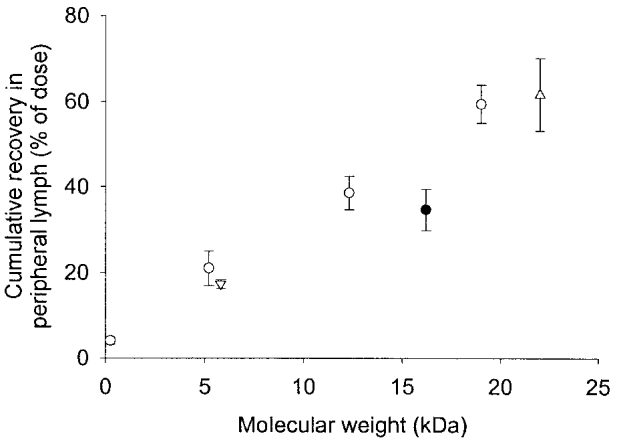

Fig. 4. Relationship between the proportions of the dose recovered in peripheral lymph (mean ± SEM for n = 3–4) and the molecular weight for selected proteins and low molecular weight compounds. Data for fluorodeoxyuridine, inulin, cytochrome *c*, and interferon α-2a (○) are from Supersaxo *et al* (6), data for human growth hormone (△) are from Charman *et al* (7), and data for insulin (▽) are from Charman *et al* (8). Data for r-metHu-Leptin recovery in peripheral lymph from this study are expressed as a proportion of the administered dose (●).

contribution of the lymphatics to the overall systemic availability for proteins with low bioavailability. Given that the subcutaneous bioavailability of many protein therapeutics is often less than complete, expression of the lymphatic contribution relative to the absorbed dose may provide a more accurate assessment of the role of the lymphatics as an absorption pathway for proteins.

In defining the absorption of proteins from the SC injection site, the properties of the interstitium and its influence on the rate and extent of absorption via the lymphatics and blood also warrants additional investigation. While the extent of blood and lymphatic absorption is most likely determined primarily by molecular weight or size, the rate of absorption may also be influenced by physicochemical characteristics of the proteins and interactions with the interstitial matrix. Estimation and subsequent comparison of absorption rate constants for other proteins will further elucidate the mechanistic behavior of the interstitium and identify the rate limiting step(s) in the absorption process.

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