

# Lymphatic Absorption of Subcutaneously Administered Proteins in Sheep: Influence of Different Injection Sites

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## Introduction

Previous reports in the literature have consistently suggested that proteins larger than approximately 16 kDa are absorbed primarily via the lymphatics (1). Much of the information to date has been generated following protein injection into either the lower region of the hind leg or the interdigital space of the hind leg in sheep. These injection sites were used for practical reasons in that they allowed cumulative and quantitative recovery of the peripheral lymph draining the injection site via cannulation of the popliteal lymph duct which collects all the lymph from the lower leg. With this experimental design (i.e. collection of peripheral lymph), the absorption process could be studied without consideration of the effect of transport through the larger lymphatics and lymph nodes (2). However, the question remains as to whether these studies provide a realistic estimation of the contribution of the lymphatics to the absorption process given that the injection sites were not representative of those typically used in humans.

## Objective

The current study was conducted to explore the role of injection site in dictating the absorption kinetics of a model 37 kDa protein, Darbepoetin alpha (DA), and to characterize the role of the lymphatics in the absorption process.

## Methods

### Parallel group study design

**Group 1:** IV Control group (blood sampling) to serve as a reference for absolute bioavailability

**Group 2:** SC Control group (blood sampling) for determination of absolute bioavailability

**Group 3:** SC Central cannulated group (blood sampling and continuous lymph collection) for determination of the individual contributions of lymph and blood to overall bioavailability

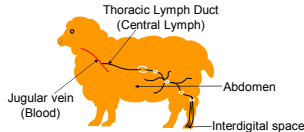
### Dose administration

Bolus IV injection into the jugular vein (0.5 µg/Kg)

Bolus SC injection in the interdigital space or the abdomen (2 µg/Kg)

### Animal Model

In order to study the influence of different injection sites, a central lymph-cannulated sheep model was used with the assumption that the majority of the lymph draining the different anatomical sites is collected in the thoracic lymph duct prior to its entry into the systemic circulation (2).



### Calculations

$F_{lymph} = \% \text{ of the dose recovered in the lymph}$

$F_{blood} = \% \text{ of the dose absorbed via the blood}$

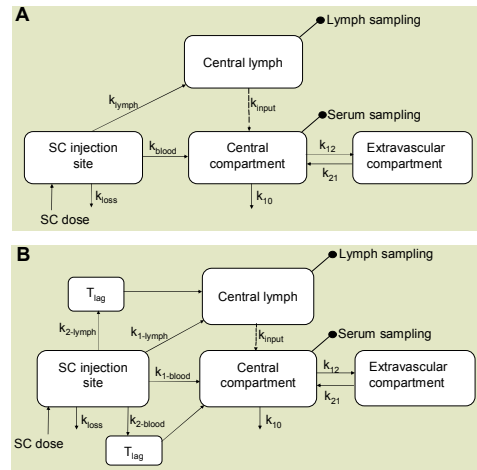
$F_{total} = F_{lymph} + F_{blood}$

### Estimation of DA in serum and lymph

The concentration of DA was determined using an erythropoietin ELISA kit (Quantikine® IVD®, R&D Systems). The lower limit of quantitation of the assay was 0.2 ng/mL. Quality control samples were consistently within ±15% of the theoretical concentration.

## Pharmacokinetic Modeling

- Pharmacokinetic modelling was performed using SAAM II software (Version 1.2, SAAM Institute, University of Washington, Seattle)
- Mean data from each group was fitted to the models (Figure 1) with fractional standard deviation (FSD) assigned to each datum based on the coefficient of variance

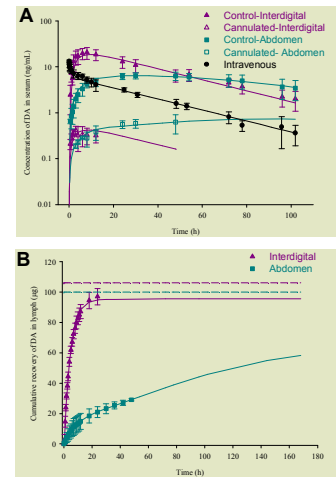


**Figure 1: Proposed pharmacokinetic model for DA following SC administration into the interdigital space (Panel A) and the abdomen (Panel B)**

- A two compartment model was used to fit the intravenous data.
- For the SC data, a model (Figure 1) was constructed to simultaneously fit the lymph and blood data.
- A transfer rate constant ( $k_{input}$ ) was included only in the control group.
- All the IV, SC Control and SC Central Lymph Cannulated data were fitted simultaneously in a single window.
- The parameters  $V_c$ ,  $k_{12}$ ,  $k_{21}$  and  $k_{10}$  were constrained to be equal to the values in the IV model.
- The absorption rate constants for the control data were constrained to be equal to the rate constants in the model for the cannulated data.
- The loss at the injection site ( $K_{loss}$ ) was constrained in the cannulated model to be equal to the value in the control model

## Results

### Serum Data – Control and Lymph Cannulated Groups



**Figure 2: Panel A: Concentration-time profiles of DA following IV injection of 0.5 µg/kg and SC injection of 2 µg/kg into the interdigital space and the abdomen (n=4 per group).**

**Panel B: Cumulative recovery of DA in µg following SC injection of 2 µg/Kg into the interdigital space and the abdomen (n=4 per group).**

Symbols represent the experimental data while the continuous lines represent the model predicted data. The discontinuous lines in panel B represent the mean administered dose in µg for each corresponding group.

- Cannulation of the thoracic lymph duct led to a decrease in serum concentrations for both the injection sites (Figure 2).
- This decrease suggests that a considerable amount of DA was absorbed by the lymphatics from both the injection sites.
- The pharmacokinetic model in Figure 1 did not adequately describe the abdomen data.
- Based on the biphasic pattern of the abdomen data (Figure 2B), an additional absorption rate constant with a lag time was added to both the lymph and blood components in the model to fit the abdomen data.

**Table 1: Pharmacokinetics of DA following SC administration of 2 µg/kg**

Parameter	Units	Interdigital	Abdomen
<b>Non Compartmental Parameters</b>			
$F_{lymph}$	%	92 ± 15	24 ± 8.8 <sup>a</sup>
$F_{blood}$	%	6.8 ± 4.1	7.6 ± 2.4 <sup>a</sup>
$F_{total}$	%	99 ± 12	32 ± 11 <sup>a</sup>
$F_{abs\_Control}$	%	110 ± 18	85 ± 21
<b>Compartmental Parameters</b>			
$F_{lymph}$	%	90 ± 1	67 ± 9
$F_{blood}$	%	2 ± 0	16 ± 2
$F_{total}$	%	92 ± 1	83 ± 9
$K_{1-Lymph}$	h	0.21 ± 0.01	0.05 ± 0.01
$K_{2-Lymph}$	h	-	0.02 ± 0.00

<sup>a</sup>based on incomplete profiles up to 48 h

- The systemic availability of DA was high for both injection sites.
- The rate of absorption of DA into the lymph was considerably slower for the abdomen group in comparison to the interdigital group (Figure 2B).
- Based on the data in Table 1, it is clear that the lymph is the predominant absorption pathway for DA following SC injection into the interdigital site.
- In the abdomen group, absorption was not complete by 48 h resulting in incomplete mass balance. Unfortunately, the lymph cannulated animals could not be maintained after this time due to the potential for dehydration.
- The pharmacokinetic model shown in Figure 1B provided a reasonable prediction of the lymphatic recovery of DA in the abdomen group in spite of the paucity of data. This model relies heavily on the SC control data for the same injection site for which a complete set of data was available.
- The two absorption rate constants for the abdomen group in Table 1 were necessary to take into account the biphasic absorption pattern evident in the lymph data (Figure 2B).

## Conclusions

Although significant differences in the rate of absorption of DA were evident after SC injection into the interdigital space and the abdomen, the lymphatics represented the predominant absorption pathway for both injection sites.

## Acknowledgement

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## References

- McLennan DN et al., Drug Discov Today: Technologies, 2 (1), 89-96
- Porter CJH et al, Advanced drug delivery reviews, 50: 157-171, 2001