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Research paper

Analysis of the absorption kinetics of macromolecules following intradermal and subcutaneous administration

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ABSTRACT

Recent years have witnessed rapid growth in the area of microneedle-assisted intradermal drug delivery. Several publications involving *in vivo* studies in humans and minipigs have demonstrated distinct change in pharmacokinetics of peptides and proteins following intradermal (ID) administration as compared to subcutaneous (SC) injections. Specifically, ID administration produced a "left-shift" in pharmacokinetic profiles i.e. shorter time to achieve maximum plasma concentrations (shorter T_{max}), and often higher maximum plasma concentrations (higher C_{max}), as compared to the SC route. In the present work differences in the kinetics of drug absorption after ID and SC administration were explored for eight peptides and proteins with the focus on obtaining quantitative information about the absorption process and identifying similarities and differences in the absorption behavior across compounds. We confirmed that systemic uptake, as judged by apparent absorption rate constants, was 2- to 20-fold higher from the dermis as compared to the subcutis. Additionally, shapes of time-resolved absorption rate profiles demonstrated notable differences in absorption kinetics between ID and SC routes. For both administration routes evaluated herein there was a general trend of small macromolecules absorbing at higher rates as compared to the large macromolecules.

1. Introduction

Several literature reports pertaining to the intradermal (ID, e.g. microneedle-mediated) delivery of peptides and proteins in humans and minipigs highlighted the fact that their pharmacokinetics (PK) differ as compared to subcutaneous (SC) injections [1–8]. Typical pharmacokinetic differences included shorter time to achieve maximum plasma concentrations (T_{max}) and higher or comparable maximum plasma concentrations (C_{max}). Bioavailability (BA) varied on an individual case basis with microneedle-mediated delivery resulting in either increased or decreased amount of macromolecule reaching the systemic circulation. Microneedles deliver drugs into the dermis, which is known to be highly perfused with blood and lymph capillary networks. Blood capillaries have low barrier properties toward small molecules due to their "leaky" endothelial structure and hence can readily absorb small molecules. Lymph capillaries, on the other hand, can accommodate molecules of different sizes, and even cellular debris, due to their unique structure and physiological function [9,10]. Lymphatic uptake after intradermal delivery has been studied experimentally using near infrared (NIR) imaging [4] demonstrating that lymphatic absorption may be a significant contributor of drug uptake from microneedle-assisted delivery. Due to the differences in the physiology of dermis and subcutis, the ID route of delivery may provide unique absorption kinetics, and as a result, pharmacokinetics compared to the SC route. These differences in PK may be potentially useful from a therapeutic point of view, e.g., when a fast onset of action is desired. However, they can also be a drawback if the goal is to mimic the PK profile of a SC-injected drug. Beyond purely pharmacokinetic considerations, it was showed that ID drug delivery can also produce a difference in the pharmacodynamic (PD) response, as compared to the SC route. For example, microneedle delivery of Insulin results in a shorter T_{max} which could arguably lead to improved postprandial glucose control as compared to SC injection in adults [5] as well as in children [8]. In another example, Parathyroid Hormone (1–34, PTH) delivered intradermally via drug-coated microneedle patch (ZP patch) [2] demonstrated significant gains in bone mineral density in the lumbar spine as well as the hip over those obtained from a SC-administered PTH (Forteo®).

Although several studies have reported PK of therapeutics delivered through the ID route [1–8] only sparse published reports are available on quantitative analysis of the PK data. A notable exception is a recent publication by Norman et al. [8] where

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authors reported fitting PK profiles of Insulin obtained after ID and SC administration to a first-order absorption model and estimated the absorption and elimination rate constants. This analysis showed that the absorption rate constant after ID administration in human was approximately 2.8-times higher than that of SC with no difference in elimination rate constant. Some other publications have shown fits to pharmacokinetic models; however, in these reports the models were not explicitly described [2,6]. It can be hypothesized that in-depth kinetic analysis of the PK data will be useful in elucidating the similarities and differences between ID absorption process and the SC absorption. Subsequently such information would be helpful in gaining insights into the mechanism of absorption from these sites and build in-silico absorption models, which can adequately describe the process and predict PK. At this point we did not consider PK data obtained from small animals given uncertainty about its relevance for human PK and limited our analysis to data obtained exclusively from pigs and humans. To this end, the objective of the present work was to gain quantitative insights about the kinetics of macromolecule absorption process from dermis and subcutis in human and minipig. The two major goals of this analysis were: (1) quantify differences in absorption kinetics from dermal and subcutaneous tissues for a given compound, and (2) summarize such absorption differences observed across eight different compounds. In this article, the PK, data analysis, and absorption kinetics of Follicle-stimulating Hormone (FSH) are described in detail, followed by an analysis of the absorption kinetics of seven additional peptides and proteins.

2. Materials and methods

All animal PK studies described herein were conducted under a protocol approved by the Merck IACUC.

2.1. Follicle-stimulating hormone pharmacokinetic studies

Puregon® (FSH) was received as a gift from MSD Oss B.V. (Molenstraat 110 Oss, 5342 CC, the Netherlands) in the form of 150 IU of lyophilized rhFSH (lyosphere) and solvent for reconstitution containing saline solution at 0.45% concentration.

2.1.1. Comparison of intradermal and subcutaneous routes ("first study")

The PK study was a sequential three-arm cross-over design using female Gottingen minipigs ($n = 3$). Minipigs were positioned in a sling and manually restrained at time of dosing. No anesthesia was administered prior to dosing. Formulation was prepared by reconstitution of rhFSH lyospheres (Puregon®) in 0.45% saline immediately prior to the PK study. FSH was injected intravenously (IV) in the marginal ear vein, injected subcutaneously (SC) in the groin area, and injected intradermally (ID) in the back using Mantoux technique with a conventional 26G intradermal needle. ID injections took up to 5 min to complete. FSH dose was 450 IU (≈45 μg) per animal, or approximately 25 IU/kg, for all routes of delivery. Volumes injected were as follows: 0.1 mL of 4500 IU/mL solution for SC and ID routes and 1 mL of 450 IU/mL solution for the IV route. Occasionally, approximately one drop of formulation was observed to be present atop of the skin wheal formed at the ID injection site. Blood samples were collected at the following pre-determined time-points: (1) IV route: 0, 0.8, 0.17, 0.25, 0.5, 1, 2, 4, 8, 24, 32, 48, 72, 96 [h]; (2) SC and ID routes: 0, 1, 2, 4, 8, 12, 24, 32, 48, 72, 96, 168 [h]. 1 mL blood samples were drawn from the jugular vein into vacutainer devoid of anti-coagulants. Blood was let clot (30–60 min) and centrifuged for 10 min at 1100G to collect serum for analysis. Serum samples were stored at −20 °C until analysis.

2.1.2. Comparison of intradermal injections at two different anatomical sites ("second study")

The PK study was a sequential two-arm cross-over design using female Gottingen minipigs ($n = 3$). (Note: these were different animals from those used in the first FSH study.) Minipigs were positioned in a sling and manually restrained at time of dosing. No anesthesia was administered prior to dosing. Formulation was prepared by reconstitution of rhFSH lyospheres (Puregon®) in 0.45% saline immediately prior to the PK study. FSH was injected intradermally (ID) in the back and in the thigh using the Mantoux technique with a conventional 26G intradermal needle. ID injections took up to 5 min to complete. FSH dose was 300 IU (≈30 μg) per animal, or approximately 25 IU/kg. Volumes injected were 0.1 mL of 3000 IU/mL solution. Occasionally, approximately one drop of formulation was observed to be present atop of the skin wheal formed at the ID injection site. Blood samples were collected at the following pre-determined time-points: 0, 0.5, 1, 2, 3, 4, 8, 12, 24, 32, 48, 72, 96, 168 [h]. 1 mL blood samples were drawn from the jugular vein into vacutainer devoid of anti-coagulants. Blood was let clot (30–60 min) and centrifuged for 10 min at 1100G to collect serum for analysis. Serum samples were stored at −20 °C until analysis.

2.1.3. Serum quantification of FSH samples

Serum samples were assayed with the FSH ELISA kit from IBL Immuno-Biological Labs, Minneapolis, MN, by a Contract Research Organization. The possibility of cross-reactivity of the human FSH ELISA with porcine FSH was investigated and it demonstrated that the IBL ELISA method was specific for human FSH without interference caused by presence of porcine FSH. None of the human FSH ELISA reacted with physiologic concentrations of porcine FSH; thus, endogenous FSH in mini-pig serum should not impact the accuracy of rhFSH measurements. The method was qualified using the following acceptance criteria: Linearity $R^2 \geqslant 0.99$; Precision CV (OD SD/Mean) ⩽20%; Accuracy Calculated [IU/L]/Theoretical [IU/L] ± 20%. LLOQ was found to be 5UI/L and the average recovery between 5 and 80 IU/L of rhFSH was 97%. Additionally, repeated freeze-thaw cycles of serum were found not to diminish recovery.

2.2. Oxytocin pharmacokinetic studies

Oxytocin powder was purchased from American Peptide Company (5 mg Oxytocin, catalogue #66-0-52). Pitocin® (injectable Oxytocin) was acquired from Myoderm (48 East Main Street, Norristown, PA).

The PK study was a sequential three-arm cross-over design using female Gottingen minipigs ($n = 3$). Minipigs were positioned in a sling and manually restrained at time of dosing. No anesthesia was administered prior to dosing. Oxytocin formulation at 50 IU/mL in 10 mM acetate buffer and 0.084% NaCl (pH 4.0) was prepared for ID and SC dosing a day prior to the PK study beginning and refrigerated overnight. For IV dosing a commercial Pitocin® formulation at 10 IU/mL was used. Oxytocin was injected intravenously (IV) in the marginal ear vein, injected subcutaneously (SC) in the thigh, and injected intradermally (ID) in the thigh using Mantoux technique with a conventional 26G intradermal needle. ID injections took approximately 1 min to complete. Oxytocin dose was 5 IU (≈10 μg) per animal which translated to approximately 0.5 IU/kg for ID and SC dosing and 0.25 IU/mg for IV dosing. Volumes injected were 0.1 mL of 50 IU/mL solution for ID and SC routes and 0.5 mL of 10 IU/mL for the IV route. In two of three pigs approximately one drop of formulation was observed to be present atop of the skin wheal formed at the ID injection site. Blood samples were collected at the following pre-determined time-points: 0, 0.8, 0.17, 0.25, 0.34, 0.5, 0.75, 1.0, 1.5, 2.0 [h]. 1 mL blood samples were drawn from the jugular vein into an EDTA vacutainer.