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Original article

# Porcine model to evaluate local tissue tolerability associated with subcutaneous delivery of protein

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ABSTRACT

**Introduction:** The conversion from intravenous (IV) to subcutaneous (SC) delivery of biotherapeutics has increased in recent years. Some of the reasons for this shift in route of delivery are due to patient convenience, reduced adverse systemic effects, lack of a need for vascular access, and reduced cost of patient care, which ultimately lead to improved patient quality of life. One caveat to SC delivery is the limited volumes that can be administered at a single site and the associated local tolerability. To characterize factors that affect subcutaneous delivery of large volumes of therapeutic proteins, a porcine model was developed. Model endpoints included measurement of interstitial pressure, assessment of local skin visco-elasticity, and the qualitative assessment of local infusion sites. **Methods:** Immunoglobulin G (IgG) was subcutaneously infused into the abdominal region of Yucatan miniature swine. Changes in interstitial pressure were measured, using an in-line pressure transducer, during and after infusions. Additionally, pre- and post-infusion changes in local skin visco-elasticity were measured using a Cutometer®. Lastly, infusion sites were assessed for post-infusion local skin reactions such as erythema and swelling. Similar assessments were made following SC IgG delivery with the permeation enhancer recombinant human hyaluronidase PH20 (rHuPH20). **Results:** Subcutaneous infusions of IgG, in the presence of rHuPH20, significantly reduced average interstitial pressures by 55% during the infusion period and by 67% during the post-infusion period, compared to the control. Infusions in the presence of rHuPH20 also maintained better local skin elasticity as seen by a 42% increase in local skin pliability compared to the control. Finally, infusions with rHuPH20 resulted in an 80% reduction in swelling area compared to the control. **Discussion:** A large animal model was developed that incorporates both quantitative and qualitative assessment methods to aid in understanding SC delivery of proteins.



## 1. Introduction

Subcutaneous (SC) delivery of protein biologics is often a preferred route of administration due to improved bioavailability when compared to other non-parenteral routes of administration (Porter, Edwards, & Charman, 2001). Specifically, among parenteral delivery routes, the SC route has some key advantages over the intravenous (IV) route, which include improved patient autonomy, decreased adverse systemic effects, and lack of a need for vascular access (Berger, 2004; Haller, 2007). A significant disadvantage of SC delivery has been the limited volumes that can be administered at a single site and at a single time, requiring multiple and frequent dosing regimens (Haller, 2007; Misbah et al., 2009).

In the past several years, the SC route of administration for delivering protein therapeutics has become more common. It has been reported that approximately two thirds of all drugs in development are biologics with the optimal delivery route for many of these drugs being subcutaneous or intramuscular injections with a delivery volume of 1 mL or less (Sharp & Whyte, 2011). However, some biologics, such as immunoglobulin G (IgG), as a replacement therapy for the treatment of primary immunodeficiency (PID), can require monthly dosages of at least 400 mg/kg, which corresponds to dosing volumes in the hundreds of milliliters (Gardulf, 2007). Patients suffering from PID are also favoring the conversion to the SC route of administration due to time and cost savings, and the comfort and convenience of self-infusions at home (Gustafson et al., 2008; Misbah et al., 2009). The current dosing regimen for SC IgG therapy for patients with PID involves multiple administrations with high infusion volumes. Hansen, Gustafson, Smith, and Gardulf (2002) reported that the most common local reactions seen in patients that received large SC infusions of IgG were swelling, redness (erythema), and soreness. Additionally, Jorgensen et al. (1996) suggested that pain associated with a SC administration is related to the infusion volume.

Preclinical studies have been conducted in rodent models with the aim of understanding the mechanisms of dispersion and absorption following SC delivery of therapeutic proteins

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(McDonald, Zepeda, Tomlinson, Bee, & Iven, 2010). However, the skin of rodents differs significantly from humans as rodent skin is loosely connected to the underlying muscle and has the ability to extensively move over most parts of the body (Kawamata, Ozawa, Hashimoto, Kurose, & Shinohara, 2003). In contrast, the pig shares many morphological and physiological similarities to human skin (Mahl et al., 2006; Svendsen, 2006). One notable similarity is that the skin is tightly attached to the SC tissue (Nunoya et al., 2007). Additionally, the skin at the abdominal region of pigs provides even greater similarity to humans in regards to thickness and structure (Mahl et al., 2006). Due to the many similarities to human skin, the miniature pig (mini-pig) was selected as the animal species to further investigate SC delivery of therapeutic proteins.

As the conversion of IV to SC delivery of therapeutic proteins increases, a potential concern with SC delivery of protein therapies, such as IgG, is the tolerability of the patient to undergo a continuous treatment regimen that consists of large volume requirements and multiple frequency of dosing. In order to better understand infusion dynamics and tolerability, we saw a need for an animal model that can assess pain or tolerability associated with SC infusions; however, it has been reported that direct tolerability evaluations cannot be made preclinically, as there is a lack of useful *in vivo* models for evaluating the extent of discomfort associated with SC injections (Brazeau, Cooper, Svetic, Smith, & Gupta, 1998). Therefore, we aimed to develop a preclinical model, in a large animal species, to evaluate the tolerability of SC delivery of therapeutic proteins by establishing endpoints which characterize the physiological effects at the local infusion site. Endpoints of the investigation included assessing interstitial pressure as measured by a remotely located pressure transducer, and the quantitative and qualitative assessment of visco-elastic changes of the skin at the local infusion site. For our model development in the pig, we selected IgG as a representative biologic to model SC delivery of large volumes of therapeutic proteins.

In order to facilitate SC delivery of IgG a recombinant human hyaluronidase PH20 (rHuPH20) enzyme was used to validate the model. Hyaluronidase has been shown to enhance the local dispersion and absorption of drugs during SC delivery (Frost, 2007). The mode of action of hyaluronidase is to transiently degrade hyaluronan, the principle glycosaminoglycan (GAG) in the hypodermis, to facilitate faster absorption of therapeutic protein into the central compartment and to enable large volume delivery of drugs and fluids (Bookbinder et al., 2006). Hyaluronan is a repeating polymer of N-acetyl glucosamine and glucuronic acid that contributes to the soluble 'gel-like' component of the extracellular matrix of the skin. The depolymerization of hyaluronan by hyaluronidase is accomplished by hydrolysis of the repeating polysaccharide. This depolymerization of hyaluronan results in a transient reduction in the viscosity of the 'gel-like' phase of the extracellular matrix. This reduction in the hyaluronan viscosity by rHuPH20 is responsible for the increased hydraulic conductance that facilitates the dispersion and absorption of injected drugs.

We describe an *in vivo* model, utilizing a relevant large animal species, to evaluate tolerability of large volume SC administration using multiple endpoints. We used rHuPH20 as a matrix altering enzyme to transiently open the interstitium and allow for improved SC dispersion of IgG; however, other delivery parameters that may be important to the drug delivery design space such as delivery hardware, formulation, viscosity, and volume with other therapeutic biologics may also be used with this animal model to characterize the physiological changes that occur during SC administration of therapeutic proteins.

## 2. Methods

All uses of animals described in this article were conducted in compliance with the National Research Council's "Guide for the Care and Use of Laboratory Animals" and performed following detailed written protocols that were approved by the Institutional Animal Care and Use Committee (IACUC) at Halozyme Therapeutics.

### 2.1. Formulation of control and test articles

Control and test articles were formulated by reconstituting lyophilized human IgG (Carimune® NF; CSL Behring, Bern, Switzerland) with vehicle buffer consisting of 10 mM L-Histidine (Ajinomoto; Raleigh, NC) and 130 mM NaCl (JT Baker; Phillipsburg, NJ) at pH 6.5 for a final 15% w/v solution. Bottles were gently rotated at 4 °C overnight to allow for solubilization of the lyophilized IgG, and then were combined to result in one large homogenous solution. An aliquot was reserved for all priming and control article infusions. A second aliquot of IgG was taken and mixed with recombinant human hyaluronidase (rHuPH20) to a final concentration of 25,000 U/mL to be used for all test article infusions. All solutions were acclimated to room temperature prior to infusion.

### 2.2. Measurement of rHuPH20 enzyme activity and rheometric characterization of immune globulin solution

rHuPH20 enzyme potency was measured for control and test article using a modified version of the original turbidimetric assay described by Dorfman and Ott (1948). In addition, rheometric analysis was performed on solutions using an AR2000ex rheometer with 60 mm cone geometry and 1 degree angle (TA Instruments; New Castle, DE). Measurements were performed using a flow step method at 25 °C and viscosity values (cP) were calculated at a shear rate of 100 1/s.

### 2.3. Measurement of interstitial pressures during and after completion of infusions

Yucatan mini-pigs (male; approximately 2–3 months of age and 8–10 kg in weight; S&S Farms, Ramona, CA), that were pink in color, were acclimated to the vivarium for at least 7 days and were fasted for 12 h prior to start of study to minimize distention of the abdomen. On day of study, the animals were anesthetized with a gas mixture of oxygen and isoflurane (Minrad Inc.; Bethlehem, PA) and placed in dorsal recumbence. The abdominal region was cleaned with isopropanol and infusion sites were measured and marked using a permanent marker. Infusion sites were located on each contralateral side, 2 cm towards the midline from the cranial end of the inguinal fold and 2–3 cm cranial. The next pair of infusion sites was located 5–6 cm cranial from the two initial sites. A total of four infusion sites were marked and labeled for each animal. To minimize resistance pressure in the system from the needle, the infusion setup consisted of an 18 ga × ¾ inch "winged" needle set (Terumo; Somerset, NJ) attached to two 5.8 inch tubing extension sets in tandem (Baxter; Deerfield, IL). The tubing extension sets were then attached to a disposable pressure transducer (Utah Medical; Midvale, UT) which was connected to a 3-way stopcock (B/Braun; Bethlehem, PA). On the other end of the closed 3-way stopcock, a 20-$cm^3$ syringe (Baxter; Deerfield, IL) pre-filled with either control or test agent was attached. The syringe was then loaded into a high pressure syringe pump (KD Scientific; Holliston, MA). The connector on the disposable pressure transducer was then attached to a cable that led to a bridge amp which in turn was connected to the PowerLab 4/30 data acquisition system (AD Instruments; Colorado Springs, CO). The data acquisition system was connected to a computer for data collection (LabChart® software; AD Instruments; Colorado Springs, CO). Once the above described infusion setup was in place, a 20-$cm^3$ syringe containing priming solution (control article) was attached to the open side port of the 3-way stopcock. The entire line was then manually primed with the priming solution (IgG alone; ~2.1 mL). Once all lines were primed, the needle was placed into the SC tissue at the marked infusion site.