Journal of Controlled Release 214 (2015) 94–102

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

A novel in vitro method to model the fate of subcutaneously administered biopharmaceuticals and associated formulation components

Hanne M. Kinnunen [a,1], Vikas Sharma [b], Luis Rodrigo Contreras-Rojas [a], Yafei Yu [a], Chlöe Alleman [a], Alavattam Sreedhara [b], Stefan Fischer [c], Leslie Khawli [e,2], Stefan T. Yohe [d], Daniela Bumbaca [e], Thomas W. Patapoff [b], Ann L. Daugherty [d], Randall J. Mrsny [a,*]

[a] Department of Pharmacy and Pharmacology, University of Bath, Bath, UK
[b] Late Stage Pharmaceutical Development, Genentech, Inc. South San Francisco, CA 94080, USA
[c] Late Stage Pharmaceutical and Processing Development, Pharmaceutical Development & Supplies, Pharma Technical Development Biologics EU F. Hoffmann-La Roche Ltd, Basel CH-4070, Switzerland
[d] Drug Delivery, Genentech, Inc. South San Francisco, CA 94080, USA
[e] Pharmacokinetics, Genentech, Inc. South San Francisco, CA 94080, USA

ARTICLE INFO

Article history:
Received 29 May 2015
Received in revised form 13 July 2015
Accepted 15 July 2015
Available online 22 July 2015

Keywords:
Subcutaneous injection
Biopharmaceuticals
In vitro model
Formulation design

ABSTRACT

Subcutaneous (SC) injection is becoming a more common route for the administration of biopharmaceuticals. Currently, there is no reliable in vitro method that can be used to anticipate the in vivo performance of a biopharmaceutical formulation intended for SC injection. Nor is there an animal model that can predict in vivo outcomes such as bioavailability in humans. We address this unmet need by the development of a novel in vitro system, termed Scissor (Subcutaneous Injection Site Simulator). The system models environmental changes that a biopharmaceutical could experience as it transitions from conditions of a drug product formulation to the homeostatic state of the hypodermis following SC injection. Scissor uses a dialysis-based injection chamber, which can incorporate various concentrations and combinations of acellular extracellular matrix (ECM) components that may affect the release of a biopharmaceutical from the SC injection site. This chamber is immersed in a container of a bicarbonate-based physiological buffer that mimics the SC injection site and the infinite sink of the body. Such an arrangement allows for real-time monitoring of the biopharmaceutical within the injection chamber, and can be used to characterize physicochemical changes of the drug and its interactions with ECM components. Movement of a biopharmaceutical from the injection chamber to the infinite sink compartment simulates the drug migration from the injection site and uptake by the blood and/or lymph capillaries. Here, we present an initial evaluation of the Scissor system using the ECM element hyaluronic acid and test formulations of insulin and four different monoclonal antibodies. Our findings suggest that Scissor can provide a tractable method to examine the potential fate of a biopharmaceutical formulation after its SC injection in humans and that this approach may provide a reliable and representative alternative to animal testing for the initial screening of SC formulations.

1. Introduction

More than forty years after the innovative application of recombinant DNA technology to produce novel protein therapeutics, the biotechnology industry has fulfilled its promise of safely and effectively treating unmet medical needs and providing life saving therapies. Indeed, many diseases can now be treated as a chronic condition rather than an acute malady with a poor prognosis [1]. As there is strong patient preference for subcutaneous (SC) injections over the more time-intensive intravenous (IV) infusion therapies [2], many biopharmaceuticals under development as well as those previously approved as IV therapies are being formulated for SC injection. While IV administration is considered to consistently provide the optimal means of delivering the entirety of a dose to a patient, molecules dosed by the SC route typically have a lower bioavailability. Currently, it is impossible to predict the extent of this diminished bioavailability and to determine the basis for these outcomes; the biophysical status of a SC injected protein or peptide and its possible interactions with ECM components of the hypodermis in man are not easily monitored. What is known is that currently approved biopharmaceutical formulations have bioavailabilities that range from 20–100% [3], depending on

* Corresponding author at: University of Bath, Department of Pharmacy and Pharmacology, Claverton Down, Bath BA2 7AY, UK.
E-mail addresses: hanne.kinnunen@durham.ac.uk (H.M. Kinnunen), Rjm37@bath.ac.uk (R.J. Mrsny).
[1] Current address: Durham University, Division of Pharmacy, Queen's Campus, Wolfson Building, Stockton-on-Tees TS17 6BH, UK.
[2] Current address: Department of Pathology, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA.

http://dx.doi.org/10.1016/j.jconrel.2015.07.016
0168-3659/

the drug, with published data on the bioavailability of monoclonal antibodies in man suggests absorption between 50–100% [4]. Efforts to find animal models that universally and reliably predict human SC bioavailabilities have been unsuccessful [5]. For example, the SC bioavailability of human epoetin-β is 80% in dogs, 76% in rats, and 70% in mice [6] but only 20–36% in man [7,8]; interferon-α has a 42% BA in dogs [9] but >80% in man [10] following SC injection.

In order to enter the systemic circulation and reach intended therapeutic targets throughout the body, SC injected biopharmaceuticals must be absorbed, presumably by uptake into the blood or lymph capillaries [11,12]. Besides specific receptors possibly being present on cells at or near the injection site, uptake into the circulation appears to be dictated by the physicochemical properties of the molecule such as hydrodynamic radius, net charge, and hydrophobic characteristics [13]. A commercially acceptable biopharmaceutical product is typically stable for two years at 5 °C and has a high drug concentration to allow for a less than 1 cm^3 injection volume; formulations are commonly at pH 5–6.5 and contains stabilizing excipients such as surfactants (e.g. polysorbates), poly-alcohols (e.g. mannitol), tonicifiers (e.g., salts and sugars), and sometime a preservative. Over a time frame of minutes to hours after SC injection, the biopharmaceutical will experience potentially stressful events; sometimes including a transition through its isoelectric point as the local environment shifts from conditions of the drug product formulation to that of the hypodermis as the SC injection site regains its homeostatic state. Diffusion of a biopharmaceutical from the SC injection site may also be affected by biophysical changes induced by this transition and interaction(s) with extracellular matrix (ECM) components. Efforts made to disorganize the ECM environment and improve the outcome of SC injections support the criticality of interactions with non-cellular ECM elements affecting the overall fate of a SC injected biopharmaceutical [14,15]. Thus, we have hypothesized that a primary factor driving SC bioavailability achieved for a given formulation involves the stability of the biopharmaceutical at physiological conditions and its interactions with ECM components.

We have established a novel in vitro system to model these early events and to simulate dynamic processes imposed on a biopharmaceutical as it transitions from a drug product formulation to the physiological conditions of the hypodermis. Our approach utilizes current information on the physiological properties of the hypodermis for factors such as ECM components, pH, ionic composition, interstitial pressure, and temperature [16]. While the hypodermis also contains adipocytes and a sparse distribution of fibroblasts and macrophages, the high concentration at which most biopharmaceuticals are administered would overwhelm the capacity of these cells to exert a significant effect, magnifying the potential impact of these acellular components. In this report we present a series of studies using this in vitro subcutaneous injection site simulator system, termed "Scissor", to model some of the events that might affect the fate of biopharmaceutical formulation components following a SC injection. Real-time measurements were made to monitor the status of the injection chamber compartment; the extent of a biopharmaceutical released into the infinite sink was determined in samples removed at specific times. The tractable nature of Scissor allows for the identification of specific factors that could affect the diffusion or stability of a biopharmaceutical after its introduction into the injection chamber and further, to assess the impact of formulation changes with the aim of developing a more rational formulation selection strategy for a biopharmaceutical that includes events that might occur after SC injection.

2. Materials and methods

2.1. Materials

Buffers were made using NaCl, KCl, $CaCl_2$, $MgCl_2$, $NaHCO_3$, K_2HPO_4, Na_2HPO_4 and Na_2SO_4 purchased from Sigma (Gillingham, UK). Acetonitrile, pyridine, ethanol, *m*-cresol, acetic acid, and hydrochloric acid were of HPLC quality and purchased from Sigma (Waltham, MA, USA.). Hyaluronic acid (HA) sodium salt isolated from *Streptococcus equi* with an average molecular weight of 1.5 to 1.8 MDa was obtained from Sigma (Gillingham, UK). Hyaluronidase (HAdase) isolated from bovine testes, fluorescently labeled dextrans, methylene blue, 4-aminofluorescein and 3-(3-Dimethylaminopropyl)-1-ethyl-carbodiimide and citric acid were all purchased from Sigma (Gillingham, UK). Bradford reagent, Pierce Slide-a-lyzer dialysis cassettes, Biodesign 14,000 molecular weight cut-off dialysis tubing, and phosphate buffered saline (PBS) tablets were purchased from Fisher (Loughborough, UK). The Dermaroller™ device was purchased from AesthetiCare (Wetherby, UK). Water was reverse osmosis purified (Millipore, France). The monoclonal antibodies anonymized as mAb 2, mAb F, mAb T and mAb A were supplied by Genentech, Inc. (South San Francisco, USA). Insuman Comb 50 (Sanofi Aventis) was purchased from AAH Pharmaceuticals (Coventry, UK). Recombinant human insulin (100 IU/mL) obtained from Sigma (Gillingham, UK) was formulated at pH 4 or at pH 7.4 in 0.1 M citric acid or 0.2 M Na_2HPO_4 buffers, respectively. In addition, each insulin formulation contained 16 mg/mL glycerol, 2.5 mg/mL m-cresol and 0.015 mg/mL Zn^{2+}; all from Sigma (Gillingham, UK).

2.2. Methods

2.2.1. Fluorescent labeling of HA

Hyaluronic acid was fluorescently labeled following the protocol of Ogamo et al. [17]. Briefly, 200 mg of sodium salt of hyaluronic acid was dissolved in 20 mL of 3:1 (v/v) mixture of 1 M HCl and pyridine. 114 mg of 5-aminofluorescein (5-AF) was dissolved in 4 mL of the same solvent and the solution was then added to the HA solution. The pH of the mixture was adjusted to pH 7.4 with 12 M HCl. 0.34 g of 3-(3-Dimethylaminopropyl)-1-ethyl-carbodiimide HCl was then added to the reaction mixture while the pH was maintained at 7.4 by addition of 6 M HCl. The reaction mixture was stirred in room temperature for 1 h. To purify the fluorescent-HA, the reaction mixture was dialyzed against 1 L of cold water for 24 h followed by a wash with 100 mL of cold ethanol, then dissolved in 100 mL of deionized water and freeze-dried. The resulting solid was reconstituted in water at 5 mg/mL for use.

2.2.2. Description of the in vitro system

Transparent 14 kDa MWCO dialysis tubing (Biodesign, Carmel, NY, USA) was modified by puncturing a series of holes of approximately 150 μm by 50 μm in a grid pattern of 3 mm × 3 mm using a stainless steel fine bore Dermaroller™ microneedle roller device. A Slide-a-lyzer® dialysis cassette was disassembled and the original membranes removed. Holes were drilled into the plastic frame of the dialysis cassette to allow replacement of the membranes with the modified Biodesign dialysis membrane and securing it in place using plastic screws and nuts. Holes drilled into the cassette were also used to facilitate the introduction of a pH probe and a needle for introduction of test formulations. The cassette was then filled with a physiological buffer containing HA at a final concentration of 1, 5, or 10 mg/mL. The physiologic buffers were composed of 6.4 g NaCl, 0.09 g $MgCl_2 \cdot 6H_2O$, 0.4 g KCl, 0.2 g $CaCl_2$ and 2.1 g $NaHCO_3$ in 1 L H_2O CO_2, having a calculated osmolarity of ~280 mOsm/L, as described by Kay et al. [18].

A glass beaker containing 300 mL of the physiological buffer solution that was heated to and maintained at 34 °C, and stabilized at pH 7.4 with gaseous CO_2, was placed in a Milton Roy Spectronic 601 spectrophotometer (Pont-Saint-Pierre, France). The modified dialysis cassette was positioned inside the beaker so that light path of the Milton Roy Spectronic 601 spectrophotometer could be used to monitor light transmittance at the simulated injection site within the cassette. A micro-pH probe (Orion Micro, Thermo Scientific, Waltham, MA) connected to a Hanna Instruments HI3220 pH meter (Woonsocket, RI, USA) was placed into the cassette to be adjacent to the site of an introduced biopharmaceutical formulation but not in the spectrophotometer light path. The pH meter