Journal of Pharmaceutical Sciences xxx (2016) 1-5

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Mini Review

Subcutaneous Injection Volume of Biopharmaceuticals—Pushing the Boundaries

Roman Mathaes, Atanas Koulov, Susanne Joerg, Hanns-Christian Mahler*

Drug Product Services, Lonza AG, Basel, Switzerland

ARTICLE INFO

Article history:
Received 19 April 2016
Revised 13 May 2016
Accepted 17 May 2016

Keywords:
subcutaneous injection
injection volume
injection pain
combination products
hypodermis
health care

ABSTRACT

Administration into the subcutaneous (SC) tissue is a typical route of delivery for therapeutic proteins, especially for frequent treatments, long-term regimens, or self-administration. It is currently believed that the maximum volume for SC injections is approximately 1.5 mL. Larger SC injection volumes are considered to be associated with injection pain and adverse events at the injection site. However, no controlled clinical studies and actual evidence exist to support this assumption. In this review, we discuss current and publically available data related to SC administration volumes. We conclude that injection volumes higher than 3.5 mL are worth exploring if required for the development of efficacious drug treatments. Studying tissue back pressure, injection site leakage, local tolerability, and injection-related adverse events, such as injection pain, should be considered for the development of higher SC injection volumes.

Introduction

Over the past decades, recombinant proteins have become a standard therapeutic modality for the treatment of severe diseases.[1] Monoclonal antibodies (mABs) and new antibody formats are dominating the biopharmaceutical market as they offer numerous benefits including target specificity[2] and long serum half-life.[3]

Administration into the subcutaneous (SC) tissue is a typical route to deliver therapeutic proteins, especially for frequent treatments, long-term regimens, or self-administration. Particularly, combination products such as prefilled syringes and autoinjectors enable self-administration, shifting the point of care from the hospital to the patient's home.[4] Prefilled syringes also improve patient compliance and safety as well as minimize dosing errors and microbial contaminations.[4] Despite these benefits, several challenges associated with the SC administration route remain.

Currently, it is believed that the maximum volume for SC injections is up to 1.5 mL and would in no case be higher than 2.5 mL.[5-8] SC injection volumes larger than 2 mL are associated with various issues including injection pain, adverse events at the injection site, and injection site leakage (i.e., backflow of injected solution).[7] However, to the best of our knowledge, no controlled clinical studies and actual evidence exist to support this assumption.

mAbs typically require high doses, usually ranging from >80 mg per patient,[5] up to 1000 mg per patient. On account of the perceived volume limitation, mAB formulations often need to be developed at very high concentrations of 150-200 mg/mL and in some cases even higher.[5,9] This leads to several specific challenges associated with highly concentrated protein solutions. For example, protein concentration may impact protein stability by increasing the propensity for aggregation and protein particle formation during shelf-life. In addition, protein concentration may impact protein stability and bioavailability after injection in the SC tissue. For example, for smaller proteins that are mainly absorbed by passive diffusion, over blood capillaries distribution in the SC tissue may impact adsorption kinetics.[10] However, controlled studies to investigate *in vivo* protein stability remain missing.

An increased viscosity (rising exponentially with protein concentration) influences processability, the ability to filter and deliver (administration)[5] the drug product. Viscosity can be influenced by formulation parameters such as pH and excipients and solution temperature[11] depending on the type of underlying molecular interactions. For example, temperature, pH, and ionic strength were found to possibly influence the viscosity of mAB

Abbreviations used: ECM, extracellular matrix; mABs, monoclonal Antibodies; SC, subcutaneous.

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* *Correspondence to:* (Telephone: +41 61 316 8322; Fax: +41 61 316 9322).
E-mail address: Hanns-Christian.Mahler@Lonza.com (H.-C. Mahler).

http://dx.doi.org/10.1016/j.xphs.2016.05.029
0022-3549/

Table 1
Factors Influencing Injection Pain

Injection Pain Influencing Factor	Explanation
Injection speed	Slow injection speed is considered to cause less injection pain[23-25]
Injection site	The hypodermis is a highly variable tissue, which significantly differs across body sites[7]
Active pharmaceutical ingredient	Limited clinical relevant data available[26,27]
Temperature of the drug product	Drug products at body temperature are considered to cause less injection pain[28,29]
Formulation parameters	Limited relevant clinical data available, viscosity: influencing spreading,[30] osmolality, pH: directly influence injection pain[31]
Patient-to-patient differences in pain tolerance	Subjective experience of pain[32]
Injection depth within the SC tissue	No relevant clinical data available
Injection needle	No relevant clinical data available; a smaller needle gauge (larger needle diameter) is considered to cause greater injection pain
Type of used combination product (prefilled syringe vs. autoinjector)	No relevant clinical data available
Skill of medical care person	No relevant clinical data available
Pretreatment	Application of topical anesthetics before actual injection is considered to reduce injection pain[33]

formulations.[12] However, for conjugated molecules, such as PEGylated peptides or proteins, these parameters do not significantly influence viscosity.[11] In contrast, polymer type, size (e.g., 20 vs. 40 kD polyethylene glycol), size distribution, and structure (e.g., linear vs. branched polyethylene glycol) matter.

For highly viscous solutions, the choice of an adequate injection needle is critical. As the inner diameter of the needle decreases, the required injection forces increase, following the Hagen–Poiseuille law to the power of 4. In cases where very thin needles such as 30G are used, the viscosity at the temperature of administration cannot exceed 10 cP, assuming a maximum injection force of 10 N[13] is desired.

An increased SC dose volume would offer the option to significantly reduce the protein concentration, which may positively influence protein stability and lower viscosity.

An alternative to larger SC injection volumes, which also allows for formulating the product at lower protein concentration, is the application of multiple SC injections of smaller volumes.

As an example: a 200-mg dose can be achieved in several ways:

- 200 mg/mL in 1-mL injection volume
- 100 mg/mL in 2 × 1–mL injections
- 100 mg/mL in a 2-mL injection volume
- 50 mg/mL in 4-mL injection volume

In this review, we discuss the current and publically available data related to SC administration volumes. We delineate the injection volume of commercialized SC administered biopharmaceuticals in the context of the current standard of care. Furthermore, tissue back pressure, local tolerability, injection site leakage, and injection-related adverse events including injection pain are discussed. Finally, we evaluate the possibilities of injecting larger volumes through the SC route by formulating the drug product with and without the functional excipient *hyaluronidase*.

Table 2
Examples of Commercialized Products for SC Injection and the Corresponding Injection Volume

Therapeutic Protein	Brand Name	Disease	Injection Volume
Adalimumab	Humira™	Anti-inflammatory	0.8 mL
Canakinumab	Ilaris™	Anti-inflammatory	1 mL
Efalizumab	Raptiva™	Anti-inflammatory	1.25 mL
Insulin	Various	Diabetes	<1 mL
Interferon alfa 2a	Roferon-A™	Antiviral	0.5 mL
Golimumab	Simponi™	Anti-inflammatory	0.5 mL
Omalizumab	Xolair™	Anti-inflammatory	1.2 mL
Ustekinumab	Stelara™	Anti-inflammatory	1 mL
Tocilizumab	Actemra™	Anti-inflammatory	0.9 mL
Certolizumab pegol	Cimzia™	Anti-inflammatory	2 × 2 mL
Secukinumab	Cosentyx™	Anti-inflammatory	2 × 1 mL

Physiological Structure of the Skin—Considerations for Subcutaneous Injection Volume

SC administration refers to the application of the drug product into the hypodermis, specifically the SC tissue.

The SC tissue is located between the muscle tissue and the skin. The hypodermis thickness differs across body sites and shows significant person-to-person variability. Absorption and thus the bioavailability of therapeutic proteins after SC administration is a complex process: An oversimplified model describes that on administration, therapeutic proteins with a molecular weight larger than 16 kDa reach systemic circulation by the lymphatic vessels.[8] However, several other molecular properties such as charge or hydrophobicity as well as formulation composition or variability between patient-to-patient and the chosen injection site may also influence absorption and bioavailability of a protein.[14,15]

Adipose tissue comprises the main part of the hypodermis and is separated by the fibrovascular network into lobules. The size and shape as well as the structure of the fibrovascular network show variability across body sites and people. For example, gender or the body mass index of a person influences the structure of the hypodermis.[16]

Fibroblasts in the SC connective tissue produce components of the extracellular matrix (ECM). The ECM mainly consists of collagen and glycosaminoglycans[7,8] and is a physiological barrier for SC-administered drugs. The ECM, specifically the collagen fibers, determines the mechanical properties of the SC tissue. The glycosaminoglycans, mainly hyaluronan, are responsible for the gel-like phase of the ECM and control its hydraulic conductivity. The low hydraulic conductivity of the SC ECM prevents rapid spreading of the SC-administered drug product. After SC administration, therapeutic proteins are transported by diffusion through the interstitial space where the lymphatic capillaries drain the injection site.[8] Administration of an injection volume of >2 mL may lead to a bulge or injection site induration. The observation of bulge formation in these cases may have contributed to the dogma of <2.5 mL maximum “allowed” injection volume. However, the injection site induration is usually considered only a cosmetic issue, and there is no relevant clinical evidence that injection site indurations are related to injection pain.

The ECM is also a contributor to injection back pressure, especially for viscous drug products and larger SC injection volumes, where the required injection forces increase significantly. While the injection needle is the main contributor to injection forces,[17] tissue back pressure is another component that requires consideration. Studies