

Subcutaneous drug delivery and the role of the lymphatics

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Subcutaneous injections are widely utilised as a delivery route for compounds with limited oral bioavailability or as a means to modify or extend the release profile. In this review, factors affecting absorption from the subcutaneous space are discussed with particular emphasis on differential drug absorption into either the underlying blood or lymphatic capillaries. Formulation and targeted delivery approaches, which utilise the subcutaneous administration route, are reviewed with reference to associated technologies and future challenges.

Introduction

Subcutaneous (SC) injections have been extensively utilised as a delivery route to circumvent low oral bioavailability, as an alternative administration route when oral dosing is not well tolerated and in some cases, to modify or extend the release characteristics in efforts to prolong systemic exposure. Despite the acceptance and frequency of utilisation of the SC route of drug administration, relatively little effort has been directed towards understanding the factors that govern the rate and extent of SC absorption and the relative roles of the lymphatics and vasculature in transporting xenobiotics to the systemic circulation.

Characterisation of the SC absorption process is crucial to both the design of improved drug delivery systems and the interpretation and development of useful pharmacokinetic–pharmacodynamic relationships. This review provides an

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overview of the factors that govern SC absorption, describes research and technologies focused on utilising or modifying SC absorption and provides insight into future challenges for lymphatic delivery after SC administration.

Absorption from subcutaneous injection sites

Drug administration by SC injection results in delivery to the interstitial area underlying the dermis of the skin. The interstitium consists of a fibrous collagen network supporting a gel-phase comprising negatively charged glycosaminoglycans (largely hyaluronan), salts and plasma-derived proteins [1,2]. The proteins present within the interstitial space are essentially the same as those in plasma although they are thought to be present at approximately 50% lower concentration [3].

The physiology of the SC environment likely dictates the patterns of absorption of both typical ‘small’ drug molecules as well as macromolecular and particulate systems after SC administration. In general, small drug molecules (<1 kDa) are thought to be preferentially absorbed by the blood capillaries due to their largely unrestricted permeability across the vascular endothelium together with the high rate of filtration and reabsorption of fluid across the vascular capillaries (in the range of 20–40 L/day in comparison to approximately 2–4 L/day of fluid drained by the lymph). By contrast, the absorption of small particulates (generally less than about 100 nm)

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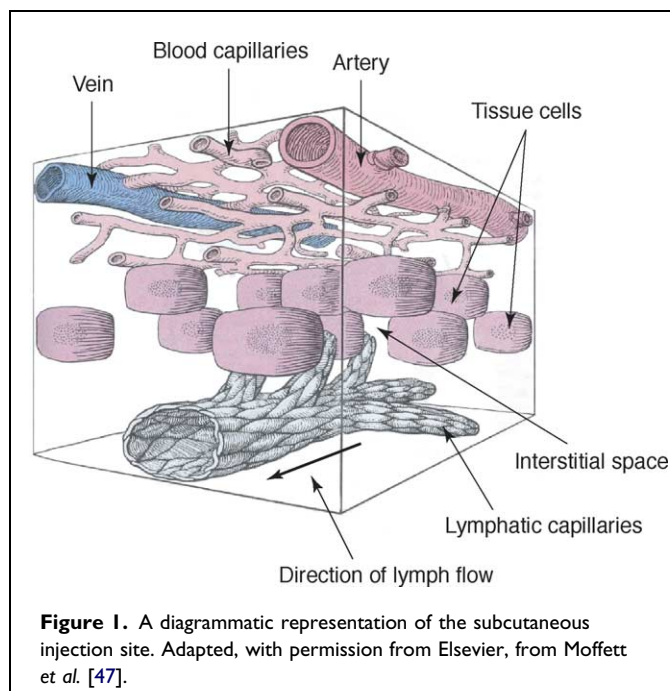
[4,5] and macromolecules into the blood is restricted by their limited permeability across the vascular endothelia and in this case, the lymphatics provide an alternative absorption pathway from the interstitial space.

Passage through the interstitium to the vascular or lymphatic capillaries can also present a barrier to efficient drug absorption after SC administration. Interstitial diffusion of macromolecular drugs is likely to be influenced by their physiochemical characteristics, including size, charge and hydrophilicity, and their interactions with endogenous components present within the interstitium. Electrostatic interactions with negatively charged glycosaminoglycans, interaction with interstitial proteins and the degree of interstitial hydration can all play a role in diffusion and absorption processes. Simple formulation characteristics, such as drug concentration, injection volume, ionic strength, viscosity and pH, together with the presence of formulation excipients can also influence the rate of diffusion from the SC injection site [6]. Other factors which can limit the extent of absorption of drugs from the interstitial space include susceptibility to enzymatic degradation at the injection site, cellular uptake by endocytic and phagocytic mechanisms [7] and simple precipitation, aggregation or poor resolubilisation.

Lymphatic structure and function in relation to drug absorption from the interstitium

In addition to its role in the absorption and transport of dietary lipids and highly lipophilic compounds (such as lipid soluble vitamins and some drugs) from the intestine to the systemic circulation, the lymphatic system plays a key role in the maintenance of an effective immune system and the dissemination of metastases from several solid tumours. The lymphatic system also provides a unidirectional pathway from the peripheral tissues to the systemic circulation for materials, such as extravasated plasma proteins, excess fluid and cellular debris. This latter function is crucial to the maintenance of homeostasis and osmotic pressure but also underpins the role of the lymphatics in the absorption of therapeutic macromolecules and particulates from SC injection sites.

Lymph originates from the interstitial fluid and plasma exudate and initially drains into the smallest of the lymphatic vessels, the lymphatic capillaries, which are extensively distributed throughout the body in close proximity to blood capillaries (Fig. 1). The lymphatic capillaries subsequently drain into larger collecting vessels that transport lymph via the lymph nodes to the thoracic duct, the largest of the lymphatic vessels. The thoracic lymph duct then ascends through the thoracic cavity and eventually empties into the systemic circulation at the junction of the left internal jugular and left subclavian veins [3]. The lymphatic capillaries are blind-ended tubules that are densely distributed within the subcutaneous tissue and mucous membranes [8].



The outer walls of the capillaries consist of a single layer of endothelial cells, which are highly attenuated and characterised by a discontinuous basement membrane [9]. Apposing endothelial cells are loosely adherent and overlap to form 'cleft-like' intercellular junctions along the surface of the capillary (Fig. 2), which are estimated to be between 15 to 20 nm and several microns wide [10]. These junctions provide an uninterrupted channel from the interstitium into the capillary lumen [11] and open and close in response to changes in interstitial volume and pressure (for review, see [12]). It is this relatively 'open' structure of the lymphatic capillaries that facilitates the absorption of small particulates and macromolecules from the interstitial space into lymph. By contrast, the vascular capillary endothelium is characterised by the presence of tight junctions between apposing endothelial cells and an underlying basement membrane, which effectively restricts the free passage of macromolecules and particulates.

The paucity of data in the literature relating to lymphatic absorption of drugs following SC administration is linked, in part, to the technical difficulties associated with the establishment of animal models to study lymphatic absorption. The physical size of the collecting lymphatics dictates that relatively large animal models are required to allow access to these extremely small vessels, and as such, the costs and complexities of the surgical preparation are a significant barrier to their widespread applicability [13]. The site of cannulation also plays an important role in determining whether the data generated describe the absorption of molecules into the lymphatics directly draining the injection site or both absorption and subsequent transfer from the periph-

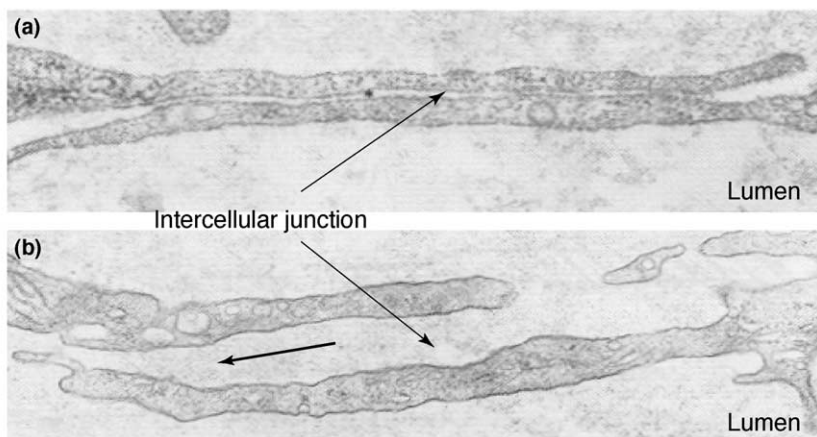


Figure 2. Microscopic view of the lymphatic capillary illustrating a closed intercellular junction (a) and an open intercellular junction (b) allowing luminal access to molecules along the pathway indicated by the arrow. Adapted, with permission from The Rockefeller University Press, from Leak [48].

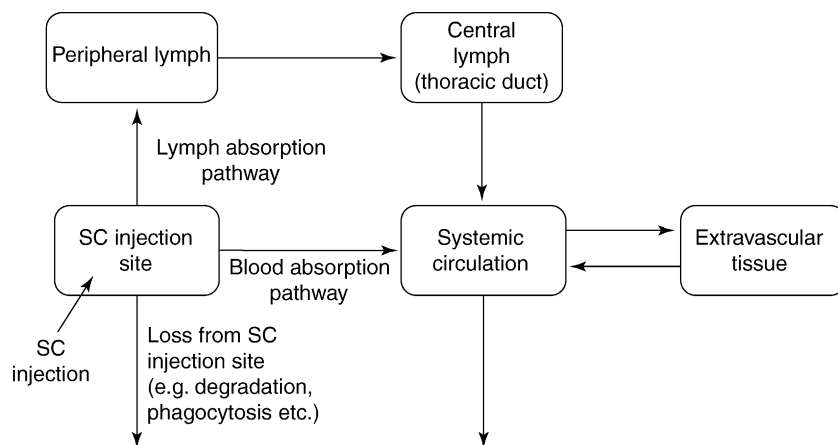
eral lymphatics to the thoracic duct ultimately emptying into the systemic circulation. The generalised model shown in Fig. 3 illustrates that both lymphatic absorption and transport processes need to be considered to more accurately develop pharmacokinetic models to describe macromolecular disposition following SC administration [14,15].

Conventional formulations for SC administration

A large number of studies have utilised the SC route for administration of drugs in aqueous or oily solutions, simple emulsions and suspension formulations (Table 1). Although isolated studies have examined the rate and extent of absorption of drug molecules following SC injection and the influence of formulation (as reviewed by [6]), relatively few studies have investigated the mechanism of drug absorption from SC injection sites, and in particular the role of the lymphatics in this process. This stems from the generally held assumption that absorption following SC injection is rapid and complete

for most 'small' drug molecules. In comparison, the absorption kinetics of therapeutic proteins have been extensively characterised because they represent a class of therapeutics that currently require parenteral administration and where SC administration can provide patient compliance advantages over intravenous or intramuscular administration routes. Following SC injection, the rate of absorption of protein drugs is typically prolonged as evidenced by a delay in the time to maximum concentration and a prolonged terminal half-life relative to that following IV administration. Both the rate and extent of absorption of protein therapeutics are variable depending on the injection site [16–18], but the basis for this variation has not been rigorously examined.

Over recent years, several groups including ours have begun to examine the role of the lymphatics in the absorption of protein therapeutics [14,15,19–22]. The combined results from these studies have demonstrated that an approximately linear relationship exists between the molecular



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Figure 3. Generalised schematic representing SC absorption via the blood and lymphatic absorption pathways into the systemic circulation.

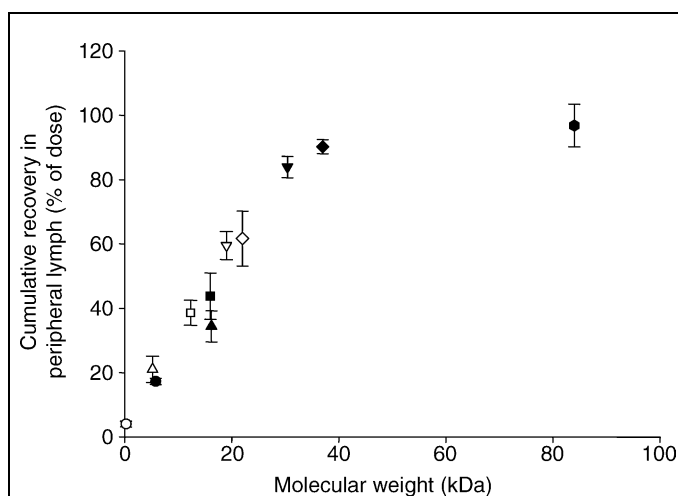
Table 1. Technologies associated with SC delivery

	Conventional SC formulations	Modified release SC formulations	Lymph-targeting SC delivery systems
Examples of specific type of technology	<ul style="list-style-type: none"> - Aqueous solutions - Oily solutions - Suspensions - Simple emulsions 	<ul style="list-style-type: none"> - Biodegradable <i>in situ</i> implants - Biodegradable microspheres - Osmotically controlled implants - Liposomes - Lipid nanoparticles 	<ul style="list-style-type: none"> - Nanoparticles, liposomes, microspheres - Dendrimers - Biodegradable polymers, conjugates, prodrugs - ISCOMs - Inorganic colloids (sulfur, iron) - Monoclonal antibodies
Names of specific technologies with associated companies and company websites	No specific technologies available	Alzamer [®] Depot [™] , DUROS [®] , Stealth [®] (ALZA Corporation, http://www.alza.com) Atrigel [®] (Atrix Laboratories, http://www.qltinc.com) SABER [®] (Durect Corp, http://www.durect.com) ProLease (Alkermes Inc, http://www.alkermes.com) DepoFoam [®] (SkyePharma Inc, http://www.skyepharma.com) SupraVail [™] (Phares Drug Delivery AG, http://www.phares.biz)	No specific technologies available
Pros	<ul style="list-style-type: none"> - Generally biocompatible - Ease of manufacture - Suitable for local and systemic exposure - Some scope for delayed release with suspensions, oily solutions - Lymphatic absorption dictated primarily by molecular size 	<ul style="list-style-type: none"> - Reduced dosing frequency, improved compliance - Improved efficacy/safety profile - Protection of labile compounds by encapsulation - Improved delivery of poorly soluble compounds - Ease of administration with <i>in situ</i> implants, micro- and nanospheres and nanoparticles - Removal of implantable devices allows immediate discontinuation of therapy 	<ul style="list-style-type: none"> - Useful for targeting, imaging, diagnostics - Approaches allow lymphatic delivery of both small molecules and macromolecules - Flexibility and versatility in carrier characteristics - Localisation of drug in tissues (lymph, lymph nodes, tumour, etc.)
Cons	<ul style="list-style-type: none"> - Rapid release from aqueous solutions - Deactivation/instability at the injections site - Variable absorption from suspension depots - Challenge of mimicking pulsatile physiological release - Hypersensitivity 	<ul style="list-style-type: none"> - Limited drug payload - Hypersensitivity - Manufacturing considerations for micro- and nanosphere preparations - Specialised insertion for implants and devices - Potential long-term biocompatibility issues of implantable devices 	<ul style="list-style-type: none"> - Limited drug payload - Polydispersity in size characteristics - Regional differences in SC blood flow and lymphatic drainage - Biological fate of carrier - Potential immunogenicity and toxicity
References	[6,14,15,19–25,49]	[27–38]	[34,35,39–46]

weight (as a surrogate for molecular size) of an injected protein and the proportion of the dose absorbed into the peripheral lymphatics draining the SC injection site in sheep (Fig. 4). This correlation reinforces the importance of the lymphatics in the absorption of proteins of increasing molecular size. Importantly, for large proteins in the molecular weight range of approximately 30–40 kDa, almost complete absorption via the peripheral lymphatics was observed in sheep. Furthermore, molecular size did not limit the extent of lymphatic absorption up to at least 84 kDa (Fig. 4)

although it is probable that there is a maximum size range above which reduced absorption from the injection site might occur.

The importance of apparent molecular size in dictating the rate of absorption following SC injection is illustrated by the effect of self-association on the rate of absorption of insulin. Using radiolabelled tracer techniques, a strong correlation has been demonstrated between the average insulin dissociation state and the rate of disappearance of radiolabelled insulin from the SC injection site [23]. This relationship



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Figure 4. Relationship between the proportion of the dose absorbed into peripheral lymph (mean \pm SEM, $n = 3-5$) and the molecular weight for selected proteins and low molecular weight compounds after SC administration in sheep. Data for fluorodeoxyuridine (\circ), inulin (\triangle), cytochrome c (\square) and interferon- α (∇) from Supersaxo *et al.* [20], human growth hormone (\diamond) [21], soluble insulin (\bullet) [22], r-metHu-Leptin (\blacktriangle) [14], an analogue of Leptin (\blacksquare) [49], epoietin alfa (\blacktriangledown) [15], darbepoetin alfa (\blacklozenge) [49] and a high molecular weight protein (\bullet) [49].

has formed the basis for research on insulin analogues with reduced tendencies to self-associate with the aim of providing a more rapid onset of action (e.g. Lispro [24] and Aspart insulin [25]). The conventional view has been that dissociation of insulin hexamers and dimers to monomeric protein is required before absorption by the blood capillaries and that absorption via the lymph does not occur to any significant extent [26]. However, recent studies conducted using a cannulated sheep model indicated first, that insulin was partially absorbed via the lymphatics following SC injection, and second, that the extent of lymphatic absorption exceeded what would be expected for a molecule the size of monomeric insulin [22]. These results raised the possibility that associated forms of insulin (and indeed other proteins) could potentially be absorbed directly via the lymphatic capillaries before dissociation.

Modified release formulations for SC administration

An increasing body of work has explored the utility of modified or sustained release formulations to extend the timescale of drug absorption from SC injection sites thus providing prolonged exposure and a reduction in the maximum plasma concentration (Table 1). SC depots and implantable delivery systems control the rate of drug release whereas the mechanism of absorption of released drug via either the blood or the lymph is expected to be dictated by the characteristics (e.g. size) of the delivered agent as highlighted in the previous section.

Suspensions represent the simplest method for creating a SC depot and rely on slow dissolution of active drug at the injection site. This approach has been utilised extensively for intermediate- and long-acting insulin preparations (e.g. NPH, Lente and Ultralente insulin) and has recently been applied to a long acting formulation for medroxyprogesterone acetate (Depo-Subq Provera 104TM). Depot liquid and microsphere formulations, containing biodegradable polymers such as poly(lactide-co-glycolic acid) (PLGA), poly(lactic acid) (PLA) or collagen, have also been utilised to provide extended release up to approximately one month [27]. Liquid depot formulations typically form gels or solids upon injection thus providing an *in situ* depot for extended release [28–30]. Microspheres prepared with biodegradable polymers [31–33] have been extensively explored for the controlled release of several agents including proteins, peptides, antigens and low molecular weight drugs and one depot formulation for human growth hormone is now commercially available (Nutropin Depot[®]). Other prolonged release delivery systems that can be administered either by SC or IV injection, such as liposomes [34–36] and nanoparticles [37], often rely on an extended circulation time for the delivery system rather than delayed release of active at the injection site.

Implantable systems including biodegradable matrices, which are injected via a large bore needle or removable devices that are surgically implanted, can provide a long duration of exposure ranging from weeks up to a year [27]. Biodegradable implants using PLGA have been the subject of considerable research, with one of the best-known implantable formulations being a commercially available preparation of goserelin acetate (Zoladex[®]). Viadur[®] is a commercial example of an osmotically driven implantable device for chronic administration of leuprolide acetate which provides reliable and consistent drug release over prolonged time periods [38].

SC delivery approaches to target the lymphatics

Specific access to the lymphatics after SC administration has potential utility in the treatment of lymph and lymph node-resident diseases such as infection and tumour metastases, and also in improved lymphatic visualisation with attendant benefits in disease detection, diagnosis and treatment. A large number of formulation approaches and delivery systems have been investigated as potential strategies to target therapeutics to the lymphatics following SC administration (Table 1). In general, these approaches utilise the differential anatomy (and more specifically permeability) of lymphatic versus blood capillaries to promote selective lymphatic access.

As previously described, molecules of increasing molecular size preferentially drain from SC injection sites into the lymphatics. Macromolecular prodrugs have therefore been used to promote selective delivery to the regional lymphatics after SC administration and in general, larger cationic com-

plexes appear to be retained in regional lymph nodes whereas smaller anionic materials appear to flow more readily through the nodes and into larger lymphatic vessels (reviewed in [39]). Radiolabelled synthetic macromolecules (such as $^{99}\text{Tc}^{\text{m}}$ -dextran) and monoclonal antibodies have also been extensively used to image the regional lymphatics (see, e.g. [40–42]).

Realisation that increased molecular size results in a larger proportion of an SC dose accessing the lymphatics has led to the investigation of the lymph-directing or lymphotropic properties of a large range of colloidal and micro- and nano-particulate systems, immune stimulating complexes (ISCOMs), dendrimers and liposomes (for reviews, see [5,7,39,40,43]). For this approach to work effectively, particles must be sufficiently large to preclude absorption across the vasculature, but sufficiently small that relatively facile absorption from the injection site is possible. Clearly this depends on the interfacial properties of the delivery system, but in general, an optimal particle size range of approximately 50 nm has been suggested [4,40]. Technological difficulties associated with the manufacture of very small particles has limited close examination of particles in the 1–10 nm size range; however, recent studies using polymeric dendrimers suggest that lymphatic transport of materials of this size is possible [44].

A relatively recent advance in the field of nanoparticle and liposome engineering has been the development of pegylated nanoparticulate systems, where adsorption or grafting of polyethylene oxide chains to the surface of a microparticulate reduces recognition by prospective opsonins, reduces uptake by the cells of the reticuloendothelial system and promotes retention in the circulation after intravenous administration [35]. Far fewer studies have examined the effect of pegylation on particle uptake into the lymph; however, enhanced drainage from the SC injection site and the ability to tailor retention (or not) in the regional lymph nodes has been described [5,7,34].

An interesting combination of molecular and colloidal approaches has been described by Supersaxo *et al.* [45], who utilised the physical size of phospholipid-bile salt micelles to promote lymphatic uptake, and in parallel synthesised a lipophilic prodrug of the anticancer compound 5-FuR to facilitate improved micellar solubilisation. This combination of approaches led to the uptake of approximately 60% of an SC-injected dose into the lymphatics and can provide an additional generic mechanism for improved access of small molecules to the lymphatics.

Conclusions

Subcutaneous delivery is a commonly utilised administration route, particularly for molecules where low bioavailability precludes oral administration and where prolonged release might be desirable. Although the factors that govern the rate

and extent of SC absorption are incompletely defined, the recent trends observed for protein drugs and particulates in animal models suggest that size is a crucial determinant of the relative roles of the blood and lymphatic absorptive pathways. While increasing the molecular size appears to enhance access to the lymph, it should be acknowledged that increasing the size is likely to eventually retard movement through the interstitial space and thus restrict lymphatic drainage. This concept is well illustrated by the lack of facile lymphatic access for larger colloidal materials (>100 nm).

Realisation that the lymphatics can play a significant or indeed primary role in the absorption of molecules of increasing molecular size has considerable therapeutic and toxicological ramifications. Indeed, the relatively small volume of fluid in the lymphatic system and apparent lack of distribution of materials out of the lymphatics on transport to the systemic circulation dictates that for drugs where lymphatic transport is significant, concentrations in the central lymph are typically one to two orders of magnitude higher than the corresponding plasma concentrations. This obviously provides exciting opportunities for the improved targeting of the lymphatics for the treatment of lymph resident diseases but also introduces complexity into the design of pharmacokinetic and toxicokinetic studies, where variation in the extent of lymphatic transport can lead to significant differences in lymphatic and systemic exposure. A further, potentially complicating factor is that variations in subcutaneous blood flow and lymphatic drainage rates throughout the body can lead to regional differences in absorption rates and corresponding differences in the relative contributions of the vascular and lymphatic absorption pathways. To further understand these processes and to provide a means for assessing lymphatic targeting of therapeutic agents, there is a clear need to examine issues of interspecies scaling and the predictability of absorption patterns in humans given that variations in lymphatic architecture can lead to differences in lymphatic transport across animal models and humans.

It is apparent that molecules, analogues or prodrugs of increasing molecular size, will increasingly access the lymphatics after interstitial injection and that even 'small' molecules can be directed to the lymph by formulation in appropriate colloidal carriers. While the benefit of this approach is evident in several diagnostic and imaging products, application of these lymph-targeting approaches to a commercial therapeutic agent has not yet been forthcoming. To this end, a recent discussion article has reiterated the challenges associated with colloidal drug delivery and in particular, the contradictory requirements for appropriate encapsulation (e.g. to facilitate efficient lymphatic targeting) and facile drug release [46]. Nonetheless, examples of successful second-generation colloidal drug delivery systems such as Doxil[®] are evident and may eventually be applied to enhance local lymphatic uptake into the regional lymphatics.

Related articles

Supersaxo, A. *et al.* (1990) Effect of molecular weight on the lymphatic absorption of water-soluble compounds following subcutaneous administration. *Pharm. Res.* 7, 167–169

Zuidema, J. *et al.* (1994) Release and absorption rates of intramuscularly and subcutaneously injected pharmaceuticals (II). *Int. J. Pharm.* 105, 189–207

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A more complete understanding of the molecular and formulation-related determinants of absorption from SC injection sites and the physiological factors which dictate SC absorption profiles has the capacity to improve the design of SC delivery systems, inform the development of pharmacokinetic–pharmacodynamic models and potentially provide for enhanced access to the local lymphatic system.

Outstanding issues

- The influence of regional variations in lymphatic and vascular architecture on SC absorption processes.
- Interspecies variations in lymphatic versus vascular absorption from SC absorption sites and the ability to extrapolate animal data to humans.
- Further evaluation of formulation technologies (e.g. implants, nano- and micro-particles, colloidal and liquid crystalline systems) designed to provide sustained or pulsatile release from SC injection sites and/or targeting to the lymphatics.
- Further assessment of the therapeutic benefit of lymphatic targeting for the treatment of lymph resident diseases.

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