Strengths, weaknesses, opportunities and challenges for long acting injectable therapies: Insights for applications in HIV therapy

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Advances in solid drug nanoparticle technologies have resulted in a number of long-acting (LA) formulations with the potential for once monthly or longer administration. Such formulations offer great utility for chronic diseases, particularly when a lack of medication compliance may be detrimental to treatment response. Two such formulations are in clinical development for HIV but the concept of LA delivery has its origins in indications such as schizophrenia and contraception. Many terms have been utilised to describe the LA approach and standardisation would be beneficial. Ultimately, definitions will depend upon specific indications and routes of delivery, but for HIV we propose benchmarks that reflect perceived clinical benefits and available data on patient attitudes. Specifically, we propose dosing intervals of $\geq 1$ week, $\geq 1$ month or $\geq 6$ months, for oral, injectable or implantable strategies, respectively. This review focuses upon the critical importance of potency in achieving the LA outcome for injectable formulations and explores established and emerging technologies that have been employed across indications. Key technological challenges such as the need for consistency and ease of administration for drug combinations, are also discussed. Finally, the review explores the gaps in knowledge regarding the pharmacology of drug release from particulate-based LA injectable suspensions. A number of hypotheses are discussed based upon available data relating to local drug metabolism, active transport systems, the lymphatics, macrophages and patient-specific factors. Greater knowledge of the mechanisms that underpin drug release and protracted exposure will help facilitate further development of this strategy to achieve the promising clinical benefits.

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1. Introduction

The HIV/AIDS epidemic remains a major public health threat and approximately 36.9 million [34.3 million–41.4 million] people worldwide are estimated to be infected. In 2014, AIDS claimed an estimated 1.2 million [980,000–1.6 million] lives globally, with 2 million [1.9 million–2.2 million] people being newly infected in the same year. Worldwide, around 15.8 million people were accessing antiretroviral therapy in June 2015, constituting ~41% of adults and ~32% of children infected around 15.8 million people being newly infected in the same year. Worldwide, 41.4 million people were accessing antiretroviral therapy in June 2015, constituting ~41% of adults and ~32% of children infected with the virus [1]. Antiretroviral therapy (ART) currently involves co-administration of drugs to simultaneously inhibit multiple viral targets, maximising inhibition of viral replication whilst minimising drug resistance. To date, 6 classes of antiretroviral drugs are available: nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (Pis), fusion inhibitors, CCR5 antagonists and integrase inhibitors (INIs). Although ART has led to a decline in mortality and morbidity, therapeutic failure and pharmacokinetic variability to drug exposure. Currently available antiretroviral agents, such as viral characteristics, immunological status, and pharmacokinetic variability to drug exposure. Currently available formulations necessitate lifelong, daily dosing and suboptimal adherence places patients at risk of treatment failure and low rates of protection for PrEP [3].

Recently, two antiretroviral drugs have entered clinical development as long-acting (LA) injectable depot formulations. The first of these, developed by Janssen is rilpivirine LA (Edurant®) [4–6] and the second, developed by ViV Healthcare is cabotegravir LA [7,8]. Both of these medicines are based upon the same nanotechnology that generates solid drug nanoparticle (SDN) suspensions via the process of wet bead milling (also known as nanomilling; see also Section 4.1 below). LA injectable formulations have previously been developed and licenced for other indications such as contraception and schizophrenia (Table 1) [9–11]. The advent of the HIV LA medicines has been greeted with great excitement within the scientific, clinical and patient communities. In the short term, since only single agent LA medicines will be available, the largest impact is likely to be made by their deployment in PrEP [12]. However, it is hoped that the arrival of these medicines will spur further development of fully LA regimens for the treatment of infection. Several factors contribute to heterogeneity in the response to antiretroviral agents, such as viral characteristics, immunological status, and pharmacokinetic variability to drug exposure. Currently available formulations necessitate lifelong, daily dosing and suboptimal adherence places patients at risk of treatment failure and low rates of protection for PrEP [3].

Table 1

Comparison of selected clinically-available long-acting injections and candidate injections under clinical development.

<table>
<thead>
<tr>
<th>Technology</th>
<th>Drug name</th>
<th>Route</th>
<th>Dosing interval</th>
<th>Condition</th>
<th>Clinical depot volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspension-based</td>
<td>Medroxyprogesterone acetate</td>
<td>SC</td>
<td>3 monthly</td>
<td>Hormone therapy</td>
<td>0.65 mL</td>
</tr>
<tr>
<td>Solid drug particle</td>
<td>Mesorocynprogestrone acetate</td>
<td>IM</td>
<td>3 monthly</td>
<td>Hormone therapy</td>
<td>1 mL</td>
</tr>
<tr>
<td>Solid drug particle</td>
<td>Olanzapine</td>
<td>SC</td>
<td>3 monthly</td>
<td>Schizophrenia</td>
<td>Max. 2.7 mL</td>
</tr>
<tr>
<td>Solid drug particle</td>
<td>Paliperidone palmilate</td>
<td>SC</td>
<td>3 monthly</td>
<td>Schizophrenia</td>
<td>Max. 2.7 mL</td>
</tr>
<tr>
<td>Solid drug particle</td>
<td>Paliperidone palmitate</td>
<td>SC</td>
<td>3 monthly</td>
<td>Schizophrenia</td>
<td>Max. 2.7 mL</td>
</tr>
<tr>
<td>Solid drug particle</td>
<td>Somatropin</td>
<td>IM</td>
<td>3 monthly</td>
<td>Hormone therapy</td>
<td>Max. 1.5 mL</td>
</tr>
<tr>
<td>Solid drug particle</td>
<td>Leuprolide acetate</td>
<td>IM</td>
<td>3 monthly</td>
<td>Hormone therapy</td>
<td>Max. 1.5 mL</td>
</tr>
<tr>
<td>Solid drug particle</td>
<td>Cabotegravir</td>
<td>IM</td>
<td>1 quarterly</td>
<td>HIV therapy and PrEP</td>
<td>2 × 2 mL split*</td>
</tr>
<tr>
<td>Solid drug particle</td>
<td>Rilpivirine</td>
<td>IM</td>
<td>1 monthly</td>
<td>HIV therapy and PrEP</td>
<td>2 × 2 mL split*</td>
</tr>
<tr>
<td>Solution-based</td>
<td>Flupenthixol decanoate</td>
<td>IM</td>
<td>2–4 weekly</td>
<td>Schizophrenia</td>
<td>Max. 2 mL</td>
</tr>
<tr>
<td>Oil-based</td>
<td>Testosterone cypionate</td>
<td>IM</td>
<td>2–4 weekly</td>
<td>Schizophrenia</td>
<td>Max. 3 mL</td>
</tr>
<tr>
<td>Oil-based</td>
<td>Estradiol valerate</td>
<td>IM</td>
<td>1 monthly</td>
<td>Hormone therapy</td>
<td>Max. 1.5 mL</td>
</tr>
<tr>
<td>In-situ implant</td>
<td>Leuprolide acetate</td>
<td>SC</td>
<td>1–6 monthly</td>
<td>Prostate cancer</td>
<td>0.375 mL</td>
</tr>
<tr>
<td>Early stage solution-based immunotherapies</td>
<td>CCR5 Monoclonal Antibody (PRO-140)</td>
<td>SC</td>
<td>1–2 weeks</td>
<td>HIV</td>
<td>2 × 1 mL split*</td>
</tr>
<tr>
<td>Aqueous concentrated protein (undergoing human trials)*</td>
<td>Broadly neutralising monoclonal antibody (VRC01)</td>
<td>SC</td>
<td>3–4 weekly</td>
<td>HIV</td>
<td>TBD</td>
</tr>
<tr>
<td>Aqueous concentrated protein (undergoing human trials)*</td>
<td>Broadly neutralising monoclonal antibody (VRC01)</td>
<td>IV</td>
<td>3–4 weekly</td>
<td>HIV</td>
<td>NA^b</td>
</tr>
<tr>
<td>Aqueous concentrated protein (undergoing human trials)*</td>
<td>Anti-CD4 binding site monoclonal Antibody (3BNC117)</td>
<td>IV</td>
<td>1 monthly</td>
<td>HIV</td>
<td>NA^b</td>
</tr>
</tbody>
</table>

Note that since these formulations are currently still in clinical development, dosing interval and volume should be considered subject to change.

Intravenous infusions have shown long-acting benefits but are not considered depot injections.
of HIV. There are a number of clinical challenges associated with the use of LA injectables, which have already been recently reviewed [13]. These challenges include the management of adverse drug reactions without the ability to easily stop therapy, concerns regarding the potential for injection site reactions (which may be exacerbated by the currently large injection volumes required), and the potential for emergence of viral resistance during protracted periods of sub-therapeutic exposure after therapy discontinuation.

A major current obstacle to effective roll-out of the LA strategy for HIV therapy is the existence of only two antiretroviral drugs in this format. However, despite the fact that an NNRTI and INI combination has not previously been routinely utilised as a dual therapy oral regimen, the combination of rilpivirine and cabotegravir is currently being intensively studied. Indeed, the LATTE study, which investigated once-daily rilpivirine and cabotegravir as a maintenance therapy in virologically-suppressed adults demonstrated similar efficacy to a conventional oral regimen of efavirenz with two NRTIs at 96 weeks [14]. The very recent announcement that at week 32 in the LATTE 2 study the dual LA combination showed comparable efficacy to oral cabotegravir with dual NRTIs is extremely encouraging, although the data are yet to be published [15].

The range of chronic diseases that are being routinely managed, and the growth in preventative treatments coupled to a continual patient demand for interventions that do not restrict quality of life, has led to a clear clinical need for LA medicines across diseases [16]. This is particularly evident within an increasingly active ageing population. The market for injectable drug delivery devices, including self-injection, is predicted to grow from approximately £8 billion in 2013 to approximately £12 billion in 2018. This is within the context of an estimated total injectable drug market rising to approximately £380 billion by 2020 [17]. LA injections offer increases in patient adherence to therapy, reduction in clinical intervention and improvement in lifestyle.

The purpose of this review is to provide a context for further LA development of antiretroviral drug regimens. Current technologies that have been applied to LA delivery across diseases and indications will be reviewed along with technological challenges that may be important in the context of future development. Special consideration is also given to perceived gaps in knowledge relating to the pharmacology of drug release from an LA depot.

2. The conceptual basis for long-acting drug delivery of antiretroviral drugs

2.1. The key need to consider potency for antiretroviral long-acting medicines

It is tempting to assume that protracted drug release to achieve sustained plasma exposure to the active pharmaceutical ingredient (API) is the predominant consideration in development of an LA drug delivery strategy. However, it should be noted that the plasma and intracellular concentrations required to achieve sustained viral suppression are key considerations for the approach. Indeed, while pharmacokinetic exposure is a prerequisite for success, ultimately it is the potency of the API that drives the concentrations that need to be achieved and for how long. Conceptually, this is described in Fig. 1. Ultimately, the amount of drug that is required is dependent upon the potency of the API and while the pharmacokinetic exposure for a drug with high potency may be sufficient to reduce the frequency of dosing, it may not be sufficient for a drug with lower potency. This is important because the technological complexity of achieving a set plasma concentration for a specific duration may or may not be equal for APIs, irrespective of the potency.

The overwhelming majority of currently available LA injectable formulations available in the clinic were developed for drugs that were already available as oral formulations. Over the past 3 decades the understanding of critical physiochemical and molecular features that are important in the context of drug development for orally delivered drugs has proliferated. This understanding has culminated in the biophysical classification system (BCS) and design rules for optimal bioavailability such as Lipinski’s rule of five and its various iterations [18,19]. Numerous nanotechnological approaches are being explored to augment oral bioavailability and the mechanisms that determine API bioavailability for such advanced formulations may differ from conventional small molecules [20]. Importantly however, the physicochemical and molecular mechanisms that determine drug release for LA injectable formulations are not as well understood (see Section 6 below). However, it is likely that the physicochemical properties of an optimal LA injectable drug are different from that of an optimal oral drug and a classification system may warrant development in this area as was recently suggested for topical drugs [21].

Putative differences in optimal physiochemistry are exemplified by the case of paliperidone palmitate. Prodrug strategies have been extensively used by pharmaceutical scientists for decades as an approach to improve aqueous solubility and thereby augment oral bioavailability [22]. However, paliperidone was first approved by the FDA on December 19th 2006 as a once daily oral treatment of schizophrenia and later, on July 31st 2009, paliperidone palmitate was approved as an LA injectable for the acute and maintenance treatment of schizophrenia in adults. Paliperidone was already poorly soluble in water (48.6 μg/mL) with the requirement for a sophisticated oral delivery technology. However, the ‘prodrugging’ of paliperidone with the palmitate moiety further reduced aqueous solubility, resulting in slow intramuscular dissolution and therefore enabling the LA approach [23].

Other examples of the use of a pro-drug to decrease solubility and improve compatibility with the LA approach also exist as for olanzapine pamoate [24]. Often during the drug development process there is a need to compromise potency as medicinal chemists utilise design rules to improve oral bioavailability potential. Importantly therefore, if LA injectable approaches are explored at the outset of development, rather than as a strategy for already licenced oral drugs, there is at least the potential to develop medicines with higher potency that require a lower pharmacokinetic exposure, thereby enabling increased duration of activity while reducing the volume of the depot required. However, the need to start patients on an oral regimen prior to initiating LA regimens (i.e. an “oral lead-in”) to mitigate putative occurrence of adverse drug reactions has been heavily debated in recent years and if the physicochemical properties really do differ markedly then this may pose a challenge for agents explicitly developed for LA.

2.2. A need for standardised nomenclature for long-acting delivery?

There are a number of terms that are commonly used to describe formulations designed to deliver exposure to therapeutic concentrations of APIs over a protracted period of time. Such terms include time-release, sustained-release, prolonged-release, extended release, controlled release and long-acting delivery to name but a few, and this nomenclature is often used interchangeably within the scientific and commercial literature. This transposable use of terminology complicates the ability to rapidly identify and utilise relevant literature and the authors would like to advocate for a standardised terminology for this particular therapeutic strategy. Therefore, for the purposes of this review, the term long-acting has been applied because it is currently the most widely applied terminology across different routes of delivery (Fig. 2), and because the emphasis should be placed upon the duration of therapeutic exposure, which depends upon clearance and potency of the molecule in addition to the “release”.

Irrespective of the specific terminology applied, it is important to recognise that none of the existing terminology specifies the actual duration of therapeutic exposure. Rather, the terms are applied to exemplify that a longer duration of exposure, or a less frequent need for dosing is achievable relative to pre-existent or conventional formulations of the same drug. However, as new technologies emerge and the
strategy gains traction, it should be noted that the development of LA formulations may be preferred to their conventional counterparts and thus the LA formulation itself is likely to set the precedent. Importantly, the desired duration is dictated by the specific application for which the formulation is being developed, and is then tempered by what is achievable in terms of the physicochemistry and pharmacology of the API, and the favoured route of administration. This is exemplified by the emergence of an insulin formulation (insulin glargine) for once daily administration that was termed LA because of the inability of its predecessors to achieve daily administration [25]. Since the term LA was expended on insulin glargine there are now reports of an ‘ultra-LA’ insulin formulation (insulin Degludec) that describe medicines with a longer duration of action [26]. Clearly, this poses the question of what an insulin medicine may be termed if ever the challenge of delivering basal insulin for an even longer duration were ever to be surmounted.

**Fig. 1.** Diagrammatic representation of the relationship between pharmacokinetics and potency for theoretical long-acting formulations. It can be seen that a prolonged higher pharmacokinetic exposure, with the associated technological challenges, will be required to achieve a long-acting strategy for an API with lower potency compared with an API with higher potency. Ultimately, potency determines how much drug is required and for how long in order to achieve long-acting delivery.

**Fig. 2.** Results of a Google search with combinations of different nomenclature used to describe long-acting delivery with different routes of delivery, illustrating the interchangeable use of currently applied terminology.
2.3. The importance of route of delivery in achieving the long-acting strategy

In the context of HIV therapy and prophylaxis, robust regimens are now already available for delivering therapeutic concentrations of multiple drugs from once-daily oral fixed dose combinations. Therefore, a prerequisite for LA delivery in a HIV context must be considered to enable less frequent dosing than once daily. There are extremely compelling clinical and patient-specific factors that are driving the enthusiasm for LA medicines in HIV. In the context of PreP and HIV therapy, adherence to medication is a major factor that influences the ultimate clinical outcome, and this has been extensively reviewed in the context of LA injectable products previously [27,28]. Adherence to medication is a major driver of LA injectable development and is in some cases heavily influenced by ‘pill fatigue’. Indeed, despite historical public caution about “nanotechnology”, a recent survey of HIV-positive patients in the USA demonstrated that 61%, 72% and 84% of patients would “definitely or probably try injectable nanoformulated antiretroviral therapy” for weekly, two-weekly or monthly dosing, respectively [29].

While oral drug development in HIV focused for many years on achieving fixed dose combinations available for once-daily dosing of drug regimens, it is difficult to see the utility of any formulation that can deliver drug for greater than a day but less than a week. This is predominantly because of the additional complexity that would be associated with remembering to take the medication in a timely and consistent fashion. Therefore, to have a truly beneficial impact for an oral regimen it is likely that a formulation would need to deliver a minimum of a weekly therapeutic exposure. However, such a formulation would also likely require some forgiveness for administration in order to circumvent issues if a medication were not administered at the same time on the same day each week. Tools such as mobile phone applications or automated calls could be brought to bear to deliver patient reminders for taking a weekly medicine. Current knowledge of the plasma half-life of existing PIs, NNRTIs, entry inhibitors and INIs make it difficult to see how a weekly administration format can be achieved without further API development. Notwithstanding, it should be noted that ultimate performance of antiretroviral therapies are thought to be highly dependent on intracellular drug concentrations in HIV target cells [30,31], and the active metabolites of some NRTIs are known to have long intracellular half-lives [32,33]. However, given the enthusiasm for the LA approach in adolescence, it is worthy of consideration that at least some of the NRTI metabolites have been reported to be almost 2-fold lower in patients under the age of 25 [34].

In the context of implants or devices for drug delivery, the invasive- ness and associated need for trained healthcare staff to conduct the implantation procedure is likely to mean that a longer duration between administrations will be required to realise this approach. Implants have proven successful in other indications such as contraception [35]. However, it should be noted that the challenge of drug combinations, which are a prerequisite for HIV therapy has not yet been met for implants. Moreover, the plasma concentrations required to mediate the desired effect are lower than those required to suppress HIV replication with currently available antiretroviral drugs. Indeed, peak plasma concentrations of etonogestrel following implantation of IMPLANON® were 813 pg/mL [36], which is lower than those required for adequate viral suppression with antiretroviral drugs, and an implant for HIV therapy would also be required to deliver at least two drugs simultaneously as a minimum. For context, the IC_{50} concentrations for dolutegravir, rilpivirine and raltegravir are 64 ng/mL (protein binding-adjusted [37]), 20.3 ng/mL (protein binding-adjusted [38]) and 15 ng/mL [39], respectively. Therefore, while conceptually the ability to deliver an efficacious drug combination for >6 months via an implant is extremely appealing, it seems unlikely to be realised in the short- to medium-term without significant advances in current device technologies and/or potency of available antiretroviral drugs. The authors direct the reader to a recent thorough review of implantable drug delivery devices [40].

Taken collectively the authors propose the following table to describe the use of LA terminology in HIV that takes into account routes of administration, patient attitudes (where available) and current clinical paradigms (Table 2).

While it is important to consider developments in oral and implantable LA strategies, the remainder of this review will focus on LA injectable medicines.

3. Technologies that have been clinically successful for LA delivery

The manufacture of products for LA delivery has been achieved using a variety of differing technologies and a range of new strategies are being investigated. In general, currently marketed products utilise one of two broad formulation approaches (Fig. 3); injection of solutions or particle suspensions [41]. Administration predominantly targets intramuscular routes despite subcutaneous injection offering the use of shorter needles and less patient discomfort. This is due to the volume of injected material of up to 5 mL in adults and 2 mL in children that can be tolerated [42], allowing a larger dose of drug to be delivered to the depot site. The current most successful LA delivery technology utilises an oil-based vehicle containing dissolved lipophilic drug; the two remaining leading formulation strategies utilise dispersed solids within a liquid matrix (Table 1).

3.1. Solution-based injections

Despite speculation concerning the actual date of the introduction of intramuscular injections [42], the early to mid-1900s saw the development of injectable solutions of drugs within the range of oil phases. The drug compounds were often palmitate or decanoate esters that facilitated dissolution [43], and oils such as castor oil, soybean oil, peanut oil, cottonseed oil, caprylic and capric triglycerides from coconut or palm seed oils, sesame oil and safflower oil have been regularly used since this time in approved intramuscular formulations [44]. Formulation additives often include small molecule solubilising agents and stabilisers such as antioxidants. These first generation LA formulations were typically developed for antipsychotic therapies [45] and relied upon the injected oil to generate a localised bolus which would slowly disperse and release drug; esterases are believed to chemically cleave the dissolved ingredients to generate the parent active compound [24]. In more recent cases, lipophilic drugs are utilised that do not require localised or plasma activation.

Solutions of lipophilic drugs within injectable oils are relatively simple to manufacture but several issues require optimisation to achieve the desired delivery profile. Release from intramuscular solutions generally allows for drug delivery over a matter of weeks and repeated injection is most commonly required each month [41]. The availability of a range of oils provides the opportunity to modify release kinetics whilst the generation of prodrugs with varying lipophilicity also adds to the available formulation variables [46]. Partition coefficients between the oil solution and the surrounding environment after injection have a direct influence on release kinetics and observed pharmacokinetics [47]. However, the injection site [48,49] and volume of injected liquid also play important roles [47]. Other non-covalent interactions may also be manipulated to enable tailored drug release, such as the variation in the degree of hydrogen bonding between drug and hydroxyl groups within castor oil through the formation of oil mixtures with varying castoric oil content [50].

<table>
<thead>
<tr>
<th>Table 2</th>
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</thead>
<tbody>
<tr>
<td>Proposed timescales to be deemed long-acting for HIV segmented by administration route.</td>
</tr>
<tr>
<td>Route of delivery</td>
</tr>
<tr>
<td>Dosing frequency</td>
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</tbody>
</table>
or copolymer functionality and polymer-drug interactions, and physical properties such as molecular weight, dispersity, microsphere porosity and diameter, degree of crystallinity, glass transition temperature, hydrophilicity and drug distribution throughout the microsphere [56–58]. Despite these variables, initial burst release profiles are often seen from microsphere injections [59] with further identifiable stages of slow and sustained release and a later stage acceleration as the microsphere degrades [60,61].

Biodegradation of the microsphere is important to allow for clearance of the polymeric matrix material and polyesters dominate the choice of material [62]. Various microsphere morphologies have been employed to optimise drug release including hollow polymer particles and solid matrices to either encapsulate the drug or evenly distribute the API throughout the spherical particle [63]. The first microsphere-containing formulation was approved by the FDA in 1989 for palliative treatment of prostate cancer patients [41].

4. Emerging technologies for LA formulation

The use of solution and suspension-based injections has been successful in introducing many clinical options for the treatment of a relatively narrow range of indications including prostate cancer, hormone moderation and schizophrenia. A number of emerging technologies for LA and injectable therapeutics are either on the brink of translation to clinical adoption or maturing through the stages of commercial development; four of these are detailed below.

4.1. Nanoparticle suspensions

Suspension-based intramuscular injections have typically utilised particles in the <10 μm diameter range. However, the development of mechanical attrition processes [64] (for example nanomilling technologies and high pressure homogenisation) and non-attrition nanoparticle fabrication (for example emulsion-templated freeze drying) has allowed access to nano-scale solid drug particles (<1 μm) [65–68]. The rilpivirine LA and cabotegravir LA options [6,7] that are in extended clinical trials (including evaluation of simultaneous combination in LATTE 2) are based upon this technology.

4.2. Injectable monoliths

Drug eluting implants have been used to provide LA delivery of various therapies for several years. LA contraception has particularly benefitted from the use of sub-dermal injectable implants, providing a ‘forgettable’ solution for several years [69]. Insertion requires trained administration and removal requires a surgical incision, often leading to scarring. In recent years, the formation of biodegradable injectable monoliths has been the subject of considerable interest for the LA delivery of biological drugs [70].

4.3. In situ-forming depots/implants

The limitations of injectable monolithic implants are being addressed using liquid formulations that solidify to form drug depots after injection [71]. These options are known as in situ-forming depot injections and are designed to degrade during use and, therefore, mitigate the need for surgical removal. Injections can be intramuscular or subcutaneous and several solidification mechanisms have been employed, including in situ precipitation, chemical crosslinking and solidification of molten liquids or assembly of liquid crystal [72]. A precipitation mechanism, driven by dispersion of a co-solvent, was employed in the first FDA-approved in situ-forming implant system in 2002, which is available in 1, 3, 4 and 6 month delivery options [73].
4.4. Microneedle delivery

To overcome the intrusive nature and patient acceptability of injections, the development of microneedle technologies has been highly active for nearly two decades [74]. Initially, inorganic and metallic needles up to 900 μm in length were developed and arrays of drug-coated microneedles have been arranged onto patches for transdermal delivery [75]. This format has been extended to include dissolvable/degradable microneedles that deliver drugs in an encapsulated form into the skin [76]. In some studies, the potential for delivery over timescales up to 2 months has been demonstrated [77]; in other cases, delivery of up to several days has been achieved [78,79]. However, as discussed above, these observations must be tempered against API potency and the resultant target concentrations that need to be achieved.

5. Technological challenges for LA delivery

Current LA technologies have been successful in addressing defined clinical needs within a window of indications. Key challenges in this regard relate to the ultimate manufacture of sterile medicines with maximum drug loading to keep the volume of administration as low as possible. A number of other therapy-specific, manufacturing and administration challenges exist in the extension of these approaches to a broader range of therapeutic needs and these are elaborated in this section of the review.

5.1. Drug combinations

The formulation and manufacturing approaches employed in LA development outlined above require optimisation towards ideal release for each drug compound. The incorporation of two or more drugs into fixed dose LA combinations, an approach that is critical to the success of HIV therapy, is a major challenge. The identification of single vehicles capable of dissolving multiple drugs for solution-based LA injections provides a number of hurdles, especially when attempting to overlay various partition coefficients to maintain the dose ratios over extended periods. Recent reports of bottom-up formation of combination SDNs suggests some potential for future LA formulations using nanosuspension approaches but the tailoring of release profiles is yet to be demonstrated [80,81]. Equally, the formation of microparticles containing drug combinations using solvent evaporation techniques requires a solvent phase able to generate appropriate drug combinations to match final therapeutic need whilst preventing unequal losses to the aqueous phase during evaporation. In addition, the triphasic release profile observed for microparticles, including the initial ‘burst’ and final increase, presents a significant hurdle to maintaining drug delivery ratios for mixed pharmaceutical payloads.

The range of alternative and emerging technologies also suffer from similar production and manufacturing concerns. For example, the precipitation of multiple drugs from an in-situ forming depot may lead to uneven distribution of drug compounds within the depot and variable or preferential release rates of individual therapy components.

5.2. “Syringeability”

The administration of any LA formulation necessitates the delivery of a dose of drug able to achieve target plasma concentrations for prolonged periods. For liquid formulations, including dispersions, high viscosities may be unavoidable which necessitate the use of wider bore needles than desired, unfavourably high volume or number of simultaneous injections. The generation of low viscosity approaches, new injection methodologies or excipients to enable reduced volumes may aid the application of LA formulations for lower potency drugs or high potency molecules with low ‘processability’.

5.3. Depot consistency, shape and size

Currently, the dimensions that an LA formulation adopts after injection may be highly variable and depend on factors such as the structure of the tissue within the injection site, the physiology of the recipient, the rate of injection and the technology format used. This impacts the drug release kinetics and generates considerable variability [82]. The use of suspension technologies can also have difficulties relating to stability of the drug particles including changes in crystal polymorph and aggregation within the suspension leading to variation in the intended particle size distribution [83].

5.4. Formulation sterility and manufacturing

Controlling the sterility of injectable pharmaceuticals is a constant challenge, although one that is overcome using a range of auditable manufacturing processes and equipment [84]. Innovation using injectable micro or nanoparticles and high concentration liquids may lead to the introduction of specialised processing needs with subsequent development of new quality controls. The recent trends towards the production of pre-filled sterile syringes for administration of biopharmaceutical products may offer opportunities to advanced LA products and minimise concerns during drug administration [85].

6. Gaps in knowledge regarding the pharmacology of solid drug nanoparticle LA injectables

As alluded to above (Section 2.1.) the key physiochemical properties and molecular mechanisms important in the context of achieved LA delivery have not been extensively discussed or explored within the scientific literature. As such, there exist unanswered questions in terms of the physiological, anatomical, and genetic factors that may influence the extent of bioavailability from a depot and the variability therein. There are also unanswered questions that relate to the impact of environmental factors on drug release. Following oral administration, a drug must survive the molecular processes in the intestinal epithelium, prior to passage via the hepatic portal vein and subsequent first-pass metabolism within the liver. Intuitively one might expect that the pharmacokinetics of a drug after intramuscular administration might be less variable than following oral administration, because these complex mechanisms that evolved to protect the body from exposure to potentially toxic dietary components are circumvented. However, for the majority of LA injectable formulations the pharmacokinetic variability appears to be as large, if not larger than the counterpart oral formulations (Table 3).

Irrespective of the route of administration for an API, it is assumed that once the molecule enters the systemic circulation the mechanisms that contribute to its distribution and clearance will remain the same. Therefore, it seems logical to conclude that the predominant reasons for the higher inter-patient variability in pharmacokinetic exposure following LA delivery are governed by the manner in which the drug is absorbed. A potential caveat to this is that there is currently a paucity

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<thead>
<tr>
<th>Drug</th>
<th>Oral PK variability (AUC CV%)</th>
<th>LA PK variability (AUC CV%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paliperidone</td>
<td>35.3</td>
<td>40%</td>
<td>[86,87]</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>26%</td>
<td>50%</td>
<td>[88,89]</td>
</tr>
<tr>
<td>Medroxyprogesterone</td>
<td>52%</td>
<td>34%</td>
<td>[90,91]</td>
</tr>
<tr>
<td>Rilpivirine</td>
<td>39%</td>
<td>52%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>[92,93]</td>
</tr>
<tr>
<td>Cabotegravir</td>
<td>27%</td>
<td>39%</td>
<td>[94]</td>
</tr>
</tbody>
</table>

<sup>a</sup> Note that the value given represents CV% at a dose of 300 mg and the same paper demonstrates a decrease in variability at higher doses falling to 32% at 600 mg and 24% at 1200 mg, which may indicate a saturable process involved in pharmacokinetic variability.
of information relating to whether intact particles enter the systemic circulation after administration as an LA injectable depot (discussed in more detail below). This is driven by the bioanalytical challenges associated with quantification of dissolved versus particulate drug within the blood — conventional methods of drug detection such as HPLC and LC–MS/MS do not discriminate because of the solvent extraction process necessary in sample preparation, which dissolves this type of nanoparticles. Recently, the authors validated a flow cytometry method for detection of intact nanoparticles in plasma [95] and developed SDNs based on fluorescence resonance energy transfer [80] that may have utility in understanding this fundamental question. Nonetheless, if the majority of the pharmacokinetic variability stems from drug release from the depot there are a number of putative depot-specific physiological, anatomical or environmental factors that may contribute and these are hypothesised in this section of the review.

6.1. A role for drug transporters and metabolic enzymes in LA bioavailability?

As discussed elsewhere in this issue of the journal [96], transporters and drug-metabolism enzymes expressed in the intestinal epithelium and hepatocytes, play a key role in modulating the bioavailability of drugs dosed via the oral route. ABCB1 (P-glycoprotein) remains the best-studied transporter in intestine and there is some evidence that it is variably expressed in skeletal muscle [87]. More robust evidence exists for expression of a number of other well known drug transporters of the ATP-binding cassette (ABC) family in skeletal muscle and these include ABCC1, ABCC4 and ABCC5 [98,99], all of which have been shown to have antiretroviral substrates [100–102]. This same manuscript demonstrated that other common drug transporters such as ABCG2 (MRP2) and ABCC2 (BCRP) were expressed at low levels in these cells. Transfection of skeletal muscle myoblasts with ABCW1 was demonstrated to protect against statin-related toxicity indicating a functional role for this transporter in skeletal muscle [98].

In liver, the organic anion transporting polypeptide isoforms 1B1 and 1B3 (OATP1B1 and OATP1B3 coded by SLC01B1 and SLC01B3) have been shown to play a key role in inter-patient variability in oral pharmacokinetics for many APIs including statins [103] and antiretroviral drugs [104]. A related drug influx transporter, OATP2B1 is also expressed in skeletal muscle and transfection of skeletal muscle myoblasts with OATP2B1 enhanced statin-related toxicity in these cells [98]. While these data are interesting it should be noted that they do not directly demonstrate a role for transporters in facilitating or mitigating drug permeation from muscle to blood. Ultimately, if drug transporters are important in regulating bioavailability from an LA injectable it seems likely that their expression in capillary endothelium, rather than muscle cells, would be a major mediator. However, while ABCC1 expression appears to vary between capillary endothelial cells from different tissues [105], the authors are not aware of any data that have explicitly investigated expression of transporters in relevant subcutaneous or skeletal muscle-derived capillary endothelium, let alone that derived from gluteal (or deltoit) muscle.

Similarly to transporters, biotransformation of APIs by drug metabolism enzymes in intestinal epithelium and liver is a key determinant of oral bioavailability and pharmacokinetic variability of antiretroviral and other drugs [106–108]. The cytochrome P450 (CYP) enzymes are widely recognised as the predominant family for catalysing phase I metabolism of drugs and the most abundant isoform in liver and intestine is CYP3A4. Other CYP isoforms that play a key role for antiretroviral drugs include CYP3A5, CYP2B6, CYP2C9, and CYP2C19 [109–111] but very little is known about expression of these in muscle or subcutaneous adipose tissue. Interestingly, CYP3A4 and CYP3A5 mRNA and protein expression have been explicitly demonstrated in childhood rhabdomyosarcoma and adjacent healthy tissue [112], as well as quadriceps skeletal muscle from children and adolescents [113]. Numerous relevant CYP isoforms have also been shown to be expressed in human subcutaneous adipose tissue explants, albeit at levels that are far lower than liver [114]. Phase II enzymes of the UDP-glucuronyl transferase (UGT) family also metabolise antiretroviral drugs [115,116] but their expression in skeletal muscle and adipose tissue is not well understood. Members of the nuclear receptor family of transcription factors that are known to regulate drug transporters and metabolism enzymes are expressed in skeletal muscle [117] and subcutaneous adipose tissue [118], which further highlights the potential metabolic function of this tissue. Moreover, if skeletal muscle (or adipose tissue) does contribute to metabolism of drugs, the presence of nuclear receptors such as PXR and CAR may mean drug–drug interactions at the depot site may warrant consideration if LA combinations are realised.

The capillary architecture and permeability varies from tissue to tissue and the endothelium may be continuous or discontinuous, and continuous endothelium may be fenestrated within tissues such as intestine, kidney or endocrine organs [119]. Muscle capillaries are predominantly continuous, meaning that they are the least permeable of capillaries with many tight junctions between the endothelial cells and fewer intercellular clefts for unrestricted small molecule passage than other types of capillary [120]. Interestingly, it has been estimated that the fractional area of the blood capillary wall that is composed of intercellular cleft and therefore exposed for free diffusion of small molecules and fluids is only 0.4% [121]. Moreover, the width of the intercellular cleft has been determined to vary but appears to be ~20 nm at the wider end [121], with a physiological upper limit pore size of 5 nm in skeletal muscle [122]. Therefore, this would be expected to restrict the direct access of LA intact nanoparticles (measurable in 100 s of nm) directly into the blood. As such, it seems reasonable to speculate that if transporters are expressed within the endothelium they may play an important role in governing the passage of drug from depot directly into blood, and variability therein. Collectively this means the study of the expression of transporters and drug metabolism enzymes specifically in gluteal muscles and their associated blood (or lymph — see below) capillary endothelium may be worthy of further investigation.

6.2. The putative role of the lymphatics in LA bioavailability?

In addition to the blood capillaries, skeletal muscle and subcutaneous tissue is also permeated by a network of lymphatic capillaries (Fig. 4) [123,124]. The lymphatic system is constructed of a web of interspersed vessels and nodes, and serves as a key organ of the immune system [125]. Indeed, early studies with liposomes showed the systemic circulation predominantly via the blood capillaries whereas structures between 10–100 nm are optimum for entry into lymphatic vessels [recently reviewed [126]]. In fact, early studies with liposomes showed the critical importance of size [129]. Importantly, particles above 100 nm are thought to be less able to permeate lymph [126]. However, it should be noted that although the SDN formulations such as rilpivirine LA and cabotegravir LA have particle diameters measured in 100 s of nanometers, they have a large polydispersity in comparison to other nanoparticle technologies meaning that at least some of the rilpivirine LA particles (for early development formulations) were between 10–100 nm in diameter [5]. Additionally,
as larger particles dissolve into molecules, they would also be expected to pass through this size range. Therefore, for this nanoformulation type uniquely, the authors speculate that a combination of mechanisms may contribute to delivery of the drugs to the systemic circulation and that the contribution of individual mechanisms may differ across the dosing interval (i.e. as particles dissolve). It should be noted that concentrations of ripivirine were reported to be 100 times higher than plasma in the local lymph node a month after intramuscular delivery of the LA formulation [4]. However, intact nanoparticles were not assessed presumably because of the bioanalytical challenges discussed above. The role of the lymphatic system in bioavailability of SDN formulations is not clear but it should be remembered that the flow rate of lymph is much slower than that of blood, which may be beneficial for LA if it is sufficient to provide a meaningful contribution to absorption.

It should be noted that the lymphatic system has been an area of interest for the delivery of anticancer nanomedicines in recent years [126, 130,131] and may be worthy of further specific development in the area of antiretroviral therapy, especially considering the role of the lymphatics in HIV pathogenesis [132], the presence of viral reservoirs within lymph nodes [133], and their identification as a key target for eradication strategies [134,135]. Importantly, subcutaneous delivery (rather than intramuscular delivery) has been at the forefront of gaining access to the lymphatics in cancer and was recently extensively reviewed [136]. It must be acknowledged that if intact nanoparticles do enter the systemic circulation then there may be other routes of elimination that require consideration beyond those for conventional small molecules, and biodistribution may also be influenced by particle-related mechanisms (recently reviewed [137,138]).

6.3. A role for macrophages in nanoparticle bioavailability?

Interesting recent work from the University of Nebraska Medical Centre demonstrated co-localisation of atazanavir SDNs with macrophages after intramuscular administration to mice [139]. Importantly, these SDNs were produced using techniques analogous to those used in the manufacture of ripivirine LA and cabotegravir LA. Similar studies have not been conducted with the antiretroviral clinical formulations available it will be necessary to determine whether such factors play an equal role in pharmacokinetic variability, or whether other as yet understudied mechanisms contribute or even predominate. As discussed above, although intuitive to expect variability to be lower for LA formulation due to avoidance of molecular processes within intestine, this is not born out by currently available data (Table 3). It is also tempting to speculate that the magnitude of some transporter- or enzyme-mediated drug–drug interactions or pharmacogenetic associations may be lower for the same reason. However, this will ultimately depend upon the contribution of the intestinal mechanisms relative to

The factors that influence the inter-patient variability in pharmacokinetic exposure to orally administered drugs have been well studied and are for the most part drug-specific, involving interrelated factors such as pharmacogenetics [148,149], body weight [150,151], gender [152,153], and drug–drug interactions [154]. As new LA agents become available it will be necessary to determine whether such factors play an equal role in pharmacokinetic variability, or whether other as yet understudied mechanisms contribute or even predominate. As discussed above, although intuitive to expect variability to be lower for LA formulation due to avoidance of molecular processes within intestine, this is not born out by currently available data (Table 3). It is also tempting to speculate that the magnitude of some transporter- or enzyme-mediated drug–drug interactions or pharmacogenetic associations may be lower for the same reason. However, this will ultimately depend upon the contribution of the intestinal mechanisms relative to
the role of the liver (or other tissues). In the case of HIV therapy this may be particularly pertinent to interactions with integrase inhibitors that are affected by pH and chelation to metal ions in gut [155,156].

Other factors for which there is a current paucity of information, relate to whether physical activity, ambient temperature, muscle density/body mass index, or accumulated scar tissue after prolonged therapy may influence depot drug release and therefore pharmacokinetic exposure after LA administration. Data from the 1980s indicated that there may be differences in pharmacokinetic exposure to insulin following exercise after subcutaneous administration, and marked differences in absorption were also noted when patients were at 30 °C relative to 10 °C ambient temperatures [157]. Although there are currently no data for the emerging HIV LA medicines, other studies have also shown an impact of exercise on pharmacokinetics following parenteral administration (reviewed previously [158,159]), and this may be worthy of further investigation. If exercise and ambient temperatures do influence drug release then it is important to consider that factors such as geographical location and occupation may need consideration. The manner in which a depot is administered may also influence the variability in exposure and it is of interest to note that even using image-guided intramuscular administration in a preclinical species, part of the dose ultimately resided subcutaneously [142].

7. Summary and conclusions

The emergence of antiretroviral LA medicines is academically exciting and early signs indicate a high desirability by the HIV patient community. However, while many technologies are discussed in the context of LA delivery it is imperative that the specific indication is considered when interpreting such data. It is important to consider that LA may refer to once daily dosing for one indication versus once monthly (or longer) for another. Therefore, the nomenclature for LA delivery would greatly benefit from standardisation to harmonise strategies in a disease-specific and technology-specific manner. For HIV, it is difficult to see an injectable medicine having real impact unless it can be administered with dose intervals of at least four weeks, preferably a quarter or even longer. There is a current paucity of knowledge regarding the mechanisms that determine ultimate drug delivery into the systemic circulation, which may be critical to future LA development and important for rationalising clinical strategies for their effective use. In addition, the challenges associated with pre-clinical evaluation of candidate LA formulations have also been reviewed recently [46,160] and the first physiologically-based pharmacokinetic (PBPK) modelling strategies are beginning to emerge [161]. Robust in vitro and in silico methods will help accelerate development while reducing the burden on animal experimentation, which is particularly difficult for LA formulations due to the timescales involved. This is extremely important in the context that a key next step will be to develop truly LA regimens including more than one drug. The recent data from the LATTE 2 study are extremely encouraging for a ripivirine and cabotegravir combination but a single intramuscular administration would be preferable and NRTIs have stood the test of time as backbone therapies. It is worth noting that if the technological challenges associated with LA formulation of an NRTI backbone can be surmounted, two LA regimens may become available shortly after. Importantly, LA regimens may be well suited to, if not dependent upon, directly-observed therapy, and therefore may be less prone to issues with compliance. The US National Institutes of Health have recently funded the Long-acting/extended-release antiretroviral resource programme (LEAP; www.longactinghiv.org), which aims to accelerate collaboration, development and translation in this area. LEAP constitutes an international network of key stakeholders from industry, academia, charitable organisations and the patient community, aimed at maintaining momentum in this truly exciting area through the provision of workshops, resources and an externally accessible PBPK modelling core.

Competing financial interests

The authors are co-inventors of patents relating to the application of nanotechnology to HIV drug delivery and are co-founders of a University of Liverpool start-up company, Tandem Nano Ltd. The authors have also received funding from Merck, Janssen, Viiv Healthcare, AstraZeneca, and Pfizer.

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