Optimising nanomedicine pharmacokinetics using PBPK modelling

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Summary

The delivery of therapeutic agents is characterised by numerous challenges including poor absorption, low penetration in target tissues and unspecific dissemination in organs, leading to toxicity or poor drug exposure. Several nanomedicine strategies have emerged as an advanced approach to enhance drug delivery and improve the treatment of several diseases. Numerous processes mediate the pharmacokinetics of nanoformulations, with the absorption, distribution, metabolism and elimination (ADME) being poorly understood and often differing substantially from traditional formulations..Understanding how nanoformulation composition and physicochemistry influences drug distribution in the human body is of central importance when developing future treatment strategies. A helpful pharmacological tool to simulate the distribution of nanoformulations is represented by physiologically based pharmacokinetics (PBPK) modelling, which integrates system data describing a population of interest with *in vitro* nanoformulation data through a mathematical description of ADME. The integration of property–distribution relationships in PBPK models may benefit nanomedicine research, giving opportunities for innovative development of nanotechnologies. This approach will not only improve our understanding of the mechanisms underpinning nanoformulation disposition and allow for more rapid and accurate determination of their kinetics, but will also help clarify interactions between different nanoformulation properties, identifying antagonistic or synergistic effects. Consequently, the design and development of nanoformulations can be informed by this modelling approach to generate novel nanoformulations with desirable pharmacokinetics.

Key words: nanoformulation, pharmacokinetics, PBPK, in silico, optimization, ADME, nanoparticle

Perspectives and opportunities in nanotechnology for drug delivery

Acceptable pharmacokinetics of drugs can be impeded by several factors, including poor absorption, low penetration into target tissues and high clearance. Insolubility of drugs, with the resulting low bioavailability, remains a serious concern for drug development programs in the pharmaceutical industry. It is estimated that >60% of new drug candidates are poorly soluble in water, inhibiting development programmes and ultimately the success of new treatments (Sareen *et al.*, 2012; Sikarra *et al.*, 2012). Moreover, the lack of drug penetration in tissues where exposure is most needed can have a detrimental influence on therapy efficacy and toxicity.

Numerous nanomedicine strategies are currently being assessed to improve drug delivery. Nanomedicines include nanoparticles (defined as solid submicron particles consisting of polymers or inorganic material) and liquid based drug nanocarriers such as nanoemulsions. Nanoformulations can be produced to contain a drug (or drugs) which may be associated with the particle in various ways (Kreuter, 1994). Many nanoformulations can effectively be absorbed and subsequently concentrated in tissues through passive targeting, exploiting both the physicochemical characteristics of the nanocarriers and the specific properties of the tissues of interest. Different strategies can also be applied for active targeting of tissues, pathogens and cancer cells.

The wide variety of nanocarrier designs means that a large, almost overwhelming, range of delivery strategies are available for research and application. Polymers can be used as containers for drug molecules, either by forming solid polymer matrix nanoparticles to encapsulate drugs, or through the construction of vehicles such as block copolymer liposomes/vesicles, micelles and nanoemulsions (Wischke and Schwendeman, 2008). Direct non-covalent or covalent conjugation of drugs to polymers have been successfully used to enhance circulatory times and deliver drugs through triggered/controlled release (Joralemon et al., 2010). A wide variety of inorganic oxides have been used to create nanoparticles, such as gold (Thakor et al., 2011), silver (Ong et al., 2013; Zhang et al., 2013), silica (Wu et al., 2013) and iron (Ittrich et al., 2013). However, the influence that these formulations can have on drug pharmacokinetics is only partly understood. In this review we describe what is known of the main processes regulating nanoformulation ADME. We also discuss

strategies to optimise the design of nanoformulations, focussing on the use of mechanistically-based ADME modelling to obtain optimal pharmacokinetics.

Importance of nanoformulation pharmacokinetics

The use of nanoformulation delivery systems has the potential to radically improve drug pharmacokinetics. However, efficacy and toxicity of drugs can also be negatively influenced by nanoformulation distribution: insufficient absorption and diffusion into tissues may compromise drug activity, while excessive nanoformulation accumulation could lead to tissue-specific toxicity (related to the drug, the nanoformulation, or potentially both). Consequently, understanding the interactions between nanoformulations and the human body is of central relevance for the engineering of future treatment strategies, and a thorough investigation of the processes regulating nanoformulation disposition is essential to optimise effective and safe nanoformulations for drug delivery. Several processes mediate the distribution of nanoformulations in the human body and the absorption, distribution, metabolism and elimination of nanoformulations can differ substantially from traditional formulations. In most cases nanoformulation ADME is not fully characterised and can vary based on the characteristics of the nanoformulations (Figure 1). The preferred routes of administration for nanoformulations are oral, transdermal, ocular, pulmonary, nasal and intravenous, which we discuss in this section.

Oral administration

Certain nanoformulations can enhance the absorption of drugs by releasing drug into the lumen in a controlled manner, thus reducing solubility issues. The intestinal wall is designed to absorb nutrients and to act as a barrier to pathogens and macromolecules. Small amphipathic and lypholic molecules can be absorbed by partitioning into the lipid bilayers and crossing the intestinal epithelial cells by passive diffusion, while nanoformulation absorption may be more complicated due to the intrinsic nature of the intestinal wall. The first physical obstacle to nanoparticle oral absorption is the mucous barrier which covers the luminal surface of the intestine and colon (Corazziari, 2009; Johansson et al., 2011). The mucus barrier contains distinct layers and is composed mainly of heavily glycosylated proteins called mucins, which have the potential to block the absorption of certain nanoformulations.

Modifications can be made to produce nanoformulations with increased mucous-penetrating properties (Ensign et al., 2012). Once the mucous coating has been traversed, the transport of nanoformulations across intestinal epithelial cells can be regulated by several steps, including cell surface binding, endocytosis, intracellular trafficking and exocytosis, resulting in transcytosis (transport across the interior of a cell) with the potential involvement of multiple subcellular structures. Moreover, nanoformulations may also travel between cells through opened tight junctions, defined as paracytosis (Tuma and Hubbard, 2003). Non-phagocytic pathways, which involve clathrin and caveolae mediated endocytosis and macropinocytosis, are the most common mechanisms of nanoformulation absorption by the oral route, although heterogeneity in the efficiency of these processes has been described for different types of nanoformulations. Consequently, it is difficult to identify a predominant process determining transcytosis of nanoformulations (He et al., 2013; Hillaireau and Couvreur, 2009; Smith et al., 2012).

Alternative administration routes

The inability of certain nanoformulations to undergo efficient oral absorption necessitates alternative administration routes. Also, the use of non-oral administrations can provide additional benefits, such as direct targeting to the desired site of action (Patel *et al.*, 2012) and an extended period of drug action (van 't Klooster *et al.*, 2010).

The skin provides a desirable route of nanoformulation administration, as it avoids the risks associated with intravenous therapy and the inconveniences associated with varying gastric pH, emptying time, and first-pass hepatic metabolism. However, administration of drugs is not easy because of the impermeable nature of the skin (Menon et al., 2012; Rehman and Zulfakar, 2013). Transdermal administration has been optimised for nanoformulations such as SLNs and NEs, which are characterised by good biocompatibility, lower cytotoxicity and desirable drug release modulation (Cappel and Kreuter, 1991; Gide et al., 2013; Khurana et al., 2013).

Topical ocular drug delivery provides a useful administration route for nanomedicines treating ocular pathologies, but utilisation is disadvantaged by the multiple defensive

barriers of the eye (de Salamanca *et al.*, 2006). Corneal and conjunctival epithelial cells are connected by intercellular tight junction complexes that limit the entrance of exogenous substances. In addition, the tear film can trap drugs and nanoformulations, removing them via the lacrimal drainage system. Consequently an efficient ocular drug delivery system has to interact with the ocular mucosa, protect the drug from chemical or enzymatic degradation and allow drug delivery to the ocular tissue. Different nanotechnologies have been utilised to overcome these barriers, helping the drug reach and target conjunctival epithelial cells (Alonso and Sánchez, 2004). Successful administration of nanoformulated intra-ocular-pressure-lowering drugs (Chen *et al.*, 2010; Hathout *et al.*, 2007) and anti-apoptotic drugs (Nkansah *et al.*, 2008) has been achieved *in vivo*. In addition, intravitreal administration of nanoformulations has been used to overcome absorption issues (Jiang *et al.*, 2007).

Nasal administration of certain nanoformulations has been assessed, hypothesising that nanoformulations may penetrate the nasal mucosal membrane. Nanoformulations can cross the membrane using a transmucosal route by endocytosis or via a carrier- or receptor-mediated transport process (Illum, 2007). Proof-of-concept has been achieved *in vivo*, for example by nasal administration of chitosan nanoparticles of tizanidine to increase brain penetration and drug efficacy in mice (Patel *et al.*, 2012).

The lungs are a promising route of administration for drug delivery due to the large surface area, ease of access and the thinness of the air-blood barrier. The lumen of the bronchial airways is lined with a thin layer of serous fluid, upon which floats a layer of mucus which helps to entrap aerosolized particles. The action of the cilia, present on the ciliated columnar epithelium, mediates the movement of the mucous layer towards the proximal airways, where it can be eliminated. The mucus barrier, metabolic enzymes in the tracheobroncial region and macrophages in the alveoli are the main barriers for penetration of drugs. Particle size is a major factor determining the diffusion of nanoformulation in the bronchial tree, with particles in the nano-sized region more likely to reach the alveolar region and particles with diameters between 1 and 5 μm expected to deposit in the bronchioles (Musante et al., 2002; Patton and Byron, 2007). A limit to absorption has been shown for larger particles, presumably due to an inability to cross the air-blood barrier (Ryan *et al.*, 2013b). Particles can

gradually release the drug which can consequently penetrate into the blood stream, or alternatively particles can be phagocyted by alveolar macrophages (Bailey and Berkland, 2009).

Certain nanoformulations have a minimal penetration through biological membranes in sites of absorption, therefore to obtain an efficient distribution in tissue an intravenous administration can be the preferred route (Wacker, 2013). Although long-term drug exposure has been demonstrated in certain cases (van 't Klooster *et al.*, 2010), the use of intravenous injection for multiple short-acting treatments is limited due to inconvenience and safety issues.

Distribution in tissues and organs

Once a drug-containing nanoformulation has entered the systemic circulation, the subsequent distribution into tissues can begin. The distribution of nanoformulations can vary widely depending on the delivery system used, the characteristics of the nanoformulation, and potentially the variability between individuals (organ size, body-fat index, etc). Another important factor to understand is the rate of drug loss from the nanoformulations, as the distribution characteristics of both the free drug and nanoformulated drug will most likely differ greatly. The main function of certain types of nanoparticles, for example SDNs, is the improvement of drug absorption, which does not require them to arrive intact in the systemic circulation. Consequently, the distribution and the clearance of these drugs would not be altered. Other nanotechnologies, however, are capable of surviving the absorption process, therefore altering the distribution and clearance of the contained drug.

On reaching the systemic circulation, nanoformulations come into contact with numerous proteins which can give rise to the formation of dynamic nanoformulation-protein coronas (Tenzer et al., 2013b). The protein corona influences nanoformulation size and physicochemical characteristics, consequently affecting processes such as nanoformulation degredation, cellular uptake (Paula *et al.*, 2013), accumulation and clearance (Peng *et al.*, 2013). Nanoformulation-protein coronas can also influence the body, potentially causing pathologies such as inflammation (Saptarshi *et al.*, 2013) and haemolysis (Tenzer et al., 2013a). Proteins can adhere

to nanoformulations through forces such as Van der Waals interactions, hydrogen bonding and solvation, thus generating protein coronas with environment-specific stability and characteristics. In human blood, a protein corona normally consists of serum albumin, immunoglobulins, fibrinogen and apolipoproteins (Ge *et al.*, 2011; Hellstrand *et al.*, 2009; Jansch *et al.*, 2012). For some nanoformulations, more abundant proteins such as albumin and fibrinogen may initially aspecifically bind to nanoformulations and subsequently can be replaced by other proteins having higher binding affinity (Saptarshi *et al.*, 2013). Therefore, the distribution of these nanoformulations is less simple to determine theoretically and further research is needed in this area.

Nanoformulations of a certain size and composition are able to diffuse in tissues through well characterised processes, such as the enhanced permeability and retention (EPR) effect, while some nanoformulations might accumulate in specific cell populations, allowing the targeting of specific organs. The EPR effect is the mechanism by which high-molecular-weight drugs, pro-drugs and nanoparticles tend to accumulate in sites of inflammation or cancer, which are tissues with increased vascular permeability (Matsumura and Maeda, 1986). Tumour vasculatures have large pores, ranging from 100 nm to several hundred nanometers in diameter, as compared to normal vessel junctions of 5–10 nm (Hobbs *et al.*, 1998). Consequently, nanoformulations can be designed to preferentially penetrate with higher efficiency in tumour tissue. As an additional factor, the lymphatic system in tumours might be impaired, increasing the retention of macromolecules and nanoformulations (Maeda *et al.*, 2000). In some cases this targeting method is not very effective, and the size-dependency, slow time frame, and variability from tumour to tumour limit treatment effectiveness (Iyer *et al.*, 2006; Maeda *et al.*, 2000)

Complex biological barriers can protect organs from exogenous compounds and the blood brain barrier (BBB) represents an obstacle for many therapeutic agents (Varatharajan and Thomas, 2009). Multiple cell populations comprising of endothelial cells, microglial cells, pericytes and astrocytes are present in the BBB which contain extremely restrictive tight junctions and efflux mechanisms, limiting the permeation of most drugs (Begley, 2004). Transport through the BBB is restricted to small lipophilic molecules and nutrients that are carried by specific transporters. One of the most important mechanisms regulating diffusion of nanoformulations into the brain is

endocytosis by brain capillary endothelial cells. Recent studies have correlated particle properties with nanoformulation entry pathways and processing in the human BBB endothelial barrier, indicating that uncoated nano-particles have limited penetration through the BBB and that surface modification can influence the efficiency and mechanisms of endocytosis (Georgieva *et al.*, 2011; Lee *et al.*, 2000). In many cases low penetration of nanoformulations into tissues can be a major barrier for the treatment of diseases. The use of ligands to enhance this process of uptake into tissue represents a promising solution (Ruoslahti, 2012). Tumour-penetrating peptides have been utilized which can activate bulk tissue-specific transport pathways, targeting receptors present in the tumour vasculature such as annexin1 (Hatakeyama *et al.*, 2011; Oh *et al.*, 2004), plectin-1, (Kelly *et al.*, 2008) and neuropilin-1 (Teesalu *et al.*, 2009).

The migration of monocytes in numerous tissues and sites of inflammation, infection, and tissue degeneration provides a unique mechanism to improve drug delivery (Lameijer *et al.*, 2013; Murphy *et al.*, 1975). Indeed, monocytes and macrophages have a central role in the pathogenesis of several diseases such as HIV (Crowe *et al.*, 2003), tuberculosis (Philips and Ernst, 2012), leishmaniasis (Farah *et al.*, 1975), cancer (Biswas and Mantovani, 2010), diabetes (Cnop *et al.*, 2005), inflammatory bowel disease (Heinsbroek and Gordon, 2009), rheumatoid arthritis (Szekanecz and Koch, 2007) and chronic obstructive pulmonary disease (Barnes, 2004), making these cells desirable drug targets in themselves. Nanoformulations can be engineered, controlling size and surface charge, to allow for their active uptake by monocytes and macrophages through phagocytosis. Monocytes and macrophages are characterised by a broad variety of receptors, which can be actively targeted using nanoformulations combined with specific ligands (Kelly *et al.*, 2011).

Elimination and Clearance

A multitude of processes can regulate the clearance of nanoformulations, from chemical and enzymatic degradation to renal and biliary elimination. Nanoformulations may undergo degradation in penetrated tissues or circulating blood, gradually releasing their content. Degradation kinetics is an important variable

that controls drug release and complicates the design of optimal drug delivery systems with predictable drug release properties (Mohammad and Reineke, 2013).

The immune system is responsible for removing foreign objects from the body, including not only pathogens but also any material it may be in contact with, including nanoformulations. It is of fundamental importance to achieve a thorough understanding of the way nanoformulations interact with immune cells and all related consequences. Macrophages in the liver are a major pool of the total number of macrophages in the body. Around $8.6 \pm 1.4 \times 10^5$ Kupffer cells are present in one gram of human liver tissue (Friedman *et al.*, 1992) and this cell population possesses numerous receptors for selective phagocytosis of opsonized particles (receptors for complement proteins and for the Fc part of IgG). Small inorganic nanoparticles are effectively phagocytosed by Kupffer cells which can have a central role in the generation of active oxygen species, tumor necrosis factor-α and nitric oxide, resulting in liver injury (Chen *et al.*, 2013; Sadauskas *et al.*, 2007). Cells with phagocytic activity are also present in the spleen which is another major site for nanoformulation elimination (Vyas and Malaiya, 1989). Nanoformulations containing polyethylene glycol (PEG) are characterised by prolonged presence in the systemic circulation by inhibiting receptor interactions and thus preventing phagocytosis by the mononuclear phagocytic system (Bazile *et al.*, 1995). Renal clearance is one of the most important mechanisms mediating nanoformulation excretion. The glomerular endothelium is characterised by fenestrations of 50-100 nm, with capillaries having a basement membrane (300nm thickness) as well as podocytes with phagocytic functionality.

Using PBPK modelling

Types of nanoformulations and pharmacokinetic challenges

The distribution of nanoformulations is influenced by multiple factors, including the nanoformulation physicochemical properties and composition, route of administration and characteristics of the individual to which the nanoformulations are administered. The most promising types of nanoformulations used for drug delivery are: inorganic nanoparticles, solid drug nanoparticles (SDN), solid lipid nanoparticles (SLNs), nanoemulsion (NEs), liposomes, polymeric nanoparticles and dendrimers (Figure 2). Hybrid nanoformulations, which contain elements of more than one nanoformulation class, are also possible, thus complicating classification.

A common goal of nanomedicine research is to increase the bioavailability of drugs and to manipulate movement of drug to target sites in the body. Table 1 gives examples of improvements in drug PK seen in selected nanoformulation studies. In this section we will review some interesting applications used for the different nanodelivery systems and the physiological and molecular processes regulating their absorption, distribution, metabolism and elimination.

Inorganic nanoparticles

A wide variety of inorganic oxides have been used to create nanoparticles, such as gold (Thakor *et al.*, 2011), silver (Ong *et al.*, 2013; Zhang *et al.*, 2013), silica (Wu *et al.*, 2013) and iron (Ittrich *et al.*, 2013). The potential uses of inorganic nanoparticles vary greatly and can include molecular diagnostics (Radwan and Azzazy, 2009), photoacoustic imaging (Lu *et al.*, 2011), targeted drug delivery (Assifaoui *et al.*, 2013; Chamundeeswari *et al.*, 2013), photothermal therapy (Huang *et al.*, 2006) and nonviral gene-delivery vectors (Sitharaman *et al.*, 2008). A particularly fascinating use of iron oxide nanoparticles has been to actively target specific tissues using an external magnetic influence (Dilnawaz *et al.*, 2010). The biodistribution, elimination and potential toxicity of inorganic nanoparticles vary wildly depending on materials used, and have been reviewed previously (Almeida *et al.*, 2011; Bachler *et al.*, 2013; Choi *et al.*, 2007; Pelley *et al.*, 2009; Waalkes, 2000). As a paradigm example we have focussed here on silver nanoparticles.

Following i.v. injection, silver nanoparticles are rapidly removed from the blood and widely distributed to organs, in particular the liver, lungs and spleen (Lankveld *et al.*, 2010). The size of the silver nanoparticles can influence distribution, with particles larger than 20 nm being more readily accumulated in tissue. The ionic silver in the body is changed to silver sulphide via mercaptan interaction, and is also metabolised to silver-glutathione for biliary secretion (Ballatori and Clarkson, 1985). The major elimination route of intact 33 nm silver nanoparticles was found to be the kidneys via tubular secretion (Malfatti *et al.*, 2012). A PBPK model has been created which predicts the exposure of silver nanoparticles in both rats and humans (Bachler *et al.*, 2013).

Solid drug nanoparticles (SDNs)

SDNs are lipid-free nanoparticles which are used to improve the oral bioavailability and exposure of poorly water-soluble drugs (Chan, 2011; Tanaka *et al.*, 2012). Constituents include drug and stabiliser, and SDNs are produced using a “top-down” (high pressure homogenisation and wet milling) or bottom-up (solvent evaporation and precipitation) approach (Zhang *et al.*, 2011). Our group has developed efavirenz SDNs which exhibit around four-fold higher pharmacokinetic exposure after oral administration to rodents, compared to free drug (Kreuter, 1994; McDonald *et al.*, 2013) (Siccardi *et al.*, 2013a). In a separate study, a single s.c. injection of rilpivirine SDN resulted in a constant release of around 25 ng/mL for 20 days, providing evidence that s.c. injections of antiretroviral SDNs could be used for long-acting therapy (Baert *et al.*, 2009).

It is not fully known whether SDNs remain intact following oral absorption, and therefore the relevance of SDN distribution and elimination *in vivo* is poorly understood.

Solid lipid nanoparticles (SLNs)

SLNs consist of a lipid (or lipids) which is solid at room temperature, an emulsifier and water. Lipids utilised include, but are not limited to, triglycerides, partial

glycerides, fatty acids, steroids and waxes (Mehnert and Mader, 2001). Different combinations of lipid and emulsifier can be used to create unique SLN properties, such as drug release rate and pH sensitivity, although the effects this has on the SLNs *in vivo* is poorly understood. Due to their lipid core, SLN's are most suited for delivery of highly lipophilic drugs, although enhanced delivery of hydrophilic drugs, such as the anti-tubercular drug isoniazid, has been achieved *in vivo* (Bhandari and Kaur, 2013a). The use of SLNs to deliver siRNA and siRNA-drug combinations has also been demonstrated (Lobovkina *et al.*, 2011; Yu *et al.*, 2012).

SLNs have successfully been used to increase the absorption of drugs. Olanzapine-loaded cationic SLNs showed a 4.3-fold increase in olanzapine exposure (Sood *et al.*, 2013) and 2.6-fold increase in tamoxifen exposure (Hashem *et al.*, 2013) compared to free drug.

The *in vivo* fate of SLNs are determined by several factors, including the inherent stability and physicochemical properties of the SLNs, the biological and enzymatic surroundings of the administration site, and the distribution process from the administration site. Using pulmonary (Videira *et al.*, 2012), subcutaneous (Harivardhan Reddy *et al.*, 2005), and oral (Cavalli *et al.*, 2000; Paliwal *et al.*, 2009; Zara *et al.*, 2002) dosing strategies, SLNs have been shown to target the lymphatic system *in vivo*.

An advantage of using SLNs is that formulations are believed to be safe and easily cleared from the body. Organic solvent is not required for SLN production, and the lipids which are used are usually biodegradable, thus reducing the risk of SLN - accumulation-associated toxicities. This degradation provides further benefits, as the size and choice of lipid influences the elimination rate of SLNs, with longer lipids generally outlasting smaller lipids and waxes lasting longer than triglycerides, allowing for controlled release of drug. Due to the solid status of SLNs, elimination is generally slower than with liquid-lipid-based nanoformulations.

Interestingly, PEGylated solid lipid particles have an increased clearance rate following repeat i.v. or s.c. administration (Zhao *et al.*, 2012a; Zhao *et al.*, 2012b). This phenomenon is caused by immune response to PEG and subsequent removal of SLNs from the circulation, referred to as the "accelerated blood clearance" (ABC)

phenomenon, although the exact immunological process is not known (Abu Lila *et al.*, 2013).

Nanoemulsion (NEs)

Liquid droplets of less than a 1000 nm dispersed in an immiscible liquid are classified as NEs. NEs represent excellent carriers for transport of hydrophobic and hydrophilic substances and can find application in intravenous (Ichikawa *et al.*, 2007), oral (Sun *et al.*, 2012), transdermal (Khurana *et al.*, 2013), nasal (Bahadur and Pathak, 2012) and ocular (Badawi *et al.*, 2008) drug delivery. The rate of lipolysis and the organ-specific elimination of nanoemulsions are influenced by the choice of constituents and route of administration, which allows for a more controlled release of drug. Oral administration is the route of choice for chronic therapy and NEs can effectively enhance oral bioavailability of small molecules, peptides and proteins. The mechanisms through which NEs mediate higher oral absorption are improved drug solubilisation, protection from enzymatic and chemical hydrolysis and increased permeability due to surfactant-induced membrane fluidity. The hydrophobic core of the NEs is an ideal environment for drugs with poor solubility in water and the surfactants present in the formulation favour the solubilised state in the GI tract. BCS class II compounds (high permeability, low solubility) are ideal candidates for NEs and their pharmacokinetics can be greatly enhanced through this nanotechnology. Paradigmatic examples of this are represented by drugs such as Ramipril, Ezetimibe (Bali *et al.*, 2010) and Anethol trithione (Han *et al.*, 2009) which the bioavalability has been increased 2.3, 3 to 4 and 2 to 3 fold, respectively, compared to traditional formulations. In a study using Balb/c mice, orally-dosed saquinavir in flax-seed oil nanoemulsion was found to have more than two-fold increased exposure in brain, compared to free drug (Vyas *et al.*, 2008).

Polymeric nanoparticles

Polymeric nanoparticles are solid particles typically around 200-800 nm in size which can be created using both synthetic and natural polymers. The natural polymers

used are generally biodegradable and can include as examples gelatine, cellulose, chitosan and gluten (Zhang *et al.*, 2007). Synthetic polymers such as polyactides, poly(d,l-lactic-*co*-glycolide) (PLGA) and PEG allow for a high level of degredation control. Different polymers are often used in combination, forming copolymers with potentially beneficial properties, such as pectin-PLGA (Liu et al., 2004) and alginate–chitosan-PLGA (Zheng et al., 2004). Polymers can also be blended with or attached to other nanoformulation types, such as polymer-liposome complexes used for targeted co-delivery of drug and gene to cancer cells (Wang et al., 2010). These properties make polymer nanoparticles an extremely versatile tool for improving drug delivery.

Polymeric nanoparticles can be used to increase the bioavailability of drugs and other substances, compared to traditional formulations (Morgen et al., 2012). The size of polymeric nanoparticle has been shown to influence oral absorption. The absorption potential of chitosan nanoparticles of sizes 300 nm to 1000 nm were assessed, with 300 nm showing greater permeation in both Caco-2 cells and rat oral dose studies (He et al., 2012). Polymer-coated nanoparticles are capable of actively targeting tissues such as hepatocytes, lymph nodes and tumours (Muthiah et al., 2013), therefore allowing for targeted therapy and avoidance of organ-specific toxicity. Clearance of polymeric nanoparticles is dependent on several factors, such as choice of polymer and co-polymers, polymer size, polymer charge and the existence of active tissue targeting. Trends in clearance have been observed, with positively charged nanoparticles larger than 100 nm being eliminated predominantly via the liver (Alexis et al., 2008).

Polymeric nanoparticles are capable, both purposefully and inadvertently, of affecting the host immunological response. As an example, PEG has been utilised to reduce the immune response to nanoformulations by shielding the particle surface from recognition (Moghimi, 2002). This technique has only been partly successful, as a long term PEG-specific immune response has been observed in subsequent studies (Ishida et al., 2007; Wang et al., 2007). Time-dependent immune system stimulation by nanoformulations may influence pharmacokinetics, as phagocytosis-driven increases in nanoformulation clearance would potentially occur.

Dendrimers

Dendrimers are tree-like, nanostructured polymers that have received significant attention as drug delivery systems, due to their well-defined size, tailorable structure, and potentially favourable biodistribution (Biricova and Laznickova, 2009). Dendrimer-based drug delivery systems can be manufactured to provide theoretically almost any size, but are commonly 10–20 nm in diameter and show promise as agents for imaging (Kobayashi and Brechbiel, 2004), gene therapy (Dufes *et al.*, 2005), drug delivery (Svenson, 2009) and biological adhesive (Joshi and Grinstaff, 2008).

Due to the near-infinite variety of possible dendrimer structures, an understanding of how these structures will relate to ADME/PK is a problematic task. Properties specific to each dendrimer, such as size, shape, charge, hydrophobicity and hydrodynamic weight, may all potentially alter disposition *in vivo*, as could attachments to the dendrimer structure such as PEG, drugs, RNA or antibodies (Kaminskas *et al.*, 2011). Further research is needed to understand these relationships to ensure optimum disposition and to avoid toxicity issues.

Liposomes

Liposomes are spherical vesicles consisting of a phospholipid bilayer. A variety of lipids can be utilised, allowing for a degree of control in degredation level. In addition to oral dosing, liposomes can be administered in many ways, including intravenously (McCaskill *et al.*, 2013), transdermally (Pierre and Dos Santos Miranda Costa, 2011), intravitreally (Honda *et al.*, 2013), pulmonary (Chattopadhyay, 2013)

Encasing drug in liposomes can dramatically increase drug exposure. In a PK study using Kunming mice, danorubicin liposomes had a 13-fold higher $AUC_{0\text{-}48h}$ compared with free drug (Ying *et al.*, 2011). Drug in liposomes often show greater PK variability than free drug, which is exacerbated when the clearance rate of the liposomes is low (Schell *et al.*, 2013). This could potentially prevent the use of liposomes to deliver drugs with a small therapeutic window.

Liposomes have the potential to radically alter tissue distribution of encapsulated drugs, which allows for targeting of tissues, such as the lymphatic system and brain (Cai *et al.*, 2011; Lai *et al.*, 2013), but this can also lead to increased toxicity. As an example, in a tumour-expressing CD1 mouse study, liposome encapsulation increased zoledronic acid 20 to 100-fold in liver, 7-10-fold in tumour and 2-fold in bone, which resulted in more than 50-fold increase in drug-associated toxicity in animals but no additional inhibition of tumour growth (Shmeeda *et al.*, 2013). Liposomes can be combined with synthetic polymers to form lipid-polymer hybrid nanoparticles (LPNs), extending their ability to target specific sites in the body (Hadinoto *et al.*, 2013).

The clearance rate of liposome-encased drugs is determined by both drug release and destruction of liposomes (uptake of liposomes by phagocyte immune cells, aggregration, pH-sensitive breakdown, etc) (Ishida *et al.*, 2002). In a PK study using Kunming mice, docetaxel clearance was reduced from 19.9 to 7.5 L/h*kg when liposome-encased, resulting in a 81% increase in $t_{1/2}$ (Zhang *et al.*, 2012). Similarly to solid lipid particles, liposomes attached to PEG also show ABC following repeat doses (Suzuki *et al.*, 2012).

PBPK and nanotechnology: challenges and limitations

PBPK requires large amounts of information.

Commonly used blood-to-tissue partition coefficients may not apply to nanoformulations.

The lymphatic system is not routinely included in PBPK models (REF). Considering that the lymphatic system has been shown to be integral to the absortion (REF) and distribution (REF) of certain nanoformulations, a full inclusion of this system

Unusual “metabolism” of nanoformulations (pH-triggered, phagocytisede etc) and in different parts of body to standard drugs (also internal distruibution in cells?). Would need integration into PBPK models for comprehensive prediction.

The huge number of potential nanoformulation to select for a particular drug/vaccine etc. There is perhaps traits within nanoformulation classes (eg SDNs unlikely to accumulate in body after absorption etc).

A minor alteration in nanoformulation size, shape, charge can potentially have large influence of the exposure and effectiveness of an encapsulated or attached drug.

Optimization of nanoformulation design

Numerous polymers and materials have been developed for the preparation of nanoformulations and the ideal components should be non-toxic, non-immunogenic, and should allow for the transport and release of sufficient amount of drug. Nanoformulation composition has been correlated with tissue distribution patterns, highlighting how the inclusion of specific polymers can have a critical effect on nanoformulation distribution. A paradigm example is Poly-ethylene glycol (PEG), which can be adsorbed or covalently attached to the surface of nanoformulations. PEG has been shown to reduce the interaction between nanoformulations and proteins due to its hydrophilicity and repulsion effect, reducing opsonisation, complement activation, phagocytosis and clearance mechanisms (Bazile *et al.*, 1995). Moreover it appears evident that the chain length, shape, and density of PEG on the particle surface are important parameters affecting nanoformulation PEG stealth activity (Gref *et al.*, 2000). In the study by Gref *et al*, the ideal molecular weight, density and content of PEG were optimised to minimise the amount of plasma protein absorbed, thus reducing uptake by polymorphonuclear leukocyte (PMN) and human monocyte (THP-1).

The physiological processes regulating nanoformulation ADME, such as hepatic filtration, tissue extravasation, tissue diffusion and kidney excretion, indicate that nanoformulation size is a key determining pharmacokinetic factor. A clear example of the importance of size is given by a study investigating polystyrene nanoparticles, where particle sizes of 50 and 500 nm showed higher levels of agglomeration of the larger nanoparticles in the liver (Nagayama *et al.*, 2007). Size and polydispersity can substantially affect the distribution of micelles which have a half-life of around 8 hours with a low hepatic and spleen uptake (Rijcken *et al.*, 2007). Considering dendrimers, size has been the best characterised property and it is thought to be a determinant predictor of *in vivo* distribution. Rapid clearance mediated by the kidney has been observed for smaller dendrimers (Generation 5 (G_5) or smaller, with a radius of less than 3.5 nm), with minimal or no renal clearance observed for larger dendrimers. Dendrimers of generation G_7, characterised by radius above 5nm, readily accumulate in the liver and spleen tissue and, consequently, are cleared by

the RES system and by biliary excretion. (Kobayashi *et al.*, 2001a; Kobayashi *et al.*, 2001b).

Characteristics of the nanoformulation surface, such as charge or functional groups, can influence the uptake of different cell populations. The effect of surface roughness and charge on the cellular uptake of polymeric/silica nanoparticles in HeLa cells has been recently investigated, and rough nanoparticles are internalized by the cells more slowly and by an unidentified uptake route compared to smooth nanoparticles. Moreover, nanoparticles with negative charges are internalised with higher efficiency compared to positively charged ones, independent of the surface roughness (Schrade *et al.*, 2012). In another study, silica-based fluorescent nanoparticles were tested in murine pre-osteoblast cell line, MC3T3-E1 and the effect of three surface modified nanoparticles were analysed: positively charged (PTMA), negatively charged (OH), and neutrally charged polyethylene glycol (PEG). Positively charged PTMA-modified nanoparticles demonstrated the most rapid uptake, within 2 hours, while PEG modified and negatively charged OH nanoparticles demonstrated slower uptake (Ha *et al.*, 2013). Preferential uptake of polystyrene nanoparticles by phagocytic cells has been recently investigated and carboxylated nanoparticles were highly phagocyted in macrophages while amino-functionalized particles had higher uptake in monocytes (Lunov *et al.*, 2011). The interaction between gold nanoparticles (with different hydrophobicity, charge density and ligand length) and lipid bilayers has been clarified investigating physicochemical properties favouring penetration through the bilayer. Hydrophobic and anionic nanoparticles did not have any significant interactions with the bilayer and different charge densities may induce pore formation or nanoparticle wrapping, resembling first stages of endocytosis. Consequently trough the tuning of charge density it can be possible to favour the internalization of nanoparticles into cells through different mechanisms such as passive translocation, (low charge density) or endocytosis (higher charge densities) (McCaskill *et al.*, 2013).

All the above mentioned factors can interact together, defining a multifactorial scenario where multiple nanoformulation properties determine pharmacokinetic processes. Consequently, choosing which nanotechnology is the best tool to improve the distribution of a defined drug, by the usage of ideal nanoformulation characteristics, is a complex problem that unquestionably ought to take into account

our current knowledge on nanoformulation ADME. This would be possible by integrating an exhaustive description of the physicochemical, physiological and molecular processes underpinning nanoformulation pharmacokinetics with the correlation between nanoformulation characteristics and their distribution.

A helpful pharmacological tool to inform the design of nanoformulations and thus optimise their pharmacokinetics is represented by physiologically based pharmacokinetics (PBPK) modelling. This modelling technique has been successfully used for traditional formulation in drug developing programs as well as simulation of relevant clinical scenarios (Karlsson *et al.*, 2013; Siccardi *et al.*, 2012; Siccardi *et al.*, 2013b). PBPK modelling is a bottom up technique which aims to simulate drug distribution by combining system data describing a population of interest (e.g. demographics, physiology, anatomy and genetics) with *in vitro* drug data (e.g. Caco-2 permeability, protein binding, intrinsic clearance, lipophilicity) through a mathematical description of absorption, distribution, metabolism and elimination (ADME). This modelling technique gives a complete overview of all the physiological and anatomical processes involved in drug distribution, offering the opportunity to identify important determinants of pharmacokinetics. For traditional formulations, absorption can be simulated considering the dynamic interplay between dissolution, passive permeability and the affinity/activity of metabolic enzymes and transporters. Drug distribution is simulated by evaluating tissue volumes and the diffusion of drugs into tissues, which is influenced by physicochemical properties (Poulin and Theil, 2002). Moreover, tissues and organs are connected by virtual blood and lymphatic flows. To simulate clearance, *in vitro* stability data can be used and integrated into the model using scaling factors. Inter-patient variability is observed in all of the above processes, and virtual populations can be simulated capturing inter-individual variability by considering anatomical and physiological characteristics, and their covariance. The application of PBPK models for nanomedicines is in its infancy and characterised by several challenges.

The first study describing a PBPK model for nanoformulations was published in 2008, predicting the pharmacokinetics of quantum dots in mouse using whole-body PBPK. The authors included a distribution coefficient to simulate the diffusion of nanoparticle in tissues based on *in vitro* data, and could predict animal pharmacokinetics with good accuracy (Lin *et al.*, 2008). Subsequently, a PBPK

model for the simulation of carbon nanoparticles was developed, integrating imaging data collected in humans using radioactive nanoparticles (Pery *et al.*, 2009). Silver nanoparticle PK has been successfully simulated which considered how the effect of size and size-dependent tissue distribution influenced toxicity and health risks. Unfortunately experimental data could not be match completely, possibly due to the effect of other nanoparticle characteristics, such as surface charge and coating, which were not included in the PBPK model (Lankveld *et al.*, 2010). PBPK modelling for five poly(lactic-co-glycolic) acid (PLGA) nanoparticle formulations prepared with different versions of monomethoxypoly (ethyleneglycol) (mPEG) (PLGA, PLGA-mPEG256, PLGA-mPEG153, PLGA-mPEG51, PLGA-mPEG34) has been generated, investigating the relationship between nanoparticle properties (size, zeta potential, and number of PEG molecules per unit surface area) and distribution parameters. The multivariate regression in the study generated significant linear relationships between nanoparticle properties and distribution parameters. Subsequently, this *in silico* model was successfully utilized to predict the distribution of a sixth nanoformulation (PLGA-mPEG 495) in mice (Li *et al.*, 2012).

Temporal exposure and elimination of 5 gold/dendrimer composite nanodevices (CNDs) in mice bearing melanoma was evaluated using a PBPK model (Mager *et al.*, 2012). The authors concluded that, since specific binding ligands ware lacking, size and charge of nanodevices governed most of their *in vivo* interactions. A PBPK model for ionic silver and nano-encaspulated silver was developed on the basis of toxicokinetic data from intravenous studies. The authors validated the model structure for both silver forms by reproducing exposure conditions (dermal, oral, and inhalation) of *in vivo* experiments and comparing simulated with real pharmacokinetic data for plasma and tissues. Interestingly, in all of the cases examined the model could successfully predict the distribution of both ionic silver and 15-150 nm silver nanoparticles not coated with PEG. The *in silico* model was also used to asses relevant scenarios of exposure to silver nanoparticles such as dietary intake, use of three separate consumer products, and occupational exposure (Bachler *et al.*, 2013).

The effect of chemical components and nanoformulation properties on the distribution of nanoformulations is surely significant, but only partially characterised and necessitates future research. Moreover, universal property–distribution relationships for all materials are unlikely, unless the effect of specific a

physicochemical property is extremely predominant. PBPK models can be applied to simulate drug and nanoformulation pharmacokinetics not only in humans but in different animals, therefore PBPK modelling may be applied in preclinical screening of nanoformulation, reducing the number of animals used for experimentations (Geenen *et al.*, 2013; Willmann *et al.*, 2010; Wong *et al.*, 2010; Yang *et al.*, 2013). Besides describing nanoformulation distribution and pharmacokinetic parameters, PBPK modelling can provide quantitative evaluation of the influence of nanoformulation properties on their absorption, diffusion and clearance. The integration of these property–distribution relationships in PBPK models may have extensive benefits in nanomedicine research, giving opportunities for innovative development of nanotechnologies. This approach will not only improve our understanding of the mechanisms underpinning nanoformulation disposition and allow for more rapid and accurate determination of their kinetics, but will also help clarify interactions between different nanoformulation properties, identifying antagonistic or synergistic effects. Consequently, the design and development of nanoformulations can be informed by this modelling approach to generate novel nanoformulations with desirable pharmacokinetics (Figure 3).

IDEAS FOR FUTURE PERSPECTIVES

Use PBPK models with nanoformulations with well described characteristics, perform sensitivity analysis to determine the key physiological and physicochemical characteristics controlling

Reduce the reliance on in vivo animal data, which is possibly unreliable for nano.

If animal use unavoidable, then PBPK can be used to bridge extrapolate animal data to inform human tox/PK studies. Since standard blood-to-tissue parameters do not apply to nanoformulations, non PBPK may not be sufficient.

Create catalogue of nanoformulations with well described characteristics in PBPK models, for “selection” when a particular trait is required for a future drug.

PBPK can be combined with PD or tox.

PBPK model of the nanoparticle can be combined with a PBPK model of the released drug, by including a degredation rate etc.

Figure 1. A selection of issues relating to the administration (green boxes), distribution (pink boxes) and elimination (orange boxes) of nanomedicines.

Figure 2. Examples of nanodelivery systems.

Figure 3. Flow chart representing an optimization process based on PBPK modelling and interactions between the different stages.

Drug	*Formulations*	*Dose*	*Outcome*	*Reference*
Tamoxifen	SLN	p.o.	↑156% plasma exposure	(Hashem *et al.*, 2013)
Olanzapine	SLN	p.o.	↑330% plasma exposure	(Sood *et al.*, 2013)
Isoniazid	SLN	p.o.	↑516% plasma exposure	(Bhandari and Kaur, 2013b)
Lopinavir	SLN	p.o.	↑95% plasma exposure	(Negi *et al.*, 2013)
Vincristine	Liposome	i.v.	↑66% plasma exposure, no increased patient toxicity	(Yan *et al.*, 2012)
Indinavir	Liposome	p.o.	200-fold higher exposure in lymph, no increased toxicity *in vivo*	(Gagne *et al.*, 2002)
Doxorubicin	Liposome	p.o.	Reduced patient toxicity	(O'Brien *et al.*, 2004)
Efavirenz	SDN	p.o.	↑301% plasma exposure	(McDonald *et al.*, 2013)
Probucol	SDN	p.o.	↑127% plasma exposure	(Nishino *et al.*, 2012)
Rosuvastatin	Nanoemulsion	p.o.	↑145% plasma exposure	(Balakumar *et al.*, 2013)
Chloambucil	Nanoemulsion	p.o.	↑91% plasma exposure and >2-fold increase in tumour growth suppression	(Ganta *et al.*, 2010)
Primaquine	Nanoemulsion	p.o.	↑28% plasma exposure and ↑40% liver exposure	(Singh and Vingkar, 2008)
Doxorubicin	Dendrimer	s.c.	682-fold and 2.7-fold higher lymph exposure than standard and liposome formulation, respectively	(Ryan *et al.*, 2013a)
Zidovudine	Dendrimer	i.v.	↑1320% lymph concentration 3hrs post-dose	(Gajbhiye *et al.*, 2013)

Table 1. Examples of improved drug exposure and tissue distribution achieved in nanoformulation studies *in vivo*.

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