Nanosized Drug Delivery Vectors and the Reticuloendothelial System

Lisa M. Kaminskas and Ben J. Boyd

Abstract Nanomaterials have potential as drug delivery vectors that can improve the chemical stability and pharmacokinetic profile of small molecule drugs or improve drug uptake into solid tumours. However, one consequence of the use of nanosized drug delivery vectors is their potential recognition by tissue macrophages and accumulation in organs of the reticuloendothelial system (RES). While in some instances the uptake of drug loaded nanomaterials or 'nanomedicines' into organs of the RES is favoured, in most instances uptake into the RES can limit systemic exposure of the nanomedicine and limit therapeutic utility. Hence, this section discusses ways in which the RES uptake of nanomedicines can either be promoted or inhibited. Specifically, the effect of various physicochemical properties and presence or absence of key RES 'recognition ligands' on RES uptake are examined.

Keywords Drug delivery • Macrophage • Nanomedicine • Opsonisation • Reticuloendothelial

Abbreviations

RES	Reticuloendothelial system
PAMAM	Polyamidoamine
PEG	Polyethylene glycol
DPPC	Dipalmitoyl phosphatidylglycerol
Succinate	Succinic acid
Ph-sulphonate	Benzene sulphonate
Ph-disulphonate	Benzene disulphonate
TIM	T cell Ig domain and mucin domain

L.M. Kaminskas and B.J. Boyd (✉)
Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, Australia
e-mail: ben.boyd@monash.edu

A. Prokop (ed.), *Intracellular Delivery: Fundamentals and Applications*, Fundamental Biomedical Technologies 5, DOI 10.1007/978-94-007-1248-5_6,

NPC	Non-parenchymal cell
HPI	Hydrogenated phosphatidylinositol
DPPG	1,2-dipalmitoyl-sn-glycero-3-phospho-rac-(1-glycerol)
DAB	Diaminobutane
Gd-DAB	Gadolinium conjugated diaminobutane dendrimer
HLA-DR	Human leukocyte antigen DR
BAI	Brain specific angiogenesis inhibitor
GM1	Monosialoganglioside GM1
DPPC	1,2-dipalmitoyl-sn-glycero-3-phosphocholine
DSPC	L-alpha-phosphatidylcholine distearoyl
DSPG	L-alpha-phosphatidyl-DL-glycerol-distearoyl

1 Introduction

A number of attributes of nanosized drug delivery particles are driving their application in a wide range of uses in medicine and as drug delivery vectors. Their circulation behaviour in plasma can be extended by controlling size to be on the one hand sufficiently small to permit intravenous administration, while on the other hand being sufficiently large to hinder passage through endothelial gap junctions, leading to extended circulation time. Drugs can be incorporated into their structures either via entrapment or surface conjugation. Particles can be engineered to permit entrapped drugs to gradually leak out of the structures down a concentration gradient, to enable an extended release-type delivery of drug to systemic circulation, or to liberate drug specifically under physiological conditions encountered at the desired site of drug action. They can be engineered for tissue-specific drug delivery either passively (for instance via the enhanced permeation and retention effect in solid tumours) or actively via the surface presentation of ligands to cell surface receptors. In addition, association of drugs with nanosized delivery vectors allows delivery into the cells in the form of a nano-drug formulation bypassing the efflux mechanism giving rise to resistance. This is particularly relevant in the treatment of cancer, where cytotoxic drugs often develop multidrug resistance phenotypes.

A wide range of potential structures based on lipids, polymers, dendrimers, solid drug particles, surfactants and other matrix materials have been described for use as particulate or macromolecular drug delivery systems for intravenous application. A range of these structures are illustrated below in Fig. 1. The two classes of particulate nanomaterials, i.e. the macromolecules and matrix-based particles associated with drug molecules, are collectively called ‘nanomedicines’ for the purpose of this contribution.

Although nanomedicines have shown a number of beneficial attributes for their application in drug delivery, they are also prone to recognition and removal via the reticuloendothelial system (RES), which can severely limit their application. Specifically, recognition of nanomedicines by the RES can result in very rapid

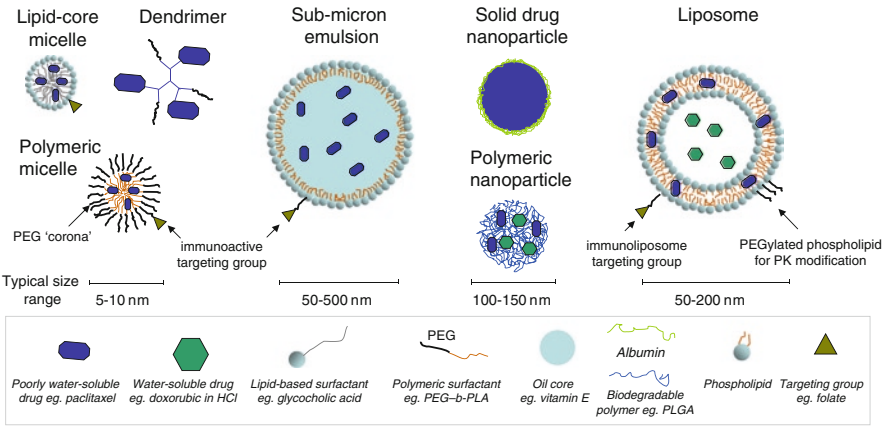


Fig. 1 Various commonly used nanostructures in drug delivery

removal of the particle from plasma within only minutes to a few hours, thus limiting the therapeutic usefulness of the nano-drug formulation where extended circulation time is important for performance. This chapter is therefore dedicated to explaining the reticuloendothelial system, its functions, pathophysiology and mechanisms of nanomedicine removal and detailing ways in which uptake via the system can either be avoided or promoted.

2 Pathophysiology of the Reticuloendothelial System

The reticuloendothelial system (commonly referred to as the RES) is comprised of a collection of organs (Fig. 2) that contain high proportions of the cells that make up the body's defence system against particulate pathogens. Specifically, it is a collection of monophagocytic cells that are largely manufactured by the bone marrow and transported throughout the body to aid in the removal of foreign organisms, damaged cells and products of cellular degradation. Immature macrophages in the systemic circulation are monocytes that migrate into tissues once matured. Mature, tissue fixed macrophages make up the Kupffer cells in the liver and the reticular cells in the spleen, lungs, lymph nodes and bone marrow. These cells remove foreign material via rapid phagocytosis. In some instances, initial priming via lymphocytes and the internalization of material into endosomes is required. The endosomes eventually fuse with lysosomes that release enzymes into the endosome that digest the engulfed material. For material that is resistant to degradation via lysosomal enzymes, the material remains trapped within the macrophage until the cell eventually dies. The largest concentration of macrophages in the body are located in the liver and lungs, however macrophages in different locations exhibit very different biochemical and functional activity that can influence their ability to scavenge various nanomedicines.

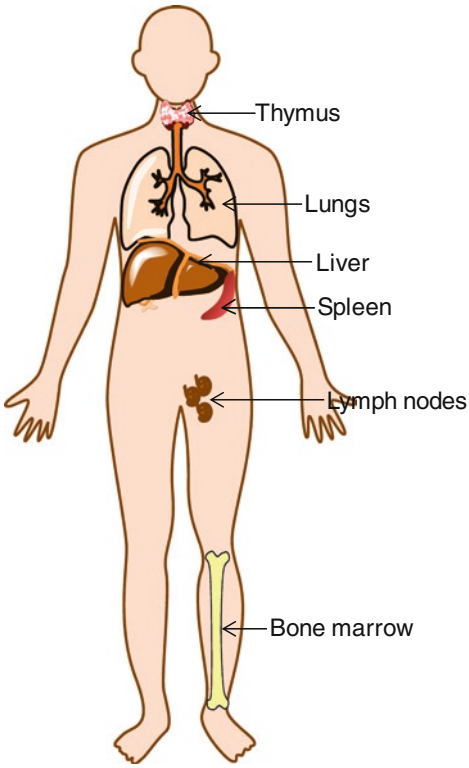


Fig. 2 Diagram of the organs that make up the reticuloendothelial system

The RES is therefore largely responsible for the removal of nanomedicines from the systemic circulation. The uptake of particles is largely size dependent. Compared to circulating biological proteins, drug delivery particles are usually large in size (Fig. 1), making them appear as foreign materials to the monophagocytic system. Opsonisation of drug delivery particles by plasma proteins and lipoproteins accelerates and enhances the recognition process. The pathophysiology of several major organs of the RES (liver, spleen, lymph nodes and lungs) and how this relates to particle uptake is therefore described in the following section.

2.1 The Liver

The liver is one of the most important metabolic organs in the body and is a part of the digestive system. The visceral surface of the liver contains the porta hepatis, the site at which vessels and ducts enter and leave the liver, including the portal vein, the hepatic artery and the common hepatic, cystic and bile ducts. At any one time, approximately 20% of the bodies blood supply resides in this organ and the liver is a major site for the removal of drugs absorbed into the blood from the gastrointestinal tract.

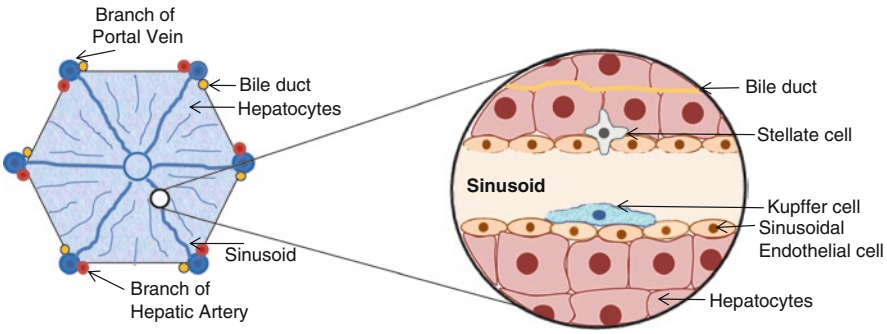


Fig. 3 Diagram of the basic lobular structure of the liver (*left*) and of the microstructure surrounding the sinusoids (*right*)

The major microstructural unit of the liver is the lobule (Fig. 3), a hexagonal structure that contains a central vein with blood tracts that protrude from the main structure (called the sinusoids that are lined with a thin layer of sinusoidal endothelial cells) and branches of the portal vein, hepatic artery and bile ducts at each corner of the hexagonal lobule. The major cell type that resides between the sinusoids are the hepatocytes. These are the major metabolic cells of the liver and are storage sites for a number of essential nutrients that occur in excess in the blood, such as iron and glycogen. The hepatocytes occur in a single layer between the liver sinusoids and bile cannaliculi and are responsible for absorbing drugs and materials from the blood and processing them for secretion into the bile ducts. Thus, the excretion of nanomedicines via the bile and subsequently via the feces requires initial uptake via the hepatocytes. Hepatic stellate cells also lie in between clusters of hepatocytes. These cells are the fat storage cells of the liver. Finally, Kupffer cells (the major phagocytic cells of the liver that account for approximately 2% of the liver volume) lie within the sinusoids. Their residence at this site optimizes their exposure to blood pathogens, foreign material and more importantly to this chapter, nanomedicines. Blood entering the liver from the hepatic artery and the portal vein flow towards the lobule via their branches, through the sinusoids where the blood is 'cleaned' and into the central vein that pools detoxified or cleaned blood into the hepatic vein. There are also two distinct types of Kupffer cells in the liver. The smaller, more immature cells are mainly located in the centrilobular region. The larger, more mature cells that play a more prominent role in phagocytosis, and hence are of more importance in the uptake of particles from the blood by the liver, are located in the periportal region.

2.2 The Spleen

The spleen is important in generating blood cells during fetal development, but it is not a vital organ in adults. It does, however, function in addition to the liver to destroy and remove damaged or aged red blood cells from the systemic circulation.

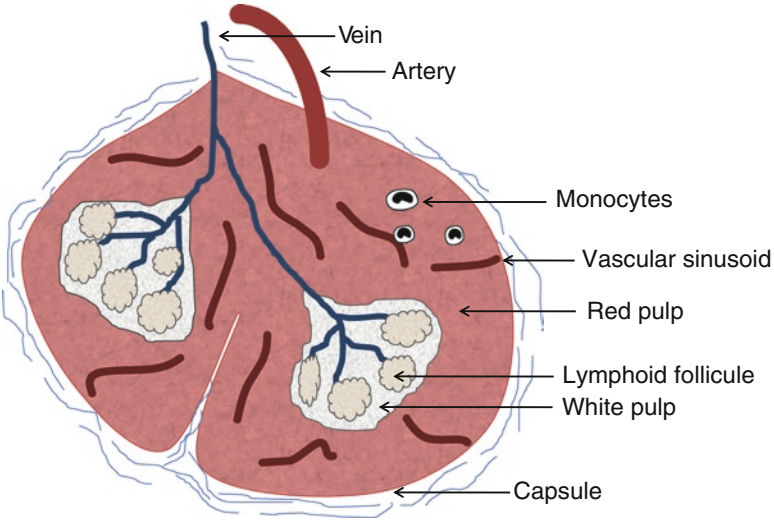


Fig. 4 Representation of the basic structure of the spleen containing red and white pulp

This function takes place in the red pulp of the spleen that comprises the majority of the organ's structural surface area (Fig. 4). The spleen is the largest organ within the lymphatic network and functions to filter blood in a similar manner to the way lymph nodes filter lymph. Blood enters the spleen via the splenic artery that extends into the white pulp and branches into the venous sinuses that remove damaged red cells. Splenic cords extend between venous sinuses that contain reticular connective tissue, macrophages and lymphocytes that act to remove dead cells and foreign material from the blood. The spleen also acts as a reservoir for red cells that can be added to the blood via splenic contractions when needed.

The spleen also has clusters of white pulp that are responsible for producing, storing and exchanging lymphocytes with the blood. Therefore, nanomedicines circulating in the blood can be removed either via macrophages and lymphocytes in both the red and white pulp of the spleen. As an example, the biodistribution of polylysine dendrimers conjugated with both polyethylene glycol and methotrexate display more avid uptake into the spleen than in the liver of athymic nu/nu rats that have a reduced number of T cells (Kaminskas et al. 2009a). Macrophages in the spleen likely act in this instance to compensate for the loss of T cell activity by becoming more phagocytically active. Also, the relative biodistribution of liposomes into liver and spleen varies according to composition, where liposomes composed of egg phosphatidylcholine show roughly equivalent uptake into liver and spleen, whereas liposomes containing phosphatidylserine or dipalmitoylphosphatidylglycerol (DPPC) are more avidly taken up into the spleen (Oussoren and Storm 1997).

2.3 *Lymph Nodes*

The lymphatic system is comprised of a series of capillaries, ducts, nodes and organs including the spleen and thymus, and is involved in the production and release of lymphocytes into the blood. Lymphatic capillaries lie in interstitial spaces along with blood capillaries. They collect excess interstitial fluid, extravasated protein and lipids absorbed from the digestive tract to form a creamy white fluid that flows from peripheral capillaries into lymphatic ducts that finally drain into the thoracic lymph duct and the right lymph duct at the base of the neck. From here, lymph fluid drains directly into the systemic circulation.

The main difference between blood and lymph capillaries is that while blood capillaries have tight junctions between endothelial cells and a fibrous basement membrane that limits the passage of macromolecules and particulate nanomedicines, lymphatic capillaries have wide intercellular junctions and a loose basement membrane. Blood flow is approximately 100–1000 times faster than that of lymph, so uptake of small molecules from the interstitium is preferentially via the blood, whereas the uptake of macromolecules is via the lymph. Prior to emptying into the systemic circulation, lymph fluid filters through lymph nodes placed at strategic positions to remove foreign particles preventing their access to the systemic circulation. The lymph nodes are therefore the main sites of deposition for nanomedicines that are absorbed from the interstitium, either after subcutaneous administration or after extravasation from the blood stream.

Lymph nodes are located at the end of afferent lymph capillaries that enable lymph flow in one direction via valves that are positioned along the entire length of the capillary system (Fig. 5). Lymph nodes contain an outer subcapsular sinus with

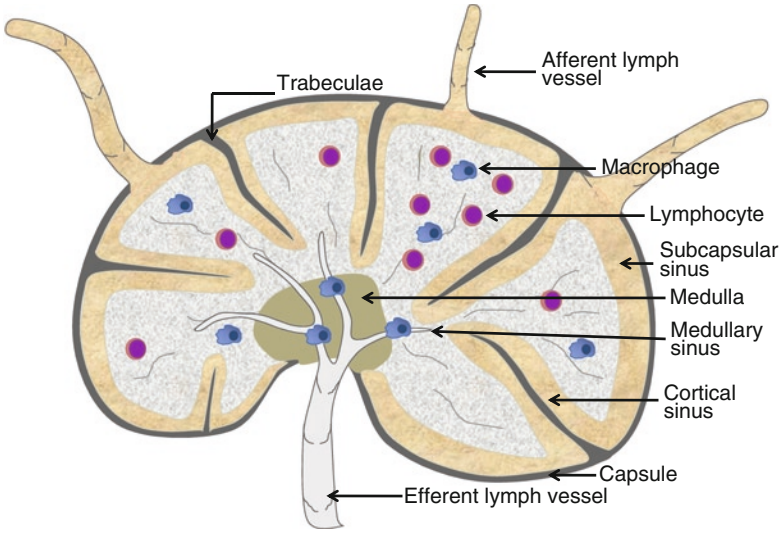


Fig. 5 Diagram of the microstructure of a lymph node

collagenised trabeculae extending deep into the node. Lymph flows from afferent lymph vessels into the subcapsular sinus where it is delivered into deep cortical sinuses that contain a network of reticular fibers, macrophages and lymphocytes that filter pathogens and foreign material from the lymph. Filtered lymph fluid then drains towards the efferent lymph vessel via medullary sinusoids that contain a high density of macrophages that represent the final filtration barrier for pathogens and particulate nanomedicines before lymph is delivered into the systemic circulation. When examining the lymph node localization of PEGylated PAMAM dendrimers after subcutaneous administration the greatest lymph node retention of the dose is in the subcapsular sinus and the medulla (Mounzer et al. 2007).

2.4 The Lungs

As the major internal organ exposed to the air, the lungs are densely populated with macrophages that act to prevent systemic exposure to airborne pathogens and particles. The airways are constructed such that larger airways (bronchi) branch out and thin down to smaller bronchioles. The bronchioles supply air to thin alveolar sacs that are composed of a single layer of alveolar endothelial cells, and a mucosal membrane that lines the inside of the alveolar sacs to prevent the alveolar membranes sticking to each other (Fig. 6). Lymphatic and blood capillaries lie on the outside of the alveoli encased by a membranous cover (the pleura) that encloses the whole organ.

There are two distinct populations of macrophages in the lungs that are located in the alveolar membrane and protrude out into the alveolus and interstitial macrophages that account for approximately half of the macrophages in the lungs. Macrophages resident in the alveolar mucosa act to phagocytose pathogens and

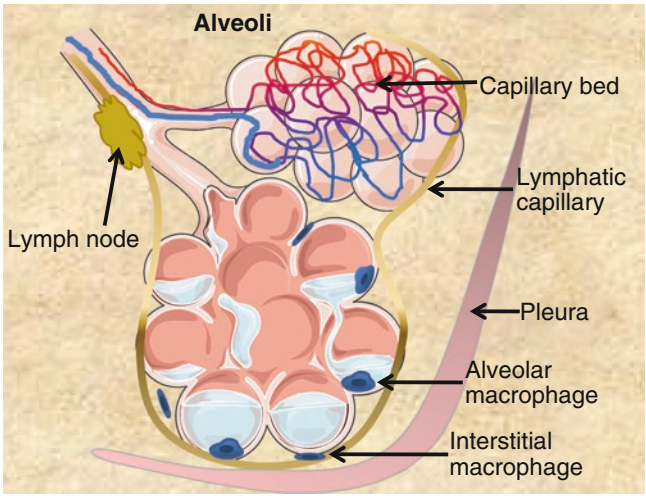


Fig. 6 Diagram of the alveoli and localisation of macrophages

particles that enter via the airways. Alveolar macrophages that trap pathogens then enter into the lymphatic capillaries where they drain into the pulmonary network of lymph nodes (both in the pleura and in the upper airways). Here they are presented to T lymphocytes that facilitate an immune response against the pathogen. The lungs therefore represent an alternate route for the delivery of vaccines that avoids the use of needles since the induction of an immune response in one mucosal membrane transfers immunity to other mucosal membranes. It is also this dense distribution of macrophages that makes the lungs a potential target for the uptake of particulate nanomedicines from the blood. This can be achieved to a certain degree by conjugating surface targeting ligands that make nanoparticles more attractive to alveolar macrophages such as mannose. For instance, the uptake of rifabutin via the lungs after IV administration can be improved by associating the drug with mannosylated lipid nanoparticles that display equivalent biodistribution to the liver and lungs (Nimje et al. 2009).

3 Particle Properties That Mediate Reticuloendothelial Uptake

3.1 Nanoparticles and Colloids That Target the Reticuloendothelial System and the Role of Opsonisation

Since the RES is designed to protect the body against invading pathogens and other foreign materials, any properties of an administered nanomedicine that indicates that the particle or macromolecule is foreign will make it susceptible for RES uptake. Nanomedicines, depending on definition, range in size from 1 to 1,000 nm in diameter. In comparison, the diameter of a virus particle can range from 25 nm to approximately 120 nm and the diameter of a bacterium can range from 200 to 250 μm. Thus, particulate nanomedicines typically have a size that mimics that of a pathogen. This alone does not determine the ultimate fate of a nanomedicine, since particulate nanomedicines have been administered that demonstrate very good biocompatibility and very limited uptake by organs of the RES. The surface characteristics of the material also strongly influence the RES targeting capacity of a nanomedicine, either by promoting opsonisation that essentially tags the particle for removal by macrophages, or by acting as ligands to receptors expressed on the macrophage surface. Receptor mediated processes will be discussed in a later section of this chapter.

It is important, however, to have an understanding of the opsonisation process. The surface of a macrophage is anionic as are the surfaces of many pathogens. This dictates that binding of a pathogen with the macrophage membrane would be unfavourable on purely electrostatic grounds. Thus, prior modification of the pathogen surface is required to enable phagocytosis of these species which would otherwise be repelled from the macrophage surface. Opsonisation is a process whereby a

plasma component coats the surface of a pathogen or target particle and presents it to macrophage cells via receptor mediated recognition of the bound component. Classical plasma opsonins include complement factors, immunoglobulins, mannose binding lectin, fibronectin and β2-glycoprotein 1. Macrophage receptors to these factors include CR1, Fc and mannose receptors. This process is highly species specific. However, in some cases the binding of plasma components does not result in RES targeting, but rather protects the particle from macrophage uptake. Although the identity of these factors is largely unknown, several plasma components have been suggested as potential dysopsonins, including IgA, α1-acidic glycoprotein and albumin. As an example, polystyrene microparticles of approximately 1 μm diameter display avid uptake into dendritic cells in culture in the absence of serum, however uptake into dendritic cells is significantly reduced by preadsorption of human serum albumin (Thiele et al. 2003).

3.2 Effect of Size

In general, the plasma pharmacokinetics of nanomaterials after intravenous administration is largely dependent on their renal clearance. Particles smaller than approximately 20 kDa (or approximately 8 nm in diameter) generally pass through glomerular filtration slits relatively unhindered, enabling ready removal from the body via the urine. Particles that are too large for effective renal clearance are then subjected to alternative clearance processes including metabolism (for biodegradable particles), biliary excretion (after uptake via hepatocytes) and biodistribution into tissues. Very large particles have the capacity to circulate in the bloodstream for extended periods of time on account of their limited capacity for extravasation via fenestrated capillaries or compromised vasculature. The limited extravasation and biodistribution results in their increased presentation to macrophages.

All other physicochemical factors aside, smaller particles have a greater tendency to avoid uptake via the RES, whereas larger particles represent better substrates. This has been demonstrated in detail when comparing the uptake of liposomes or dendrimers into draining lymph nodes after subcutaneous administration. For example, increasing the size of small (<20 nm) dendrimers, results in both increased uptake via the lymph, and increased uptake into draining lymph nodes (Kaminskas et al. 2009b). However in the case of larger liposomes (40–400 nm), increasing size results in slower clearance of the dose from the injection site into the lymphatic system, but an increase in the proportion of the absorbed dose recovered in lymph nodes (Oussoren and Storm 2001). The complexity of examining RES uptake in this way is that retention of nanomedicines in lymph nodes is dependent both on uptake by macrophages as well as physical filtration, both of which have been demonstrated to play a role in the retention of relatively biologically inert particles.

For particles that show relatively limited renal clearance and that do not contain targeting groups intended to direct particles to particular organs, increasing particle size results in long term accumulation in the liver and spleen. This has been

demonstrated for PEGylated dendrimers, although the cellular targets have not been specifically identified (Kaminskas et al. 2008). Thus it cannot be stated with certainty that targeting towards the liver and spleen over time is a result of cumulative uptake via macrophages. Given that increasing dendrimer size beyond approximately generation 7 (>100 kDa) results in a dramatic decrease in blood retention and an increase in uptake via the liver and spleen 15 min after an IV dose (Kobayashi et al. 2001a), it can be suggested that very large particles are recognized more avidly by RES macrophages than smaller ones. For dendrimers, uptake of large particles is preferentially via the liver, whereas for large liposomes uptake of particles larger than approximately 70 nm is preferentially via the spleen (on a percentage of injected dose per gram of tissue basis) (Litzinger et al. 1994). For liposomes based on phosphatidylcholine, although smaller particles show less uptake via the liver and spleen, uptake into these organs is almost entirely via the macrophages (Gabizon et al. 1990). Uptake via the lungs, however, is decreased, likely reflecting either the different function of spleen, liver and lung macrophages or the relative difficulty in accessing macrophages in the interstitium and alveolus from the vascular side.

Increasing the size of polystyrene nanospheres from 60 to 225 nm also resulted in a change in biodistribution from preferential absorption by liver Kupffer cells (no uptake via the hepatocytes or sinusoidal cells was evident) to uptake via macrophages in the red pulp of the spleen (Moghimi 2002).

3.3 Effect of Surface Charge

As discussed above, macrophages have an overall intrinsic anionic charge on their surface that prevents direct adsorption of anionic particles. Although one would assume that this means that cationic particles should be distributed more avidly to macrophages of the RES, this is not necessarily true in the majority of cases. Particles carrying a cationic charge bind with little tissue specificity to all organs and to the blood vasculature on account of ionic interactions with anionic charges present on the glycoproteins present in vascular membranes. Somewhat preferential targeting of a more metabolically stable D-lysine capped poly-L-lysine dendrimer towards the liver, spleen and kidneys was observed in rats, suggesting some capacity of very small nanometer particles to target the RES (Boyd et al. 2006). This was not seen for dendrimers composed entirely of L-lysine as the dendrimer was rapidly degraded to free L-lysine that was subsequently incorporated into protein resynthetic pathways (Boyd et al. 2006). Thus in general, cationic particles have a somewhat limited chance for specific uptake via macrophages in either the RES or elsewhere.

Interestingly, however, increasing the surface cationic charge on liposomes from 0% to 25% surface cationic lipid has no influence on the uptake of the dose in either the liver, spleen or the lungs. At a 1:1 ratio of cationic lipid: uncharged lipid, however, uptake of liposomes via the lungs is increased by almost an order of magnitude, but again, accumulation in the liver and spleen does not change. The reason

for this lung specific accumulation is not clear although a similar pattern of biodistribution has been observed with several cationic nanoparticles, where most of the injected dose is recovered in the lungs, with accumulation in the liver being the second most common fate of particles 10 min after initial IV administration (Al Jamal et al. 2009).

Anionic particles (both lipid and non-lipidic), as discussed previously, are substrates for opsonins and therefore are targeted more avidly towards liver and spleen macrophages. Furthermore, increasing the anionic strength of the particle increases both the degree of opsonisation and the extent of liver targeting. This was demonstrated using arylsulphonate and succinic acid capped polylysine dendrimers (Kaminskas et al. 2007). A generation 4 dendrimer containing 32 surface succinate groups (weakly acidic) did not show any evidence of opsonisation and was excreted via the urine (Fig. 7). A small generation 3 dendrimer containing 16 surface benzene sulphonate groups (stronger anion) showed some evidence of plasma opsonisation and was targeted more avidly towards the liver. Increasing anionic surface charge further by increasing the number of anionic groups to 32 benzene sulphonates on a generation 4 dendrimer resulted in progressively increasing degree of opsonisation plus increased uptake via the liver. The dendrimer with the strongest surface anionic charge (the generation 4 benzene disulphonate dendrimer) also showed a massive increase in uptake via the spleen such that the proportion of the injected dose recovered in the spleen was higher than the proportion of the dose in liver tissue (on a recovered dose per gram of tissue basis). A similar observation is seen with lipid colloids where increasing anionic surface charge increases uptake by the spleen more so than the liver.

3.4 Effect of Particle Hydrophobicity

The hydrophobicity of administered particles has a significant influence on the RES targeting of the system, in particular to the liver. For example, by modifying the structural hydrophobicity of dendrimers, the extent of liver targeting of systems with similar size and surface charge can be altered significantly. An example is a comparison between PAMAM-core dendrimers and more hydrophobic diaminobutane (DAB) – core dendrimers labeled with Gd contrast agent. Intravenous administration of the DAB-core dendrimer resulted in preferential accumulation of the MRI contrast agent in the liver 15 min after dosing, whereas PAMAM dendrimers of the same size were located in the blood vasculature and bladder/kidneys (Kobayashi et al. 2001b) (Fig. 8). Similarly, subcutaneous administration of Gd-DAB dendrimers resulted in better retention and resolution of the contrast agent in draining lymph nodes (Kobayashi et al. 2003, 2006). The reason for this effect may be due to increased capacity for hydrophobic interactions of the particles with plasma proteins, resulting in the recognition of particles as being opsonised by liver Kupffer cells. Another mechanism may be the receptor mediated recognition of more hydrophobic particles by macrophage receptors that recognize low density lipoproteins.

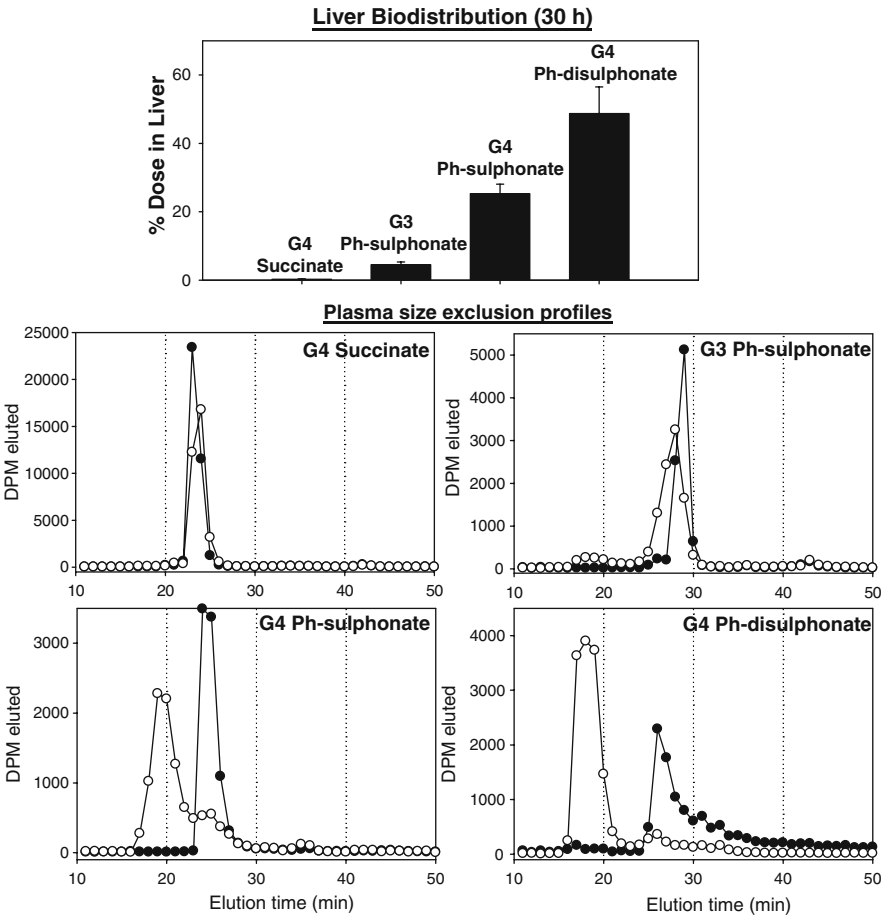


Fig. 7 Proportion of an IV dose of ^{3}H-labelled generation 3 (*G3*) and 4 (*G4*) polylysine dendrimers conjugated with succinic acid (*succinate*), benzene sulphonate (*Ph-sulphonate*) or benzene disulphonate (*Ph-disulphonate*) identified in the liver of rats 30 h after dosing (5 mg/kg, *top panel*) and size exclusion chromatography of each dendrimer diluted in phosphate buffered saline (*closed symbols*) or preincubated in plasma at 37°C for 1 h (*open symbols*). The shift in the ^{3}H-peak to an earlier elution time represents the formation of protein-bound dendrimer in plasma (Data taken from Kaminskas et al. (2007))

3.5 Presentation of Ligands to Organs of the Reticuloendothelial System

The previous discussion has focused mainly on particle properties that result in intrinsic targeting towards macrophages of the RES. However, in some instances macrophage targeting represents a therapeutic aim, rather than a negative consequence of various particle properties. Examples include drug delivery to macrophages involved in microbial or viral propagation (such as HIV infection), cancer

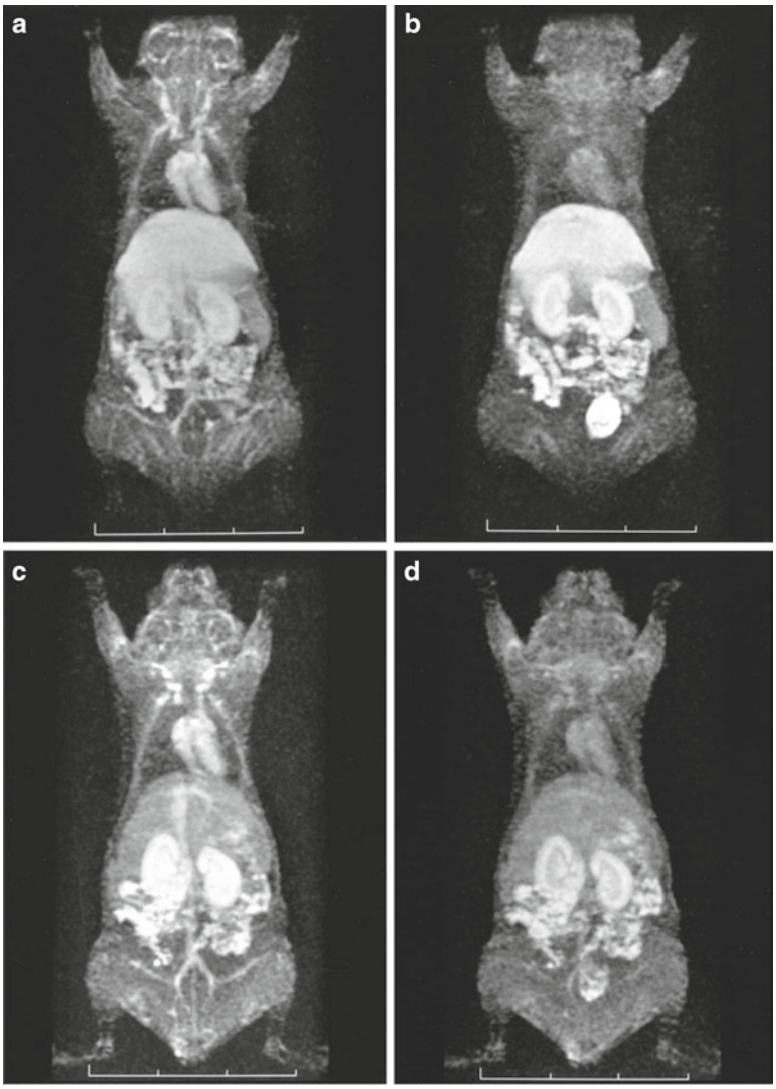


Fig. 8 Whole body 3D-micro MR imaging of mice injected with 0.033 mmol Gd/kg of generation 4 polypropyleneimine diaminobutane dendrimer conjugated with 64 IB4M-Gd (panel **a** and **b**) or generation 4 polyamidoamine dendrimer conjugated with 64 IB4M-Gd (panel **c** and **d**). Panels (**a**) and (**c**) represent imaged taken within 2 min of IV dosing of dendrimer and panels (**b**) and (**d**) represent images taken 10 min after IV injection (Reproduced from Kobayashi et al. (2001b). With permission)

or improved drug delivery to lymph nodes, specific delivery of toxic agents to prevent macrophage activity and delivery of antigens to promote immunization. For instance, Amphotericin B liposomes are used to target macrophage resident fungal infections. Each of these goals requires specific internalization within target

macrophages and avoidance of other cell types. Thus, although targeting to RES macrophages can be achieved by the administration of nanomedicines containing the agent of interest that are readily opsonised and endocytosed, the use of targeting ligands can achieve this effect for non-RES targeted particles that otherwise have been optimized for the proposed delivery.

Macrophage targeting ligands are specifically ligands to receptors that are ether specifically or over-expressed on the surface of the target macrophages. In addition to targeting towards the specific cells that make up the RES, other ligands may be used to enhance the delivery and cellular internalization of materials to other cells within organs of the RES, such as hepatocytes. Galactose is an example of a hepatocyte targeting ligand. This section, however, is focused specifically on the targeted delivery of particles to reticuloendothelial cells (specifically the macrophages). Table 1 summarizes the different ligands and their target receptors that have been used to improve nanomedicine targeting towards macrophages.

Phosphatidylserine is commonly incorporated into liposomes to decrease the surface charge of the colloid or to effect targeting towards macrophages. In mice, targeting of phosphatidylserine liposomes to liver Kupffer cells occurs without the need for prior opsonisation by plasma components (Liu and Liu 1996). However,

Table 1 Ligands used to improve targeting of nanoparticles towards reticuloendothelial organs and macrophages and the target receptors

Ligand	Receptor	Conjugated nanomedicine	References
Phosphatidylserine	TIM-4, stablin-1 & 2, BAI-1 plus others	Liposomes	Liu et al. (1995a)
Mannose/mannan	Mannose receptors (multiple subtypes)	Liposomes, dendrimers, nanoparticles, niosomes, antigens. micelles, metal colloids, emulsions	Nag and Ghosh (1999); Kaur et al. (2008); Vyas et al. (2000); Irache et al. (2008)
RVG peptide	Nicotinic acetylcholine receptor	RVG peptide-siRNA	Kim et al. (2010)
Stromal cell derived factor 1	CXCR4	Peptide-DNA complex	Egorova et al. (2009)
Anti-HLA-DR	HLA-DR	Liposomes	Dufresne et al. (1999)
N-formyl-methionyl leucyl phenylalanine	Formyl peptide receptor 1	Nanoparticle, liposomes	Wan et al. (2008); Morikawa et al. (1988)
Fc region of antibody	Fc receptor	Opsonised nanomedicines	
Decadeoxyguanine	Scavenger receptor class 1	Liposomes	Rensen et al. (2006)
Tuftsin	Tuftsin receptor (uncloned)	Dendrimer, liposomes	Dutta et al. (2008); Agarwal et al. (1994)

in other species, prior opsonisation is often required to optimize uptake of liposomes by Kupffer cells, highlighting a species difference in the mechanism of macrophage targeting. Similarly, after subcutaneous injection of phosphatidylserine containing liposomes into rats the retention of the lymphatically available dose in regional lymph nodes is over 300% of the injected dose per gram, approximately three-fold higher than subcutaneous administration of phosphatidylcholine or phosphatidylglycerol based liposomes (Oussoren and Storm 1997).

By far the most common method of targeting drugs, DNA and cytotoxic agents to the RES is by conjugation of sugars, particularly mannose (mannan, mannose polymer) to the surface. These target the many mannose receptors present on the surface of macrophages and monocytes. Although in general good targeting can be achieved towards many organs of the RES via the use of sugars as targeting ligands, the cellular targets vary widely. This was demonstrated by Kawakami et al. (Kawakami et al. 2000) who prepared liposomes of approximately 90 nm in diameter with 5% galactose-cholesterol, mannose-cholesterol or fructose-cholesterol. Each of these liposomes rapidly distributed to the liver and spleen after IV dosing to mice when compared to control liposomes without sugar, but distribution of these liposomes into parenchymal (hepatocytes) versus non-parenchymal (Kupffer, sinusoidal and stellate) cells was 15.1:1, 0.5:1 and 0.2:1 when compared to control liposomes that were 1:1 (Fig. 9). Hence, the choice of conjugated sugar can dictate both the tissue biodistribution of the nanomedicine and the cellular disposition. Others have reported over tenfold greater accumulation of galactose-conjugated liposomes in hepatocytes than non-parenchymal cells, and seven to ten times greater accumulation of mannose-conjugated liposomes in hepatocytes compared

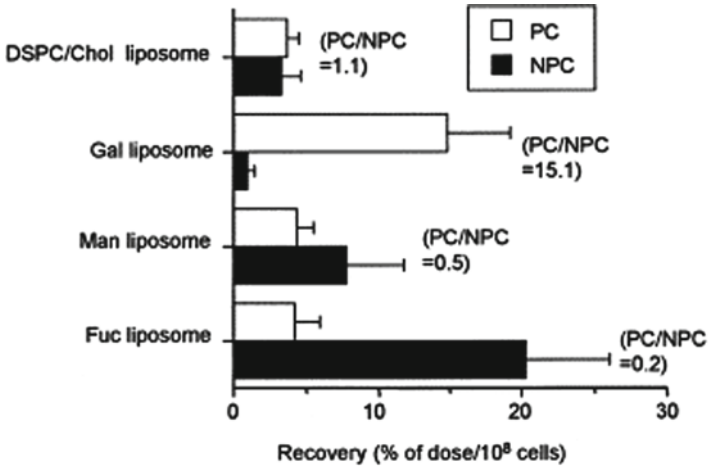


Fig. 9 Hepatocellular localization of ^{3}H CHE from *DSPC/cholesterol liposomes* (85–95 nm diameter) that were glycosylated, galactosylated, mannosylated or fucosylated 30 min after IV administration in mice. Data represent mean ± s.d. (n = 3) in parenchymal cells (*PC*) and non parenchymal cells (*NPC*) in the liver (Reproduced from Kawakami et al. (2000). With permission)

to non-parenchymal cells after IV administration in mice, depending on whether the liposomes were conventional or PEGylated (Nag and Ghosh 1999).

Mannose-conjugated generation 5 polypropyleneimine dendrimers also distribute preferentially and rapidly to the liver and kidneys (due to renal filtration) after IV administration in mice, and to a lesser extent in the spleen and lungs. By contrast, conjugation of lactose to dendrimers increased uptake via the liver and spleen (Agashe et al. 2007). It is therefore clear that galactose promotes cellular targeting towards hepatocytes, whereas mannose specifically targets liver and spleen resident macrophages after intravenous dosing.

Using a mannose targeting approach to improve delivery towards the lungs has been limited in success, however improved delivery of the anti-HIV drug didanosine into alveolar macrophages has been observed for mannosylated gelatin nanoparticles after IV administration in rats (Jain et al. 2008). Similarly, mannose conjugation improved the delivery of the gelatin nanoparticles into the spleen, liver and lymph nodes. Mannose conjugation has also been demonstrated to improve the delivery of the anti-HIV drug zidovudine entrapped within liposomes, to draining lymph nodes when compared to administration of the drug in non-targeted liposomes by subcutaneous administration (Kaur et al. 2008). In this respect, subcutaneous administration of mannose-targeted nanomedicines is expected to improve targeting and cellular internalization in both lymph node resident macrophages and in the lymphatic endothelium that similarly express mannose receptors that mediate lymphocyte and cancer cell trafficking.

4 Avoiding Reticuloendothelial Targeting

4.1 Reducing Surface Anionic Charge

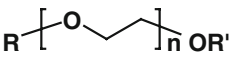
As demonstrated previously, reduction in the extent and strength of surface anionic charge results in reduced particle opsonisation and subsequent uptake via the liver and spleen. This can be achieved either by changing the surface materials (either by incorporating less anionic lipids into colloid membranes or changing the surface functionality on nanoparticles) or by masking surface anionic charges. Lipids such as phosphatidylserine have a permanent anionic charge and incorporation of increasing amounts of these lipids into colloidal membranes increases the magnitude of the negative zeta potential and increases RES targeting. Alternatively, anionic groups may be masked by the inclusion of long, uncharged polymers into the particle surface. A classical example of this is the use of polyethylene glycol that coils around the particle surface, masking surface charge and neutralizing zeta potential. Since anionic charges are prone to recognition by plasma opsonins, a greater load of PEG (i.e., higher MW PEG) is needed to increase plasma residence and decrease RES targeting than for cationic particles. In theory, however, any uncharged polymer that can coat the particle surface has the potential to mask surface charges.

4.2 PEGylation

Polyethylene glycol (Fig. 10) was identified in the 1970s to be a highly biocompatible polymer. Since then, its use in improving the pharmacokinetic and biodistribution properties of nanomedicines has become widespread. The structure is composed of multiple repeating units of ethylene glycol that complex many water molecules resulting in the biological 'masking' properties of the polymer. The polymer is also relatively non-toxic; the administration of several grams per kg of body weight is required before toxicity becomes evident. Although it is slowly metabolized in the body, metabolism of PEG is not considered a major mechanism in the clearance of conjugated nanomedicines. However, for very large, long circulating systems, gradual metabolism of PEG chains with a resulting reduction in particle size and exposure of surface groups may eventually alter the biodistribution of the particles.

In the pharmaceutical industry, PEGylation has been used to increase the circulation times of proteins and liposomes, reduce RES uptake by liposomes and reduce the susceptibility of proteins to enzymatic degradation. Although PEGylation of proteins masks key receptor binding sites and therefore decreases *in vitro* activity compared to the native protein, *in vivo* therapeutic efficacy is generally increased as a result of the increased exposure of target receptors to the protein due to improved residence time in circulation. PEGylation works in a similar manner to mask biologically incompatible surface properties on nanomedicines, thus reducing both opsonisation and uptake via macrophages of the RES.

Although increasing particle size generally acts to increase RES targeting, conjugation of PEG, resulting in increased particle molecular weight and hydrodynamic size, does not have a similar effect. Increasing the size of conjugated nanomedicines via PEGylation instead acts to increase circulation times and reduce excretion via the urine. Increased eventual uptake by the RES (in particular the liver and spleen) for large PEGylated materials is more a consequence of increased exposure to macrophages that slowly phagocytose the material rather than increased uptake of larger materials. This is demonstrated by data showing that increasing the extent of PEGylation on polylysine dendrimers (resulting in increasing dendrimer sizes due to increased PEG molecular weight) results in slow uptake of the particles by the liver and spleen, such that approximately 12% of the injected dose of 68 kDa dendrimers with terminal plasma half lives of 3 days are recovered in the liver and spleen after 1 week (Kaminskas et al. 2008).



R - nanoparticle
R' - H (hydroxy PEG)
CH_3 (methoxy PEG)
Functional group attachment point

Fig. 10 Polyethylene glycol

4.3 Alternatives to PEGylation

In addition to PEG, several other materials have been used to improve plasma circulation times and avoid reticuloendothelial uptake of liposomes, including monosialoganglioside GM1, hydrogenated phosphatidylinositol and glucuronide conjugates. Studies in mice and *in vitro* in isolated murine bone marrow macrophages have demonstrated that the inclusion of only 5% GM1 into the liposome structure has the capacity to significantly reduce uptake of the liposome into RES organs (Allen et al. 1991a, b). This is reportedly due to the capacity of GM1 to prevent opsonisation of the liposome. *In vitro*, as the molar ratio of GM1 increases, so too does uptake of the liposomes by macrophages (Allen et al. 1991a). *In vivo*, uptake into the liver and spleen over 24 h can be halved when compared to liposomes of similar composition (Allen et al. 1991b). At molar ratios of GM1 or PEG in the range 5–10, however, PEG appears to be superior to GM1 *in vitro* at reducing the macrophage uptake of liposomes, although interestingly, this is not reflected by *in vivo* observations that show equivalent RES avoiding capacity (Allen et al. 1991a, b).

Although hydrogenated phosphatidylinositol (HPI) has not been widely incorporated into the design of long circulating liposomes, some research has been conducted by Alberto Gabizon who has shown in general that increasing the proportion of HPI into the liposome structure increases blood retention and decreases uptake by the liver and spleen up to a molar ratio of 41% HPI which results in increased uptake by the liver with a concomitant decrease in uptake by the spleen on account of the massive increase in anionic charge (Gabizon and Papahadjopoulos 1992). Thus, on account of the long circulating behavior of HPI-liposomes, Gabizon has demonstrated improved tumour biodistribution and anti-tumour efficacy of chemotherapeutic drug loaded liposomes composed of approximately 5 mol% of HPI in tumour bearing mice (Gabizon 1992; Gabizon et al. 1989; Gabizon and Papahadjopoulos 1988).

The glucuronide conjugate palmityl-D-glucuronide has demonstrated some capacity to improve the blood circulation time of liposomes in mice by reducing liver uptake, however it also increases the proportion of a dose taken up by the spleen by approximately 15%, 12 h after IV dosing (Oku et al. 1992). On account of the increased plasma blood exposure, tumour accumulation of modified liposomes is increased when compared to unmodified liposomes. However, this effect of the glucuronide is only seen in mice, since administration of palmityl-D-glucuronide modified liposomes in rats results in an increase in blood clearance and an increase in uptake by the liver and spleen, presumably via the action of complement factors (Liu et al. 1995b).

4.4 Choice of Lipids for Construction of Colloids

As described in the previous sections, certain structural features of a nanoparticle can render it a target for uptake by reticuloendothelial organs. This applies both to surface and structural features. Although a number of surface modifications have

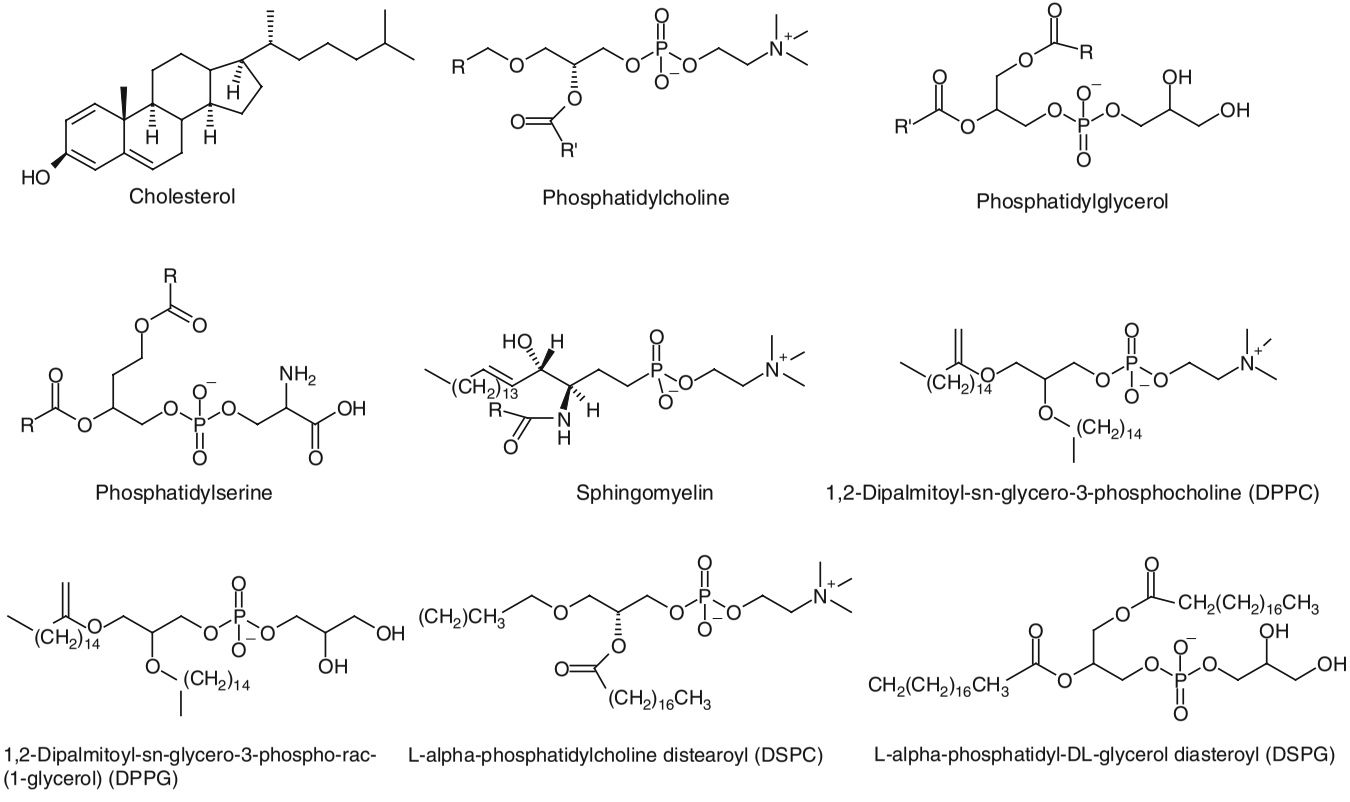


Fig. 11 Structure of lipids commonly used to formulate colloidal nanoparticles

proven useful in reducing the uptake of a variety of nanoparticles by the RES organs, the appropriate choice of lipid in the construction of the particle can similarly minimize or indeed increase the extent of RES targeting. This is particularly relevant for intravenous delivery systems that require extended plasma circulation times and subcutaneous delivery systems that may be used to improve drug uptake into the lymphatic system or specifically into regional lymph nodes. Much of the literature surrounding the influence of lipid composition on the affinity of colloids for the RES is based on liposomes. Hence, Fig. 11 depicts some of the lipids commonly used to generate liposomes.

As mentioned previously, the biodistribution of liposomes to the liver and spleen can be increased via the incorporation of phosphatidylserine into the lipid bilayer that increases the uptake of liposomes via fixed macrophages (Oussoren and Storm 1997). However, liposomes based entirely on DPPC also display avid uptake into both lymph nodes and the spleen after subcutaneous administration, even though most of the dose remains at the injection site after more than 2 days (Oussoren and Storm 1997). This effect is abrogated via the concurrent use of DPPC and cholesterol (Oussoren and Storm 1997). In general, however, increased plasma circulation times and minimized RES uptake appear to be achieved when using lipid compositions that include phosphatidylcholine, phosphatidylglycerol and sphingomyelin, although in each situation, RES uptake is generally best minimized by the incorporation of a hydrophilic or alternate biocompatible polymer into the outer surface (Oussoren and Storm 1997; Spanjer et al. 1986).

5 Summary

In the majority of applications of nanomedicines, extended blood circulation times are required. Consequently RES uptake is a major drawback in the use of nanoparticles as drug delivery systems, and increased uptake by the RES organs has the potential to be associated with increased delivery of drug to organs that may be sensitive to the toxic effects of the drug. As a general rule, the more ‘foreign’ the particle looks the greater the potential for RES uptake. Examples include increased particle surface charge (that may mediate particle opsonisation or increased adhesion to the surface of macrophages and other tissues), increased size and the use of structural materials that promote receptor mediated recognition of the particle by one or more cells within the RES. The flip side to this rule is in instances where improved nanoparticle targeting to particular cells within the RES, particularly macrophages, are desired. Examples of these are in the improved delivery of antigens to immune cells in order to enhance the immunization process or where delivery of antiretrovirals to macrophages may improve the resistance of the cells to viral attack or replication. Examples of such modifications have been given. However, where RES uptake is not desired, steps can be taken to minimize the susceptibility of nanoparticle systems for uptake via these organs. These include

reducing surface charge and size or incorporating polymers and other functions into the surface of nanoparticles to improve their biocompatibility. To date, the most widely utilized method of avoiding RES uptake of nanoparticles is via the attachment of PEG chains onto the surface. However, several other systems are showing promise as alternatives to the use of PEG.

References

A. Agarwal, H. Kandpal, H.P. Gupta, N.B. Singh, C.M. Gupta, Tuftsin-bearing liposomes as rifampin vehicles in treatment of tuberculosis in mice, Antimicrobial Agents and Chemotherapy, 38 (1994) 588–593.

H.B. Agashe, A.K. Babbar, S. Jain, R.K. Sharma, A.K. Mishra, A. Asthana, M. Garg, T. Dutta, N.K. Jain, Investigations on biodiostribution of technetium-99 m-labelled carbohydrate-coated poly (propylene imine) dendrimers, Nanomedicine: Nanotechnology, Biology and Medicine, 3 (2007) 120–127.

W.T. Al Jamal, K.T. Al Jamal, A. Cakebread, J.M. Halket, K. Kostarelos, Blood circulation and tissue biodistribution of lipid-quantum dot (L-QD) hybrid vesicles intravenously administered in mice, Bioconjugate Chemistry, 20 (2009) 1696–1702.

T.M. Allen, G.A. Austin, A. Chonn, L. Lin, K.C. Lee, Uptake of liposomes by cultured mouse bone marrow macrophages: influence of liposome composition and size, Biochimica et Biophysica Acta, 1061 (1991a) 56–64.

T.M. Allen, C. Hansen, F. Martin, C. Redemann, A. Yau-Young, Liposomes containing synthetic lipid derivatives of poly(ethylene glycol) show prolonged circulation half lives in vivo, Biochimica et Biophysica Acta, 1066 (1991b) 29–36.

B.J. Boyd, L.M. Kaminskas, P. Karellas, G. Krippner, R. Lessene, C.J.H. Porter, Cationic poly-L-lysine dendrimers: Pharmacokinetics, biodistribution and evidence for metabolism and bioresorption after intravenous administration in rats., Molecular Pharmaceutics, 3 (2006) 614–627.

I. Dufresne, A. Desormeaux, J. Bestman-Smith, P. Gourde, M.J. Tremblay, M.G. Bergeron, Targeting lymph nodes with liposome bearing anti-HLA-DR-Fab' fragments, Biochimica et Biophysica Acta, 1421 (1999) 284–294.

T. Dutta, M. Garg, N.K. Jain, targeting of efavirenz loaded tuftsin conjugated poly(propyleneimine) dendrimers to HIV infected macrophages in vitro, European Journal of Pharmaceutical Sciences, 34 (2008) 181–189.

A. Egorova, A. Kiselev, M. Hakli, M. Ruponen, V. Baranov, A. Urtti, Chemokine-derived peptides as carriers for gene delivery to CXCR4 expressing cells, Journal of Gene Medicine, 11 (2009) 772–781.

A.A. Gabizon, Selective tumor localization and improved therapeutic index of anthracyclines encapsulated in long-circulating liposomes, Cancer Research, 52 (1992) 891–896.

A. Gabizon, D. Papahadjopoulos, Liposome frmulations with prolonged circulation time in blood and enhanced uptake by tumors, Proceedings of the National Academy of Science USA, 85 (1988) 6949–6953.

A. Gabizon, D. Papahadjopoulos, The role of surface charge and hydrophilic groups on liposome clearance in vivo, Biochimica et Biophysica Acta, 1103 (1992) 94–100.

A. Gabizon, R. Shiota, D. Papahadjopoulos, Pharmacokinetics and tissue distribution of doxorubicin encapsulated in stable liposomes with long circulation times, Journal of the National Cancer Institute, 81 (1989) 1484–1488.

A. Gabizon, D.C. Price, J. Huberty, R.S. Bresalier, D. Papahadjopoulos, Effect of liposome composition and other factors on the targeting of liposomes to experimental tumors: biodistribution and imaging studies, Cancer Research, 50 (1990) 6371–6378.

J.M. Irache, H.H. Salman, C. Gamazo, S. Espuelas, Mannose-targeted systems for the delivery of therapeutics, Expert Opinion in Drug Delivery, 5 (2008) 703–724.

S.K. Jain, Y. Gupta, A. Jain, A.R. Saxena, P. Khare, A. Jain, Mannosylated gelatin nanoparticles bearing an anti-HIV drug didanosine for site-specific delivery, Nanomedicine, 4 (2008) 41–48.

L.M. Kaminskas, B.J. Boyd, P. Karellas, S.A. Henderson, M.P. Giannis, G. Krippner, C.J. Porter, Impact of surface derivatisation of poly-L-lysine dendrimers with anionic arylsulphonate or succinate groups on intravenous pharmacokinetics and disposition, Molecular Pharmaceutics, 4 (2007) 949–961.

L.M. Kaminskas, B.J. Boyd, P. Karellas, G.Y. Krippner, R. Lessene, B. Kelly, C.J.H. Porter, The impact of molecular weight and PEG chain length on the systemic pharmacokinetics of PEGylated poly-L-lysine dendrimers, Molecular Pharmaceutics, 5 (2008) 449–463.

L.M. Kaminskas, B. Kelly, V. McLeod, B.J. Boyd, G.Y. Krippner, E.D. Williams, C.J.H. Porter, Pharmacokinetics and tumour disposition of PEGylated methotrexate conjugated poly-L-lysine dendrimers, Molecular Pharmaceutics, 6 (2009a) 1190–1204.

L.M. Kaminskas, J. Kota, V.M. McLeod, B.D. Kelly, P. Karellas, C.J.H. Porter, PEGylation of polylysine dendrimers improves absorption and lymphatic targeting following SC administration in rats, Journal of Controlled Release, 140 (2009b) 108–116.

C.D. Kaur, M.H. Nahar, N.K. Jain, Lymphatic targeting of zidovudine using surface-engineered liposomes, Journal of Drug Targeting, 16 (2008) 798–805.

S. Kawakami, J. Wong, A. Sato, Y. Hattori, F. Yamashita, M. Hashida, Biodistribution characteristics of mannosylated, fucosylated and galactosylated liposomes in mice, Biochimica et Biophysica Acta, 1524 (2000) 258–265.

S.S. Kim, C. Ye, P. Kumar, I. Chiu, S. Subramanya, P. Shankar, N. Manjunath, Targeted delivery of siRNA to macrophages for anti-inflammatory treatment, Molecular Therapeutics, 18 (2010) 993–1001.

H. Kobayashi, S. Kawamoto, T. Saga, N. Sato, A. Hiraga, J. Konishi, K. Togashi, M.W. Brechbiel, Micro-MR angiography of normal and intratumoral vessels in mice using dedicated intravascular MR contrast agents with high generation of polyamidoamine dendrimer core: reference to pharmacokinetic properties of dendrimer-based MR contrast agents, Journal of Magnetic Resonance Imaging, 14 (2001a) 705–713.

H. Kobayashi, S. Kawamoto, T. Saga, N. Sato, A. Hiraga, T. Ishimori, Y. Akita, M.H. Mamede, J. Konishi, K. Togashi, Novel liver macromolecular MR contrast agent with a polypropylenimine diaminobutyl dendrimer core: Comparison to the vascular MR contrast agent with the polyamidoamine dendrimer core, Magnetic Resonance in Medicine, 46 (2001b) 795–802.

H. Kobayashi, S. Kawamoto, P.L. Choyke, N. Sato, M.V. Knopp, R.A. Star, T.A. Waldmann, Y. Tagaya, M.W. Brechbiel, Comparison of dendrimer-based macromolecular contrast agents for dynamic micro-magnetic resonance lymphangiography, Magnetic Resonance in Medicine, 50 (2003) 758–766.

H. Kobayashi, S. Kawamoto, M. Bernardo, M.W. Brechbiel, M.V. Knopp, P.L. Choyke, Delivery of gadolinium-labeled nanoparticles to the sentinel lymph node: comparison of the sentinel node visualization and estimations of intra-nodal gadolinium concentration by the magnetic resonance imaging, Journal of Controlled Release, 111 (2006) 343–351.

D.C. Litzinger, A.M. Buiting, N. Van Rooijen, L. Huang, Effect of liposome size on the circulation time and intraorgan distribution of amphipathic poly(ethylene glycol)-containing liposomes, Biochimica et Biophysica Acta, 1190 (1994) 99–107.

F. Liu, D. Liu, Serum independent liposome uptake by mouse liver, Biochimica et Biophysica Acta, 1278 (1996) 5–11.

D. Liu, F. Liu, Y.K. Song, Recognition and clearance of liposomes containing phosphatidylserine are mediated by serum opsonin, Biochimica et Biophysica Acta, 1235 (1995a) 140–146.

D. Liu, F. Liu, Y.K. Song, Monosialoganglioside GM1 shortens the blood circulation of liposomes in rats, Pharmaceutical Research, 12 (1995b) 508–512.

S.M. Moghimi, Chemical camouflage of nanospheres with a poorly reactive surface: towards development of stealth and target-specific nanocarriers, Biochimica et Biophysica Acta, 1590 (2002) 131–139.

K. Morikawa, R. Nayar, I.J. Fidler, In vitro activation of tumoricidal properties in mouse macrophages using the chemotactic peptide N-formyl-methionyl-phenylalanine (FMLP) incorporated in liposomes, Cancer Immunology and Immunotherapy, 27 (1988) 1–6.

R. Mounzer, P. Shakarin, X. Papademetris, T. Constable, N.H. Ruddle, T.M. Fahmy, Dynamic imaging of lymphatic vessles and lymph nodes using a bimodal nanoparticulate contrast agent, Lymphatic Research and Biology, 5 (2007) 151–158.

A. Nag, P.C. Ghosh, Assessment of targeting potential of galactosylated and mannosylated sterically stabilized liposomes to different cell types of mouse liver, Journal of Drug Targeting, 6 (1999) 427–438.

N. Nimje, A. Agarwal, G.K. Saraogi, N. Lariya, G. Rai, H. Agarwal, G.P. Agarwal, Mannosylated nanoparticulate carriers of rifabutin for alveolar targeting, Journal of Drug Targeting, 17 (2009) 777–787.

N. Oku, Y. Namba, S. Okada, Tumor accumulation of novel RES-avoiding liposomes, Biochimica et Biophysica Acta, 1126 (1992) 255–260.

C. Oussoren, G. Storm, Lymphatic uptake and biodistributions of liposomes after subcutaneous injection: III. influence of surface modification with poly(ethyleneglycol), Pharmaceutical Research, 14 (1997) 1479–1484.

C. Oussoren, G. Storm, Liposomes to target the lymphatics by subcutaneous administration, Advanced Drug Delivery Reviews, 50 (2001) 143–156.

P.C. Rensen, J.C. Gras, E.K. Lindfors, K.W. van Dijk, J.W. Jukema, T.J. van Berkel, E.A. Biessen, Selective targeting of liposomes to macrophages using a ligand with high affinity for the macrophage scavenger receptor class A, Current Drug Discovery Technologies, 3 (2006) 135–144.

H.H. Spanjer, M. van Galen, F.H. Roerdink, J. Regts, G.L. Scherphof, Intrahepatic distribution of small unilamellar liposomes as a function of liposome lipid composition, Biochimica et Biophysica Acta, 863 (1986) 224–230.

L. Thiele, J.E. Deiederichs, R. Reszka, H.P. Merkle, E. Walter, Competitive adsorption of serum proteins at microparticles affects phagocytosis by dendritic cells, Biomaterials, 24 (2003) 1409–1418.

S.P. Vyas, Y.K. Katare, V. Mishra, V. Sihorkar, Ligand directed macrophage targeting of amphotericin B loaded liposomes, International Journal of Pharmaceutics, 210 (2000) 1–14.

L. Wan, X. Zhang, S. Pooyan, M.S. Palombo, M.J. Leibowitz, S. Stein, P.J. Sinko, Optimizing size and copy number for PEG-fMLF (N-formyl-methionyl-leucyl-phenylalanine) nanocarrier uptake by macrophages, Bioconjugate Chemistry, 19 (2008) 28–38.