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Factors Controlling Pharmacokinetics of Intravenously Injected Nanoparticulate Systems

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Introduction

Particulate nanosystems, such as liposomes, polymeric micelles, and nanospheres have long been used for site-specific delivery of therapeutic and diagnostic agents following intravenous injection (Moghimi et al., 2005a). Additionally, there is a catalogue of nanoparticulate entities exhibiting unique physical and chemical properties, such as high rigidity, high thermal and electrical conductivity, and superparamagnetism, which have applications in experimental imaging, cell ablation, and even drug delivery following introduction into the vasculature (Moghimi et al., 2005a; Moghimi & Kissel, 2006). Examples include semiconductive single- and multi-walled carbon nanotubes (SWNT and MWNT, respectively) and iron/iron oxide core–shell nanoclusters (Klumpp et al., 2006; Qiang et al., 2006).

The biological performance of intravenously injected nanoparticles is controlled by a complex array of physicochemical and physiopathological factors (Moghimi et al., 2001, 2005a, 2006c). Physicochemical considerations include nanoparticle size distribution, shape, density, rigidity or deformability, and surface characteristics (e.g. surface electric charge, surface density, and conformation of adsorbed or grafted synthetic polymers and biological ligands). These factors not only control the flow properties of nanoparticles within the blood vessels and at bifurcations in vascular and capillary systems, but also modulate nanoparticle circulation times, tissue deposition patterns, mode of entry into cells (as in *Rho*-dependent phagocytosis, clathrin-mediated endocytosis, internalization through membrane rafts, and uptake mechanisms independent of phagocytosis, clathrin, and caveolae), intracellular trafficking, contents release, and toxicity (Andresen et al., 2004; Bhatia et al., 2003; Decuzzi & Ferrari, 2006; Harush-Frenkel et al., 2007; Lovrić et al., 2005; Moghimi et al., 2001, 2004, 2005a, 2006c; Patil et al., 2001; Poznansky & Juliano, 1984). For instance, oblate ellipsoidal particles have been proposed to adhere more effectively to the biological substrates than their corresponding classical spherical particles of the same volume (Decuzzi & Ferrari, 2006). In addition, non-spherical nanoparticles can carry more drugs and

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contrast agents than their corresponding spherical particles of the same adhesive strength, which may improve therapeutic and diagnostic efficacy.

Biological considerations that control nanoparticle circulation times in the blood and tissue distribution include determinants of phagocytic/endocytic recognition and ingestion, the "state-of-responsiveness" of the host immune system, and escape routes from the vasculature (Moghimi, 2003; Moghimi et al., 2001, 2005a).

A clear understanding of the above-mentioned events is crucial for optimization and development of complex multi-functional nanoparticles for investigative, diagnostic, and therapeutic needs. This article briefly examines the interplay between physicochemical and biological factors that control nanoparticle pharmacokinetics following intravenous route of injection.

Protein Adsorption and Opsonization Events

Numerous studies have confirmed that, following contact with the blood, nanoparticles acquire a coating of plasma proteins (Chonn et al., 1992, 1995; and reviewed in Moghimi et al., 2001; Moghimi & Szebeni, 2003). This coating differs considerably in amount and in pattern depending on the dose, the physicochemical characteristics of the nanoparticles, and the exposure time. The protein coating has a number of important ramifications for pharmacokinetics and tissue distribution of nanoparticles. One aspect concerns the extent of nanoparticle aggregation and trapping in the first capillary bed encountered. Another consequence of nanoparticle–protein interaction is opsonization that is the adsorption of those plasma proteins subsequently capable of interacting with their receptors expressed by macrophages in contact with the blood (Moghimi et al., 2001; Moghimi & Szebeni, 2003). Examples of opsonic proteins include various subclasses of immunoglobulins, certain components of the complement system (e.g. C1q, C3b, iC3b), fibronectin, C-reactive protein, lipopolysaccharide-binding protein, and von Willebrand factor. Non-specific protein adsorption may also play an important role in particle clearance. Following adsorption, non-specific proteins could undergo conformational changes and expose chemical structures that could either be recognized directly by macrophage receptors or could act as a template for subsequent recognition by opsonic proteins.

The largest population of macrophages in contact with blood is located in the liver sinuses (Kupffer cells). Therefore, it is not surprising to see that after intravenous injection opsonized particles are rapidly, and predominantly, intercepted by Kupffer cells. In addition, nanoparticle clearance from the blood by splenic marginal zone and red-pulp macrophages, persinal phagocytes of the bone marrow, and blood monocytes also occurs to some extent. Thus, following intravenous injection of drug-encapsulated nanoparticles, drug exposure becomes limited to lysosomal and cytoplasmic compartments of macrophages, and is advantageous for treatment of disease and disorders of the reticuloendothelial system (Moghimi, 2003; Moghimi et al., 2005a). Macrophage recognition and clearance of nanoparticles from the blood can be further accelerated by surface attachment of substrates/ligands for macrophage scavenger, integrin, dectin,

galactose/fucose, mannose, and Toll-like receptors. Here, associated signalling events will be different, depending on the target receptor.

Blood monocytes can also migrate out of the circulation into various locations such as lungs and the brain, and differentiate into macrophages. The specific targeting of one or more immunomodulators to monocytes could, therefore, significantly enhance host resistance against cancer in specific locations; for instance, the lungs are a major site of disseminated metastatic disease, and an organ that is not rich in reticuloendothelial activity.

Differential Opsonization and Macrophage Heterogeneity

Of particular interest is the concept of "differential opsonization", which may account for the observed differences in clearance rates and phagocyte sequestration of nanoparticles and vesicles (Moghimi et al., 2001, 2005a; Moghimi & Hunter, 2001; Yan et al., 2004). For example, certain liposomes of 200–500 nm in size are more efficient in activating the complement system, and hence are cleared faster from the blood than their smaller counterparts (50 nm) (Devine et al., 1994). This is a reflection of the surface dynamic and geometric effects on the assembly of proteins and proteases involved in complement activation. The extent and the mode of complement activation by liposomes also depend on vesicular lipid composition, bilayer packing, and surface characteristics (Moghimi & Hunter, 2001). For instance, in the absence of anti-phospholipid and anti-cholesterol antibodies, liposomes containing anionic phospholipids (e.g. cardiolipin and phoshpatidylglycerol) in their bilayer interact with C1q, a process that leads to activation of the classical pathway of the complement system in rats and humans and fixation of complement opsonic C3 fragments (Marjan et al., 1994). On the other hand, cationic liposomes tend to activate the human complement system via the alternative pathway. In contrast to charged lipids, liposomes composed of zwitterionic egg phosphatidylcholine seem to activate complement only after prolonged exposure to serum, presumably via C-reactive protein-binding pathway (Volanakis & Wirtz, 1979). Elevated levels of plasma lipoproteins can also modulate the extent of complement activation. For example, we (Moghimi et al., 2006b) have shown that elevated levels of serum LDL and HDL can significantly suppress cholesterol-rich liposome-mediated complement activation, Figure 9.1.

Depending on surface characteristics of nanoparticles, non-specific adsorption of intact C3 may initiate complement activation in the presence of factors B and D (Moghimi & Szebeni, 2003). Activation of the alternative pathway may even involve antibodies via their F(ab) portion (Moore et al., 1982), the binding of which to C3b is most likely to depend on a two antigenic subsite-fixed orientation of IgG.

Hydrophobic entities such as carbon nanotubes also activate complement, but the activation process is dependent on nanotube morophology. Both hydrophobic SWNTs and MWNTs activate the human complement system via the classical pathway through surface adsorption of C1q, but DWNTs further activate complement through the alternative pathway (Salvador-Morales et al., 2006). In addition, binding of other plasma proteins to carbon nanotubes is a highly selective process; remarkably, there is no IgG, IgM, pentraxin, and fibronectin binding, but protein

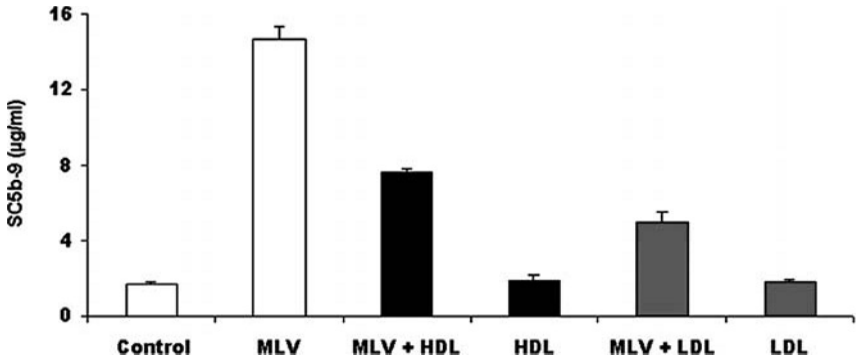

Figure 9.1 The effect of elevated levels of HDL and LDL in human serum on liposome-mediated complement activation. Multilamellar vesicles (MLV) were composed of dimyristoylphosphatidylcholine, dimyristoylphosphatidylglycerol, and cholesterol (50:5:45 mole ratios). The final concentration of liposomes in serum was 5 mg/mL. The cholesterol and triglyceride levels of the serum were normal, and approximately 180 and 200 mg/dL, respectively. The addition of purified HDL to serum increased cholesterol levels by 25–30%. The addition of purified LDL doubled serum cholesterol levels, a situation similar to that encountered in heterozygous familial hypercholesterolemia. Liposome-mediated complement activation, through both calcium-sensitive and alternative pathways, in undiluted human serum was monitored by measuring the production of the S-protein-bound form of the terminal complex, SC5b-9, using an enzyme-linked immunosorbent assay kit (Moghimi et al., 2006b).

adsorption is exclusive to apolipoprotein AI, AIV, C-III, albumin, and fibrinogen. These observations are also in line with rapid Kupffer cell deposition of intravenously injected carbon nanotubes of 1 nm diameter and 300 nm length (Cherukuri et al., 2006). Longer nanotubes (spanning from several to tens of micrometer in length), however, may be too large to be phagocytosed, and as a result of complement activation, inflammatory responses and granuloma formation may follow.

The concept of differential opsonization is also important as macrophages are heterogeneous with respect to physiological function and phenotype, even within the same organ (Moghimi, 2003; Moghimi et al., 2001). Therefore, some populations of macrophages may utilize one particular predominant recognition mechanism. In relation to this statement, a recent study has demonstrated the predominant localization of intravenously injected liposomes, which were surface modified with the anionic 1,5-dihexadecyl-L-glutamate-*N*-succinic acid, to persinal macrophages of the rabbit bone marrow (Sou et al., 2006). The dynamic and differential processes of protein/opsonin binding to nanoparticles could even indicate an arrangement based on a recognition hierarchy. Thus a specific macrophage receptor might recognize the earliest changes associated with a particle surface, whereas other receptors might recognize particles at a later stage, ensuring their complete removal from the systemic circulation.

The Role of Protein Adsorption on Particle Binding to Non-phagocytic Cells

Adsorption of blood proteins on to nanoparticles may even affect nanoparticle binding to and clearance by non-phagocytic cells. For instance,

following complement activation and binding of C3b, C4b, or C3b breakdown products iC3b and C3d, nanoparticles may further interact with platelets and erythrocytes. In rats, platelets express type I complement receptors (CR1) that bind C3b-opsonized liposomes (Loughrey et al., 1990), whereas in primates erythrocytes express CR1 (Cornacoff et al., 1983), which could play a critical role in pharmacokinetic of certain C3b-opsonized nanoparticles. Another example is surface enrichment of polysorbate-80-coated nanospheres within the blood by apolipoproteins-B and E, resulting in some nanoparticle recognition by the LDL receptors of the rat blood–brain barrier endothelial cells (Kreuter et al., 2003). Apolipoprotein-E was also shown to play an exclusive role in recognition of electrically neutral liposomes by murine hepatocytes (Scherphof & Kamps, 2001; Yan et al., 2005).

Suppression of Opsonization Events

The surface of nanoparticles, nanotubes, and even viruses can be manipulated with a wide range of polymeric materials that are capable of generating a hydrophilic steric barrier (Liu et al., 2006; reviewed in Moghimi, 2006; Moghimi et al., 2001, 2005a; Moghimi & Szebeni, 2003; Romberg et al., 2007). In principle, the engineered steric barrier should combat surface opsonization events, and hence minimize nanoparticle clearance by macrophages in contact with the blood (Moghimi et al., 1993b). As a result, the circulation time of nanoparticles in the blood is expected to be prolonged, and nanoparticles may be targeted "passively" or "actively" to accessible non-macrophage elements. The pharmacokinetics of such engineered entities, however, depends on density and conformation of surface-projected hydrophilic polymers, Figure 9.2 (Al-Hanbali et al., 2006; Gbadamosi et al., 2002). For example, the tetrafunctional polyethylene oxide/polypropylene oxide ethylenediamine block copolymer poloxamine 908 can adsorb on to the surface of hydrophobic nanoparticles via its central polypropylene oxide chains, which leaves the relatively hydrophilic polyethylene oxide segments to extend outwards from the nanoparticle surface (Moghimi et al., 1993b). We (Al-Hanbali et al., 2006) recently demonstrated that accommodation of at least 11,500 poloxamine molecules on the surface of a polystyrene nanoparticle of 230 nm diameter was necessary for suppressing nanoparticle-mediated complement consumption (but not totally inhibiting the process) and conferring longevity in the blood when compared with a naked nanoparticle. These conditions generated a sufficient density of polyethylene oxide on nanoparticle surface, where projected chains assumed a brush-like configuration to combat rapid complement activation and particle clearance by the hepatic macrophages. At a lower surface density, while maintaining the brush configuration of the projected polyethylene oxide chains, complement consumption was increased and nanoparticles were more prone to phagocytic clearance. At a critical surface density of 3,700 poloxamine 908 molecules per nanoparticle, polyethylene oxide chains assumed a mushroom-like configuration as supported by biophysical characterization. Here, complement consumption proceeded rapidly in a manner comparable to uncoated nanoparticles, and the engineered particles circulated in the blood for considerably shorter periods of time.

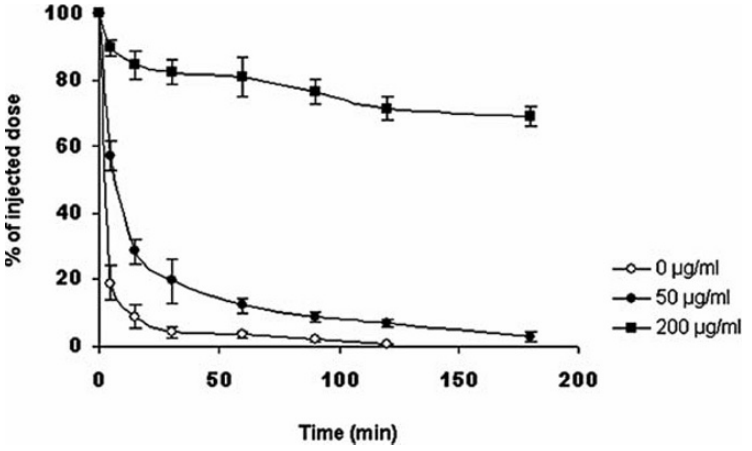

Figure 9.2 The effect of poloxamine 908 surface coverage on polystyrene nanoparticle circulation profile in rats. The core size of polystyrene nanoparticles was 60 nm in diameter. Particles were surface labelled with [^{125}I]Na prior to poloxamine coverage. Surface modification was achieved by incubating nanoparticles with poloxamine 908 at room temperature overnight. The equilibrium poloxamine concentration was either 50 or 200 µg/ml, respectively. At the lower equilibrium poloxamine concentration, the surface-projected poly(ethylene oxide) chains are spread laterally with portions in close contact with the surface of nanoparticles (mushroom configuration) and correspond to the first plateau region on the adsorption isotherm (Al-Hanbali et al., 2006). At an equilibrium poloxamine 908 concentration of 200 µg/ml, which corresponds to the top plateau region of the adsorption isotherm, poly(ethylene oxide) chains assume a laterally compressed elongated random coil conformation in a direction perpendicular to the plane of particle surface. Particles (3.5 mg polystyrene/kg body weight) were injected intravenously via tail vein and the blood concentration of nanoparticles was evaluated at various intervals.

Methoxy(polyethylene glycol)$_{2000}$-grafted liposomes (PEGylated liposomes) are also known to exhibit prolonged circulation time in the blood (reviewed in Moghimi et al., 2001; Moghimi & Szebeni, 2003). For example, a clinical formulation of PEGylated liposomes (Doxil®) shows a biphasic circulation half-life of 84 min and 46 h in humans. Contrary to the view that poly(ethylene glycol) grafting should suppress opsonization processes, we have (Moghimi et al., 2006a) shown that PEGylated liposomes can efficiently activate the human complement system via both classical and alternative pathways and fix complement proteins (Figure 9.3). Our studies indicated that complement activation was due to the expression of the net anionic charge on the oxygen phosphate moiety of phospholipid-methoxy (polyethylene glycol) conjugate; methylation of the phosphate oxygen, and hence the removal of the negative charge, totally prevented generation of complement activation products (e.g. SC5b-9 complexes), anaphylatoxins, and complement split products (e.g. Bb and C4d) in human serum (Moghimi et al., 2006a). Remarkably, complement fixation by PEGylated liposomes seemed to play a minor role in macrophage clearance via complement receptors; the surface-projected methoxy(polyethylene glycol) chains sterically interfered with the binding of surface-bound iC3b to the macrophage complement receptor. This process seems to explain the prolonged circulation times of "stealth" liposomes.

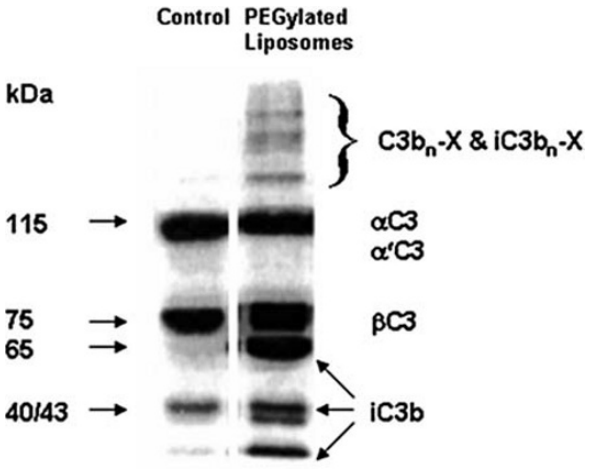

Figure 9.3 SDS-PAGE analysis of PEGylated liposome-mediated complement activation in 20% (v/v) human serum supplemented with [^{125}I]-labelled C3. Generation of the scission products of iC3b (40/43 kDa bands) together with the lack of α′C3 band implies efficient inactivation of C3b to iC3b by factors H and I (Moghimi et al., 2006b). The presence of C3b$_n$-X/iC3b$_n$-x complexes suggests that C3 processing has occurred in a similar fashion to immune aggregate-mediated complement activation.

Long-circulating entities are further amenable to surface modification with biological ligands for targeting to blood elements (e.g. lymphocytes or blood clots) and vascular endothelial cells (Moghimi, 2006; Moghimi et al., 2005a; Allen, 2002; Murray & Moghimi, 2003). Pharmacokinetics and biodistribution of these entities will be modulated by their size and stability in the blood as well as surface density, location (direct attachment to the particle surface, or to distal end of surface-projected polymer chains, or both), and conformation of the attached ligands (Bendas et al., 2003).

Nanoparticle Escape from the Vasculature

Splenic Red-Pulp

If confinement of long-circulating nanoparticles to the vascular system is necessary (e.g. for slow and controlled drug release), then splenic filtration processes in the red-pulp regions must be considered. In sinusoidal spleen (as in rat and human) blood flow is mainly through the open route of circulation (little or no endothelial continuity from arterial capillaries to venous vessels, resulting in direct blood contact with the reticular meshwork of marginal zone and the red-pulp), where particulate filtration at inter-endothelial cell slits of venous sinuses become predominant. Splenic filtration is frequently observed for non-deformable entities whose size exceeds the width of cell slits (200–250 nm) (Moghimi et al., 1991). Thus, long-circulating "rigid" nanoparticles will be cleared efficiently by the splenic filter, providing that their size exceeds 200 nm. Otherwise, opportunities are there for gaining access to the splenic red-pulp regions, which are rich in macrophages. Indeed, we (Moghimi et al., 1991, 1993a) have shown that within a few hours of intravenous injection, poloxamine 908-coated polystyrene nanoparticles of 230 nm size predominantly localize to the splenic red-pulp compartments. Remarkably, resident macrophages of the red-pulp phagocytosed filtered nanoparticles (Moghimi et al., 1993a). This was a surprising observation, since poloxamine-coated nanoparticles were expected to resist ingestion by quiescent macrophages. The phagocytic

process, presumably had occurred after intrasplenic loss of some surface-adsorbed poloxamine molecules (as a result of inter-endothelial cell slit resistance to blood flow through the reticular meshwork and altered dynamic flow properties of red blood cells and plasma), thus triggering local opsonization events at the nanoparticle surface. This notion is in agreement with significant levels of complement activation and fixation with nanoparticles bearing a critical number of poloxamine molecules (see above) (Al-Hanbali et al., 2006).

The meshwork of the red-pulp also expresses an enormous contact surface area for particulate matters; its large cross-sectional area for flow can slow down red blood cell and particle velocity and facilitate their trapping. Here, factors influencing particulate retention are principally physicochemical and are related to surface properties such as surface charge and adhesiveness.

Bone Marrow Sinuses

The medullary sinuses in the bone marrow are formed from an uninterrupted layer of spindle-shaped endothelial cells, an outer discontinuous layer of supporting adventitial cells, and a discontinuous basal lamina. Macrophages abound in the vicinity of marrow sinuses, and in some species they are considered as a component of the marrow–blood barrier. Systemic nanoparticles, however, may reach the bone marrow compartment through transport mechanisms across the blood–bone marrow barrier, which includes transcellular and intracellular routes. The former route is through diaphragmed fenestrae of endothelial walls, whereas the intracellular route is associated with the formation of bristle-coated pits on the luminal surface of the endothelium (Moghimi, 1995).

A remarkable case, however, was reported with poloxamer 407-coated polystyrene particles of 60 nm size after intravenous injection into rabbits (Porter et al., 1992). These particles were resistant to clearance by scavengers of the liver and the spleen, but within 12 h of injection predominant nanoparticle localization to sinus endothelial cells of the bone marrow was notable without evidence of transcytosis. Thus exquisite levels of recognition and specificity are achievable with surface modification, either directly or through a time-dependent differential protein-binding phenomenon.

Open Fenestrations in the Liver and Pathological Vessels

Nanoparticle escape from the circulation may further occur at sites where the blood capillaries have open fenestrations, or when the integrity of the endothelial barrier is perturbed by inflammatory processes, or dysregulated angiogenesis. For example, open fenestrations are found in the sinus endothelium of the liver, where the size of fenestrae can be as large as 150 nm. A significant fraction of some deformable particles and vesicles (even larger than 150 nm) may get access to the hepatic parenchyma through the space of Disse, between the lining cells and the hepatocyte surface, by “endothelial massaging” (Romero et al., 1999). Although this may provide an opportunity for targeting of particles to hepatocytes and

hepatic stellate cells, the number and frequency of endothelial fenestrae is significantly decreased in chronic liver diseases.

Hyper-permeability of tumour microvessels to macromolecules as well as to nanoparticles is long established (reviewed in Moghimi, 2007a; Munn, 2003). Detailed morphological investigations have demonstrated heterogeneity in pore sizes along a typical tumour blood vessel; tumours that were grown subcutaneously exhibited a characteristic pore cut-off size ranging from 200 to 1200 nm with the majority ranging between 380 and 780 nm (Hobbs et al., 1998). The pore cut-off size in tumours is also modulated by microenvironmental factors such as hormones and growth factors. For instance, the pore cut-off size is reduced when tumours are grown in the cranium (Hobbs et al., 1998). In androgen-dependent mouse mammary tumour, testosterone withdrawal also reduced the pore cut-off size from 200 nm to less than 7 nm within 48 h (Hobbs et al., 1998). Nevertheless, the idea of exploiting the vascular abnormalities of the tumours, restricting penetration into normal tissue interstitium while allowing better access to that of the tumour with long-circulatory and multi-functional nanoparticles and vesicles of different shape, size, and composition, is an attractive one, and has received considerable attention (reviewed in Moghimi et al., 2001, 2005a; Moghimi, 2006, 2007a). These approaches should, therefore, be used before hormone ablation therapies or other therapies that may reduce transvascular transport. In the case of macromolecules, transport across tumour blood vessels may further occur via caveolae and vesicular vacuolar organelles.

Following extravasation into tumour interstitium, nanoparticulate carrier systems must be able to release its cargo at a rate that maintains free drug levels in the therapeutic range; a process also amenable to modulation by nanoengineering (Andresen et al., 2004). While passively targeted long-circulating carriers can show anti-tumour activity, higher and more selective anticancer activity is achievable by surface decoration of long-circulating nanoparticles with a plethora of tumour-specific ligands, such as monoclonal antibodies (e.g. anti-HER2 antibodies), folate, transferrin, vasoactive intestinal peptide, and sigma-1 selective substrates (Allen, 2002; Sudimack & Lee, 2000).

Lymph Nodes

Small fractions of particles of 1–20 nm size range may slowly extravasate from the vasculature into the interstitial spaces, from which they are transported to the regional lymph nodes by way of patent junctions of the lymphatic vessels (Moghimi & Bonnemain, 1999). Also, depending on the species type, some nanoparticles may leave the vascular compartment through post-capillary venules and adjoining capillaries, where blood flow is sluggish and endothelial permeability is controlled by a number of mediators, and reach the lymph nodes. Indeed, these modes of particle extravasation from the vascular system have been exploited in medical imaging using dextran-coated superparamagnetic iron oxide nanocrystals. For instance, such nanocrystals have aided visualization of micrometastases in lymph nodes by magnetic resonance imaging (Harisinghani et al., 2003).

The Glomerular Filter

The capillary endothelium of glomerular filter contains large round fenestrations of 50–100 nm diameter, which occupies approximately 20% of the endothelial surface. These fenestrations do not exhibit diaphragms. However, nanoparticle passage across these fenestrations is hampered by the presence of a continuous and thick (240–340 nm) basement membrane as well as by the primary processes of podocytes, which embrace the capillaries, giving rise to pedicels, which interdigitate with those of the primary processes. The openings between the pedicels, or the filtration slits, are approximately 25 nm in width and bridged by a delicate electron-dense diaphragm of 4 nm thick. Podocytes also possess phagocytic function, that is to ensure the removal of large molecules and small particles that may have been trapped in the outer layers of the filter. Macromolecules and particles trapped on the endothelial side are cleared by mesangial cells.

In spite of these barriers, a recent study involving carbon nanotubes has claimed that following intravenous injection into mice, water-soluble functionalized SWNTs (20–40 nm in width and variable long lengths) are cleared rapidly intact from the systemic circulation through the renal excretion route (Singh et al., 2006). These observations presumably indicate that carbon nanotubes are capable of damaging the endothelial barrier of the glomerular capillaries (primarily the filtration slits) following insertion through their hydrophilic termini. The damage may even extend to fenestrated capillaries of proximal- and distal-convoluted tubule regions. Unfortunately, kidney function tests and morphological studies were not conducted to assess the integrity of glomerular filter following nanotube administration. In the absence of such studies, the proposed therapeutic applications of intravenously injected carbon nanotubes cannot be perceived safe.

The capillary endothelium of glomerular filter and the outer surface of the podocytes, including the filtration slits, exhibit a strong negative electric charge. These sites may play a role in binding to small entities that exhibit cationic charge in plasma. For example, efficient kidney accumulation of generation 3 poly(amidoamine) Starburst dendrimers was reported by Roberts et al. (1996).

Dosing Regimen

It is now apparent that particulate nanocarriers exert substantial effects on pharmacokinetics and body distribution of their entrapped cargo. However, limited information is available for differing dose schedules and dose intensities. This is of particular importance where patients are expected to receive multiple injections, as in delivery of anticancer agents with long-circulating nanoparticles. Here, one should ensure that the first dose has had time to clear before subsequent doses.

A recent study (Charrois & Allen, 2003) has evaluated the effect of dosing schedule on the pharmacokinetics of doxorubicin entrapped in long-circulating liposomes (Doxil®) following multiple intravenous injections into mice (four injections of liposomes at a dose of 9 mg/kg and dose intervals of either 1, 2, or 4 weeks). Interestingly, plasma pharmacokinetics

of doxorubicin in each injection cycle was independent of the next cycle. Initially, these observations, however, would appear to contradict those of Ishida et al. (2003, 2006b) who demonstrated that an injection of PEGylated liposomes in rats could elicit the production of a poly(ethylene glycol)-specific IgM in the spleen (independent of T-cells), resulting in opsonization (complement activation) and rapid hepatic elimination of a subsequent liposome dose given 5 days later. Increasing the time interval between the two injections, however, weakened the effect and by third dose, hardly any effect on pharmacokinetics relative to the first injection was seen. In addition, the altered pharmacokinetics was dose dependent (Ishida et al., 2006a). For instance, no rapid clearance of the second liposome dose occurred when the first liposome dose exceeded 5 μmol phospholipid/kg body weight. Further, it was shown that doxorubicin encapsulation could also control pharmacokinetics of subsequent liposome injections (Ishida et al., 2006a). The first dose of doxorubicin-encapsulated liposomes failed to dramatically increase plasma levels of poly(ethylene glycol)-specific IgM antibodies, presumably as a consequence of doxorubicin-mediated macrophage death and inhibition of B cell-proliferation and/or killing of proliferating B cells, resulting in prolonged circulation times of subsequent liposome doses. Altered pharmacokinetics of long-circulating liposomes following repeated administration has also been reported by other investigators (Dams et al., 2000; Romberg et al., 7007).

The most notable dose-limiting toxicity associated with continuous and repeated infusion of Doxil® is doxorubicin accumulation in the skin, resulting in palmar–plantar erythrodysesthesis (PPE) (Lyass et al., 2000). The incidence of PPE can be lowered substantially by reducing the dose intensity as well as the interval between subsequent dosing. For example, recent studies in mice have demonstrated that skin pharmacokinetics of one injection cycle of Doxil® was independent of the next injection cycle when the dose interval was 4 weeks, with little evidence of symptoms of PPE (Charrois & Allen, 2003).

In contrast to liposomes, repeated intravenous injection of long-circulating polymeric nanospheres generates different responses. For instance, a single intravenous dose of poloxamine 908-coated polystyrene nanospheres of 60 nm size dramatically affected the circulation half-life and body distribution of a second subsequent dose in a time-dependent, but opsonic-independent, manner (Moghimi & Gray, 1997). At 3 days after a single intravenous dose, Kupffer cells and splenic macrophages could clear a second dose of long-circulating nanospheres from the blood. When the interval between the two injections was increased to 14 days, the second dose behaved as long circulatory. Remarkably, the coating material (poloxamine 908) was shown to trigger nanosphere clearance by resident Kupffer cells and certain sub-populations of splenic macrophages, since a single intravenous injection of an endotoxin-free solution of poloxamine 3 days before the administration of long-circulating nanospheres induced similar effects (Moghimi & Gray, 1997). The observations may be due to altered macrophage functions through gene activation and signalling pathways, leading to increased mobility of some plasma membrane receptors or expression of new receptors capable of directly recognizing surface characteristics of long-circulating nanospheres.

The State of Macrophage Responsiveness

Macrophage priming and activation can be induced experimentally (e.g. by lipopolysaccharide or zymosan challenge), and is also known to occur in certain physiopathological conditions. Such macrophages can proceed with recognition and internalization of long-circulating nanoparticles via both opsonic-dependent and opsonic-independent modes (reviewed in Moghimi et al., 2001). These observations are of importance in therapeutic protocols that utilize long-circulating carriers for targeting to non-macrophage sites in clinical conditions associated with globally or regionally enhanced macrophage activity.

Conclusions

The biomedical applications of nanoparticle carriers for site-specific delivery of therapeutic and contrast agents are well established. This trend will surely continue with the advent of nanotechnology and parallel developments in design of functional entities (e.g. metal and rod nanoshells, porous silicon nanoparticles) as well as multi-functional nanomedicines for simultaneous sensing, signalling, and drug release. Such innovations and novel approaches are already the focus of the US National Institute of Health's Nanomedicine Roadmap Initiative as well as the European Science Foundation. Nanoparticles, however, do not behave similarly when injected intravenously. Their pharmacokinetics is controlled by complex and inter-related physicochemical, anatomical, pathophysiological, and immunobiological factors, as well as the dosing regimen. These parameters must be studied individually for each specific nanoparticle. In the case of intravenous gene therapy with polyplexes and cationic nanoparticles, issues such as poor transfection efficiency, polycation-mediated cell death, and insufficient distribution to target cells are substantial and need to be resolved (Moghimi, 2007b; Moghimi et al., 2005b). A clear understanding of these issues could lead to design and engineering of multi-functional nanomedicines with optimal biological performances in relation to specific clinical conditions.

References

Al-Hanbali, O., Rutt, K. J., Sarker, D. K., Hunter, A. C., & Moghimi, S. M. (2006). Concentration dependent structural ordering of poloxamine 908 on polystyrene nanoparticles and their modulatory role on complement consumption. *J. Nanosci. Nanotechnol.*, *6*, 3126–3133.

Andresen, T. L., Jensen, S. S., & Jørgensen, K. (2004). Advanced strategies in liposomal cancer therapy: problems and prospects of active tumor specific drug release. *Prog. Lipid Res., 44*, 68–97.

Allen, T. M. (2002). Ligand-targeted therapeutics in anticancer therapy. *Nat. Rev. Cancer, 2*, 750–763.

Bendas, G., Rothe, U., Scherphof, G. L., & Kamps, J. A. A. M. (2003). The influence of repeated injections on pharmacokinetics and biodistribution of

different types of sterically stabilized immunoliposomes. *Biochim. Biophys. Acta, 1609*, 63–70.

Bhatia, S. K., King, M. R., & Hammer, D. A. (2003). The state diagram for cell adhesion mediated by two receptors. *Biophys. J., 84*, 2671–2690.

Charrois, G. J. R., & Allen, T. M. (2003). Multiple injections of PEGylated liposomal doxorubicin: pharmacokinetics and therapeutic activity. *J. Pharmacol. Exp. Ther., 306*, 1058–1067.

Cherukuri, P., Gannon, C. J., Leeuw, T. K., Schmidt, H. K., Smalley, R. E., Curley, S. A., & Weisman, R. B. (2006). Mammalian pharmacokinetics of carbon nanotubes using intrinsic near-infrared fluorescence. *Proc. Natl. Acad. Sci. USA, 103*, 18882–18886.

Chonn, A., Semple, S. C., & Cullis, P. R. (1992). Association of blood proteins with large unilamellar liposomes in vivo. Relation to circulation lifetimes. *J. Biol. Chem., 267*, 18759–18765.

Chonn, A., Semple, S. C., & Cullis, P. R. (1995). β2-Glycoprotein I is a major protein associated with very rapidly cleared liposomes in vivo, suggesting a significant role in the immune clearance of 'non-self' particles. *J. Biol. Chem., 270*, 25845–25849.

Cornacoff, J. B., Hebert, L. A., Smead, W. L., & Van Aman, M. E. (1983). Primate erythrocyte-immune complex-clearing mechanism. *J. Clin. Invest., 71*, 236–247.

Dams, E. T. M., Laverman, P., Oyen, W. J. G., Storm, G., Scherphof, G. L., Van der Meer, J. W. M., Corstens, F. H., & Boerman, O. C. (2000). Accelerated blood clearance and altered biodistribution of repeated injections of sterically stabilized liposomes. *J. Pharmacol. Exp. Ther., 292*, 1071–1079.

Decuzzi, P., & Ferrari, M. (2006). The adhesive strength of non-spherical particles mediated by specific interactions. *Biomaterials, 27*, 5307–5314.

Devine, D. V., Wong, K., Serrano, K., Chonn, A., & Cullis, P. R. (1994). Liposome-complement interactions in rat serum: implications for liposome survival studies. *Biochim. Biophys. Acta, 1191*, 43–51.

Gbadamosi, J. K., Hunter, A. C., & Moghimi, S. M. (2002). PEGylation of microspheres generates a heterogeneous population of particles with differential surface characteristics and biological performance. *FEBS Lett., 532*, 338–344.

Harisinghani, M. G., Barentsz, J., Hahn, P. F., Deserno, W. M., Tabatabaei, S., Hulsbergen van de Kaa, C., de la Rosette, J., & Weissleder, R. (2003). Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. *New Engl. J. Med., 348*, 2491–2499.

Harush-Frenkel, O., Debotton, N., Benita, S., & Altschuler, Y. (2007). Targeting of nanoparticles to the clathrin-mediated endocytic pathway. *Biochem. Biophys. Res. Commun., 353*, 26–32.

Hobbs, S. K., Monsky, W. L., Yuan, F., Roberts, W. G., Griffith, L., Torchilin, V. P., & Jain, R. K. (1998). Regulation of transport pathways in tumor vessels: Role of tumor type and microenvironment. *Proc. Natl. Acad. Sci. USA, 95*, 4607–4612.

Ishida, T., Atobe, K., Wang, X., & Kiwada, H. (2006a). Accelerated blood clearance of PEGylated liposomes upon repeated injections: effect of doxorubicin-encapsulation and high-dose first injection. *J. Control. Rel., 115*, 251–258.

Ishida, T., Ichihara, M., Wang, X., & Kiwada, H. (2006b). Spleen plays an important role in the induction of accelerated blood clearance of PEGylated liposomes. *J. Control. Rel., 115*, 243–250.

Ishida, T., Maeda, R., Ichihara, M., Irimura, K., & Kiwada, H. (2003). Accelerated clearance of PEGylated liposomes in rats after repeated injections. *J. Control. Rel., 88*, 35–42.

Klumpp, C., Kostarelos, K., Prato, M., & Bianco, A. (2006). Functionalized carbon nanotubes as emerging nanovectors for the delivery of therapeutics. *Biochim. Biophys. Acta, 1758*, 404–412.

Kreuter, J., Ramge, P., Petrov, V., Hamm, S., Gelperina, S. E., Engelhardt, B., Alyautdin, R., von Briesen, H., & Begley, D. J. (2003). Direct evidence that polysorbate-80-coated poly(butylcyanoacrylate) nanoparticles deliver drugs to the CNS via specific mechanisms requiring prior binding of drugs to the nanoparticles. *Pharm. Res., 20*, 409–416.

Liu, Z., Cai, W., He, L., Nakayama, N., Chen, K., Sun, X., Chen, X., & Dai, H. (2006). In vivo distribution and highly efficient tumour targeting of carbon nanotubes in mice. *Nat. Nanotechnol., 2*, 47–52.

Loughrey, H. C., Bally, M. B., Reinish, L. W., & Cullis, P. R. (1990). The binding of phosphatidylglycerol liposomes to rat platelets is mediated by complement. *Thromb. Haemost., 64*, 172–176.

Lovrić, J., Cho, S. J., Winnik, F. M., & Maysinger, D. (2005). Unmodified cadmium telluride quantum dots induce reactive oxygen species formation leading to multiple organelle damage and cell death. *Chem. Biol., 12*, 1227–1234.

Lyass, O., Uziely, B., Ben-Yosef, R., Tzemach, D., Heshing, N. I., Lotem, M., Brufman, G., & Gabizon, A. (2000). Correlation of toxicity with pharmacokinetics of PEGylated liposomal doxorubicin (Doxil) in metastatic breast carcinoma. *Cancer, 89*, 1037–1047.

Marjan, J., Xie, Z., & Devine, D. V. (1994). Liposome-induced activation of the classical complement pathway does not require immunoglobulin. *Biochim. Biophys. Acta, 1192*, 35–44.

Moghimi, S. M. (1995). Exploiting bone marrow microvascular structure for drug delivery and future therapies. *Adv. Drug Deliv. Rev., 17*, 61–73.

Moghimi, S. M. (2003). Exploitation of macrophage clearance functions. In S. Gordon (Ed.), *Hand-Book of Experimental Pharmacology: The macrophage as therapeutic target,* Volume 158 (pp. 41–54). Berlin: Springer-Verlag.

Moghimi, S. M. (2006). Recent development in polymeric nanoparticle engineering and their applications in experimental and clinical oncology. *Anti-cancer Agent. Med. Chem., 6*, 553–561.

Moghimi, S. M. (2007a). Passive targeting of solid tumors: pathophysiological principles and physicochemical aspects of delivery systems. In M. M. Amiji (Ed.), *Nanotechnology for cancer therapy* (p. 11–18). Boca Raton: CRC Press.

Moghimi, S. M. (2007b). Nanotoxicology of synthetic gene-transfer vectors: poly(ethylenimine)- and polyfectin-mediated membrane damage and apoptosis in human cell lines. In C. S. S. R. Kumar (Ed.), *Nanotechnologies for Life Sciences: Nanomaterials for medical diagnosis and therapy*, Volume 10 (pp. 629–643). Berlin: Wiley-VCH Verlag.

Moghimi, S. M., & Bonnemain, B. (1999). Subcutaneous and intravenous delivery of diagnostic agents to the lymphatic system: applications in lymphoscintigraphy and indirect lymphography. *Adv. Drug Deliv. Rev., 37*, 295–312.

Moghimi, S. M., & Gray, T. (1997). A single dose of intravenously injected poloxamine-coated long-circulating particles triggers macrophage clearance of subsequent doses in rats. *Clin. Sci. (Lond.) 93*, 371–379.

Moghimi, S. M., Hamad, I., Andresen, T. L., Jørgensen, K., & Szebeni, J. (2006a). Methylation of the phosphate oxygen moiety of phospholipids-methoxy(polyethylene glycol) conjugate prevents PEGylated liposome-mediated complement activation and anaphylatoxin production. *FASEB J., 20*, 2591–2593 (doi: 10.1096/fj.06-6186fje, electronic pages: E2057–E2067).

Moghimi, S. M., Hamad, I., Bünger, R., Andresen, T. L., Jørgensen, K., Hunter, A. C., Baranji, L., Rosivall, L., & Szebeni, J. (2006b). Activation of the human complement system by cholesterol-rich and PEGylated liposomes–Modulation of cholesterol-rich liposome-mediated complement activation by elevated serum LDL and HDL levels. *J. Liposome Res., 16*, 167–174.

Moghimi, S. M., Hedeman, H., Muir, I. S., Illum, L., & Davis, S. S. (1993a). An investigation of the filtration capacity and the fate of large filtered sterically-stabilized microspheres in rats. *Biochim. Biophys. Acta, 1157*, 233–240.

Moghimi, S. M., & Hunter, A. C. (2001). Recognition by macrophages and liver cells of opsonized phospholipids vesicles and phospholipids headgroups. *Pharm. Res., 18*, 1–8.

Moghimi, S. M., Hunter, A. C., & Murray, J. C. (2001). Long-circulating and target-specific nanoparticles: theory to practice. *Pharmacol. Rev., 53*, 283–318.

Moghimi, S. M., Hunter, A. C., & Murray, J. C. (2005a). Nanomedicine: current status and future prospects. *FASEB J., 19*, 311–330.

Moghimi, S. M., Hunter, A. C., Murray, J. C., & Szewczyk, A. (2004). Cellular distribution of non-ionic micelles. *Science, 303*, 626–627.

Moghimi, S. M., & Kissel, T. (2006). Particulate nanomedicines. *Adv. Drug Deliv. Rev., 58*, 1451–1455.

Moghimi, S. M., Muir, I. S., Illum, L., Davis, S. S., & Kolb-Bachofen, V. (1993b). Coating particles with a block copolymer (poloxamine-908) suppresses opsonization but permits the activity of dysopsonins in the serum. *Biochim. Biophys. Acta, 1179*, 157–165.

Moghimi, S. M., Porter, C. J. H., Muir, I. S., Illum, L., & Davis, S. S. (1991). Non-phagocytic uptake of intravenously injected microspheres in rat spleen: influence of particle size and hydrophilic coating. *Biochem. Biophys. Res. Commun., 177*, 861–866.

Moghimi, S. M., Symonds, P., Murray, J. C., Hunter, A. C., Debska, G., & Szewczyk, A. (2005b). A two-stage poly(ethylenimine)-mediated cytotoxicity: implications for gene-transfer/therapy. *Mol. Ther., 11*, 990–995.

Moghimi, S. M., & Szebeni, J. (2003). Stealth liposomes and long circulating nanoparticles: critical issues in pharmacokinetics, opsonization and protein-binding properties. *Prog. Lipid Res., 42*, 463–478.

Moghimi, S. M., Vegas, E., Garcia, M. L., Al-Hanbali, O. A. R., & Rutt, K. J. (2006c). Polymeric nanoparticles as drug carriers and controlled release implant devices. In V. P. Torchilin (Ed.), *Nanoparticles as drug carriers* (p. 29–42). London: Imperial College Press.

Moore, Jr. F. D., Austen, K. F., & Fearon, D. T. (1982). Antibody restores human alternative complement pathway activation by mouse erythrocytes rendered functionally deficient by pre-treatment with pronase. *J. Immunol., 128*, 1302–1306.

Munn, L. L. (2003). Aberrant vascular architecture in tumors and its importance in drug-based therapies. *Drug Discov. Today, 8*, 396–403.

Murray, J. C., & Moghimi, S. M. (2003). Endothelial cells as therapeutic targets in cancer: new biology and novel delivery systems. *Crit. Rev. Ther. Drug Carr. Syst., 20*, 139–152.

Patil, V. R. S., Campbell, C. J., Yun, Y. H., Slack, S. M., & Goettz, D. J. (2001). Particle diameter influences adhesion under flow. *Biophys. J., 80*, 1733–1743.

Porter, C. J. H., Moghimi, S. M., Illum, L., & Davis, S. S. (1992). The polyoxyethylene/polyoxypropylene block co-polymer poloxamer-407 selectively redirects intravenously injected microspheres to sinusoidal endothelial cells of rabbit bone marrow. *FEBS Lett., 305*, 62–66.

Poznansky, M., & Juliano, R. L. (1984). Biological approaches to controlled delivery of drugs: a critical review. *Pharmacol. Rev., 36*, 277–336.

Qiang, Y., Antony, J., Sharma, A., Nutting, J., Sikes, D., & Meyer, D. (2006). Iron/iron oxide core-shell nanoclusters for biomedical applications. *J. Nanoparticle Res., 8*, 489–496.

Roberts, J. C., Bhalgat, M. K., & Zera, R. T. (1996). Preliminary biological evaluation of polyamidoamine (PAMAM) Starburst dendrimers. *J. Biomed. Mat. Res., 30*, 53–65.

Romberg, B., Oussoren, C., Snel, C. J., Carstens, M. G., Hennink, W. E., & Storm, G. (2007). Pharmacokinetics of poly(hydroxyethyl-L-asparagine)-coated liposomes is superior over that of PEG-coated liposomes at low lipid dose and upon repeated administration. *Biochim. Biophys. Acta,* doi: 10.1016/j.bbamem.2006.12.005, *1768*, 2655–2666.

Romero, E. L., Morilla, M. J., Regts, J., Koning, G. A., & Scherphof, G. L. (1999). On the mechanism of hepatic transendothelial passage of large liposomes. *FEBS Lett., 448*, 193–196.

Salvador-Morales, C., Flahaut, E., Sim, E., Sloan, J., Green, M. L. H., & Sim, R. B. (2006). Complement activation and protein adsorption by carbon nanotubes. *Mol. Immunol*., *43*, 193–201.

Scherphof, G. L., & Kamps, J. A. A. M. (2001). The role of hepatocytes in the clearance of liposomes from the blood circulation. *Prog. Lipid Res., 40*, 149–166.

Singh, R., Pantarotto, D., lacerda, L., Pastorin, G., Klumpp, C., Prato, M., Bianco, A., & Kostarelos, K. (2006). Tissue distribution and blood clearance rates of intravenously administered carbon nanotube radiotracers. *Proc. Natl. Acad. Sci. USA, 103*, 3357–3362.

Sou, K., Goins, B., Takeoka, S., Tsuchida, E., & Phillips, W. T. (2006). Selective uptake of surface-modified vesicles by bone marrow macrophages in vivo. Biomaterials, *28*, 2655–2666.

Sudimack, J., & Lee, R. J. (2000). Targeted drug delivery via the folate receptor. *Adv. Drug Deliv. Rev., 41*, 147–162.

Volanakis, J. E., & Wirtz, K. W. A. (1979). Interaction of C-reactive protein with artificial phosphatidylcholine bilayers. *Nature, 281*, 155–157.

Yan, X., Kuipers, F., Havekes, L. M., Havinga, R., Dontje, B., Poelstra, K., Scherphof, G. L., & Kamps, J. A. A. M. (2005). The role of lipoprotein E in the elimination of liposomes from blood by hepatocytes in the mouse. *Biochem. Biophys. Res. Commun., 328*, 57–62.

Yan, X., Morselt, H. W. M., Scherphof, G. L., Poelstra, K., & Kamps, J. A. A. M. (2004). The role of beta(2)-glycoprotein I in liposome-hepatocyte interaction. *Biochim. Biophys. Acta, 1667*, 208–214.