JPP Journal of Pharmacy and Pharmacology

JPP 2005, 57: 411–421

Received October 11, 2004
Accepted January 11, 2005
DOI 10.1211/0022357055894
ISSN 0022-3573

Review Article

Tumour targeting: biological factors and formulation advances in injectable lipid nanoparticles

V. S. Shenoy, I. K. Vijay and R. S. R. Murthy

Abstract

Cancer chemotherapeutic agents are often administered systemically. Following systemic administration, numerous biological factors associated with the tumours influence the delivery of the drugs to the tumours. These factors have been extensively studied for the last 2 decades. The influence of these biological factors has brought about a drastic change in the design of drug delivery systems to solid tumours. This review discusses the various biological factors influencing drug delivery to tumours and the subsequent development of injectable delivery systems (i.e., lipid-based nanoparticles (SLNs)) for adequate delivery of drug to solid tumours.

Introduction

Cancer is a disease characterized by the formation of abnormal tissue (neoplasm) – basically a change in the way cells proliferate and differentiate. Tumours (collection of abnormally growing cells) are either benign (non-cancerous) or malignant (cancerous). The term solid tumour is used to distinguish between a localized mass of tissue and leukaemia (tumour with fluid properties). Over 85% of human cancers are solid tumours. The effectiveness of cancer therapy in solid tumours depends on the adequate delivery of the therapeutic agent to tumour cells. Inadequate delivery of drugs to the tumour cells would result in residual tumour cells, which in turn would lead to regrowth of tumours and even result in the development of resistant cells. Recent advances in the molecular targeting approach have led to the discovery of novel therapeutics, including monoclonal antibodies, cytokines, sense or antisense oligonucleotides, viral and non-viral gene vectors and genetically engineered cells (Adams 1998; Harper et al 1999; Muggia 1999; Schwarze et al 1999; Vasey et al 1999; Wolf et al 1999; Pagnan et al 2000). Delivery of newer agents to solid tumours, because of the relatively large size of these agents, poses new challenges beyond those encountered with traditional small-molecule cytotoxic agents. Following systemic administration, drug delivery to cells in solid tumours involves three processes – transport within a vessel, transport across vasculature walls into surrounding tissues and transport through interstitial spaces within a tumour (Jain 1989). These processes are determined by the physicochemical properties of a drug or particle (e.g., molecular or particle size, diffusivity, drug binding to cellular macromolecules) and the biological properties of a tumour (e.g., tumour vasculature, extracellular matrix components, interstitial fluid pressure (IFP), tumour cell density and tissue structure and composition).

Role of membrane transport in drug transport and accumulation in the tumour

Blood vessels, as well as lymphatic tissues, effect the perfusion and drainage of tissues, including solid tumours. Differences between the tumour vasculature and that of the normal tissues play an important role in the delivery of bioactive agents to the solid tumour. Some of the important biological factors of tumours, which are different from those of the normal tissues, to be considered while designing the delivery of drugs to solid tumours are described below.

New Drug Delivery Systems Laboratory, Pharmacy Department, Donors Plaza, Opp. University Main Office, M S University of Baroda, Fatehgunj, Vadodara-390 002, India

V. S. Shenoy, I. K. Vijay, R. S. R. Murthy

Correspondence: R. S. R. Murthy, New Drug Delivery Systems Laboratory, Pharmacy Department, Donors Plaza, Opp. University Main Office, M S University of Baroda, Fatehgunj, Vadodara-390 002, India.
E-mail: murthyrsr@sify.com

Tumour vasculature

Blood supply to the tumour plays an important role in the delivery of therapeutic agents to solid tumours (Liotta et al 1974; Yamaura & Sato 1974; Folkman 1990, 1995). Small tumours, which are less than 2 mm in diameter, receive a blood supply from the surrounding host tissues. The growth and enlargement of these tumours are usually accompanied by newly formed microvessels (Folkman 1995). Tumour vasculature is functionally and morphologically different from the vasculature in normal tissues. Tumour blood vessels are generally more heterogeneous in distribution, larger in size and more permeable (Yamaura & Sato 1974). Similarly, there are notable quantitative differences in the vasculature in transplanted animal tumours and spontaneous human tumours (e.g., higher vascular density and better blood circulation in transplanted tumours because of the absence of sinuses) (Falk 1982; Lauk et al 1989). The vascularization of the implanted tumour is also likely to be different from that in spontaneous tumours. Neovascularization would be required to support the growth of the relatively large number of implanted tumour cells, whereas early-stage spontaneous tumours can be supported by the normal tissues until the tumour size exceeds 2mm in diameter, a process that frequently requires months or years (Folkman 1995). Tumour vasculature is highly heterogeneous in terms of density, length and diameter; the distribution of vessels depends on the location within a tumour and the tumour size. Four regions are categorized on the basis of tumour vasculature: avascular necrotic regions with no vasculature; seminecrotic regions characterized by capillaries, precapillaries and post capillaries extended, without branching, toward the avascular necrotic region; stabilized microcirculation regions characterized by many venular and venous drainage vessels and few arteriolar vessels; and tumor advance front regions where flow is similar to percolation in porous medium (Endrich et al 1979). Generally, the peripheral regions of a tumour show higher blood-vessel density than the central regions (Tannock & Steel 1969). The ratio of avascular and seminecrotic regions to well-perfused region is also a function of tumour size (i.e., larger avascular regions in larger tumours), which partly explains the lower average drug concentration in larger tumours (Endrich et al 1979; Jain et al 1984; Jain 1989). Heterogeneity in tumour vasculature contributes to uneven drug distribution within solid tumours. One of the unique features of the tumour microvessels is their leakiness as a result of the discontinuity of endothelium (Tannock & Steel 1969; Hori et al 1990). Unlike normal tissues, tumours show elevated levels of growth factors, such as vascular endothelial growth factor or VEGF, also called vascular permeability factor (Collins et al 1993; Roberts & Palade 1995), basic fibroblast growth factors or bFGF (Dellian et al 1996; Hobbs et al 1998) and other vasoactive factors (bradykinin and nitric oxide). High levels of bradykinin result in vasodilatation and enhanced extravasation of large molecules and their retention in tumours (Maeda et al 1988; Matsumura et al 1988).

Tumour blood flow

Tumour blood flow affects drug transport through the vascular spaces in a tumour. Blood flow is determined by the difference between arterial and venous pressure and flow resistance. The latter, in turn, is affected by the viscosity of blood and geometry of the blood vessels (Folkman 1990). Unlike normal tissues, tumours show a greater blood viscosity due to the presence of tumour cells and large molecules (e.g., protein and collagen) drained from the extravascular space, a larger vessel diameter and a longer vessel length. The net result is a greater flow resistance in tumour blood vessels. Also unlike normal tissues, tumour tissues have similar arterial pressure and a lower venous pressure (Peters et al 1980). Most blood vessels in the internal regions of a tumour are veins or venules, whereas the peripheral regions of a tumour have few arteries or arterioles (Hori et al 1990, 1991). Therefore, the arteriole–venule pressure difference as a driving force for blood flow is negligible in the central region of a tumour, but is greater in the periphery. This, in part, explains the heterogeneous blood flow within a solid tumour; blood flow is lower in the centre but higher in the periphery of the tumour relative to the blood flow in the surrounding normal tissues (Straw et al 1974; Endrich et al 1979; Beaney et al 1984; Hori et al 1991). On the whole, the average blood flow in tumours is lower than in normal tissues.

Drug transport through blood vessels. Drug molecules are extravasated from blood vessels after being transported to a tumour via the blood circulation. The extravasation of molecules is associated with fluid movement across the vasculature wall. Since the exchange of fluid is dependent on the hydrostatic and osmotic pressure difference between blood vessels and interstitial space, the microvascular pressure (MVP) plays an important role as a determinant of transvascular drug transport as well as blood flow in tumour tissues (Folkman 1990). Tumour vasculature is leaky and highly permeable, hence the major pathway of drug transport across the tumour microvascular wall is by extravasation via diffusion or convection through the discontinuous endothelial junctions. Transcytosis plays a comparatively minor role (Drummond et al 1999). The pore size of tumour microvessels limits the distribution of molecules or particles bigger than 1 μm across the tumour vasculature. The difference in vascular permeability between tumour and normal tissues partly explains the passive tumour targeting (i.e., the tumour-selective delivery) of macromolecules such as liposomes and drug-conjugated high-molecular-weight polymers (Folkman 1990).

Lymphatic drainage

Lymphatic vessels have wide distribution throughout the body and are more permeable to fluid and solutes than the blood capillaries. The major function of the lymphatic system is to return the interstitial fluid to the blood circulation. In most normal and inflammatory tissues, macromolecules are cleared from tissues via the lymphatic system (Beaney et al 1984). An impaired lymphatic system is characteristic of solid tumours. Larger particles, such as tumour cells detached from a primary tumour, can enter the lymph by passing between the endothelial cells of the lymphatic capillaries (O'Driscoll 1992).

Drug transport through the lymphatic system. The lack of lymphatic drainage in solid tumours has two effects on drug delivery and retention in solid tumours. Defective lymphatic flow in solid tumours decreases the clearance of high-molecular-weight compounds from tumour interstitium (Iwai et al 1984; Sinn et al 1990; Wu et al 1993; Seymour et al 1994; Northfelt et al 1996; Noguchi et al 1998; Muggia 1999). This, together with leaky tumour blood vessels, results in enhanced accumulation and retention of high-molecular-weight compounds in solid tumours, a phenomenon recognized as the enhanced permeability and retention (EPR) effect (Vasey et al 1999). EPR is predominant for compounds with molecular weights larger than 40 kDa but negligible for smaller molecules that readily redistribute to the blood circulation via diffusion or convection (Muggia 1999). EPR is affected by tumour size (Kerbel 2000). Studies show that enhanced retention as a result of impaired lymphatic drainage is considered more important than enhanced extravasation from greater blood-vessel permeability for the accumulation of high-molecular-weight compounds in tumours (Noguchi et al 1998). The lack of lymphatic system in solid tumours increases interstitial fluid pressure (IFP). This may be a major reason for the limited extravasation of macromolecules in spite of leaky microvasculature in tumours. Enhanced IFP induces outward convective flow, inhibiting the transvascular transport of molecules as well as transport in tumour interstitial space.

Drug transport through interstitial space. Transport of small molecules in interstitial space is mainly by diffusion, whereas transport of large molecules is mainly by convection (Jain 1994). Drug diffusion depends on diffusivity. Concentration gradient and convection depends on hydraulic conductivity and pressure difference. The net convection flow in the tumour interstitium is outward from the core of a tumour due to the higher IFP in tumours compared with normal tissues. After extravasation, drugs move through the interstitial space to reach tumour cells located distal to blood vessels (Jain 1989). Vascularization is a function of tumour size. The ratio of avascularization and poorly vascularized regions to well-vascularized region increases with tumour stage, which is indicative of size (Endrich et al 1979; Jain & Ward-Hartley 1984). The intercapillary distance in solid tumours also increases with tumour size in mouse mammary tumour (Dellian et al 1996) and rat tumors (Vaupel 1977).

Tissue components

In addition to physiological and biological factors, factors such as tissue components, including cellular molecules, extracellular matrix components and tumour structure and components, also affect drug transport, accumulation and retention in tumours. These are discussed briefly below.

Cellular macromolecules

Most anti-cancer drugs target macromolecules such as proteins and nucleic acids. Hence, they extensively bind to intracellular or extracellular macromolecules. The relationship between cellular drug binding and drug penetration into solid tumours has been studied using three-dimensional spheroids (Sasaki et al 1984). Tumour cell spheroid studies show drug binding, penetration and spatial distribution within spheroids. Drugs that do not bind to cellular macromolecules or cannot cross cell membranes readily penetrate spheroids. 5-Fluorouracil, cisplatin, thymidine-5′-triphosphate, sucrose, inulin and monoclonal antibody against anti-carcinoembryonic antigen are evenly distributed in thyroid cancer cell spheroids within 15 min (Erlichman & Viogen 1984; Nederman & Carlsson 1984; Nederman et al 1988). In contrast, drugs like doxorubicin, daunomycin, actinomycin D, methotrexate, vinblastine and paclitaxel bind to the cellular macromolecules and remain localized in the periphery of spheroids (West et al 1980; Erlanson et al 1992). Despite uneven intratumoral distribution, these high-binding drugs, because of their extensive binding and retention in cells, show a higher average concentration per spheroid as compared with low-binding drugs.

Extracellular matrix composition

The extracellular matrix of solid tumours is composed of macromolecules such as fibrous proteins (e.g., collagen and elastin) and polysaccharides (hyaluronan and proteoglycan). These macromolecules are produced by the host cells, but their production is regulated by tumour cells (Gullino & Grantham 1963; Muggia 1999). The physiological functions of extracellular matrix in normal tissues are to maintain homoeostasis, stabilize the spatial and functional regulation between cells (e.g., generating tissue cohesiveness), pose as a barrier to bacterial invasion and regulate macromolecule transport through the interstitium (Winlove & Parker 1995). In a tumour, the extracellular matrix proteins are a source of physical resistance to drug transport (Liotta & Rao 1985). The presence of collagen in the tumour extracellular matrix contributes to drug transport resistance in the interstitium (Netti et al 2000). Studies reveal that collagen is a major determinant of resistance to drug transport in solid tumours and suggest reduction of collagen content in tumours as a method for enhancing drug delivery to solid tumours. As the studies concentrated on the diffusivity of the macromolecules and not on the spatial drug distribution, it may not follow that the reduction in collagen would bring an even distribution of macromolecules throughout a tumour (Pluen et al 2000).

Tumour structure and composition

Tumor cell density. Diffusion through the tumour interstitial space is a major mode of drug transport in solid tumours. A larger fraction of interstitial space or a decrease in tortuosity would result in more rapid drug diffusion. Tumour histocultures have been used to study the spatial relationships between interstitial space, tumour cell density and drug penetration in solid tumours (Kuh et al 1999; Jang et al 2001; Zheng et al 2001). Stromal tissues and interstitial space in tumours are important determinants of drug delivery and transport.

Angiogenesis. In the initial growth phase (up to 1–2 mm in diameter), tumour cells can obtain oxygen and nutrients from the existing blood supply to the surrounding normal tissues. Angiogenesis is required to support the further growth of tumours beyond the microscopic stage. During angiogenesis, new blood vessels sprouting from mature blood vessels in the surrounding normal tissues grow toward tumour cells. The maintenance of these new vessels requires the presence of growth factors such as VEGF, which is recognized as the key inducer of tumour angiogenesis (Kerbel 2000).

Drug-induced cell death. Many anti-cancer drugs act by inducing apoptosis. The apoptosis process involves a sequence of events including cell shrinkage, increased cytoplasmic density, chromatic condensation and segregation into sharply circumscribed masses and the formation of membrane-bound surface apoptotic bodies. Apoptotic cells are phagocytosed from the midst of living tissues by neighbouring cells or macrophages without eliciting an inflammatory reaction. Studies in histoculture systems have shown that drug-induced apoptosis leads to decreased tumour cell density and expanded interstitial space, which in turn results in an enhanced rate of drug penetration to the inner layers of a solid tumor (Kuh et al 1999; Jang et al 2001; Zheng et al 2001). In addition to apoptosis, anti-cancer drugs can also induce necrosis. Whether a drug induces apoptosis or necrosis appears to be dependent on the intensity of the initial drug-induced insult, with necrosis occurring at higher intensity. Although both apoptosis and necrosis produce cell death and thereby reduce tumour cell density, there are significant differences in the nature of cell death by these two processes. Apoptosis occurs in an orderly fashion and does not elicit inflammation, whereas necrotic cell death is accompanied by extensive inflammation. Whether inflammation and the resulting pathological changes (e.g., accumulation of cells and fluid) alter drug transport in tumours is unknown. Apoptosis, because it occurs in cells scattered throughout a tumour, would result in expansion in interstitial space throughout a tumour. This is more desirable than space expansion in isolated areas of a tumour, as would be expected in the case of necrosis, where cell death occurs in large groups of contiguous cells. Finally, apoptosis induction typically requires lower drug concentrations and is therefore more readily attainable as a result of clinically relevant doses (Nicotera et al 1999).

Experimental approaches to enhance drug delivery

Altering tumour blood flow

These methods depend on the existing vasculature and may improve the drug delivery to vascular regions of tumours but will not improve the delivery to avascular regions. Local hyperthermia enhances the delivery of radioimmunoconjugates and monoclonal antibodies in animals (Cope et al 1990; Gridley et al 1991; Wilder et al 1993; Schuster et al 1995; Mittal et al 1996; Kinuya et al 1999) and in man (Chen et al 2001), presumably through an initial increase in tumour blood flow. However, further studies have shown that hyperthermia leads to dilatation of precapillary arterioles and results in a decrease in the arteriolar–venular pressure gradient and thereby in a decrease in tumour blood flow. Hence, enhanced drug delivery by local hyperthermia results from factors other than increased blood flow (Endrich & Hammersen 1986; Folkman 1990). The efficiency of vasopressors to increase tumour blood flow has been tested: angiotensin II was effective, whereas adrenergic vasopressors (adrenaline and methoxamine) were not effective (Edlich et al 1966; Suzuki et al 1981; Trotter et al 1991; Hori et al 1993). At a systemic blood pressure of 100–150 mmHg, angiotensin II enhances tumour blood flow without changing the blood flow to normal organs such as the liver, brain or bone marrow (Suzuki et al 1981; Trotter et al 1991). The selective increase in tumour blood flow results from the loss of autoregulation of blood flow and homoeostasis in tumour blood vessels (Suzuki et al 1981), presumably because tumour blood vessels lack both smooth muscle cells surrounding the endothelial cells and angiotensin II receptors (Vaupel 1977). In contrast, other vasopressors, such as adrenaline and methoxamine, reduce rather than enhance tumour blood flow. This is because these molecules act on different sites of the arteriole network (Hori et al 1993). The concept of using angiotensin II to improve the delivery or efficacy of chemotherapeutic agents to solid tumours has been verified experimentally. Studies have shown that the anti-tumour effects of mitomycin C against a subcutaneously implanted hepatoma tumour in rats, including reduction of tumour size, reduction of lymph node metastases and prolongation of survival time, were significantly improved by angiotensin II-induced hypertension (Suzuki et al 1981).

Drug retention in tumour

The EPR effect is being evaluated as a passive tumour-targeting approach to deliver macromolecules. Tumour-selective accumulation of soluble macromolecules, such as polymeric drug conjugates (e.g., poly(styrene-co-maleic acid-half-n-butylate)-conjugated neocarzinostatin (Iwai et al 1984; Seymour et al 1994; Noguchi et al 1998; Muggia 1999) and PK1[N-(2-hydroxypropyl)-methacrylate amide copolymer doxorubicin] (Muggia 1999), proteins (Sinn et al 1990) and liposomes (Wu et al 1993; Northfelt et al 1996)), has been demonstrated. Some of these compounds are currently in clinical evaluation (Northfelt et al 1996; Vasey et al 1999). Theoretically, increasing the levels of vasoactive factors such as VEGF, bFGF, bradykinin and nitric oxide may enhance vessel permeability. However, this approach may have limited practicality because of the instability of these molecules. Furthermore, these molecules have biological activity that may counteract the advantage of increased drug delivery. For example, VEGF is associated with enhancing tumour growth and metastasis and bFGF is associated with tumour resistance to chemotherapy .The use of an angiotensin-converting enzyme inhibitor such as enalapril or tempocapril also inhibits the degradation of bradykinin,

to increase the EPR effect. However, this principle has not been verified experimentally or clinically.

Modulating vascular and interstitial pressure

A high MVP results in an increase in transvascular fluid filtration (i.e., convection flow across the vascular wall), and enhances transvascular drug transport to tumours (Nicotera et al 1999). Similarly, lower IFP results in the same effects. Hence, a larger difference between MVP and IFP may result in a greater convection flow and fluid extravasation and thereby enhance the delivery of macromolecules (Jain 1989; Netti et al 1999).

Theoretically, either decreasing IFP or increasing MVP may enhance drug delivery to solid tumours. This approach has been evaluated in experimental model systems (Jain 1989; Griffon-Etienne et al 1999; Netti et al 1999) but has yet to be tested in patients. So far, there are no practical methods to successfully increase MVP.

Apoptosis-inducing pretreatment

Drug transport in the tumour interstitium increases with expansion of the interstitial space and reduction in tumour cell density. The use of apoptosis-inducing pretreatment has been investigated for highly-protein-bound drugs (paclitaxel, doxorubicin) to increase tumour transport (Kuh et al 1999; Zheng et al 2001). These drugs, as such, induce apoptosis. In-vitro studies using histocultures of xenograft and human patient's tumours, as well as in-vivo studies in tumour-bearing animals, have shown that tissue priming with these drugs enhances the rate and extent of drug delivery and eliminates the steep drug concentration gradient between the periphery and the core of solid tumours. The ability of tissue priming to enhance drug delivery to solid tumours may explain the finding that pretreatment with intravenous diphtheria toxin enhanced the delivery of a 36-kDa polymeric contrast agent, administered 44 h later, to human BRO melanoma xenograft in mice. The pretreatment resulted in a more even distribution and enhanced delivery to the vascular and avascular regions of the tumour by about two fold (Ranney et al 1988; Ranney 2000).

The above biological characteristics and their impact on drug delivery to the tumour have been summarized in Table 1.

Formulation design based on biological factors

Research on the role of the above-mentioned factors on drug transport and the accumulation of drugs in tumour cells has been ongoing for the last two decades. Drug transport efficiencies utilizing biological factors such as EPR effect (i.e., the enhanced permeability and retention), which is affected due to the leaky tumour vasculatures, resulting in cellular uptake of particles of the size of 400 nm, have resulted in the design of particulate or colloidal drug delivery systems (Nomura et al 1998; Monsky et al 1999). This colloidal drug delivery system is comprised of various novel carrier systems, such as fat emulsions, liposomes, microparticles and nanoparticles, prepared from different biodegradable polymers. The conventional systemic administration of bioactive compounds poses many problems, among which are their short in-vivo half-lives and their toxic side effects. In some cases, multiple injections of therapeutic agents are needed for the required dose to reach the desired target. Over the past few years, researchers have paid great attention to improving drug efficacy by developing new drug delivery systems. The major goals of drug delivery systems are to reduce the total dose of drug and concentrate it at the

Table 1 Biological characteristics of tumour and significance in drug delivery

Tumour features	Characteristics	Impact on drug delivery
Anatomical		
Vasculature	Heterogeneous distribution, large size and permeable Leaky microvessels Elevated levels of growth factors and bradykinin	Facilitates extravasation of large molecules and retention in tumours
Lymphatic system	Impaired lymphatic system	Enhanced permeability and retention effect of the drug in tumours
Interstitial pressure	Increased intra fluid pressure	Increases transport of large molecules to the tumours
Tissue components		
Cellular matrix	Presence of protein and nucleic acids	Ensures retention of high binding drugs to the tumours
Extracellular matrix	Presence of fibrous protein (collagen) and polysaccharide	Resistance of drug delivery to the tumours
Tumour structure		
	Increased interstitial space and decreased tortuosity	Rapid drug diffusion into the tumours
	Angiogenesis and presence of VEGF	Facilitates the drug delivery to the tumour due to extravasation of molecules
	Apoptosis	Expands the interstitial spaces in the tumours for increased drug transport

target site by using targeting and controlled release. Drug delivery systems are needed to control the location in which a drug is deposited and to manage the time of drug delivery. Incorporation of a drug within biodegradable, biocompatible injectable colloidal particles has been shown to be a promising approach for drug delivery. Fat emulsions (Wretline 1981), liposomes (Lasie 1998) and polymeric nanoparticles (Allemann et al 1993) have been some of the formulations that have been developed from the 1960s. Issues such as physical stability, difficulties in up-scaling, lack of specific tumour targeting (Collin-Gold et al 2000) and cytotoxicity of the polymers (Smith & Hunneyball 1986) have been the reasons for the limitation of the delivery systems.

In recent years, due to these drawbacks of the carrier systems, research groups have focused on nanoparticles prepared using lipid matrices. This system has the combined advantages of other innovative carrier systems, such as physical stability, protection of incorporated labile drugs from degradation, controlled release and excellent tolerability, and at the same time minimizes the associated problems.

Solid lipid nanoparticles (SLNs) in tumour targeting

These are particles made from solid lipids, which are solid at room temperature and also at body temperature. The main features of SLNs with regard to parenteral application are the excellent physical stability, protection of incorporated labile drugs from degradation, controlled drug release depending on the incorporation model, good tolerability and site-specific targeting. The potential disadvantages include insufficient loading capacity, drug expulsion after polymeric transition during storage and relatively high water content of the dispersions.

Due to their size, SLNs may be injected intravenously and used to target drugs to particular organs. The particles, as with all intravenously injected and colloidal particles, are cleared by the liver and spleen. To facilitate drug targeting in tumour tissue, a reticuloendothelial system avoidance (stealth) facility may be incorporated. In polyoxyethylene–polypropylene copolymers, like pluronic F188, the hydrophobic portion of the molecule forms the nanoparticle matrix, while the water-soluble polyoxyethylene block forms a hydrophilic coating on the particle. Stealth SLNs increase accumulation in the tumour (Chen et al 2001) and also allow brain delivery of anti-cancer drugs not capable of crossing the blood–brain barrier (Yang et al 2001).

Administration of SLNs and drug release. The SLNs are generally injected either intravenously, intramuscularly or subcutaneously or to the target organ. Because of their minimum size below 1 μm, SLN formulations can be used for systemic body distribution with a minimized risk of blood clotting and aggregation leading to embolism. SLNs also provide a sustained release depot of the drug when administered subcutaneously or accumulated in the mononuclear phagocytic systems (MPS). Incorporated drug is gradually released on erosion (e.g., degradation by enzymes) or by diffusion from the particles. The rate of release may be controlled by the nature of the lipid material (Fundaro et al 2000), particles size (Scholer et al 2001) and choice of surfactant (Scholer et al 2001, 2002) and also by the inner structure of the SLNs. The particle size of intravenously administered drug must be below 5 μm to avoid blocking fine capillaries, leading to embolism. The size of the SLNs can be brought much lower than 5 μm by using appropriate production techniques and controlling the parameters.

Pharmacokinetic profiles of lipid-encapsulated drugs. Comparison of drugs incorporated in SLNs versus unincorporated drug leads to different pharmacokinetic profiles, as has been described for doxorubicin (Zara et al 1999; Fundaro et al 2000) and paclitaxel (Migiglitta et al 2000). Drug-loaded SLNs showed three- to five-fold enhancement of plasma peaks. Interestingly, plasma concentrations for doxorubicin SLNs showed an exponential curve with high AUC, a lower rate of clearance and a small volume of distribution compared with free drug (Zara et al 1999; Fundaro et al 2000). The biphasic behaviour is probably due to the slow distribution of doxorubicin in SLNs. SLN formulations reduced the cardiotoxic side effects of doxorubicin in Wistar rats and prolonged the metabolization of doxorubicin to doxorubicinol. Uptake of the SLNs in cells of the MPS differed with the size and the composition of the particles. Uptake of SLNs can be avoided by PEGylation, leading to long-circulating particles called stealth SLNs. Though much work has been done on stealth SLNs, only a few reports on their in-vivo behaviour have been published. Interestingly, stealth doxorubicin SLNs showed similar circulating time and pharmacokinetic behaviour to unmodified nanoparticles (Fundaro et al 2000). The main reason might be the similar low surface hydrophobicity of both types of particles, avoiding adsorption of blood proteins mediating liver uptake. The modified tissue distribution of doxorubicin in SLNs was related to slower distribution and passage of particles through biological barriers and slow drug release from the lipid matrix into the blood. The biopharmaceutical behaviour may explain the lower blood concentration and reduction of severe side effects.

Tissue distribution and drug targeting. Concentration of SLNs within the Kupfer cells of the liver is predominantly found after intravenous injection of non-stealth SLNs. The systemic use of colloidal carriers is limited by the presence of MPS, and it is consequently necessary to avoid such recognition. SLN carriers are mostly recognized by macrophages due to the physicochemical characteristics of particle size, surface charge and surface hydrophobicity. Investigation of the anti-cancer drug camptothecin incorporated for intravenous administration to target brain has shown promising sustained release, reduced dosing and decreased systemic toxicity when administered in SLN form (Yang et al 1999).

Interactions with constituents of blood. Cellular binding of SLN formulations with emphasis on erythrocytes is important because it affects not only blood clotting and

embolic effects but also may change pharmacokinetic behaviour. SLNs have distinct affinity for red blood cells depending on the surfactant used. Flow cytometric studies showed that a SLN formulation consisting of Compritol as matrix material and Tween 80 and Poloxamer 188 did not bind to erythrocytes (SLN binding $< 10.0\%$). In contrast, when Span 85 was used, the blood cell affinity of labelled SLNs was increased, leading to significant aggregation of red blood cells (75.3%) (Weyhers et al 1995).

The interaction of SLN with the major circulatory protein, serum albumin, has been investigated recently. By photon correlation spectroscopy (PCS) and atomic force microscopy (AFM), albumin adsorption onto the particle surface formed a capping layer of 17 nm, increasing the size of the tested particle population only slightly (Olbrich et al 1999). AFM imaging revealed that the SLNs are protected by this layer against flattening on surfaces. At physiological albumin concentrations (35, 50 g L^{-1}) the increased size was not important enough to explain blood cell aggregation (Olbrich et al 1999).

Excipients used in parenteral SLNs and their safety. So far no SLN parenteral product has been used commercially but intensive research on SLNs in bioassays have been reported recently (Muller et al 2000). Most of the studies have been conducted with glycerides composed of fatty acids, which are mostly accepted as safe. The type of excipient (i.e., the surfactants and the lipids) account for the tolerability. For the formulation of parenteral SLNs, the surfactant has to be of the GRAS status. Many surfactants, such as lecithin, Tween 80, Poloxamer 188, Span 85 and sodium glycocholate, have been used in bolus injections. These have been found to have good tolerability in mice. Cetyl palmitate SLNs prepared with different surfactants caused no acute toxicity and no increase in liver and spleen weight (Muller et al 2000). No significant histological abnormality was found after autopsy.

The efficiency of the lipid nanoparticles administered parenterally in-vivo depends on the particles size. The particle size is, in turn, affected by the factors such as preparation, stability and sterilization. The effect of these parameters has been studied extensively and some of the important references for detailed information have been tabulated in Table 2.

In-vitro release of drugs from SLNs. For SLNs loaded with different drugs, varied release profiles have been observed. Stealth and non-stealth tripalmitin SLNs loaded with paclitaxel have been prepared as an alternative parenteral administration, as the commercially available product Taxol is a toxicologically critical micellar solution of the drug in Cremaphor EL (Cavalli et al 1993). During sustained in-vitro release, 0.1% of the paclitaxel was released into the receptor medium (phosphate buffer, pH 7.4) after 120 min; this correlates to first pseudo zero-order kinetics. Similar results have been obtained in the in-vitro release profiles for doxorubicin and idarubicin

Table 2 Various formulation aspects and features that influence size and eventually the efficiency of the SLNs

Formulation aspects	Features	References
Preparation		
High pressure homogenization	Preparation performed at both room and cold temperatures	Schwarz et al 1994; Cortesi et al 2002
	Cold homogenization employed for thermolabile and hydrophilic drugs	
Microemulsion method	Yields narrow particles size	Gasco 1993
	Performed by dispersing warm drug Microemulsion in excess of cold water	
Recent methods		
Solvent emulsification	Excess water removed by lyophilization /ultrafiltration	
W/O/W double emulsion	Gives narrow distribution with particle size of 100 nm	Eldem et al 1991
	Can incorporate hydrophilic drugs	Cortesi et al 2002
High-speed stirring	Yields average particle size in μm, hence needs to be reduced to nm size	
	Gives broad size distribution Short physical instability	Westensen et al 1997
	Product cannot be used for parenteral use	
Stability	More than 1 year, can be stable up to 3 years	Westensen 1994; Shahgaldian et al 2003
	Stability can be increased by spray drying and lyophilization	
Sterilization	Procedures like autoclaving and gamma filtration have been used successfully	Schwarz et al 1994; Lim & Kim 2002; Shahgaldian et al 2003

(0.1% after 120 min) in comparison with the reference solutions of the drugs, which exhibited burst release (Yang et al 1999). In-vitro release of camptothecin from stearic acid SLNs in conjunction with potential targeting to the brain, using a dialysis bag technique at 37 °C, revealed a sustained release that could be fitted to a Weibull distribution ($t½ = 23.1$ h) (Chen et al 2001).

Conclusions

The biological properties of a tumour, such as microvessels density, IFP and interstitial space, are dynamic properties that change with time and are affected by drug-induced apoptosis or necrosis. This is especially true for drugs that show high binding to macromolecules, because their transport is mainly by convection, which is affected more by the above dynamic biological properties as compared with small molecules, which are transported by simple diffusion. Some of a tumour's biological factors have opposite effects on drug delivery and transport in solid tumours and these factors are interdependent. A better understanding of the contribution of these various factors may lead to therapeutic strategies that permit passive or active tumour targeting. Most anti-cancer drugs belong to the classical low-molecular-weight drugs. In the near future, a number of therapeutic molecules like proteins or nucleotides, and drugs in gene therapy (Olbrich et al 2001), may be expected to be delivered. Due to their physical and chemical instability in the gastrointestinal tract, these drugs must be administered parenterally. Very few publications exist about protein or peptide formulations with SLNs. Alternative gene delivery system called lipoplexes have been developed. The efficiency of lipoplexes in the transfection of cos-1 cells with the β-galactosidase gene has been discussed and found to be effective (Olbrich et al 2001). For the last four decades, the field of drug delivery systems has witnessed drastic changes from the use of polymers to the use of lipid matrices in the preparation of stable injectable formulations. With research now focused on nanoparticles prepared from lipid matrices, this is expected to be a viable system in the delivery of the newer molecules as described above. As the technology used in the SLNs has various advantages, such as easy production, higher physical stability, lower cytotoxicity and increased transfection, we can in the near future expect an increasing contribution in the effective treatment of cancer.

References

Adams, G. P. (1998) Improving the tumor specificity and retention of antibody-based molecules. *In vivo* **12**: 11–12

Allemann, E., Gurny, R., Doelker, E. (1993) Drug loaded nanoparticles—preparation methods and drug targeting issues. *Eur. J. Pharm. Biopharm.* **39**: 173–191

Beaney, R. P., Lammertsma, A. A., Jones, T., McKenzie, C. G., Halnan, K. E. (1984) In vivo measurement of regional blood flow, oxygen utilization and blood volume in patients with carcinoma of the breast using positron tomography. *Lancet* **1**: 131–134

Cavalli, R., Caputo, O., Gasco, M. R. (1993) Solid lipospheres of doxorubicin and idarubicin. *Int. J. Pharm.* **89**: R9–R12

Chen, D. B., Yang, T. Z., Lu, W. L., Zhang, Q. (2001) In vitro and in vivo study of two types of long-circulating solid lipid nanoparticles containing paclitaxel. *Chem. Pharm. Bull.* **49**: 1444–1447

Collins, P. D., Connolly, D. T., Williams, T. J. (1993) Characterization of the increase in vascular permeability induced by vascular permeability factor in vivo. *Br. J. Pharmacol.* **109**: 195–199

Collin-Gold, L., Feiehtinger, N., Wamheim, T. (2000) Are lipid emulsions the drug delivery solutions? *Mod. Drug. Discov.* **3**: 44–48

Cope, D. A., Dewhirst, M. W., Friedman, H. S., Bigner, D. D., Zalutsky, M. R. (1990) Enhanced delivery of a monoclonal antibody $F(ab')_2$ fragments to subcutaneous human glioma xenografts using local hyperthermia. *Cancer Res.* **50**: 1803–1809

Cortesi, R., Esposito, E., Luca, G., Nastruzzi, C. (2002) Production of lipospheres as carriers for bioactive compounds. *Biomaterials* **23**: 2283–2294

Dellian, M., Witwer, B. P., Salehi, H. A., Yuan, F., Jain, R. K. (1996) Quantitative and physiological characterization of angiogenic vessels in mice: effect of basic fibroblast growth factor/vascular permeability factor and host microenvironment. *Am. J. Pathol.* **149**: 59–71

Drummond, D. C., Meyer, O., Hong, K., Kirpotin, D. B., Papahadjopoulos, D. (1999) Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. *Pharmacol. Rev.* **51**: 691–743

Edlich, R. F., Rogers, W., DeShazo, C. V., Aust, J. B. (1966) Effect of vasoactive drugs on tissue blood flow in the hamster melanoma. *Cancer Res.* **26**: 1420–1424

Eldem, T., Speiser, P., Hincal, A. (1991) Optimization of spray dried and congealed lipid micropellets and characterization of their surface morphology by scanning electron microscopy. *Pharm. Res.* **8**: 47–54

Endrich, B., Hammersen, F. (1986) Morphologic and hemodynamic alterations in capillaries during hyperthermia. In: Anghileri, L. J., Robert, J. (eds) *Hyperthermia in cancer treatment*. CRC Press, Boca Raton, FL, pp 17–47

Endrich, B., Reinhold, H. S., Gross, J. F., Intaglietta, M. (1979) Tissue perfusion inhomogeneity during early tumor growth in rats. *J. Natl Cancer Inst.* **62**: 387–395

Erlanson, M., Daniel-Szolgay, E., Carlsson, J. (1992) Relations between the penetrations, binding and average concentration of cytostatic drugs in human tumour spheroids. *Cancer Chemother. Pharmacol.* **29**: 343–353

Erlichman, C., Viogen, D. (1984) Cytotoxicity of adriamycin in MGH-U1 cells grown as monolayer cultures, spheroids and xenografts in immune-deprived mice. *Cancer Res.* **44**: 5369–5375

Falk, P. (1982) Differences in vascular pattern between the spontaneous and the transplanted C3H mouse mammary carcinoma. *Eur. J. Cancer Oncol.* **18**: 155–165

Folkman, J. (1990) What is the evidence that tumors are angiogenesis dependent? *J. Natl Cancer Inst.* **82**: 4–6

Folkman, J. (1995) Tumor angiogenesis. In: Mendelsohn, P. M., Howley, M. A. P., Liotta, L. A. (eds) *The molecular basis of cancer*. W. B. Saunders, Philadelphia, PA, pp 206–232

Fundaro, A., Cavalli, R., Bargoni, A., Vighetto, D., Zara, G. P., Gasco, M. R. (2000) Non stealth and stealth solid lipid nanoparticles and tissue distribution alter IV administration of rats. *Pharm. Res.* **42**: 337–343

Gasco, M. R. (1993) Method for producing solid lipid microspheres having a narrow size distribution. US Patent No 5250236

Gridley, D. S., Ewart, K. L., Cao, J. D., Stickney, D. R. (1991) Hyperthermia enhances localization of ^{111} In-labeled hapten to bifunctional antibody in human colon tumor xenografts. *Cancer Res*. **51**: 1515–1520

Griffon-Etienne, G., Boucher, Y., Brekken, C., Suit, H. D., Jain, R. K. (1999) Taxane-induced apoptosis decompresses blood vessels and lowers interstitial fluid pressure in solid tumors: clinical implications. *Cancer Res*. **59**: 3776–3782

Gullino, P. M., Grantham, F. H. (1963) The influence of the host and the neoplastic cell population on the collagen content of a tumor mass. *Cancer Res*. **23**: 648–653

Harper, E., Dang, W., Lapidus, R. G., Garver, R. I. (1999) Enhanced efficacy of a novel controlled release paclitaxel formulation (PACLIMER delivery system) for local-regional therapy of lung cancer tumor nodules in mice. *Clin. Cancer Res*. **5**: 4242–4248

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

Hori, K., Suzuki, M., Tanda, S., Saito, S. (1990) In vivo analysis of tumor vascularization in the rat. *Jpn. J. Cancer Res*. **81**: 279–288

Hori, K., Suzuki, M., Tanda, S., Saito, S. (1991) Characterisation of heterogeneous distribution of tumor blood flow in rat. *Jpn. J. Cancer Res*. **82**: 109–117

Hori, K., Zhang, Q. H., Saito, S., Tanda, S., Li, H. C., Suzuki, M. (1993) Microvascular mechanisms of change in tumor blood flow due to angiotensin II, epinephrine, and methoxamine: a functional morphometric study. *Cancer. Res*. **53**: 5528–5534

Iwai, Y., Maeda, H., Konno, T. (1984) Use of oily contrast medium for selective drug targeting to tumor: enhanced therapeutic effect and X-ray image. *Cancer Res*. **44**: 2115–2121

Jain, R. K. (1989) Delivery of novel therapeutic agents in tumors: physiological barriers and strategies. *J. Natl Cancer Inst*. **81**: 570–576

Jain, R. K. (1994) Transport phenomena in tumors. *Adv. Chem. Eng*. **19**: 129–200

Jain, R. K., Ward-Hartley, K. A. (1984) Tumor blood flow – characterization, modification and role in hyperthermia. *IEEE Trans. Sonics Ultrasonics* **31**: 504–524

Jang, S. H., Weintjes, M. G., Au, J. L.-S. (2001) Enhancement of paclitaxel delivery to solid tumors by apoptosis-inducing pretreatment: effect of treatment schedule. *J. Pharmacol. Exp. Ther*. **296**: 1035–1042

Kerbel, R. S. (2000) Tumor angiogenesis: past, present and the near future. *Carcinogenesis* **21**: 505–515

Kinuya, S., Yokoyama, K., Hiramatsu, T., Tega, H., Tanaka, K., Konishi, S., Shuke, N., Aburano, T., Watanabe, N., Takayama, T., Michgishi, T., Tonami, N. (1999) Combination radioimmunotherapy with local hyperthermia: increased delivery of radioimmunoconjugate by vascular effect and its retention by increased antigen expression in colon cancer xenografts. *Cancer Lett*. **140**: 209–218

Kuh, H. J., Jang, S. H., Wientjes, M. G., Weaver, J. R., Au, J. L.-S. (1999) Determinants of paclitaxel penetration and accumulation in human solid tumor. *J. Pharmacol. Exp. Ther*. **290**: 871–880

Lasie, D. D. (1998) Novel applications of liposomes. *Tibtech* **16**: 307–321

Lauk, S., Zeitman, A., Skates, S., Fabian, R., Suit, H. D. (1989) Comparative morphometric study of tumor vasculature in human squamous cell carcinomas and their xenotransplants in athymic nude mice. *Cancer Res*. **49**: 4557–4561

Lim, S.-J., Kim, C.-K. (2002) Formulation parameters determining the physicochemical characteristics of solid lipid nanoparticles loaded with all-trans retinoic acid. *Int. J. Pharm*. **244**: 135–146

Liotta, L. A., Rao C. N. (1985) Role of the extracellular matrix in cancer. *Ann. NY Acad. Sci*. **460**: 333–344

Liotta, L. A., Kileinerman, J., Saide, G. M. (1974) Quantitative relationships of intravascular tumor cells, tumor vessels, and pulmonary metastases following tumor implantation. *Cancer. Res*. **34**: 997–1004

Maeda, H., Matsumura, Y., Kato, H. (1988) Purification and identification of [hydroxyprolyl$_3$] bradykinin in ascitic fluid from a patient with gastric cancer. *J. Biol. Chem*. **263**: 16051–16054

Matsumura, Y., Kimura, M., Yamamoto, T., Maeda, H. (1988) Involvement of the kinin-generating cascade in enhanced vascular permeability in tumor tissue. *Jpn. J. Cancer Res*. **79**: 1327–1334

Migiglitta, A., Cavalli, R., Bocca, C., Gabriel, L., Gasco, M. R. (2000) Cellular uptake and cytotoxicity of solid lipid nanospheres (SLN) incorporating doxorubicin or paclitaxel. *Int. J. Pharm*. **210**: 61–67

Mittal, B. B., Zimmer, M. A., Sathiaseelan, V., Benson, A. B., Mittal, R. R., Dutta, S., Rosen, S. T., Spies, S. M., Mettler, J. M., Groch, M.W. (1996) Phase I/II trial of combined ^{131}I anti-CEA monoclonal antibody and hyperthermia in patients with advanced colorectal adenocarcinoma. *Cancer* **78**: 1861–1870

Monsky, W. L., Fukumura, D., Gohongi, T. (1999) Augmentation of transvascular transport of macromolecules and nanoparticles in tumors using vascular endothelial growth factors. *Cancer Res*. **59**: 4129–4135

Muggia, F. M. (1999) Doxorubicin-polymer conjugates: further demonstration of the concept of enhanced permeability and retention. *Clin. Cancer Res*. **5**: 7–8

Muller, R. H., Maassen, S., Weyhers, H., Mehnert, W. (1996) Phagocytic uptake and cytotoxicity of solid lipid nanoparticles (SLN) sterically stabilized with poloxamine 908 and poloxamer 407. *J. Drug Target*. **4**: 161–170

Muller, R. H., Mader, K., Gohla, S. (2000) Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art. *Eur. J. Pharm. Biopharm*. **50**: 161–177

Nederman, T., Carlsson, J. (1984) Penetration and binding of vinblastine and 5-fluorouracil in cellular spheroids. *Cancer Chemother. Pharmacol*. **13**: 131–135

Nederman, T., Carlsson, J., Kuoppa, K. (1988) Penetration of substances into tumour tissues. Model studies using saccharides, thymidine and thymidine-5′-triphosphate in cellular spheroids. *Cancer Chemother. Pharmacol*. **22**: 21–25

Netti, P. A., Hamberg, L. M., Babich, J. W., Kierstead, D., Graham, W., Hunter, G. J., Wolf, G. L., Fischman, A., Boucher, Y., Jain, R. K. (1999) Enhancement of fluid filtration across tumor vessels: implications for delivery of macromolecules. *Proc. Natl Acad. Sci. USA* **96**: 3137–3142

Netti, P. A., Berk, D. A., Swartz, M. A., Grodzinsky, A. J., Jain, R. K. (2000) Role of extracellular matrix assembly in interstitial transport in solid tumors. *Cancer Res*. **60**: 2497–2503

Nicotera, P., Leist, M., Ferrando-May, E. (1999) Apoptosis and necrosis: different execution of the same death. *Biochem. Soc. Symp*. **66**: 69–73

Noguchi, Y., Wu, J., Duncan, R., Strohalm, J., Ulbrich, K., Akaike, T., Maeda, H. (1998) Early phase tumor accumulation of macromolecules: a great difference in clearance rate between tumor and normal tissues. *Jpn. J. Cancer Res*. **89**: 307–314

Nomura, T., Koreeda, N., Yamashita, F., Takakura, Y., Hashida, M. (1998) Effect of particles size and charge on the deposition of lipid carriers after intra-tumoral injection into tissue — isolated tumors. *Pharm. Res*. **15**: 128–132

Northfelt, D. W., Martin, F. J., Working, P., Volberding, P. A., Russel, J., Newman, M., Amantea, M. A., Kaplan, L. D. (1996) Doxorubicin encapsulated in liposomes containing surface-bound polyethylene glycol: pharmacokinetics, tumor

localization, and safety in patients with AIDS-related Kaposi's sarcoma. *J. Clin. Pharmacol.* **36**: 55–63

O'Driscoll, C. M. (1992) Anatomy and physiology of the lymphatics. In: Charman, W. N., Stella, V. J. (eds) *Lymphatic transport of drugs*. CRC Press, Boca Raton, FL, pp 1–36

Olbrich, C., Kayser, O., Kiderlen, A. F., Muller, R. H. (1999) Flowcytometry as a possible tool for assessing cellular binding capacities of solid lipid particles (SLN). *Proc. 26th Int. Sym. Contolled Release Bioact. Mater.* 519–520

Olbrich, C., Bakowsky, U., Lehr, C. M., Muller, R. H., Kneuer, C. (2001) Cationic solid lipid nanoparticles can efficiently bind and transfect plasmid DNA. *J. Control. Release* **13**: 345–355

Pagnan, G., Stuart, D. D., Pastorino, F., Raffaghello, L., Montaldo, P. G, Allen, T. M., Calabretta, B., Ponzoni, M. (2000) Delivery of c-myb antisense oligodeoxynucleotide to human neuroblastoma cells via disialoganglioside GD(2)-targeted immunoliposomes: antitumor effects. *J. Natl Cancer Inst.* **92**: 253–261

Peters, W., Teixeira, M., Intaglietta, M., Gross, I. F. (1980) Microcirculatory studies in a rat mammary carcinoma. I. Transparent chamber method, development of microvasculature and pressures in tumor vessels. *J. Natl Cancer Inst.* **65**: 631–642

Pluen, A., Ramanujan, S., Boucher, Y., Unemori, E., Seed, B., Jain, R. K. (2000) Relacin increases the transport of large molecules in high collagen content tumors. *Proc. Am. Assoc. Cancer. Res.* **38**: 604

Ranney, D. F. (2000) Biomimetic transport and rational drug delivery. *Biochem. Pharmacol.* **59**: 105–114

Ranney, D., Antich, P., Cohen, J., Erdman, W., Kulkarni, P., Giovanella, B. (1988) Submillimeter monitoring of tumor treatment response by contrast-enhanced magnetic resonance imaging (MRI): implications for tumor access of antibodies lymphokines and macromolecular drugs. *Proc. Am. Assoc. Cancer Res.* **29**: 495

Roberts, W. G., Palade, G. E. (1995) Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor. *J. Cell. Sci.* **108**: 2369–2379

Sasaki, T., Yamamoto, M., Yamaguchi, T., Sugiyama, S. (1984) Development of multicellular spheroids of HeLa cells co-cultured with fibroblasts and their response to X-irradiation. *Cancer Res.* **44**: 345–351

Scholer, N., Hahn, H., Muller, R. H., Leiscufeld, O. (2001) Surfactant and not the size of Solid Lipid Nanoparticles (SLN) influences the viability and cytokine production of macrophages. *Int. J. Pharm.* **221**: 57–67

Scholer, N., Hahn, H., Muller, R. H., Leiscufeld, O. (2002) Effect of lipid matrix and size of solid lipid nanoparticles (SLN) on viability and cytokine production of macrophages. *Int. J. Pharm.* **231**: 167–176

Schuster, J. M., Zalutsky, M. R., Noska, M. A., Dodge, R., Friedman, H. S., Bigner, D. D., Dewhirst, M. W. (1995) Hyperthermic modulation of radiolabelled antibody uptake in a human glioma xenograft and normal tissues. *Int. J. Hyperthermia* **11**: 59–72

Schwarz, C., Mehnert, W., Lucks, J. S., Muller, R. H. (1994) Solid lipid nanoparticle (SLN) for controlled drug delivery: I production, characterization and steriliszation. *J. Control. Release* **30**: 83–96

Schwarze, S. R., Ho, A., VoceroAkbani, A., Dowdy, S. F. (1999) In vivo protein transduction: delivery of a biologically active protein into the mouse. *Science* **285**: 1569–1572

Seymour, L. W., Ulbrich, K., Steyger, P. S., Brereton, M., Subr, V., Strohalm, J., Duncan, R. (1994) Tumor tropism and anti-cancer efficacy of polymer-based doxorubicin prodrugs in the treatment of subcutaneous murine B16F10 melanoma. *Br. J. Cancer* **70**: 636–641

Shahgaldian, P., Da Silva, E., Coleman, A. W., Rather, B., Zaworotko, M. J. (2003) *Para*-acyl-calyx-arene based solid lipid nanoparticles (SLN): a detailed study of preparation and stability parameters. *Int. J. Pharm.* **253**: 23–38

Sinn, H., Schrenk, H. H., Friedrich, E. A., Schilling, U., Mair-Borst, W. (1990) Design of compounds having an enhanced tumour uptake, using serum albumin as carrier. Part I. *Int. J. Rad. Appl. Instrum B* **17**: 819–827

Sjostrom, B., Kaplon, A., Talmon, Y., Cabanc, B. (1995) Structure of nanoparticles prepared from oil-in-water emulsions. *Pharm. Res.* **21**: 39–48

Smith, A., Hunneyball, I. M. (1986) Evaluation of Polylactid as a biodegradable drug delivery system for parenteral administration. *Int. J. Pharm.* **30**: 215–230

Straw, J., Hart, M., Klubes, P., Zaharko, D., Dedrick, R. L. (1974) Distribution of anticancer agents in spontaneous animal tumors. I Regional blood flow and methotrexate distribution in canine lymphosarcoma. *J. Natl Cancer Inst.* **52**: 1327–1331

Suzuki, M., Hori, K., Abe, I., Saito, S., Sato, H. (1981) A new approach to cancer chemotherapy: selective enhancement of tumor blood flow with angiotensin II. *J. Natl Cancer Inst.* **67**: 663–669

Tannock, I. F., Steel, G. G. (1969) Quantitative techniques for study of the anatomy and function of small blood vessels in tumors. *J. Natl Cancer Inst.* **42**: 771–782

Trotter, M. J., Chaplin, D. J., Olive, P. L. (1991) Effect of angiotensin II on intermittent tumour blood flow and acute hypoxia in the murine SCCVII carcinoma. *Eur. J. Cancer* **27**: 887–893

Vasey, P. A., Kaye, S. B., Morrison, R., Twelves, C., Wilson, P., Duncan, R., Thomson, A. H., Murray, L. S., Hilditch, T. E., Murray, T., Burtles, S., Fraier, D., Fringerio, E., Cassidy, J. (1999) Phase I clinical and pharmacokinetic study of PK1 [N-(2-hydroxypropyl) methacrylamide copolymer doxorubicin]: first member of new class of chemotherapeutic agents — drug–polymer conjugates. *Clin. Cancer Res.* **5**: 83–94

Vaupel, P. (1977) Hypoxia in neoplastic tissue. *Microvasc. Res.* **13**: 399–408

West, G. W., Weichselbaum, R., Little, J. B. (1980) Limited penetration of methotrexate into human osteosarcoma spheroids as a proposed model for solid tumor resistance to adjuvant chemotherapy. *Cancer Res.* **40**: 3665–3668

Westensen, K. (1994) Novel lipid-based colloidal dispersions as potential drug administration systems – expectations and reality. *Coll. Surf. Polym. Sci.* **278**: 609–618

Westensen, K., Bunjes, H., Koch, M. J. (1997) Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. *J. Control. Release* **48**: 223–236

Weyhers, H., Ehlers, S., Mehnert, W., Hahn, H., Muller, R. H. (1995) Solid lipid nanoparticles — determination of an in vivo toxicity. Proceedings of first world Meeting APGI/APV, 489–490

Wilder, R. B., Langmuir, V. K., Mendonca, H. L., Goris, M. L., Knox, S. J. (1993) Local hyperthermia and SR 4233 enhance the anti-tumor effects of radioimmunotherapy in nude mice with human colonic adenocarcinoma xenografts. *Cancer Res.* **53**: 3022–3027

Winlove, C. P., Parker, K. H. (1995) The physiological function of the extracellular matrix. In: Reed, R. K., Laine, G. A., Bert, J. L., Winlove, C. P., McHale, N. (eds) *Interstitium, connective tissue and lymphatics*. Portland Press, London, pp 137–165

Wolf, J. K., Mills, G. B., Bazzet, L., Bast, R. C., Roth, J. A., Gerhenson, D. M. (1999) Adenovirus-mediated p53 growth

inhibition of ovarian cancer cell line is independent of endogenous p53 status. *Gynecol. Oncol.* **75**: 261–266

Wretlind, A. (1981) Development of fat emulsions. *J. Parenter. Enter. Nutr.* **5**: 230–235

Wu, N. Z., Da, D., Ruoll, T. L., Needham, D., Whorton, A. R., Dewhirst, M. W. (1993) Increased microvascular permeability contributes to preferential accumulation of stealth liposomes in tumor tissues. *Cancer Res.* **53**: 3765–3770

Yamaura, H., Sato, H. (1974) Quantitative studies on the developing vascular system on rat hepatoma. *J. Natl Cancer Inst.* **53**: 1229–1240

Yang, S. C., Lu, L. F., Cai, Y., Zhu, J. B., Liang, B. W., Yang, C. Z. (1999) Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. *J. Control. Release* **59**: 299–307

Zara, G. P., Cavalli, R., Fundaro, A., Bargoni, A., Caputo, O., Gasco, M. R. (1999) Pharmacokinetics of doxorubicin incorporated in solid lipid nanospheres (SLN). *Pharm. Res.* **42**: 337–343

Zheng, J. H., Chen, C. T., Au, J. L.-S., Wientjes, M. G. (2001) Time and concentration-dependent penetration of doxorubicin in prostrate tumors. *AAPS PharmSci.* **3**: article 15 (http://www.pharmsci.org/journal)