

Tumor targeting using liposomal antineoplastic drugs

Jörg Huwyler¹
 Jürgen Drewe²
 Stephan Krähenbühl²

¹University of Applied Sciences Northwestern Switzerland, Institute of Pharma Technology, Muttenz, Switzerland; ²Department of Research and Division of Clinical Pharmacology, University Hospital Basel, Basel, Switzerland

Abstract: During the last years, liposomes (microparticulate phospholipid vesicles) have been used with growing success as pharmaceutical carriers for antineoplastic drugs. Fields of application include lipid-based formulations to enhance the solubility of poorly soluble antitumor drugs, the use of pegylated liposomes for passive targeting of solid tumors as well as vector-conjugated liposomal carriers for active targeting of tumor tissue. Such formulation and drug targeting strategies enhance the effectiveness of anticancer chemotherapy and reduce at the same time the risk of toxic side-effects. The present article reviews the principles of different liposomal technologies and discusses current trends in this field of research.

Keywords: tumor targeting, antineoplastic drugs, liposomes, pegylation, steric stabilization, immunoliposomes

Passive targeting of solid tumors using liposomal carriers

Conventional liposomes

For highly lipophilic drugs, such as many antineoplastic agents, specific formulation strategies are needed to allow for oral or parenteral administration. Liposomes have been used traditionally as a formulation strategy to assist in formulation of poorly-soluble therapeutic agents. They can be defined as particulate drug carriers, which are formed spontaneously by dispersion of phospholipids in aqueous media. The resulting closed membrane structures can accommodate amphiphilic or lipophilic drugs incorporated into or associated with the lipid bilayer, as opposed to direct encapsulation or active entrapment of hydrophilic compounds within the aqueous inner compartment of the vesicles. Stability of the membrane bilayer as well as retention of incorporated drugs depends thereby on lipid composition and cholesterol content of the liposomal membranes. Liposomes with a defined and uniform size can be produced by different methods such as sonication or extrusion through polycarbonate filter membranes. Their minimal size of 25–100 nm is determined by the maximum possible packing of head-groups in the inner leaflet of the membrane bilayer as the curvature of the membrane increases with decreasing radius. Potential advantages of liposomal formulations are twofold: First, concentrations of lipophilic drugs in aqueous media can be increased considerably using liposomal formulations. Second, liposomal carriers have a protective effect on incorporated drugs by preventing their enzymatic degradation (Krishna and Mayer 1999). The antifungal antibiotic amphotericin B is one of the first examples of a marketed drug, which made use of this formulation principle for intravenous infusion (Gulati et al 1998). The stability and shelf-life of such drug formulations can be extended from several months to years by lyophilization (Stevens and Lee 2003).

Liposomal carriers have a strong impact on pharmacokinetics and tissue distribution of incorporated drugs. This may lead to enhanced efficacy as well as reduced

Correspondence: Jörg Huwyler
 University of Applied Sciences
 Northwestern Switzerland, Institute
 of Pharmaceutical Technology,
 Gründenstrasse 40, 4132 Muttenz,
 Switzerland
 Tel +41 61 467 46 89
 Fax +41 61 467 47 01
 Email joerg.huwyler@fhnw.ch

toxic side-effects of antitumor drugs. Clinical trials have demonstrated a reduced risk of cardiotoxicity of liposomal doxorubicin as compared to the free drug while preserving antitumor activity (Ewer et al 2004). A major draw-back of conventional liposomes is their rapid uptake and accumulation by phagocytic cells of the mononuclear phagocyte system (reticuloendothelial system or RES) after systemic administration (Frank 1993). The major organs of accumulation are the liver and the spleen due to their rich blood supply and the abundance of tissue-resident phagocytic cells. Such an unwanted macrophage targeting during chemotherapy may be problematic since it may lead to partial depletion of macrophages and interfere with important host-defense functions of this cell type (Daemen et al 1995). On the other hand, passive targeting of organs such as spleen and liver may offer as well some advantages with respect to tumor chemotherapy: First, the marked increase in tissue retention and accumulation of liposomal drugs may lead in the case of lipophilic anticancer drugs to retarded removal of the drugs from the circulation (Juliano and Stamp 1978). In these studies, two- to tenfold higher plasma exposures of the antitumor agents vinblastine, actinomycin, cytosine arabinoside and daunomycin were observed in rats 3 hours after intravenous administration as compared to control rats treated with conventional formulations of these drugs. Second, cytokines and other immunomodulators have been incorporated in liposomes and were used to activate macrophages and to render them tumoricidal (Daemen 1992). The application of such liposome-encapsulated macrophage activators for the treatment of metastatic tumors was explored recently in clinical trials (Worth et al 1999).

Sterically stabilized liposomes

Different methods have been proposed to increase the half-life of liposomes in the circulation. They include the use of synthetic phospholipids, which are conjugated to gangliosides (such as monosialoganglioside GM₁ derived from bovine brain (Allen and Chonn 1987)) or polyethylene glycol (PEG) (Klibanov et al 1990; Papahadjopoulos et al 1991; Woodle et al 1992; Uster et al 1996). Grafting of the liposome with the inert and biocompatible polymer PEG leads to the formation of a protective, hydrophilic layer on the surface of the liposomes. This modification prevents the recognition of liposomes by opsonins (ie, antibodies or components of the complement system) and therefore reduces their clearance by cells of the RES (Moghimi and Patel 1992). Such pegylated liposomes are therefore often referred to as 'sterically stabilized' or 'stealth' liposomes

(Lasic and Papahadjopoulos 1995). In humans, pegylation of liposomes results in an up to 50-fold decrease in the volume of distribution to values similar to the plasma volume (from 200 to 4.5 l), a 200-fold decrease in systemic plasma clearance from 22 to 0.1 l/hour and a nearly 100-fold increase in area under the time-concentration curve (Allen 1994). Using pegylated phospholipids, the apparent terminal half-life of such long-circulating liposomes can be extended in humans from a time-scale in minutes to days (Lasic 1996).

The protective effect of pegylation and the resulting extension of the plasma half-life *in vivo* correlates with the thickness of the PEG-coating. Experiments with polymerosomes composed of synthetic pegylated block polymers demonstrates that plasma half-life of pegylated nanoparticles scales indeed with the length of the PEG polymer chain (Photos et al 2003). On theoretical grounds, a thickness of a PEG coating of 5 to 10 per cent of the particle diameter is needed to achieve effective steric stabilization (Lasic 1996). Other studies explored the thickness of a PEG coating by direct measurement of PEG-tethered ligand-receptor interaction potentials using a surface forces apparatus (Wong et al 1997). The length of an extended PEG chain with a molecular weight of 2000 Da (PEG-2000) was thereby demonstrated to be in the range of 16 nm whereas the thickness of a coiled PEG-2000 chain was 5 nm. Based on these considerations, it can be concluded that coating of 100 nm liposomes with PEG-2000 should lead to effective steric stabilization *in vivo*.

Pegylated liposomes are biocompatible, inert and are characterized by a long half-life in the plasma compartment *in vivo*. As outlined above, they show minimal interactions with tissues and organs after systemic administration. Due to their big particulate size, long-circulating PEG-liposomes can not penetrate across continuous or fenestrated normal blood vessels since permeability in these vessels is restricted to molecules with a molecular weight of more than 5 kDa in peripheral tissues and 70 kDa in the kidney (corresponding to a glomerular filtration cut-off for cationized proteins such as ferritin of 14 nm) (Kanwar et al 1991; Maeda 2001), respectively. However, within pathological tissues such as inflammatory or solid tumor tissues, the vascular permeability increases and therefore allows for extravasation of macromolecules including plasma proteins and pegylated liposomes. In such tissues, macromolecules up to a molecular weight of approximately 4000 kDa (corresponding to a particulate size of 500 nm) are trapped within the interstitial tissue space (Yuan et al 1995). This phenomenon has been studied extensively and has been termed the tumor-selective

enhanced permeability and retention (EPR) effect (Maeda et al 2000). These unique properties of solid tumor tissue in combination with the extended circulation half-life of sterically stabilized liposomes have been exploited clinically for passive tumor tissue targeting (Gabizon et al 1994).

Clinical use of pegylated liposomes

Liposomal drug formulations offer the possibility to increase efficacy while reducing toxic side effects of cytotoxic chemotherapeutic drugs. At present, several liposomal anticancer drugs are available in the clinic or are in advanced stages of clinical development (Park et al 2004; Hofheinz et al 2005). Approved drugs include pegylated liposomal doxorubicin (Doxil/Caelyx by Alza/Johnson and Johnson in the US and Schering-Plough outside the US), non-pegylated liposomal doxorubicin (Myocet by Elan), liposomal daunorubicin (DaunoXome by Gilead), liposomal cytarabine (DepoCyte by Skye Pharma/Enzon/Mundipharma) and liposomal cisplatin (Lipoplatin by Regulon). Liposomal formulations of anthracyclines are used for the treatment of ovarian and breast cancer or HIV associated Kaposi's sarcoma. DepoCyte was approved for the treatment of lymphomas with meningeal spread and is the only liposomal drug administered by intrathecal infusion. Lipoplatin is used for the treatment of epithelial malignancies (Stathopoulos et al 2005). The clinical use of liposomal formulations of conventional cytostatic drugs was focused initially on anthracyclines since these cationic amphiphiles allow for an efficient and stable liposomal entrapment. More importantly, anthracyclines bear a high risk for acute and cumulative cardiotoxicity (resulting in cardiomyopathy) limiting their use. This problem may be addressed using appropriate liposomal formulations (Gabizon 2001; Waterhouse et al 2001) since an altered pharmacokinetics of liposomal anthracyclines offers the possibility to avoid high plasma peaks owing to the drug retention within the liposomal formulation. In addition, a reduced distribution of the liposomal anthracyclines to the heart muscle is observed using pegylated liposomes. Table 1 provides a summary of pharmacokinetic properties of commercial pegylated and non-pegylated liposomal doxorubicin in comparison to the free drug demonstrating the significant differences between the different formulation principles. As outlined above, pegylated liposomes show minimal interactions with non-diseased tissues leading to both a low systemic plasma clearance as well as a low volume of distribution of 0.03–0.05 L/kg (Table 1), which corresponds to values obtained for commonly used plasma volume markers or human IgG antibodies (Lobo et al 2004). Consequently, pegylated liposomal anthracyclines show a significantly lower risk of

Table 1 Pharmacokinetic properties in human of commercial preparations of doxorubicin (DOX). Free doxorubicin is compared to doxorubicin encapsulated in conventional liposomes (Myocet) and doxorubicin encapsulated in pegylated liposomes (Doxil, Caelyx). Liposome diameter: 85 to 150 nm. Data normalisation using an average body surface area of 1.7 m² and an average body weight of 70 kg. Examples of representative studies (Hamilton et al 2002; Gabizon et al 2003; Mross et al 2004).

| | Free DOX | Myocet (non-pegylated liposomal DOX) | Doxil/Caelyx (pegylated liposomal DOX) |
|---------------------|------------------------|--|--|
| Dose (mg/kg) | 1.2 | 1.8 | 1.5 |
| AUC (mg.h/L) | 3.5 | 19.4 | 4082 |
| Clearance (ml/h) | 25'300 | 9'520 | 23 |
| V _{ss} (L) | 365 | 139 | 3.0 |
| Half-life (h) | 0.06/10.4 ^a | <1/52.6 ^a | 84 |

^aTwo elimination phases.

cardiotoxicity (Ewer et al 2004). This site avoidance of a drug sensitive tissue is paralleled by an enhanced drug deposition in tumor tissue (passive tumor targeting) leading to a pharmacodynamic advantage as compared to the free drug (Gabizon et al 2006). Thus, the improved therapeutic index results in this case from both enhanced efficacy and reduced toxicity.

Vector-mediated tumor targeting using liposomal carriers

Receptor-mediated tumor targeting

Tumor cells are often characterized by a specific expression pattern of membrane associated proteins such as receptors, membrane transport systems or adhesion molecules. Provided that these structures are accessible from the extracellular space, such properties can be exploited for an active targeting of diseased cells and tissues using specific effector molecules. The concept of active targeting has the potential to combine the advantage of an increased therapeutic efficacy with a reduced risk for adverse side-effects in non-diseased tissues. With the arrival of genetic engineering technologies, which made it possible to design chimeric mouse-human monoclonal antibodies or recombinant peptidic receptor ligands, the clinical use of these active tumor targeting strategies has become reality. During the last years, several monoclonal antibodies were developed and FDA-approved for the active targeting of various tumors (Imai and Takaoka 2006). Examples include Trastuzumab (Herceptin), a monoclonal antibody for the treatment of HER-2/neu-positive breast cancer (Baselga 2000), Rituximab (Mabthera) for the treatment of CD20 expressing lymphoproliferative cells (McLaughlin

et al 1998) or Alemtuzumab (Campath) for the treatment of B- and T-cell hematological tumors being characterized by the expression of the CD52 surface antigen (Flynn and Byrd 2000). The mechanisms of an antibody-based cancer therapy can be twofold: First, a direct action by blocking or stimulating the function of target receptors, eg, inhibition of signaling by the human epidermal growth factor receptor 2 (HER-2/neu) by Herceptin leading to cell growth inhibition and apoptosis of the target cell. Second, immune-mediated elimination of tumor cells by IgG mediated mechanisms including antibody-dependent cellular toxicity, complement-dependent cytotoxicity and cell mediated cytotoxicity (eg, phagocytosis by macrophages or cytolysis by natural killer cells after recruitment of these immune-effector cells) (Imai and Takaoka 2006). The efficacy of such therapeutic antibodies can be increased by combination with a conventional chemotherapy. Alternatively, the antibodies can be linked directly to a toxin in order to guide the cytotoxic drug to the target tumor tissue. Experimental systems were used to study conjugates between targeting antibodies and small molecules such as the antineoplastic drug daunomycin (Sinkule et al 1991). Clinical trials have explored the pharmacological effects of conjugates between antibodies and potent plant toxins such as a deglycosylated ricin A-chain (Pastan and Kreitman 1998; Schnell et al 2003). Such targeting strategies using specific monoclonal antibodies as targeting vectors are of great interest. However, a major draw-back of these technologies is the limited carrying capacity of the monoclonal antibody vector since a very limited amount of effector molecules only can be coupled directly to a targeting vector without interfering with the antigen-recognition by the antibody.

Vector-conjugated liposomes

The pharmacokinetic properties of liposomes can be modulated by specific modifications of the liposome surface. Besides direct chemical modifications of the phospholipid headgroups (such as the introduction of surface charges or hydrophilic groups (Gabizon and Papahadjopoulos 1992)), conjugation of proteins, peptides or other macromolecules to the liposome surface can be achieved. Chemical conjugation techniques provide thereby a stable link between the liposomal phospholipids and a specific targeting vector (Hansen et al 1995; Torchilin 2005). The availability of pegylated liposomes made the development of vector-conjugated liposomes possible since the unique properties of these long-circulating liposomes can be combined with those of a targeting vector of choice within one preparation. These properties include ideally:

- Favorable pharmacokinetic properties due to minimal interactions with non-targeted tissues or organs
- High selectivity towards a biological target increasing drug efficacy and safety
- A high transport capacity since high concentrations of drug molecules can be achieved within the liposomal carrier to be transported using a limited number of conjugated targeting vectors
- Protection from enzymatic degradation of the liposomal cargo within the liposome
- High biocompatibility and therefore a presumably low immunogenicity of the liposomal carrier.

Initial attempts to realize the potential of this technology used coupling procedures where a targeting receptor was conjugated directly to the surface of the pegylated liposome. Such a co-immobilization of PEG and the vector on the same liposome, however, can lead to poor target recognition due to steric hindrance by the hydrophilic PEG corona (Schnyder and Huwyler 2005). It has therefore been proposed to use PEG as a spacer by coupling targeting vectors to the distal end of pegylated phospholipids (Blume et al 1993; Allen et al 1995; Shahinian and Silvius 1995; Huwyler et al 1996). This design increases the flexibility and accessibility of the PEG-tethered vector and therefore facilitates its interaction with the biological target.

Vector-conjugated PEG-liposomes were used widely for tumor targeting. The specificity and characteristics of these liposomal carriers is thereby given mainly by the used targeting vectors. Such vectors include small molecules, peptides or monoclonal antibodies. Representative examples for each of these targeting principles will be provided in the following sections.

Many tumor cells are characterized by an overexpression of the folate receptor. The fact that this receptor is responsible for the receptor-mediated endocytosis, and thus the cellular internalization of the vitamin folic acid, has established the possibility to deliver antineoplastic drugs, macromolecules as well as liposomes by this pathway (Wang and Low 1998; Gosselin and Lee 2002; Gabizon et al 2004). Delivery of daunomycin (Pan and Lee 2005) as well as doxorubicin (Shmeeda et al 2006) using folate-conjugated liposomes increased the cytotoxicity of the encapsulated anticancer drugs in various tumor cells. In the latter study (Shmeeda et al 2006), mouse J6456 lymphoma tumor cells up-regulated for the folate receptor were targeted using long-circulating liposomes, where folate was coupled to the distal end of PEG-grafted phospholipids. Using folate-conjugated liposomes, increased intracellular accumulation of the liposomal cargo was observed

in vitro as well as in a mouse ascitic tumor model. It remains to be elucidated, if the accumulation of liposomal carriers within the endosomal compartment of the target cell will be associated with an increased tumoricidal pharmacological effect. So far, efforts to accelerate intracellular drug release have focused on the incorporation of pH sensitive phospholipids and peptides in the liposomal membranes. Such pH sensitive liposomes are stable at physiological pH in the circulation, however, they disintegrate and thus release the transported drug upon exposure to the acidic environment of the endosomal compartment (Connor and Huang 1986; Drummond et al 2000; Hilgenbrink and Low 2005). Another approach to modulate in vivo release kinetics is the use of magnetoliposomes for active targeting as well as magnetic particle induced hyperthermia (for a review see (Ito et al 2005)).

An alternative receptor, which is of interest for tumor targeting due to overexpression on the surface of various cancer cells, is the transferrin receptor. The natural ligand of the receptor, ie, transferrin, can be coupled to the surface of pegylated liposomes to achieve tumor targeting (Ishida et al 2001). It is important to note, however, that the transferrin receptor (which has a binding constant K_D of 5.6 nM) is heavily saturated in vivo by the μ M endogenous plasma transferrin concentrations (Pardridge 1993). This strong competition with endogenous transferrin leads to poor in vivo receptor targeting after intravenous injection. However, efficient tumor targeting is possible using alternative routes of administration. This has been shown for the photodynamic therapy of carcinoma cells in vitro (Gijzen et al 2002) or in vivo in an orthotopic human AY-27 rat bladder tumor model, where transferrin-conjugated liposomes were instilled directly into the bladder of the experimental animals (Derycke et al 2004). Alternative indications might be the treatment of lung cancer, where transferrin-conjugated liposomes could be used to deliver cytostatic drugs by inhalation (Anabousi et al 2006). The limitations of the endogenous receptor ligand transferrin can be addressed by the use of specific monoclonal antibodies (mAb). Examples include the OX26 mAb directed against the rat transferrin receptor (Friden et al 1991). The OX26 recognizes an epitope on the transferrin receptor, which is distant to the transferrin binding site leading to minimal competition with plasma transferrin and therefore allows for an intravenous administration of this targeting vector (Skarlatos et al 1995). Pegylated liposomes conjugated to the OX26 mAb (ie, OX26-immunoliposomes) were used previously to target the brain vascular endothelium in vivo (Huwlyer et al 1996) and to transport incorporated drugs across the blood-brain

barrier by receptor-mediated transcytosis (Cerletti et al 2000; Zhang et al 2003).

Similar targeting strategies, which make use of immunoliposomes, can be applied to the targeting of various tumors in vivo using tumor-specific antibody-vectors. The used antibodies can be directed against various receptors or surface antigens, including antibodies against the transferrin receptor (Suzuki et al 1997; Xu et al 2002) or clinically used monoclonal antibodies (as discussed above). Examples include the use of Fab' fragments of a humanized recombinant MAb against the extracellular domain of HER2/neu, which were conjugated to sterically stabilized immunoliposomes and used for the targeting of HER2-overexpressing breast cancer cells (Kirpotin et al 1997). The significantly increased anticancer activity in several animal xenograft tumor models of the immunoliposomal preparations can be attributed to the fact, that the immunoconjugates (as well as the free antibody) are internalized rapidly by the target cells by receptor-mediated endocytosis (Park et al 2001; Park et al 2002). The importance of this observation is emphasized by studies, where liposomes conjugated to the monoclonal antibody OV-TL3 were used for the treatment of ovarian carcinoma cells in an intraperitoneal animal xenograft model (Vingerhoeds et al 1996). Despite efficient targeting of the OA3 surface receptor on the ovarian tumor cells, no superior antitumor effects could be demonstrated in vitro or in vivo as compared to non-targeted liposomal formulations. This lack of enhanced efficacy was attributed in part to the fact, that the cell-bound liposomes were not internalized by the target cells (Mastrobattista et al 1999). An interesting approach to overcome these limitations of surface-bound tumor markers and to exploit them for a targeting strategy is the use of immuno-enzymosomes (Vingerhoeds et al 1993; Bailey 1994). Immunoliposomes are thereby not used to deliver a liposomal drug to its site of action but rather to transport pro-drug activating enzymes on their surface. Subsequent to liposomal tumor targeting, an anticancer prodrug matched with the enzyme is given, which will be converted to a cytotoxic compound at the tumor site. At least in different in vitro systems, immuno-enzymosomes were able to induce a marked cytotoxicity, which was superior to the one observed for immunoliposomes or the non-targeted liposomal enzyme (Fonseca et al 2003).

Perspectives

Reversal of multidrug resistance

There are two main protein superfamilies of drug transporting proteins, which have been reported to interfere with the

pharmacokinetics and tissue distribution of pharmaceuticals and in particular anticancer drugs: Members of the solute carrier (SLC) protein family have been classified as secondary or tertiary active drug transporters, which are driven by an exchange of intracellular ions (Mizuno and Sugiyama 2002). ATP hydrolysis is the driving force for primary drug transporters, belonging to the class of ATP-binding cassette transporters (ABC transporters). The human ABC transporter gene superfamily comprises currently 49 members belonging to eight subfamilies (Klein et al 1999; Schinkel and Jonker 2003). A prominent and well characterized member of the ABC transporters is P-glycoprotein (Juliano and Ling 1976). The gene coding for P-glycoprotein (ABCB1, MDR1) has been localized in several human tissues including the liver, kidney, intestine and the brain (Thiebaut et al 1987). Expression of this ATP-dependent drug efflux pump by tumor cells is associated with a defined pattern of multidrug-resistance (MDR or multidrug-resistance phenotype) against anticancer drugs including anthracyclines, anthracenes, *vinca*-alkaloids, camptothecin derivatives (topotecan), tubulin polymerizing drugs (colchicine and taxanes), actinomycin D, and epipodophyllotoxins (eg, etoposide) (Litman et al 2001). P-glycoprotein is expressed frequently in clinical cancers. The mean expression frequency of the MDR1 gene product, as shown by statistical meta-analysis, is 38% with a range from 0% (prostate carcinoma) to 88% (endometrial carcinoma) (Efferth and Osieka 1993). Cytostatic treatment leads to an increase in P-glycoprotein expression in all tumor types analyzed in the range from 4% (sarcoma) to 51% (lung carcinoma) (Efferth and Osieka 1993). Inherent or acquired multidrug-resistance in cancer has been shown to be associated with a poor prognosis at the time of diagnosis and is thus a major challenge in cancer treatment.

Pharmacological reversal of MDR activity by the use of specific inhibitors of drug carriers is problematic (Sikic 1997). The use of compounds such as the P-glycoprotein antagonist SDZ PSC 833, a non-immunosuppressant analogue of cyclosporin A, leads to a higher susceptibility of tumors towards chemotherapy (Boesch et al 1991). However, this beneficial effect is neutralized by the fact, that such compounds potentiate toxic side-effects of the used cancer drugs in non-diseased tissues (Advani et al 1999). This phenomenon is a consequence of inhibition of endogenously expressed P-glycoprotein, which has an important protective function in these tissues (Lemaire et al 1996; Song et al 1999). An alternative approach to overcome MDR could be the use of immunoliposomes, since this technology allows to by-pass drug transporters located

in the plasma membrane (Suzuki et al 1997). Using anti transferrin receptor antibody-conjugated immunoliposomes, it could be shown that cellular uptake of the P-glycoprotein substrate digoxin by P-glycoprotein competent endothelial RBE4 cells was indeed increased by a factor of 25 as compared to the free drug (Huwyler et al 2002). In contrast to the free drug, cellular accumulation of liposomal digoxin was thereby insensitive to co-administration of the P-glycoprotein inhibitor ritonavir but sensitive to nocodazole, a reversible inhibitor of endocytosis. Other liposome-based targeting strategies, such as the use of immunoliposomes conjugated with a monoclonal antibody directed against P-glycoprotein (Matsuo et al 2001), demonstrated enhanced cytotoxic effects in P-glycoprotein expressing tumor cell lines (Mamot et al 2003).

Gene therapy

Traditionally, cationic liposomes have been used for the transfection of cells in vitro. DNA can be complexed with cationic lipids leading to the formation of condensed aggregates of DNA and multilamellar lipid bilayers (Spector and Schnur 1997). An overall positive charge of these complexes enhances transfection of anionic animal target cells. However, a use of such cationic liposomal carriers in vivo is hardly possible due to their very unfavorable pharmacokinetic properties: On one hand, an unspecific and rapid binding and transfection of every tissue is observed, which comes in contact with the cationic DNA complexes (Liu et al 1995). On the other hand, precipitation and flocculation into large aggregates of cationic DNA-lipid complexes occurs at their isoelectric point (Rädler et al 1997). After intravenous application, a massive retention by passive filtration of these aggregates is observed within the lung, which is the first tissue to be perfused after injection (Osaka et al 1996; Liu et al 1997). Very recent and exciting experiments did overcome these problems by using pegylated immunoliposomes to deliver DNA expression plasmids to rodent or primate brain tissue (Shi and Pardridge 2000; Zhang et al 2003). Unlike cationic liposomes, this neutral and long circulating liposomal formulation is stable and not trapped in the lung. This high selectivity offers the possibility of a nonviral gene therapy of tumor and possibly other tissues. To this end, pegylated immunoliposomes were conjugated to two different monoclonal antibodies, which were used to target the construct to U87 human glioma cells implanted into the brain of immunodeficient (scid) mice (Zhang et al 2003). The used antibodies were the rat 8D3 mAb to the mouse transferrin receptor to promote transfer across the mouse blood-brain barrier and

the 83–14 mAb to the human insulin receptor to target the implanted human glioma cells within the brain parenchyma. The transported DNA expression plasmid did encode for a short hairpin RNA fragment (shRNA) designed to silence the expression of an oncogenic gene (human epidermal growth factor receptor EGFR) by RNA interference (RNAi) or post-transcriptional gene silencing. This gene therapy resulted in almost 90% increase in survival time of mice with advanced intracranial brain cancer (Zhang et al 2003).

Clinical use of vector-conjugated liposomes

In view of the rapid and promising advances in the field of specific liposomal tumor targeting during the last years, a clinical use of vector-conjugated liposomes or immunoliposomes should be envisaged. Recent reports indicate that an anti-HER2 immunoliposomal formulation was developed towards clinical trials using optimized protocols supporting large-scale production and clinical use (Park et al 2001). Matsumura et al (Matsumura et al 2004) have published the first clinical trial where doxorubicin encapsulated in pegylated immunoliposomes was administered to twenty-three patients suffering from advanced or recurrent gastric cancer refractory to conventional therapy. As a targeting vector, a F(ab)' fragment of a human monoclonal antibody directed against a cancer cell surface antigen was used (Hosokawa et al 2003), which was coupled directly to the liposomal surface of pegylated liposomes without using a molecular spacer. The used PEG had an average molecular weight of 5 kDa. The immunoliposomal doxorubicin was well tolerated during a treatment regimen of up to six cycles. The volume of distribution (V_D of approx. 40 ml/kg, ie, 50% of blood volume) and the low plasma clearance (Cl of approx. 3 ml/h/kg) are comparable to the ones of doxorubicin encapsulated in sterically stabilized liposomes (Gabizon et al 2003). It remains to be elucidated in future studies, if the proposed immunoliposomal formulation of doxorubicin might offer therapeutic advantages for the treatment of gastric cancer.

During the last years, liposomes as pharmaceutical drug carriers have received a lot of attention. Successful clinical applications in the field of drug delivery and passive targeting of solid tumors have demonstrated the potential of the technology. Once optimized production processes are available, a new generation of vector-conjugated liposomal carriers will allow for an active targeting of metastatic or chemoresistant tumors, for which at present no efficient therapeutic options are available. Further investigations and clinical trials are

now required to optimize existing technologies and to make them available to cancer patients.

References

- Advani R, Saba HI, Tallman MS, et al. 1999. Treatment of refractory and relapsed acute myelogenous leukemia with combination chemotherapy plus the multidrug resistance modulator PSC 833 (Valspodar). *Blood*, 93:787–95.
- Allen TM. 1994. Long-circulating (sterically stabilized) liposomes for targeted drug delivery. *Trends Pharmacol Sci*, 15:215–20.
- Allen TM, Brandeis E, Hansen CB, et al. 1995. A new strategy for attachment of antibodies to sterically stabilized liposomes resulting in efficient targeting to cancer cells. *Biochim Biophys Acta*, 1237:99–108.
- Allen TM, Chonn A. 1987. Large unilamellar liposomes with low uptake into the reticuloendothelial system. *FEBS Lett*, 223:42–6.
- Anabousi S, Bakowsky U, Schneider M, et al. 2006. In vitro assessment of transferrin-conjugated liposomes as drug delivery systems for inhalation therapy of lung cancer. *Eur J Pharm Sci*, 29:367–74.
- Bailey AL. 1994. Antibody-directed enzyme prodrug therapy (Adept). *J Control Release*, 28:187–93.
- Baselga J. 2000. Clinical trials of single-agent trastuzumab (Herceptin). *Semin Oncol*, 27:20–6.
- Blume G, Cevc G, Crommelin MD, et al. 1993. Specific targeting with poly(ethylene glycol)-modified liposomes: coupling of homing devices to the ends of the polymeric chains combines effective target binding with long circulation times. *Biochim Biophys Acta*, 1149:180–4.
- Boesch D, Gavériaux C, Jachez B, et al. 1991. In vivo circumvention of P-glycoprotein-mediated multidrug resistance of tumor cells with SDZ PSC 833. *Cancer Res*, 51:4226–33.
- Cerletti A, Drewe J, Fricker G, et al. 2000. Endocytosis and transcytosis of an immunoliposome-based brain drug delivery system. *J Drug Targeting*, 8:435–47.
- Connor J, Huang L. 1986. pH-sensitive immunoliposomes as an efficient and target-specific carrier for antitumor drugs. *Cancer Res*, 46:3431–5.
- Daemen T. 1992. Activation of Kupffer cell tumoricidal activity by immunomodulators encapsulated in liposomes. *Res Immunol*, 143:211–4.
- Daemen T, Hofstede G, Ten Kate MT, et al. 1995. Liposomal doxorubicin-induced toxicity: depletion and impairment of phagocytic activity of liver macrophages. *Int J Cancer*, 61:716–21.
- Derycke A, Kamuhabwa A, Gijsens A, et al. 2004. Transferrin-conjugated liposome targeting of the photosensitizer AlPcS4 to rat bladder carcinoma cells. *Journal of the National Cancer Institute*, 96:1620–30.
- Drummond DC, Zignani M, Leroux J. 2000. Current status of pH-sensitive liposomes in drug delivery. *Prog Lipid Res*, 39:409–60.
- Efferth T, Osieka R. 1993. Clinical relevance of the MDR-1 gene and its gene product, P-glycoprotein, for cancer chemotherapy: a meta-analysis. *Tumordiagn u Ther*, 14:238–43.
- Ewer MS, Martin FJ, Henderson C, et al. 2004. Cardiac safety of liposomal anthracyclines. *Semin Oncol*, 31:161–81.
- Flynn JM, Byrd JC. 2000. Campath-1H monoclonal antibody therapy. *Curr Opin Oncol*, 12:574–81.
- Fonseca MJ, Jagtenberg JC, Haisma HJ, et al. 2003. Liposome-mediated targeting of enzymes to cancer cells for site-specific activation of prodrugs: comparison with the corresponding antibody-enzyme conjugate. *Pharm Res*, 20:423–8.
- Frank MM. 1993. The reticuloendothelial system and bloodstream clearance. *J Lab Clin Med*, 122:487–8.
- Friden PM, Walus LR, Musso GF, et al. 1991. Anti-transferrin receptor antibody and antibody-drug conjugates cross the blood-brain barrier. *Proc Natl Acad Sci USA*, 88:4771–5.
- Gabizon A, Catane R, Uziely B, et al. 1994. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. *Cancer Res*, 54:987–92.
- Gabizon A, Papahadjopoulos D. 1992. The role of surface charge and hydrophilic groups on liposome clearance in vivo. *Biochim Biophys Acta*, 1103:94–100.

- Gabizon A, Shmeeda H, Barenholz Y. 2003. Pharmacokinetics of pegylated liposomal Doxorubicin: review of animal and human studies. *Clin Pharmacokinet*, 42:419–36.
- Gabizon A, Shmeeda H, Horowitz AT, et al. 2004. Tumor cell targeting of liposome-entrapped drugs with phospholipid-anchored folic acid-PEG conjugates. *Adv Drug Deliv Rev*, 56:1177–92.
- Gabizon AA. 2001. Pegylated liposomal doxorubicin: metamorphosis of an old drug into a new form of chemotherapy. *Cancer Invest*, 19:424–36.
- Gabizon AA, Shmeeda H, Zalipsky S. 2006. Pros and cons of the liposome platform in cancer drug targeting. *J Liposome Res*, 16:175–83.
- Gijzen A, Derycke A, Missiaen L, et al. 2002. Targeting of the phototoxic compound ALPcS4 to HeLa cells by transferrin conjugated PEG-liposomes. *Int J Cancer*, 101:78–85.
- Gosselin MA, Lee RJ. 2002. Folate receptor-targeted liposomes as vectors for therapeutic agents. *Biotechnol Annu Rev*, 8:103–31.
- Gulati M, Bajad S, Singh S, et al. 1998. Development of liposomal amphotericin B formulation. *J Microencapsul*, 15:137–51.
- Hamilton A, Biganzoli L, Coleman R, et al. 2002. EORTC 10968: a phase I clinical and pharmacokinetic study of polyethylene glycol liposomal doxorubicin (Caelyx, Doxil) at a 6-week interval in patients with metastatic breast cancer. European Organization for Research and Treatment of Cancer. *Ann Oncol*, 13:910–8.
- Hansen CB, Kao GY, Moase EH, et al. 1995. Attachment of antibodies to sterically stabilized liposomes: evaluation, comparison and optimization of coupling procedures. *Biochim Biophys Acta*, 1239:133–44.
- Hilgenbrink AR, Low PS. 2005. Folate receptor-mediated drug targeting: from therapeutics to diagnostics. *J Pharm Sci*, 94:2135–46.
- Hofheinz RD, Gnad-Vogt SU, Beyer U, et al. 2005. Liposomal encapsulated anti-cancer drugs. *Anticancer Drugs*, 16:691–707.
- Hosokawa S, Tagawa T, Niki H, et al. 2003. Efficacy of immunoliposomes on cancer models in a cell-surface-antigen-density-dependent manner. *Br J Cancer*, 89:1545–51.
- Huwylar J, Cerletti A, Fricker G, et al. 2002. By-passing of P-glycoprotein using immunoliposomes. *J Drug Targeting*, 10:73–9.
- Huwylar J, Wu D, Pardridge WM. 1996. Brain drug delivery of small molecules using immunoliposomes. *Proc Natl Acad Sci USA*, 93:14164–9.
- Imai K, Takaoka A. 2006. Comparing antibody and small-molecule therapies for cancer. *Nat Rev Cancer*, 6:714–27.
- Ishida O, Maruyama K, Tanahashi H, et al. 2001. Liposomes bearing polyethyleneglycol-coupled transferrin with intracellular targeting property to the solid tumors in vivo. *Pharm Res*, 18:1042–8.
- Ito A, Shinkai M, Honda H, et al. 2005. Medical application of functionalized magnetic nanoparticles. *J Biosci Bioeng*, 100:1–11.
- Juliano RL, Ling V. 1976. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta*, 455:152–62.
- Juliano RL, Stamp D. 1978. Pharmacokinetics of liposome-encapsulated anti-tumor drugs. Studies with vinblastine, actinomycin D, cytosine arabinoside, and daunomycin. *Biochem Pharmacol*, 27:21–7.
- Kanwar YS, Liu ZZ, Kashihara N, et al. 1991. Current status of the structural and functional basis of glomerular filtration and proteinuria. *Semin Nephrol*, 11:390–413.
- Kirpotin D, Park JW, Hong K, et al. 1997. Sterically stabilized anti-HER2 immunoliposomes: design and targeting to human breast cancer cells in vitro. *Biochemistry*, 36:66–75.
- Klein I, Sarkadi B, Varadi A. 1999. An inventory of the human ABC proteins. *Biochim Biophys Acta*, 1461:237–62.
- Klibanov AL, Maruyama K, Torchilin VP, et al. 1990. Amphipathic polyethyleneglycols effectively prolong the circulation time of liposomes. *FEBS Lett*, 268:235–7.
- Krishna R, Mayer LD. 1999. The use of liposomal anticancer agents to determine the roles of drug pharmacodistribution and P-glycoprotein (PGP) blockade in overcoming multidrug resistance (MDR). *Anticancer Res*, 19:2885–91.
- Lasic DD. 1996. Doxorubicin in sterically stabilized liposomes. *Nature*, 380:561–2.
- Lasic DD, Papahadjopoulos D. 1995. Liposomes revisited. *Science*, 267:1275–6.
- Lemaire M, Bruelisauer A, Guntz P, et al. 1996. Dose-dependent brain penetration of SDZ PSC 833, a novel multidrug resistance-reversing cyclosporin, in rats. *Cancer Chemother Pharmacol*, 38:481–6.
- Litman T, Druley TE, Stein WD, et al. 2001. From MDR to MXR: new understanding of multidrug resistance systems, their properties and clinical significance. *Cell Mol Life Sci*, 58:931–59.
- Liu Y, Liggitt D, Zhong W, et al. 1995. Cationic liposome-mediated intravenous gene delivery. *J Biol Chem*, 270:24864–70.
- Liu Y, Mounkes LC, Liggitt HD, et al. 1997. Factors influencing the efficiency of cationic liposome-mediated intravenous gene delivery. *Nat Biotechnol*, 15:167–73.
- Lobo ED, Hansen RJ, Balthasar JP. 2004. Antibody pharmacokinetics and pharmacodynamics. *J Pharm Sci*, 93:2645–68.
- Maeda H. 2001. The enhanced permeability and retention (EPR) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. *Adv Enzyme Regul*, 41:189–207.
- Maeda H, Wu J, Sawata T, et al. 2000. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *J Control Release*, 65:271–84.
- Mamot C, Drummond DC, Hong K, et al. 2003. Liposome-based approaches to overcome anticancer drug resistance. *Drug Resist Updates*, 6:271–9.
- Mastrobattista E, Koning GA, Storm G. 1999. Immunoliposomes for the targeted delivery of antitumor drugs. *Adv Drug Deliv Rev*, 40:103–27.
- Matsumura Y, Gotoh M, Muro K, et al. 2004. Phase I and pharmacokinetic study of MCC-465, a doxorubicin (DXR) encapsulated in PEG immunoliposome, in patients with metastatic stomach cancer. *Ann Oncol*, 15:51–25.
- Matsuo H, Wakasugi M, Takanaga H, et al. 2001. Possibility of the reversal of multidrug resistance and the avoidance of side effects by liposomes modified with MRK-16, a monoclonal antibody to P-glycoprotein. *J Control Release*, 77:77–86.
- McLaughlin P, Grillo-Lopez AJ, Link BK, et al. 1998. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *J Clin Oncol*, 16:2825–33.
- Mizuno N, Sugiyama Y. 2002. Drug transporters: their role and importance in the selection and development of new drugs. *Drug Metab Pharmacokinet*, 17:93–108.
- Moghimi SM, Patel HM. 1992. Opsonophagocytosis of liposomes by peritoneal macrophages and bone marrow reticuloendothelial cells. *Biochim Biophys Acta*, 1135:269–74.
- Mross K, Niemann B, Massing U, et al. 2004. Pharmacokinetics of liposomal doxorubicin (TLC-D99; Myocet) in patients with solid tumors: an open-label, single-dose study. *Cancer Chemother Pharmacol*, 54:514–5124.
- Osaka G, Carey K, Cuthbertson A, et al. 1996. Pharmacokinetics, tissue distribution, and expression efficiency of plasmid [33P]DNA following intravenous administration of DNA/cationic lipid complexes in mice: use of a novel radionuclide approach. *J Pharm Sci*, 85:612–8.
- Pan XQ, Lee RJ. 2005. In vivo antitumor activity of folate receptor-targeted liposomal daunorubicin in a murine leukemia model. *Anticancer Res*, 25:343–6.
- Papahadjopoulos D, Allen TM, Gabizon A, et al. 1991. Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. *Proc Natl Acad Sci USA*, 88:11460–4.
- Pardridge WM. 1993. Brain drug delivery and blood-brain barrier transport. *Drug Delivery*, 1:83–101.
- Park JW, Benz CC, Martin FJ. 2004. Future directions of liposome- and immunoliposome-based cancer therapeutics. *Semin Oncol*, 31:196–205.
- Park JW, Hong K, Kirpotin DB, et al. 2002. Anti-HER2 immunoliposomes: enhanced efficacy attributable to targeted delivery. *Clin Cancer Res*, 8:1172–81.

- Park JW, Kirpotin DB, Hong K, et al. 2001. Tumor targeting using anti-her2 immunoliposomes. *J Control Release*, 74:95–113.
- Pastan I, Kreitman RJ. 1998. Immunotoxins for targeted cancer therapy. *Adv Drug Deliv Rev*, 31:53–88.
- Photos PJ, Bacakova L, Discher B, et al. 2003. Polymer vesicles in vivo: correlations with PEG molecular weight. *J Control Release*, 90:323–34.
- Rädler JO, Koltover I, Salditt T, et al. 1997. Structure of DNA-cationic liposome complexes: DNA intercalation in multilamellar membranes in distinct interhelical packing regimes. *Science*, 275:810–4.
- Schinkel AH, Jonker JW. 2003. Mammalian drug efflux transporters of the ATP binding cassette (ABC) family: an overview. *Adv Drug Deliv Rev*, 55:3–29.
- Schnell R, Borchmann P, Staak JO, et al. 2003. Clinical evaluation of ricin A-chain immunotoxins in patients with Hodgkin's lymphoma. *Ann Oncol*, 14:729–36.
- Schnyder A, Huwyler J. 2005. Drug transport to brain with targeted liposomes. *NeuroRx*, 2:99–107.
- Shahinian S, Silviu JR. 1995. A novel strategy affords high-yield coupling of antibody Fab' fragments to liposomes. *Biochim Biophys Acta*, 1239:157–67.
- Shi N, Pardridge WM. 2000. Noninvasive gene targeting to the brain. *Proc Natl Acad Sci USA*, 97:7567–72.
- Shmeeda H, Mak L, Tzemach D, et al. 2006. Intracellular uptake and intracavitary targeting of folate-conjugated liposomes in a mouse lymphoma model with up-regulated folate receptors. *Mol Cancer Ther*, 5:818–24.
- Sikic BI. 1997. Pharmacologic approaches to reversing multidrug resistance. *Semin Hematol*, 34:40–7.
- Sinkule JA, Rosen ST, Radosevich JA. 1991. Monoclonal antibody 44-3A6 doxorubicin immunoconjugates: comparative in vitro anti-tumor efficacy of different conjugation methods. *Tumour Biol*, 12:198–206.
- Skarlatos S, Yoshikawa T, Pardridge WM. 1995. Transport of [125I]transferrin through the rat blood-brain barrier. *Brain Res*, 683:164–71.
- Song S, Suzuki H, Kawai R, et al. 1999. Effect of PSC 833, a P-glycoprotein modulator, on the disposition of vincristine and digoxin in rats. *Drug Metab Dispos*, 27:689–94.
- Spector MS, Schnur JM. 1997. DNA ordering on a lipid membrane. *Science*, 275:791–2.
- Stathopoulos GP, Boulikas T, Vougiouka M, et al. 2005. Pharmacokinetics and adverse reactions of a new liposomal cisplatin (Lipoplatin): phase I study. *Oncol Rep*, 13:589–95.
- Stevens PJ, Lee RJ. 2003. Formulation kit for liposomal doxorubicin composed of lyophilized liposomes. *Anticancer Res*, 23:439–42.
- Suzuki S, Inoue K, Hongoh A, et al. 1997. Modulation of doxorubicin resistance in a doxorubicin-resistant human leukaemia cell by an immunoliposome targeting transferrin receptor. *Br J Cancer*, 76:83–9.
- Thiebaut F, Tsuruo T, Hamada H, et al. 1987. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissue. *Proc Natl Acad Sci*, 84:7735–8.
- Torchilin VP. 2005. Recent advances with liposomes as pharmaceutical carriers. *Nat Rev Drug Discov*, 4:145–60.
- Uster PS, Allen TM, Daniel BE, et al. 1996. Insertion of poly(ethylene glycol) derivatized phospholipid into pre-formed liposomes results in prolonged in vivo circulation time. *FEBS Lett*, 386:243–6.
- Vingerhoeds MH, Haisma HJ, van MM, et al. 1993. A new application for liposomes in cancer therapy. Immunoliposomes bearing enzymes (immuno-enzymosomes) for site-specific activation of prodrugs. *FEBS Letters*, 336:485–90.
- Vingerhoeds MH, Steerenberg PA, Hendriks JJ, et al. 1996. Immunoliposome-mediated targeting of doxorubicin to human ovarian carcinoma in vitro and in vivo. *Br J Cancer*, 74:1023–9.
- Wang S, Low PS. 1998. Folate-mediated targeting of antineoplastic drugs, imaging agents, and nucleic acids to cancer cells. *J Control Release*, 53:39–48.
- Waterhouse DN, Tardi PG, Mayer LD, et al. 2001. A comparison of liposomal formulations of doxorubicin with drug administered in free form: changing toxicity profiles. *Drug Saf*, 24:903–20.
- Wong JY, Kuhl TL, Israelachvili JN, et al. 1997. Direct measurement of a tethered ligand-receptor interaction potential. *Science*, 275:820–2.
- Woodle MC, Matthay KK, Newman MS, et al. 1992. Versatility in lipid compositions showing prolonged circulation with sterically stabilized liposomes. *Biochim Biophys Acta*, 1105:193–200.
- Worth LL, Jia SF, An T, et al. 1999. ImmTher, a lipophilic disaccharide derivative of muramyl dipeptide, up-regulates specific monocyte cytokine genes and activates monocyte-mediated tumoricidal activity. *Cancer Immunol Immunother*, 48:312–20.
- Xu L, Huang CC, Huang W, et al. 2002. Systemic tumor-targeted gene delivery by anti-transferrin receptor scFv-immunoliposomes. *Mol Cancer Ther*, 1:337–46.
- Yuan F, Dellian M, Fukumura D, et al. 1995. Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size. *Cancer Res*, 55:3752–6.
- Zhang Y, Calon F, Zhu C, et al. 2003. Intravenous nonviral gene therapy causes normalization of striatal tyrosine hydroxylase and reversal of motor impairment in experimental parkinsonism. *Hum Gene Ther*, 14:1–12.
- Zhang Y, Schlachetzki F, Pardridge WM. 2003. Global non-viral gene transfer to the primate brain following intravenous administration. *Mol Ther*, 7:11–8.

