

Liposomal drug delivery systems – Clinical applications

PARVEEN GOYAL^{1*}
KUMUD GOYAL¹
SENGODAN GURUSAMY VIJAYA KUMAR²
AJIT SINGH²
OM PRAKASH KATARE³
DINA NATH MISHRA²

¹*Department of Pharmaceutics
Lord Shiva College of Pharmacy
Near Civil Hospital, Post Box No. 63
Sirsa-125055, Haryana, India*

²*Department of Pharmaceutical Sciences
Guru Jambheshwar University
Hisar-125001, Haryana, India*

³*Department of Pharmaceutics
University Institute of Pharmaceutical
Sciences, Punjab University
Chandigarh-160014, India*

Received July 12, 2004
Accepted March 3, 2005

Liposomes have been widely investigated since 1970 as drug carriers for improving the delivery of therapeutic agents to specific sites in the body. As a result, numerous improvements have been made, thus making this technology potentially useful for the treatment of certain diseases in the clinics. The success of liposomes as drug carriers has been reflected in a number of liposome-based formulations, which are commercially available or are currently undergoing clinical trials. The current pharmaceutical preparations of liposome-based therapeutic systems mainly result from our understanding of lipid-drug interactions and liposome disposition mechanisms. The insight gained from clinical use of liposome drug delivery systems can now be integrated to design liposomes that can be targeted on tissues, cells or intracellular compartments with or without expression of target recognition molecules on liposome membranes. This review is mainly focused on the diseases that have attracted most attention with respect to liposomal drug delivery and have therefore yielded most progress, namely cancer, antibacterial and antifungal disorders. In addition, increased gene transfer efficiencies could be obtained by appropriate selection of the gene transfer vector and mode of delivery.

Keywords: liposomes, clinical applications, cancer therapy, gene therapy, amphotericin B, doxorubicin

INTRODUCTION

Today, clinical medicine possesses an extremely long list of different pharmaceutical products and every year many new drugs are added to the list with the understanding of molecular mechanisms of diseases. Scientists and physicians are never satisfied only with a favorable drug action against the disease under treatment. The task of avoiding undesirable drug actions on normal organs and tissues and minimizing side effects of the therapy is very important. Thus, screening of biologically active compounds became necessary, permitting the choice of drug with selective action on the appropriate organs

* Correspondence, e-mail: parveenlscp@yahoo.com

or tissues. At the same time, many pharmacologically effective compounds cannot be used as drugs due to their undesirable action on normal tissues. Their »specificity« for the drug of choice, is not based on their ability to accumulate selectively in the target organs. Normally, they are more or less evenly distributed in the whole body and to reach the target zone the drug has to cross many other organs, cells, intracellular compartments, *etc.*, where it can be partially inactivated. To overcome this problem, a high concentration of drug has to be administered, which has a potential to cause undesirable complications and is sometimes expensive too. The ideal solution to such problems is the targeting of drugs using suitable carriers like serum proteins, immunoglobulins, synthetic polymers, liposomes, niosomes, microspheres, erythrocytes, reverse micelles, pharmacosomes, monoclonal antibodies, *etc.* (1, 2). Among these carriers, liposomes show great potentials of effective delivery of drugs to the site of action and of controlling the release of these drugs at a predetermined rate.

Liposomes are lyotropic liquid crystals composed of relatively biocompatible and biodegradable materials and consist of an aqueous core entrapped by one or more bilayers of natural and/or synthetic lipids. Drugs with widely varying lipophilicities can be encapsulated in liposomes either in the phospholipid bilayer, in the entrapped aqueous core or at the bilayer interface. Reformulation of drugs in liposomes has provided an opportunity to enhance the therapeutic indices of various agents mainly through the alteration of biodistribution. They are versatile drug carriers, which can be used to control retention of entrapped drugs in the presence of biological fluids, controlled vesicle residence in the systemic circulation or other compartments in the body and enhanced vesicle uptake by target cells (3). Liposomes composed of natural lipids are biodegradable, biologically inert, weakly immunogenic (4), produce no antigenic or pyrogenic reactions and possess limited intrinsic toxicity (5). Therefore, drugs encapsulated in liposomes are expected to be transported without rapid degradation and minimum side effects to the recipients. Moreover, efforts have been made to assess the specificity of drug carriers to the target organs, cells or compartments within the cells (1). Liposomes are better suited for assessing their targetable properties because of the ease of modifying their surface when compared to other drug carriers such as nanoparticles (6, 7) and microemulsions (8, 9). Many approaches have been attempted to achieve targetable properties, including noncovalent association of cell specific antibodies with liposomes (10), coating of liposomes with heat aggregated immunoglobulins M (IgM) (11), covalent attachment of poly and monoclonal antibodies to the liposomes (12–20), glycoprotein bearing liposomes (21) and natural (22–24) and synthetic (25–29) glycolipid containing liposomes. The compounds entrapped into the liposomes are protected from the action of external media, particularly enzymes (30) and inhibitors. Moreover, liposomes afford a unique opportunity to deliver the drugs into cells by fusion or endocytosis mechanism and practically any drug can be entrapped into liposomes irrespective of its solubility.

CLINICAL APPLICATIONS

New drug delivery systems such as liposomes are developed when an existing formulation is not satisfactory and reformulation in liposomes offers clear benefits with respect to targetability, therapeutic efficacy and safety compared to the existing formula-

tions. Lack of specificity in pharmacologically active agents is an obstacle to their effective use in biological research and medicine. It follows that any approach enabling an agent to reach its target selectively and in a controlled fashion would contribute to the elimination of problems inherent in conventional methods. One such approach was the development of a non-toxic and biodegradable carrier capable of containing a variety of substances of biological interest that the carrier could, upon contact with the leaving entity, direct to the site of action and subsequently allow to perform their task. It has been well established that liposomes can meet many of these requirements, *i.e.*, liposome formulations of some drugs have shown a significant increase in therapeutic efficacy and/or therapeutic indices in preclinical models and in humans compared to conventional formulations. Encouraging results of liposomal drugs in the treatment or prevention of a wide spectrum of diseases in experimental animals and in human, indicate that more liposome-based products for clinical and veterinary applications may be forthcoming (31). These could include treatment of skin and eye diseases, antimicrobial and anticancer therapy, metal chelation, enzyme and hormone replacement therapy, vaccine and diagnostic imaging, *etc.* Below, we discuss some of the liposome applications with realistic prospects of being developed for clinical use.

Cancer therapy

Cytotoxic drugs can distribute non-specifically throughout the body, lead to death of normal as well as malignant cells, thereby giving rise to a variety of toxic side effects. Entrapment of these drugs into liposomes resulted in increased circulation lifetime, enhanced deposition in the infected tissues, protection from the drug metabolic degradation, altered tissue distribution of the drug, with its enhanced uptake in organs rich in mononuclear phagocytic cells (liver, spleen and bone marrow) and decreased uptake in the kidney, myocardium and brain. To target tumors, liposomes must be capable of leaving the blood and accessing the tumor. However, because of their size liposomes cannot normally undergo transcapillary passage. In spite of this, various studies have demonstrated accumulation of liposomes in certain tumors in a higher concentration than found in normal tissues (32, 33). One or more of the following factors may account for this: (i) higher endocytic activity of some tumor cells combined with augmented local permeability of capillaries allowing the passage of small liposomes, a phenomenon also described as »the enhanced permeability and retention effect« which can facilitate liposome uptake, (ii) diffusion of drugs from liposomes either during circulation or after they have been lodged in tissues adjacent to tumors, followed by preferential drug entry into the tumor mass, (iii) liposomes may be phagocytosed by circulation monocytes, which subsequently migrate to tumors, (iv) sustained release effect of liposomes may enhance the drugs cytotoxic effect. Passive targeting of long circulating liposomes to tumor tissues may be useful for delivering cancer chemotherapeutic agents to the target tumors, since there is a positive correlation between the circulation time of liposomes and their localization in tumors.

Many research efforts have been directed towards improving the safety profile of the anthracycline cytotoxics, doxorubicin (DXR) and daunorubicin (DNR), along with vincristine (VCR), which are associated with severe cardiotoxic side effects, although acute gastrointestinal effects and other toxicities may also occur. Liposomal entrapment of these drugs showed reduced cardiotoxicity, dermal toxicity and better survival of ex-

perimental animals compared to the controls receiving free drugs (32). Such beneficial effects of liposomal anthracyclines have been observed with a variety of liposomal formulations regardless of their lipid composition provided that lipids used [high cholesterol (Cho) concentration or phospholipids with high phase transition temperature (T_c)] are conducive to drug retention by the vesicles in the systemic circulation (34).

DXR is a potent antineoplastic agent active against a wide range of human cancer including lymphomas, leukemia and solid tumors. However, administration of this drug produces acute toxicity in the form of bone marrow depression, alopecia and oral ulceration (35–37). DXR entrapped in liposomes shows reduced non-specific toxicity and maintains or enhances anticancer effect. DXR hydrochloride constitutes the first liposomal product (Doxil™) to be licensed in the United States. Surface grafted methoxypolyethylene glycol (MPEG) provides the hydrophilic »stealth« coating, which allows the Doxil™ liposomes to circulate in the blood stream for prolonged periods. The lipid matrix and an internal buffer system combine to keep virtually all the DXR encapsulated during liposome residence in the circulation. This means that the drug is not free to exert its toxic effects (38, 39). Liposome association alters the drug pharmacokinetics and thus the liposome has a half-life of approximately 55 hours in humans, whereas the free drug distributes to the tissues within a few minutes and is entirely cleared from circulation within 24 hours (40). Liposomal formulation showed decreased toxic effects of DXR; a dose higher than the LD_{50} could be administered without acute toxicity, which suggests that these liposomes extravasate from the endothelium of tumor tissues and reside around tumor cells where they release the drug into the interstitial fluid. Therefore, the therapeutic effect was achieved by a slow and sustained release of the drug at the target site. Furthermore, liposomal DXR has substantial activity against ovarian cancer in patients that failed to respond to platinum and paclitaxel-based regimens. The responses achieved with liposomal DXR were durable and maintained with minimal toxicity (41). Encapsulation of DXR in stealth liposomes showed significant accumulation, enhanced therapeutic efficacy and reduced toxic effect against human pancreatic carcinoma AsPC-1, implanted into nude Swiss mice compared to DXR suspension. Increased penetration into the tumor and a long presence with a slow drug release from liposomes in the tumor account for the enhanced therapeutic effects (42).

A new lipid formulation containing DXR that has been optimized for both mild hyperthermic temperatures and rapid drug release times has been developed (43). This new liposome formulation, in combination with mild hyperthermia, was found to be significantly more effective than the free drug or current liposome formulations for reducing tumor growth in a human squamous cell carcinoma xenograft line. Ishida *et al.* (44) developed pH-sensitive liposomes, targeted at the CD19 epitope on B-lymphoma cells, which showed enhanced DXR delivery into the nuclei of the target cells and increased cytotoxicity compared to non-pH-sensitive liposomes. This suggested that the targeted pH-sensitive formulations of drugs might be able to increase the therapeutic efficacy of entrapped drugs. Moreover, a unique liposomal formulation of DXR (TLC D-99) is active against AIDS (acquired immunodeficiency syndrome) related Kaposi's sarcoma and resulted in improved safety and efficacy profile, and the response is dose-dependent (45).

Ten Hagen *et al.* (46) reported that systemic administration of low-dose tumor necrosis factor-alpha (TNF-alpha) augments the antitumor activity of a liposomal formulation of DXR. Addition of TNF-alpha to the DXR liposomes regimen, which by itself in-

duced some tumor growth delay, resulted in massive necrosis and regression of tumors. Furthermore, a significant increase of the liposomal drug in tumor tissue was observed when TNF- α was co-administered. The antitumor effects against solid tumors, such as Meth A sarcoma, MH-134 hepatoma and colon 26 adenocarcinoma, were examined after intratumoral administration of liposomes and TNF solution. The antitumor effects of liposomes against solid tumors were superior to those of TNF solution. In particular, the antitumor effect of positively charged liposomes was superior to that of negatively charged liposomes and TNF solution. Further, positively charged liposomes containing a higher dose of TNF than the solution could be administered without killing the mice because of reduced side effects (47).

The ability to selectively target liposomal anticancer drugs via specific ligands against antigens expressed on malignant cells could improve the therapeutic effectiveness of liposomal preparations as well as reduce the adverse side effects associated with chemotherapy. Immunoliposomes conjugated with S5A8 monoclonal antibody, an anti-idiotypic antibody to 38C13 murine B-cell lymphoma, when loaded with DXR exhibited a long circulation time and showed more effectiveness for prolonging the survival of mice bearing 38C13 tumor than non-targeted liposomal DXR or free DXR plus empty immunoliposomes (48). Lopes de Menezes *et al.* (49) reported that targeted monoclonal antibody, anti-CD19, liposomes containing DXR may be selectively cytotoxic to malignant B cells expressing CD19 surface antigens, compared to DXR entrapped in nontargeted stealth liposomes/free DXR, and may be useful for selective elimination of circulating malignant B cells *in vivo*. Neuroblastoma (NB) tumor, but not normal tissues, overexpresses the disialoganglioside GD (2) at the cell surface. Anti-GD (2) whole antibodies [aGD (2)] or their corresponding Fab' fragments were covalently coupled to stealth immunoliposomes (aGD (2)-SIL or Fab'-SIL) containing DXR and showed higher cytotoxicities than untargeted liposomes. DXR loaded Fab'-SIL also showed specific binding, uptake, and cytotoxic effects on several GD (2)-positive NB cells *in vitro*. Fab'-SIL encapsulated DXR formulations led to total inhibition of metastatic growth of human NB in a nude mouse metastatic model (50). Furthermore, long-term survivors were obtained when treated with Fab'-SIL encapsulated DXR but not among untreated mice and those treated with free anti-GD (2) Fab' fragments or free-DXR. Fab'-SIL containing DXR also prevented the establishment and metastatic growth of tumor cells in different organs (51).

A new type of long-circulating immunoliposomes, *i.e.*, polyethyleneglycol (PEG) immunoliposome attached antibodies at the distal end of PEG chain, the so called pendant type immunoliposomes, showed much higher targetability than the ordinary immunoliposomes on both targeting sites of lung endothelial cells and solid tumor tissue. This is due to the free PEG chains (not linked to the antibody) effectively avoiding the reticuloendothelial systems (RES) uptake of liposomes, resulting in elevated blood concentration and enhanced target binding of immunoliposomes. The presence of free PEG does not interfere with the binding of the terminally linked antibody to the antigen. For targeting on the vascular endothelial surface in the lungs, 34A antibody, which is highly specific to mouse pulmonary endothelial cells, was conjugated to make the pendant type immunoliposomes (34A-PEG-ILP). 34A-PFG-ILP showed a significantly higher targeting degree than the ordinary type of immunoliposomes (19, 20, 52). For targeting on the solid tumor tissue, the Fab' fragment of 21B2 antibody and transferrin pendant type immunoliposomes (Fab'-PFG-ILP and TF-PEG-ILP) were used, which showed a low

RES uptake and a long circulation time, and resulted in enhanced accumulation of the liposomes in the solid tumor. TF-PEG-ILP was internalized into tumor cells with receptor-mediated endocytosis after extravasation into tumor tissue. The pendant type immunoliposomes can escape from the gaps between adjacent endothelial cells and openings at the vessel termini during tumor angiogenesis by passive convective transport much higher than ligand directed targeting. Active targeting on tumor tissue with the pendant type immunoliposomes is particularly important for many highly toxic anticancer drugs in cancer chemotherapy. The ultimate goal of pendant type immunoliposome is the incorporation of a fusogenic molecule, which would induce liposome fusion following their binding to the target cells or their internalization by endocytosis (52).

DXR also plays an important role in the treatment of breast cancer, both in the adjuvant and metastatic settings. However, the benefits of conventional DXR in terms of antitumor activity are limited by its therapeutic index. Pegylated liposomal DXR provides tumor-targeted efficacy without many of the toxicities associated with conventional DXR, including myelosuppression, alopecia, nausea and vomiting, and most importantly, cardiac toxicity. It has also demonstrated efficacy in combination with other agents or modalities, including cyclophosphamide, paclitaxel, docetaxel, gemcitabine, vinorelbine, and hyperthermia. Owing to its comparable efficacy and favorable safety profile, pegylated liposomal DXR may be a useful alternative to conventional DXR, as well as other agents commonly used in the treatment of breast cancer (53, 54). Sterically stabilized liposomes derived from the antitumor agent hexadecyl phosphocholine showed reduced uptake by the mononuclear phagocyte system (MPS) and improved antitumor activity in breast carcinoma in nude mice compared to conventional hexadecyl phosphocholine liposomes or free hexadecyl phosphocholine (55). Cisplatin when encapsulated in polyethyleneglycol-coated long-circulating liposomes results in prolonged circulation time and enhanced tumor uptake in different mouse tumor models. In spite of these results, due to the extremely slow release rate, no superior antitumor activity is seen for liposomal cisplatin over plain cisplatin. Results demonstrated that improvement in release kinetics of the prepared liposomes would lead to higher therapeutic efficacy of entrapped cisplatin (56).

Preclinical and clinical investigations have demonstrated significantly increased efficacy and decreased toxicity of liposomes containing DNR (DaunoXome™) in comparison with free DNR (57) in the treatment of acute leukemia (58). However, in the treatment of hepatocellular carcinoma and liver cirrhosis liposomal DNR showed mild haematological toxicities and significant hepatic toxicity, which warns against further assessment of these liposomes in patients with hepatocellular carcinoma and liver cirrhosis (59). However, liposomal DNR showed encouraging results in the treatment of advanced cutaneous T-cell lymphoma (60). Furthermore, liposomal DNR and carboplatin plus etoposide, used to treat children with recurrent high-grade glioma after surgery and with progressive teratoid/rhabdoid tumor, showed encouraging results with only little and transient hematological toxicity (61). Liposomal encapsulation of VCR resulted in increased and prolonged plasma concentration, which is associated with increased antitumor activity (murine P388 ascitic tumor) but not increased drug toxicities compared to the unencapsulated drug (62). Guthlein *et al.* (63) found that VCR entrapped into a vesicular phospholipid gel consisting of densely packed liposomes was an effective delivery system with superior antitumor activity compared to conventional VCR against human small

cell lung carcinoma LXFS 650 and the human mammary carcinoma MX1. Sustained release and passive tumor targeting can explain the enhanced efficacy.

Administration of magnetic adriamycin (ADR) liposomes under magnetic force using a permanent magnet (0.4 Tesla) implanted in solid tumor produced an approximately 4-fold higher ADR concentration in the tumor than free ADR solution. Liposomal formulation resulted in an increased ADR concentration in the liver and lungs and a decreased concentration in the heart. The results indicated that intravenously administered magnetic ADR liposomes can be used to effectively deliver ADR to osteosarcoma, Os515, implanted with a magnet, as well as to the lungs, a common site of osteosarcoma metastases (64). Furthermore, it is possible to achieve complete regression of osteosarcoma in hamster following injection of magnetite cationic liposomes in osteosarcoma and generation of hyperthermia by an alternating magnetic field (65). The antitumor effect of melphalan encapsulated in thermosensitive small unilamellar vesicles administered in combination with hyperthermia was studied in C57B1/6 mice bearing B16F10 melanoma. It was found that *in vivo* efficacy of liposome-encapsulated melphalan in combination with hyperthermia was higher than that of an equivalent concentration of free melphalan with or without heating. These results suggested that the combination of drug-loaded natural lipid-derived thermosensitive liposomes with local hyperthermia at the tumor site could be useful in enhancing drug delivery to tumors and improving its therapeutic efficacy in the treatment of solid tumors (66). Furthermore, animals receiving multimodality therapy involving irradiation followed by injection of thermosensitive liposomal melphalan and hyperthermic treatment of the tumor-bearing leg at 42 ± 0.5 °C for 1 hour showed marked tumor regression compared to untreated controls or animals treated with a combination of radiation and hyperthermia or radiation and free-drug melphalan. The study showed more extensive tumor cell killing, tumor growth delay and prolonged survival produced by a combination of radiation, thermosensitive-liposome-entrapped melphalan and hyperthermia compared to animals receiving single-modality or bimodality treatments (67).

Overexpression of lectins by malignant cells was applied for *in vitro* targeting of liposomes equipped with a saccharide vector and loaded with a lipid derivative of the anticancer agent sarcolysine. It was shown that active saccharide ligands increased the level of the vectored liposome binding to malignant cells by 50–80% as compared to liposomes without vector. Targeted drug-loaded liposomes had the cytotoxic activity much higher than the vector-free ones (68). When given by intravenous route, mannobiose mono arachidic acid esters (MAE) modified liposomes were eliminated from the systemic circulation more rapidly than control liposomes without modification. Whilst the modification did not affect the distribution of liposomes to the kidney, lungs, or thymus, it increased the distribution to liver and spleen. The uptake in the hepatic parenchymal cell fraction was not influenced by MAE incorporation. Taking into account the fact that endothelial cells do not take up particles larger than 100 nm, the increase in the distribution to liver was ascribed to an increase in the uptake by Kupffer's. These results suggest that mannobiose mono fatty acid esters are useful in the targeting of liposomes on Kupffer's and other macrophages and thereby can be a useful tool for treating tumors of these cells (69). Another application in tumor therapy includes liposome entrapped mitoxantrone (LEM) of significantly decreased toxicity, which showed altered pharmacokinetics and enhanced efficacy. This suggests that LEM may provide a viable alternative to the clinical use of conventional mitoxantrone against the human hormone-independent prostate

carcinoma (70). A monoclonal antibody against the rat colon carcinoma CC531 was covalently coupled to liposomes containing a dipalmitoylated derivative of the anti-cancer drug 5-fluoro-2'-deoxyuridine (FUdR-dP), as a prodrug, in their bilayers. The parent drug was released intracellularly and showed a much stronger inhibition of CC531 cell growth *in vitro* than in non-targeted liposomes (18).

Etoposide exerts its antineoplastic effect by forming a ternary complex with topoisomerase II and deoxyribonucleic acid (DNA), leading to DNA breaks and cell death. However, it causes myelosuppression and its lipophilicity poses a major limitation during administration. Encapsulation of etoposide in liposomes significantly delayed tumor growth compared to non-liposomal etoposide. The maximum tolerated dose was significantly higher in the group treated with liposomal etoposide, which also exhibited a lesser degree of myelosuppression than the animals treated with non-liposomal etoposide (71). Sudimack *et al.* (72) designed pH-sensitive liposomes that showed excellent stability at pH 7.4 and underwent rapid destabilization upon acidification. These novel liposomes were evaluated for intracellular delivery of entrapped cytosine-beta-D-arabino-furanoside (araC) in human oral cancer cells with elevated folate receptor (FR) expression. The FR, which is amplified in many types of human tumors, has been shown to mediate the internalization of folate-derivatized liposomes into an acidic intracellular compartment. FR-targeted oleyl alcohol-based pH-sensitive liposomes showed approximately 17-times higher FR-dependent cytotoxicity in cancer cells compared to araC delivered via FR-targeted non-pH-sensitive liposomes. This data indicates that pH-sensitive liposomes based on oleyl alcohol combined with FR-mediated targeting are promising delivery vehicles for membrane impermeable therapeutic agents.

A new boron cholesterol-carborane conjugate (BCH) has been synthesized as a potential targeting agent for boron neutron capture therapy (BNCT) of cancers. BCH encapsulated in liposomes delivered sufficient levels of boron to 9L rat glioma cells *in vitro*, indicating that BCH is a promising approach for BNCT. However, the uptake appeared to depend upon BCH concentration in the media as well as on the confluence of cells. Higher boron uptake by nonconfluent cells indicated that active growth of cells was a factor in the uptake of this compound (73). Liposomes containing high levels of exogenous natural ceramide lipid in the bilayer delivered the ceramide at a controlled rate and showed antitumor activity *in vivo* against the J774 ascites tumor model. Shabbits and Mayer (74) demonstrated the potential utility of ceramide-based liposomes as a novel strategy for cancer chemotherapy based on controlled ceramide delivery. Ozpolat *et al.* (75) suggest that intravenous administration of liposomal *all-trans-retinoic acid* (L-ATRA) maintains higher and stable plasma ATRA concentrations than oral ATRA in healthy subjects after repetitive administration. L-ATRA with a favorable pharmacokinetic profile may have potential advantages over oral ATRA and may be more efficacious in the treatment of acute promyelocytic leukemia or other retinoid-responsive cancers.

Encapsulation of c-myc antisense oligodeoxynucleotides into immunoliposomes appears to enhance their toxicity toward targeted cells while shielding non-targeted cells from antisense effects and may be efficacious for the delivery of drugs with broad therapeutic applications to tumor cells (76). Moreover, coupling of an anti-CD19 targeted antibody with charge neutralized liposome-antisense oligodeoxynucleotides (asODN) was effective in delivering an asODN to a multidrug-resistant human B-lymphoma cell line *in vitro*, decreasing the activity of P-glycoprotein. No such activity was observed for non-targeted liposomes and free asODN (77, 78).

Photodynamic therapy (PDT) as a cancer treatment is notable for its quite low side effects in comparison with those of chemotherapy and radiotherapy. However, the accumulation of porphyrin derivatives used in PDT in tumor tissues is rather low. Therefore, the benzoporphyrin derivative monoacid ring A (BPD-MA) was encapsulated in long circulating liposomes to enhance targeting on tumors. Tumor regression in mice bearing Meth A sarcoma and complete tumor curing were observed when these liposomes were injected and laser-irradiated, as compared to BPD-MA solution or BPD-MA entrapped in conventional liposomes. These results suggest that a long-circulating liposomal formulation of photosensitive agents is useful for PDT (79). Enhanced *in vitro* cytotoxic activity against leukaemic cells was found for combinations of the ether lipids, octadecyl phosphocholine and ET-18-OCH₃, with both teniposide and paclitaxel. The benefit of the liposomal formulation form for ether lipids was supported by the fact that their haemolytic activity was much reduced when they were incorporated into liposomes (80).

Antimicrobial therapy

Treatment of mycobacterial infections differs from that of other bacterial diseases because of several properties possessed by the mycobacteria and the host. A hallmark of mycobacteria is the complex lipid-rich cell envelope that protects the organism from both the host response and antimycobacterial therapy. In addition, mycobacteria are facultative intracellular parasites, which generally cause a more chronic type of disease. These properties put greater constraints on efficient therapy. To be effective, drugs must be able to penetrate the host macrophage, infected intracellular sites and preferably have reduced toxicity and be effective at low doses to allow prolonged therapy (32, 81).

Antibiotics can only act against intracellular infections if they can penetrate the phagocytic cells. It is a well known fact that liposomes are able to localize in the liver and spleen, especially the RES component, where many pathogenic microorganisms reside; they can be therefore used for targeting of antibiotics on these organs. In a simple *in vitro* culture, liposomal neomycin (82) and penicillin (83) were found to be active against bacteria, whereas liposome entrapment markedly reduced the antimicrobial activity of chloramphenicol (84). Liposome encapsulation alters the tissue distribution of gentamicin when given by intravenous route to rabbits (85); however, when administered by intramuscular route, it resulted in sustained release from the injection site, providing prolonged plasma concentrations of the drug (86). Moreover, Lutwyche *et al.* (87) demonstrated that encapsulation of membrane-impermeable antibiotics such as gentamicin in appropriately designed lipid-based delivery systems can enable their use in treating intracellular infections.

Incorporation of rifabutin in liposomes resulted in a significant enhancement of activity against *Mycobacterium avium* infection compared to free rifabutin (88). Moreover, the antitubercular activity of rifampin was considerably increased when encapsulated in egg phosphatidylcholine liposomes. A further increase in the activity was observed when the macrophage activator tetrapeptide tuftsin was grafted on the surface of drug-loaded liposomes. Rifampin delivered twice weekly for two weeks in tuftsin-bearing liposomes was at least 2,000 times more effective than the free drug in lowering the load of lung bacilli in infected animals (89). Liposome encapsulated clarithromycin may be more effective than the free form against *Mycobacterium avium* intracellular (MAI) infections *in*

in vivo, and the use of a combination therapy with ethambutol could further enhance the efficacy (90). Furthermore, when the activity of TLC G-65 (liposomal gentamicin preparation), alone and in combination with rifapentine, clarithromycin, clofazimine and ethambutol, was evaluated in the beige mouse model of disseminated *Mycobacterium avium* infection showed that the combination of rifapentine and TLC G-65 was more active than either agent alone. The activity of clarithromycin in combination with TLC G-65 was similar to that of either agent alone. Clofazimine improved the activity of TLC G-65 with respect to the spleen, while ethambutol improved the activity with respect to the liver (91). Entrapment of ciprofloxacin in liposomes increases the circulation half-life of the drug when given by intravenous route in mice, which is associated with enhanced delivery of the drug to the liver, spleen, kidneys, and lungs. Furthermore, liposomal entrapment was associated with increased therapeutic efficacy against the *Salmonella typhimurium* infection model in mice (92). Stevenson and coworkers (93) showed enhanced activity of streptomycin and chloramphenicol against *Escherichia coli* in the cells of the J774 murine macrophage line mediated by liposome delivery. The apparent intracellular antibacterial activity of antibiotics was increased more than tenfold by entrapment in liposomes. Khalil *et al.* (94) demonstrated a higher accumulation of streptomycin sulfate in the liver and spleen when encapsulated in liposomes than that exhibited by the free drug. Furthermore, streptomycin liposomes, when administered in two intravenous injections, caused greater reduction of the colony forming unit in the spleen, lungs and liver, when compared with the free drug, which was given in a much higher dose by intramuscular route (95). Moreover, long circulating liposomes and conventional liposomes encapsulating streptomycin, when given twice weekly, showed bactericidal activity against *Mycobacterium avium* complex (MAC) strain 101 in the spleen when the level of infection after treatment was compared to that before treatment (96).

The current treatment of immuno-compromised patients (AIDS patients) infected with MAC microorganisms is ineffective, probably because the organism resides intracellularly, mostly in monocytes. The rationale for liposome encapsulation of aminoglycosides is the possibility to increase the therapeutic index of this class of antibiotics by increasing aminoglycosides concentration at the site of infection and/or by reducing the toxicity of these drugs (97). To this end, effective treatment of MAC in mice was achieved with liposomal amikacin (98). Furthermore, amikacin in small, low-clearance liposomes (MiKasome™) has prolonged plasma and tissue residence and *in vivo* activity against extracellular infections, including *Klebsiella pneumoniae* and *Pseudomonas endocarditis* (99). Further, encouraging results were previously obtained with liposomal ampicillin in the treatment of *Listeria monocytogenes* (100), liposomal cefalothin against *Salmonella typhimurium* (101), liposomal benzyl penicillin in *Staphylococcus aureus* infection in mice (102) and with liposomal dihydrostreptomycin against intracellular *Staphylococcus aureus* (103). The efficacy of liposome-encapsulated ciprofloxacin or azithromycin in the therapy of intracellular *Mycobacterium avium* infection showed increased cellular delivery of these antibiotics and suggested that efficiency of intracellular targeting was sufficient to mediate enhanced antimycobacterial effects (104).

Another group of microbial diseases, severe disseminated fungal infections, has benefited substantially from the use of liposomes as drug delivery system. Polyene antibiotics such as amphotericin B (AmB) and nystatin are useful in systemic fungal infections such as candidosis and aspergillosis. The mechanism of AmB action involves intercala-

tion of the drug molecules into fungal cell membranes. Several molecules associate within the membrane, forming a barrel pore through which cell constituents are lost; this leads to metabolic disruption, osmotic imbalance and cell death. AmB selectivity of action is due to its higher affinity for the ergosterol of fungal membranes, although it binds with cholesterol as well (105).

The conventional amphotericin B (CAB) therapy, fungizone, a mixed colloidal dispersion of AmB with deoxycholate as surfactant, is associated with toxicity problems, since some binding of AmB to mammalian cells is inevitable (106). The most serious adverse effect of CAB therapy is nephrotoxicity, although clinical efficacy is also limited by a high incidence of haematological adverse effects (107). Data from both animal and human studies clearly indicate that both renal and hematological toxicity with the liposomal formulations of AmB are significantly reduced compared to CAB (108, 109).

Three liposomal and lipid-based formulations of AmB have been commercially introduced; they are capable of attenuating the toxicity of AmB, *i.e.*, lipid-based AmB preparations, AmB lipid complex (ABL-C, Abelcet®), AmB colloidal dispersion (ABCD, Amphotec® or Amphocil®), and liposomal AmB (AmBisome®). These formulations have shown that antifungal activity is maintained while toxicity is reduced (110). Abelcet® and Amphocil®, which do not even possess the conventional concentric phospholipid bilayer structure, provide examples of liposomal/lipid-carrier versatility. In animal models, AmBisome® is effective in treating both intracellular (leishmaniasis and histoplasmosis) and extracellular (candidosis and aspergillosis) systemic infections. Because of its low toxicity at the organ level, intravenous AmBisome® can be safely delivered in a markedly high dose for the treatment of systemic fungal infections. AmBisome® appears to localize at sites of infection in the brain (cryptococcosis, aspergillosis, coccidioidomycosis), lungs (blastomycosis, paracoccidioidomycosis, aspergillosis) and kidneys (candidosis) in animal models, delivering AmB, which remains bioavailable in tissues for several weeks following treatment (111). Multicentre studies with AmBisome® have shown that augmented concentration of the drug in the blood and tissues of mice and rats could be achieved with non-toxic doses (112). Using the same formulation, Adler-Moore *et al.* (113) were able to show, in preclinical trials, superior efficacy of AmBisome® against murine candidosis and cryptococcosis as compared to CAB. A large number of clinical trials have confirmed the effectiveness of AmBisome® as a safe alternative to CAB in the majority of patients with invasive or superficial fungal infections (114–116). Moreover, in patients with moderate to severe histoplasmosis associated with AIDS, liposomal AmB was found to be a less toxic alternative to AmB and was associated with improved survival (117). AmBisome® has shown a significant reduction in fungal colonization and invasive *Candida* infections compared to placebo in a prospective, double blind study in bone marrow transplantation, and eradication of invasive fungal infections in children undergoing bone marrow transplantation (118). Compared to AmBisome®, another liposomal formulation of AmB (L-AMP-LRC-1) was found to be more effective at a lower dose in neonatal candidiasis (119).

A number of factors appear to be responsible for the improved safety profile achieved with these three lipid-based preparations of AmB (120). The drug is associated with the lipid carrier, and is therefore unavailable to interact with mammalian cells and to exert its toxic effects. The carriers remain stable in the circulation and no uncontrolled drug leakage from the carriers occurs. The drug pharmacokinetic profile is also

altered, which plays an integral role in reducing drug toxicity (120). Association with lipid carriers facilitates the uptake of the system by the MPS organs of the liver and spleen, the main sites of systemic fungal infections. Engulfment of liposomes by circulating monocytes, and the migration of the latter to sites of infection, represents a further mechanism of increasing AmB concentration at the sites of infections.

The improved antifungal therapy with liposomal AmB resulted in incorporation of nystatin into liposomes (19, 121, 122). Liposomal nystatin formulation is under development and the studies have provided encouraging data. Furthermore, lipid-based formulations of hamycin, miconazole, and ketoconazole have been developed but remain experimental (110). Besides their antifungal use, liposomal polyene antibiotics are expected to disrupt HIV (human immunodeficiency virus) since the lipid envelope of HIV has a higher cholesterol concentration than most normal human cells (123). *In vitro* screening indicated that liposomal nystatin is effective to suppress the expression of HIV at concentrations that are not toxic to uninfected cells (124).

Using the mannosyl-fucosyl receptors on macrophages, mannosylated liposomes loaded with an indigenous drug, andrographolide, were made to target this antileishmanial drug to treat experimental leishmaniasis in the hamster model. These liposomes were found to be most potent in reducing the parasitic burden in the spleen as well as in reducing hepatic and renal toxicity (125). Similarly, the efficacy of freeze-dried empty liposomes encapsulated with an antimonial drug, meglumine antimoniate, was evaluated in hamsters experimentally infected with *Leishmania chagasi*. A significant reduction of liver parasite burdens was observed in animals treated with these liposomes compared to control animals treated with free meglumine antimoniate. This novel liposome-based meglumine antimoniate formulation appears to be promising as a pharmaceutical product for the treatment of visceral leishmaniasis (126). When DXR was targeted on macrophages infected by *Leishmania donovani*, by incorporating it in immunoliposomes prepared by grafting Fab'(2) of anti-51-kDa antibody onto the liposomal surface, it showed complete elimination of the spleen parasite burden in mice compared to 45% and 84% parasite suppressive effects with similar doses of free and liposomal drugs (doxosome), respectively. Furthermore, immunodoxosome and doxosome were generally less toxic than the free drug in the treatment of visceral leishmaniasis (127).

Gene therapy

A number of systemic diseases are caused by lack of enzymes, factors due to missing or defective genes. Gene delivery systems are designed to control the location of administered therapeutic genes within the patient's body. Successful *in vivo* gene transfer may require: (i) condensation of the plasmid and its protection from nuclease degradation, (ii) cellular interaction and internalization of condensed plasmid, (iii) escape of the plasmid from endosomes (if endocytosis is involved), and (iv) plasmid entry into cell nuclei (128). Gene therapy methods involve introduction of genetic material into the patient's cells to synthesize the therapeutic protein. Direct administration of genes to patients may be virally or non-virally mediated. As viruses represent a highly suitable vector for gene transfer, several viruses including retrovirus, adenovirus, adeno-associated-virus and herpes virus have been investigated for their potentials in gene delivery. However, there is always the potential risk that the viruses would become replication competent

and therefore infectious, immune and inflammatory responses have also been reported in clinical trials. In recent years, several attempts have been made to restore the gene expression by delivery of relevant exogenous DNA or genes to cells (129).

The drawbacks associated with the use of viral vectors, namely those related to safety problems, have prompted investigators to develop alternative methods of gene delivery, cationic lipid-based systems being the most representative ones. Plasmid liposome complexes have many advantages as gene transfer vehicles over viral based vectors (129): (i) these complexes are relatively nonimmunogenic because they lack proteins, (ii) liposomes or lipid complexes can be used for transfection of large-sized genetic material, and (iii) viruses, unlike plasmid liposome complexes, may replicate and cause infection. Despite extensive research in the last decade on the use of cationic liposomes as gene transfer vectors and the development of elegant strategies to enhance their biological activity, these systems are still far from being viable alternatives to the use of viral vectors in gene therapy (130).

Cationic liposomes are considered to be a potential non-viral human gene delivery system (131–137). These liposomes are usually composed of cationic lipid derivatives and a neutral phospholipid such as dioleoylphosphatidyl ethanolamine (DOPE). Widely accepted cationic liposome formulations are lipofectin, lipofectamine, transfectace, transfectam and DC-Cho [3BN-(*N,N'*-dimethylaminoethane) carbamoyl-cholesterol]. Cationic liposomes based on dioleoyloxypropyl trimethylammonium chloride (DOTMA), such as lipofectin, and several other types have demonstrated success both in *in vitro* and *in vivo* gene delivery (138, 139). The negatively charged genetic material, for example, plasmid, is not encapsulated in liposomes but complexed with cationic lipids by electrostatic interactions. Plasmid liposome complexes are thought to enter the cells through fusion with the plasma or endosome membrane (140). These liposomes were generally more effective in transfecting genes than micelles of the same lipid composition, thus suggesting a role for the bilayer structure in facilitating transfection. In addition, the transfection efficiency of liposome-delivered genes was highly dependent upon the lipid composition, lipid/DNA ratio, particle size of the liposome-DNA complex, and cell lines used (141). They can be prepared with defined physicochemical properties, such as size, shape and surface charge, which in turn control the stability, distribution and uptake of DNA *in vivo*.

Different ratios of DNA phosphate to polyethylenimine amine were used for encapsulation and delivery to liver cells of chloramphenicol acetyl transferase (CAT) or luciferase expression plasmids in cationic, neutral and anionic liposomes with the galatocerebroside amount fraction of 8% for targeting on the hepatocyte asialoglycoprotein receptor. All the liposome formulations demonstrated increased transfection efficiency and significantly lower toxicity compared to nonencapsulated polyethylenimine complexes. These formulations represent feasible systems for optimizing *in vivo* delivery systems to hepatocytes (142). Moreover, liposomes composed of dipalmitoyl phosphatidylcholine, soybean-derived sterylglucoside mixture (SG) and Cho, showed greater accumulation in hepatocytes than in non-parenchymal cells after intravenous injection (143). Kawaura and coworkers (144) studied the effect of monosialoganglioside containing cationic liposomes with cationic Cho on the liposome mediated gene transfection into mammalian culture cells. They found that both cationic liposomes with either a cationic Cho derivative of a hydrophobic amino head group (I) or a hydrophilic amino head

group (II) promoted the transfection of luciferase plasmids (pGL3) into HeLa and CHO-K1 cells more than the control cationic liposomes without monosialoganglioside. Furthermore, cationic liposomes with a cationic Cho derivative (II) were about ten times as effective as the commercially available cationic liposome lipofectin. Confocal fluorescence microscopy showed that the liposome/DNA complex was transferred more efficiently into the target cells by the monosialoganglioside-containing liposomes than by the liposomes without monosialoganglioside (144).

Liposomes consisting of *O,O'*-ditetradecanoyl-*N*-(alpha-trimethyl ammonioacetyl) diethanolamine chloride (DC-6-14) as a cationic lipid, phospholipid and Cho showed effective gene transfection activity in cultured cells. DC-6-14 liposome-DNA complexes were usually thought to have positive surface charge. However, depending on the ratio of DNA to liposomes, zeta-potential of the complexes became negative. Surface charge of these liposomes determine their biodistribution pattern, *i.e.*, positively charged complexes showed immediate lungs accumulation whereas negatively charged complexes did not show such accumulation. Therefore, some surface modification of DC-6-14 liposomes may improve the biodistribution and hence the targetability of their DNA complexes (135). Transfer of interferon beta gene via cationic liposomes has been found to induce regression of malignant glioma; this suggested the feasibility and safety of interferon beta gene therapy, which may become an important treatment option for patients with malignant glioma (137). Transfection efficiency of ultradeformable cationic liposome/DNA complexes and their retention time within the organs by applying the formulation onto hair-removed dorsal skin of mice was evaluated by Kim and coworkers (136). The study demonstrated that genes were transported into several organs for six days when applied once onto intact skin.

Recent advances have shown that it is possible to enhance the gene expression levels of cationic liposomes. The main problem of cationic liposomes seems to be a lack of organ or cell selectivity. Therefore, application of cell-specific targeting technology to cationic liposomes would improve *in vivo* gene delivery and reduce unexpected side effects. Both liver parenchymal and non-parenchymal cells exclusively express large numbers of high-affinity asialoglycoprotein and mannose receptors, respectively. Receptor-mediated gene delivery systems are able to introduce foreign DNA into specific cell types *in vivo*. However, not only the nature of the ligands grafted onto carriers but also the overall physicochemical properties of the complexes need to be optimized for effective cell-selective targeting of plasmid DNA (145).

The transfection efficiency of transferrin conjugated cationic (Tf-DDAB) liposomes was found to be much higher than that of unconjugated cationic liposomes. Target-oriented Tf-dimethyl-dioctadecyl ammonium bromide (DDAB) liposomes were proven to be very efficient in DNA delivery into the cervical cancer cells in culture (146). Gene therapy of the brain is hindered by the presence of the blood-brain barrier (BBB). Shi and Pardridge (147) reported the expression of an exogenous gene in the brain after noninvasive intravenous administration of a 6- to 7-kb expression plasmid encoding either luciferase or beta-galactosidase packaged in the interior of neutral pegylated immunoliposomes. Such liposomes are conjugated with the OX26 mAb (147) peptidomimetic mAb (148) to the rat transferrin receptor, which enables targeting of the plasmid DNA on the brain via the endogenous BBB transferrin receptor. These studies indicate that tissue-specific gene expression in the brain is possible after intravenous administra-

tion of a nonviral vector with combined use of the gene targeting technology and tissue-specific gene promoters (148). Unlike cationic liposomes, this neutral liposome formulation is stable in blood and does not result in selective accumulation in the lungs.

Nabel *et al.* (150) studied Allovectin-7TM, a gene transfer liposomal product for the treatment of metastatic melanoma, renal cell and colorectal carcinoma and demonstrated that intralesional injection of Allovectin-7TM can be given safely and showed anti-tumor activity in some patients. Yoshida *et al.* (137) studied the transfer of interferon beta gene via cationic liposomes in patients with malignant glioma. They found that the prepared liposomal formulation induced regression of experimental glioma. The study indicated the feasibility and safety of interferon beta gene therapy, which may help treat the patients with malignant glioma.

The clinical significance of hydroxyapatite (HAP) as a bone substitute has become apparent in recent years and bone morphogenetic protein (BMP), a substance that induces bone formation, has attracted much attention. Ono *et al.* (151) created a bone defect on rabbit cranium and treated it with the BMP-2 gene (cDNA plasmid) combined with cationic liposomes as a vector and confirmed the clinical usefulness of gene therapy for bone formation. Antisense oligodeoxynucleotides (ODNs) possess a great potential as sequence-specific therapeutic agents. Sufficient concentration of intact ODN must bypass membrane barriers and access the cytosol and nucleus for ODNs to be therapeutically effective. Methew *et al.* (152) designed a liposome-based formulation that utilizes listeriolysin O (LLO), the endosomolytic hemolysin from *Listeria monocytogenes*, to mediate the escape of ODN from endocytic compartments into the cytosol. It was found that ODN specific to murine intercellular adhesion molecule-1 (ICAM-1) encapsulated in LLO-liposomes was released to the cytosol and trafficked to the nucleus, efficiently and specifically suppressing the activation-induced expression of ICAM-1 at both protein and mRNA levels. Delivery without LLO resulted in sequestration of ODN in vesicular compartments leading to little inhibition of ICAM-1 expression, which supports the LLO requirement of efficient cytosolic delivery using this system. It has been demonstrated that LLO-mediated escape of ODN from intracellular vesicles is an effective approach to achieve full therapeutic antisense activity in cultured macrophages.

Liposomes have been shown to potentiate DNA mediated vaccination. Intramuscular immunization of mice with DNA encoding the S region of hepatitis B antigen entrapped into cationic liposomes has led to improved humoral and cell mediated immunity, as compared to the naked DNA or DNA complexed with preformed similar liposomes. It is assumed that immunization with liposomes entrapped plasmid DNA involves antigen-presenting cells (APC), either locally or in the regional draining lymph nodes (153).

CONCLUSIONS

Since the discovery of liposomes or lipid vesicles derived from self-forming enclosed lipid bilayers upon hydration, liposome drug delivery systems have played a significant role in reformulation of potent drugs to improve their therapeutics. Thirty-four years long research in liposomal drug delivery has led to vast improvement of the technology in terms of drug entrapment efficiency, vesicle stability in storage and in the

body, design of vesicles for controlled release, site specific targeting and scale up production. In parallel, noteworthy advances have been made in understanding and controlling liposomal behavior *in vivo*. This has facilitated the application of a wide range of liposomal drugs in the treatment and prevention of diseases in experimental animals and clinically. Commercial introduction of the various liposomal formulations represents a milestone in the history of liposomal drug delivery. Many more liposome-based drug formulations can be expected in the near future both for delivery of conventional drugs and for new biotechnology therapeutics such as recombinant proteins, antisense oligonucleotides and cloned genes. With the recent development in the field, several companies are already actively engaged in expansion and evaluation of liposome products for anticancer, antifungal therapy and for prophylaxis. The future of drug therapeutics may not lie in the development of new chemical entities but in the modification of the existing drug molecules using suitable carriers to eliminate toxicity and improve activity, the principle of new lives for old drugs (149).

Acknowledgments. – The authors thank Dr. N. Udupa, Dr. S. Pandey and Dr. S. P. Vyas for their technical assistance, valuable suggestions and comments during the preparation of this review article.

Abbreviations, acronyms and codes. – ABCD – amphotericin B colloidal dispersion, ABLC – amphotericin B lipid complex, ADR – adriamycin, AIDS – acquired immuno deficiency syndrome, AmB – amphotericin B, APC – antigen-presenting cells, araC – cytosine-beta-D-arabinofuranoside, asODN – antisense oligodeoxynucleotides, BBB – blood-brain barrier, BCH – cholesterol-carborane conjugate, BMP – bone morphogenetic protein, BNCT – boron neutron capture therapy, BPD-MA – benzoporphyrin derivative monoacid ring A, CAB – conventional amphotericin B, CAT – chloramphenicol acetyl transferase, Cho – cholesterol, DNA – deoxyribonucleic acid, DNR – daunorubicin, DOPE – dioleoylphosphatidyl ethanolamine, DOTMA – dioleoyloxypropyl trimethylammonium chloride, DXR – doxorubicin, FR – folate receptor, HAP – hydroxyapatite, HIV – human immunodeficiency virus, IgM – immunoglobulins M, ILP – pendant type immunoliposomes, L-ATRA – liposomal all-trans-retinoic acid, LEM – liposome entrapped mitoxantrone, LLO – Listeriolysin O, MAC – Mycobacterium avium complex, MAE – mannobiose mono arachidic acid esters, MAI – Mycobacterium avium intracellulare, MPEG – methoxypolyethylene glycol, MPS – mononuclear phagocyte system, NB – neuroblastoma, ODNs – oligodeoxynucleotides, PDT – photodynamic therapy, PEG – polyethyleneglycol, RES – reticuloendothelial systems, SG – sterylglucoside, SIL – stealth immunoliposomes, T_c – phase transition temperature, Tf-DDAB – transferrin conjugated dimethyl-dioctadecyl ammonium bromide, TNF-alpha – tumor necrosis factor-alpha, VCR – vincristine.

List of liposome products

<i>Product name</i>	<i>Drug</i>	<i>Manufacturer (country)</i>
Abelcet®	amphotericin B	The Liposome Company (USA)
Allovectin-7™	HLA-B7 Plasmid	Vical Incorporation (USA)
AmBisome®	amphotericin B	NeXatar Pharmaceuticals (USA)
Amphocil®	amphotericin B	SEQUUS Pharmaceuticals (USA)
Amphotec®	amphotericin B	SEQUUS Pharmaceuticals (USA)
DaunoXome™	Daunorubicin	NeXatar Pharmaceuticals (USA)
Doxil™	Doxorubicin	SEQUUS Pharmaceuticals (USA)
Doxosome	Doxorubicin	Indian Institute of Chemical Biology (India)
L-AMP-LRC-1	amphotericin B	Seth G.S. Medical College and K.E.M. Hospital (India)
MiKasome™	Amikacin	NeXatar Pharmaceuticals (USA)

REFERENCES

1. G. Gregoriadis, Targeting of drug, *Nature* **265** (1977) 407–411.
2. M. J. Pozanansky and R. L. Juliano, Biological approaches to the controlled delivery of drugs: a critical review, *Pharmacol. Rev.* **36** (1984) 277–336.
3. G. Gregoriadis and A. T. Florence, Liposomes in drug delivery: Clinical, diagnostic and ophthalmic potential, *Drugs* **45** (1993) 15–28.
4. N. van Rooijen and R. van Nieuwmegen, Liposomes in immunology: multilamellar phosphatidylcholine liposome as a simple biodegradable and harmless adjuvant without any immunogenic activity of its own, *Immunol. Commun.* **9** (1980) 243–256.
5. P. I. Campbell, Toxicity of some charged lipids used in liposome preparation, *Cytobios.* **37** (1983) 21–26.
6. L. Grislain, P. Couvreur, V. Lenaerts, M. Roland, D. Depreg-Decampeneere and P. Speiser, Pharmacokinetics and distribution of a biodegradable drug-carrier, *Int. J. Pharmacol.* **15** (1983) 335–338.
7. L. Illum, P. D. E. Gones, J. Kreuker, R. W. Daldwin and D. D. Davis, Adsorption of monoclonal antibodies to polyhexylcyanoacrylate nanoparticles and subsequent immunospecific binding to tumor cells, *Int. J. Pharm.* **17** (1983) 65–69.
8. M. Hashida, Y. Takahashi, S. Muranishi and H. Sezaki, An application of water in oil and gelatin microsphere in oil emulsions to specific delivery of anticancer agents into stomach lymphatics, *J. Pharmacokin. Biopharmacol.* **5** (1977) 241–244.
9. Y. Mizushima, T. Hamano and K. Yokoyama, Use of a lipid emulsion as a novel carrier for corticosteroids, *J. Pharm. Pharmacol.* **34** (1982) 49–53.
10. G. Gregoriadis and E. D. Neerunjun, Homing of liposomes to target cells, *Biochem. Biophys. Res. Commun.* **65** (1975) 537–544.
11. G. Weissmann, D. Bloomgerden, R. Kaplan, C. Cohen, S. Hoffstein, T. Collins, A. Gotlieb and D. Nagle, A general method for introduction of enzymes, by means of immunoglobulin coated liposomes into lysosomes of deficient cells, *Proc. Natl. Acad. Sci. USA* **72** (1975) 88–94.
12. V. P. Torchilin, B. A. Khaw, V. N. Smirnow and E. Haber, Preservation of antimyosin antibody activity after covalent coupling to liposomes, *Biochem. Biophys. Res. Commun.* **89** (1979) 1114–1119.
13. A. Huang, L. Huang and S. J. Kennel, Monoclonal antibody covalently coupled with fatty acid, *J. Biol. Chem.* **255** (1980) 8015–8018.
14. M. Harsch, P. Walther, H. G. Weder and H. Hengartner, Targeting of monoclonal antibody coated liposomes to sheep red blood cells, *Biochem. Biophys. Res. Commun.* **103** (1981) 1069–1074.
15. Y. Hashimoto, M. Sugawara and H. Endoh, Coating of liposomes with subunits of monoclonal IgM antibody and targeting of the liposomes, *J. Immunol. Meth.* **62** (1983) 155–162.
16. P. A. H. M. Tooaen and D. J. A. Crommelin, Immunoglobulins as targeting agent for liposomes encapsulated drugs, *Pharm. Weekbl. Sci. Ed.* **5** (1983) 269–275.
17. M. Sugano, N. K. Egilmez, S. J. Yokota, F. A. Chen, J. Harding, S. K. Huang and R. B. Bankert, Antibody targeting of doxorubicin-loaded liposomes suppresses the growth and metastatic spread of established human lung tumor xenografts in severe combined immunodeficient mice, *Cancer Res.* **60** (2000) 6942–6949.
18. G. A. Koning, J. A. Kamps and G. L. Scherphof, Efficient intracellular delivery of 5-fluorodeoxyuridine into colon cancer cells by targeted immunoliposomes, *Cancer Detect. Prev.* **26** (2002) 299–307.
19. K. Moribe and K. Maruyama, Pharmaceutical design of the liposomal antimicrobial agents for infectious disease, *Curr. Pharm. Des.* **8** (2002) 441–454.

20. S. Maesaki, Drug delivery system of anti-fungal and parasitic agents, *Curr. Pharm. Des.* 8 (2002) 433–440.
21. Y. Banuo, K. Ohai and Y. Nozawa, Targeting of asialofetuin sugar chain-bearing liposomes to liver lysosomes, *Biochem. Int.* 7 (1983) 455–459.
22. F. C. Szoka, Jr. and E. Mayhew, Alteration of liposome disposition *in vivo* by bilayer situated carbohydrates, *Biochem. Biophys. Res. Commun.* 110 (1983) 140–146.
23. H. H. Spanzer and G. L. Scherphof, Targeting of lactosyl-ceramide-containing liposomes to hepatocytes *in vivo*, *Biochim. Biophys. Acta* 734 (1983) 40–43.
24. P. Soriano, J. Dijkstra, A. Legrand, H. Spanjer, D. Lodos-Gagliardi, F. Roerdink, G. Scherphof and C. Nicolau, Targeted and non-targeted liposomes for *in vivo* transfer to rat liver cells of a plasmid containing the preproinsulin I gene, *Proc. Natl. Acad. Sci. USA* 80 (1983) 7128–7131.
25. M. R. Mauk, R. C. Gamble and J. D. Baldeschwieler, Targeting of lipid vesicles: Specificity of carbohydrate receptor analogues for leukocytes in mice, *Proc. Natl. Acad. Sci. USA* 77 (1980) 4430–4434.
26. M. R. Mauk, R. C. Gamble and J. D. Baldeschwieler, Vesicle targeting: timed release and specificity for leukocytes in mice by subcutaneous injection, *Science* 207 (1980) 309–311.
27. M. S. Wu, J. C. Robbins, R. L. Bugianesi, M. M. Pompom and T. Y. Shen, Modified *in vivo* behavior of liposomes containing synthetic glycolipids, *Biochim. Biophys. Acta* 674 (1981) 19–29.
28. P. S. Wu, H. M. Wu, G. W. Tin, J. R. Schuh, W. R. Croasmun, J. D. Baldeschwieler, T. Y. Shen and M. M. Pompom, Stability of carbohydrate-modified vesicles *in vivo*: comparative effect of ceramide and cholesterol glycoconjugates, *Proc. Natl. Acad. Sci. USA* 79 (1982) 5490–5493.
29. M. M. Pompom, M. S. Wu, J. C. Robbins and T. Y. Shen, Synthesis of 6-(5-cholesten-3 β -yloxy)hexyl-4-o-(6-deoxy- β -D-galactopyranosyl)-1-thio- β -D-glucopyranoside and derivatives thereof for *in vivo* liposome studies, *Carbohydr. Res.* 118 (1983) 47–51.
30. B. Chaize, J. P. Colletier, M. Winterhalter and D. Fournier, Encapsulation of enzymes in liposomes: high encapsulation efficiency and control of substrate permeability, *Artif. Cells Blood Substit. Immobil. Biotechnol.* 32 (2004) 67–75.
31. G. Gregoriadis, *Fate of Injected Liposomes: Observations on Entrapped Solute Retention, Vesicle Clearance and Tissue Distribution in vivo*, in *Liposomes as Drug Carrier: Recent Trends and Progress* (Ed. G. Gregoriadis), Wiley, Chichester 1988, pp. 3–18.
32. G. Gregoriadis, Overview of liposomes, *J. Antimicrob. Chemother.* 28 (1991) 39–48.
33. G. Gregoriadis and A. T. Florence, Liposomes and cancer therapy, *Cancer Cells* 4 (1991) 144–146.
34. L. D. Mayer, L. C. Tai, M. B. Bally, G. N. Mitilenes, R. S. Ginsberg and P. R. Cullis, Characterization of liposomal system containing doxorubicin entrapped in response to pH gradients, *Biochim. Biophys. Acta* 1025 (1990) 143–151.
35. F. Olson, E. Mayhew, D. Maslow, Y. Rustum and F. Szoka, Characterization, toxicity and therapeutic efficacy of adriamycin encapsulated in liposomes, *Eur. J. Cancer Clin. Oncol.* 18 (1982) 167–176.
36. E. H. Herman, A. Rahman, V. J. Ferrans, J. A. Visk and P. S. Schien, Prevention of chronic doxorubicin cardiotoxicity in beagles by liposome encapsulation, *Cancer Res.* 43 (1983) 5427–5432.
37. Q. G. C. M. Van Hoesel, P. A. Steerenberg, D. J. A. Crommelin, A. Van Dijk, W. Van Oost, S. Klein, J. M. C. Douze, D. J. Wildt and F. C. Hillen, Reduced cardiotoxicity and nephrotoxicity with preservation of antitumor activity of doxorubicin entrapped in stable liposomes in the LOU/M Wsl rat, *Cancer Res.* 44 (1984) 3698–3705.
38. D. D. Lasic, P. M. Frederic, M. C. A. Stuart, Y. Berenholz and I. T. McIntosh, Gelation of liposomes interior: novel method of drug encapsulation, *FEBS Lett.* 312 (1992) 255–258.
39. Y. Barenholz and G. Haran, Loading and controlled-release of amphiphilic pharmaceuticals to and from liposomes, *Eur. Pat. Appl. EP 361,894* (Cl. A61K9/127) 4 Apr. 1990, US Appl. 250, 687, 28 Sep 1988; ref. *Chem. Abstr.* 114 (1991) 12199g.

40. A. Gabizon, R. Catane and B. Uziely, Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene glycol-coated liposomes, *Cancer Res.* **54** (1994) 987–992.
41. F. M. Muggia, J. D. Hainsworth, S. Jeffers, P. Miller, S. Groshen, M. Tan, L. Roman, B. Uziely, L. Muderspach, A. Garcia, A. Burnett, F. A. Greco, C. P. Morrow, L. J. Paradiso and L. J. Liang, Phase II study of liposomal doxorubicin in refractory ovarian cancer: antitumor activity and toxicity modification by liposomal encapsulation, *J. Clin. Oncol.* **15** (1997) 987–993.
42. J. Vaage, D. Donovan, P. Uster and P. Working, Tumor uptake of doxorubicin in polyethylene glycol-coated liposomes and therapeutic effect against a xenografted human pancreatic carcinoma, *Br. J. Cancer* **75** (1997) 482–486.
43. D. Needham, G. Anyarambhatla, G. Kong and M. W. Dewhirst, A new temperature-sensitive liposome for use with mild hyperthermia: characterization and testing in a human tumor xenograft model, *Cancer Res.* **60** (2000) 1197–1201.
44. T. Ishida, M. J. Kirchmeier, E. H. Moase, S. Zalipsky and T. M. Allen, Targeted delivery and triggered release of liposomal doxorubicin enhances cytotoxicity against human B lymphoma cells, *Biochim. Biophys. Acta* **1515** (2001) 144–158.
45. T. W. Cheung, S. C. Remick, N. Azarnia, J. A. Proper, J. R. Barrueco and B. J. Dezube, AIDS-related Kaposi's sarcoma: a phase II study of liposomal doxorubicin, *Clin. Cancer Res.* **5** (1999) 3432–3437.
46. T. L. Ten Hagen, A. H. Van Der Veen, P. T. Nooijen, S. T. Van Tiel, A. L. Seynhaeve and A. M. Eggermont, Low-dose tumor necrosis factor- α augments antitumor activity of stealth liposomal doxorubicin (DoxilTM) in soft tissue sarcoma-bearing rats, *Int. J. Cancer.* **87** (2000) 829–837.
47. K. Yasui and Y. Nakamura, Positively charged liposomes containing tumor necrosis factor in solid tumors, *Biol. Pharm. Bull.* **23** (2000) 318–322.
48. Y. L. Tseng, R. L. Hong, M. H. Tao and F. H. Chang, Sterically stabilized anti-idiotypic immunoliposomes improve the therapeutic efficacy of doxorubicin in a murine B-cell lymphoma model, *Int. J. Cancer* **80** (1999) 723–730.
49. D. E. Lopes de Menezes, L. M. Pilarski and T. M. Allen, *In vitro* and *in vivo* targeting of immunoliposomal doxorubicin to human B-cell lymphoma, *Cancer Res.* **58** (1998) 3320–3330.
50. F. Pastorino, C. Brignole, D. Marimpietri, P. Sapra, E. H. Moase, T. M. Allen and M. Ponzoni, Doxorubicin-loaded Fab' fragments of anti-disialoganglioside immunoliposomes selectively inhibit the growth and dissemination of human neuroblastoma in nude mice, *Cancer Res.* **63** (2003) 86–92.
51. C. Brignole, D. Marimpietri, C. Gambini, T. M. Allen, M. Ponzoni and F. Pastorino, Development of Fab' fragments of anti-GD(2) immunoliposomes entrapping doxorubicin for experimental therapy of human neuroblastoma, *Cancer Lett.* **197** (2003) 199–204.
52. K. Maruyama, O. Ishida, T. Takizawa and K. Moribe, Possibility of active targeting to tumor tissues with liposomes, *Adv. Drug Del. Rev.* **40** (1999) 89–102.
53. J. A. O'Shaughnessy, Pegylated liposomal doxorubicin in the treatment of breast cancer, *Clin. Breast Cancer* **4** (2003) 318–328.
54. T. C. Chao, W. S. Wang, C. C. Yen, T. J. Chiou, J. H. Liu and P. M. Chen, A dose-escalating pilot study of sterically stabilized, pegylated liposomal doxorubicin (Lipo-Dox) in patients with metastatic breast cancer, *Cancer Invest.* **21** (2003) 837–847.
55. D. Arndt, R. Zeisig, I. Eue, B. Sternberg and I. Fichtner, Antineoplastic activity of sterically stabilized alkyl phosphocholine liposomes in human breast carcinomas, *Breast Cancer Res. Treat.* **43** (1997) 237–246.
56. S. Bandak, D. Goren, A. Horowitz, D. Tzemach and A. Gabizon, Pharmacological studies of cisplatin encapsulated in long-circulating liposomes in mouse tumor models, *Anticancer Drugs* **10** (1999) 911–920.

57. E. A. Forssen and M. E. Ross, Daunoxome™ treatment of solid tumors: Preclinical and clinical investigations, *J. Liposome Res.* **4** (1994) 481–512.
58. F. Pea, D. Russo, M. Michieli, M. Baraldo, A. Ermacora, D. Damiani, M. Bacarani and M. Furlanut, Liposomal daunorubicin plasmatic and renal disposition in patients with acute leukemia, *Cancer Chemother. Pharmacol.* **46** (2000) 279–286.
59. B. Daniele, R. DeVivo, F. Perrone, S. Lastoria, R. Tambaro, F. Izzo, F. Fiore, P. Vallone and S. Pignata, Phase-I clinical trial of liposomal daunorubicin in hepatocellular carcinoma complicating liver cirrhosis, *Anticancer Res.* **20** (2000) 1249–1251.
60. U. Wollina, K. Hohaus, J. Schonlebe, E. Haroske and E. Kostler, Liposomal daunorubicin in tumor stage cutaneous T-cell lymphoma: report of three cases, *J. Cancer Res. Clin. Oncol.* **129** (2003) 65–69.
61. A. Fiorillo, G. Maggi, N. Greco, R. Migliorati, A. D'Amico, M. D. DeCaro, M. S. Sabbatino and F. Buffardi, Second-line chemotherapy with the association of liposomal daunorubicin, carboplatin and etoposide in children with recurrent malignant brain tumors, *J. Neurooncol.* **66** (2004) 179–185.
62. M. S. Webb, P. Logan, P. M. Kanter, G. St-Onge, K. Gelmon, T. Harasym, L. D. Mayer and M. B. Bally, Preclinical pharmacology, toxicology and efficacy of sphingomyeline/cholesterol liposomal vincristine for therapeutic treatment of cancer, *Cancer Chemother. Pharmacol.* **42** (1998) 461–470.
63. F. Guthlein, A. M. Burger, M. Brandl, H. H. Fiebig, R. Schubert, C. Unger and U. Massing, Pharmacokinetics and antitumor activity of vincristine entrapped in vesicular phospholipid gels, *Anticancer Drugs* **13** (2002) 797–805.
64. T. Kubo, T. Sugita, S. Shimose, Y. Nitta, Y. Ikuta and T. Murakami, Targeted delivery of anti-cancer drugs with intravenously administered magnetic liposomes in osteosarcoma-bearing hamsters, *Int. J. Oncol.* **17** (2000) 309–315.
65. F. Matsuoka, M. Shinkai, H. Honda, T. Kubo, T. Sugita and T. Kobayashi, Hyperthermia using magnetite cationic liposomes for hamster osteosarcoma, *Biomagn. Res. Technol.* **2** (2004) 3.
66. T. P. Chelvi, S. K. Jain and R. Ralhan, Hyperthermia-mediated targeted delivery of thermosensitive liposome-encapsulated melphalan in murine tumors, *Oncol. Res.* **7** (1995) 393–398.
67. T. P. Chelvi and R. Ralhan, Hyperthermia potentiates antitumor effect of thermosensitive-liposome-encapsulated melphalan and radiation in murine melanoma, *Tumour Biol.* **18** (1997) 250–260.
68. E. L. Vodovozova, G. P. Gayenko, V. I. Razinkov, E. Y. Korchagina, N. V. Bovin and J. G. Molotkovsky, Saccharide-assisted delivery of cytotoxic liposomes to human malignant cells, *Biochem. Mol. Biol. Int.* **44** (1998) 543–553.
69. K. Yachi, H. Kikuchi, H. Yamauchi, S. Hirota and M. Tomikawa, Distribution of liposomes containing mannobiose esters of fatty acid in rats, *J. Microencaps.* **12** (1995) 377–388.
70. P. C. Gokhale, J. Pei, C. Zhang, I. Ahmad, A. Rahman and U. Kasid, Improved safety, pharmacokinetics and therapeutic efficacy profiles of a novel liposomal formulation of mitoxantrone, *Anticancer Res.* **21** (2001) 3313–3321.
71. S. Sengupta, P. Tyagi, S. Chandra, V. Kochupillai and S. K. Gupta, Encapsulation in cationic liposomes enhances antitumor efficacy and reduces the toxicity of etoposide, a topo-isomerase II inhibitor, *Pharmacology* **62** (2001) 163–171.
72. J. J. Sudimack, W. Guo, W. Tjarks and R. J. Lee, A novel pH-sensitive liposome formulation containing oleyl alcohol, *Biochim. Biophys. Acta* **1564** (2002) 31–37.
73. G. F. Peacock, B. Ji, C. K. Wang and D. R. Lu, Cell culture studies of a carborane cholesteryl ester with conventional and PEG liposomes, *Drug Deliv.* **10** (2003) 29–34.
74. J. A. Shabbits and L. D. Mayer, High ceramide content liposomes with *in vivo* antitumor activity, *Anticancer Res.* **23** (2003) 3663–3669.

75. B. Ozpolat, G. Lopez-Berestein, P. Adamson, C. J. Fu and A. H. Williams, Pharmacokinetics of intravenously administered liposomal all-trans-retinoic acid (ATRA) and orally administered ATRA in healthy volunteers, *J. Pharm. Pharm. Sci.* **6** (2003) 292–301.
76. G. Pagnan, D. D. Stuart, F. Pastorino, L. Raffaghello, P. G. Montaldo, T. M. Allen, B. Calabretta and M. Ponzoni, Delivery of c-myc antisense oligodeoxynucleotides to human neuroblastoma cells via disialoganglioside GD(2)-targeted immunoliposomes: antitumor effects, *J. Natl. Cancer Inst.* **92** (2000) 253–261.
77. D. D. Stuart, G. Y. Kao and T. M. Allen, A novel, long-circulating and functional liposomal formulation of antisense oligodeoxynucleotides targeted against MDR1, *Cancer Gene Ther.* **7** (2000) 466–475.
78. D. D. Stuart and T. M. Allen, A new liposomal formulation for antisense oligodeoxynucleotides with small size, high incorporation efficiency and good stability, *Biochim. Biophys. Acta* **1463** (2000) 219–229.
79. N. Oku, N. Saito, Y. Namba, H. Tsukada, D. Dolphin and S. Okada, Application of long-circulating liposomes to cancer photodynamic therapy, *Biol. Pharm. Bull.* **20** (1997) 670–673.
80. B. B. Lundberg, Ether lipids enhance the cytotoxic effect of teniposide and paclitaxel in liposomes against leukaemic cells in culture, *Anticancer Drug Res.* **12** (1997) 503–513.
81. W. W. Barrow, Treatment of mycobacterial infections, *Rev. Sci. Tech.* **20** (2001) 55–70.
82. N. A. Hodges, R. Mounajed, C. J. Olliff and J. M. Padfield, The enhancement of neomycin activity on *Escherichia coli* by entrapment in liposomes, *J. Pharm. Pharmacol.* **31** (1979) 85.
83. M. K. R. Chowdhury, R. Goswami and P. Chakbarti, Liposomes trapped penicillins in growth inhibition of some penicillin resistant bacteria, *J. Appl. Microbiol.* **51** (1981) 223–226.
84. M. Stevenson, A. J. Baillie and R. M. E. Richards, Antimicrobial activity of liposome entrapped chloramphenicol, *J. Pharm. Pharmacol.* **33** (1981) 31–36.
85. J. R. Morgan and K. E. Williams, Preparation and properties of liposome associated gentamicin, *Antimicrob. Agents Chemother.* **17** (1980) 544–548.
86. A. Cabanes, F. Reig, J. M. Garcia-Anton and M. Arboix, Evaluation of free and liposome-encapsulated gentamycin for intramuscular sustained release in rabbits, *Res. Vet. Sci.* **64** (1998) 213–217.
87. P. Lutwyche, C. Cordeiro, D. J. Wiseman, M. St-Louis, M. Uh, M. J. Hope, M. S. Webb and B. B. Finlay, Intracellular delivery and antibacterial activity of gentamicin encapsulated in pH-sensitive liposomes, *Antimicrob. Agents Chemother.* **42** (1998) 2511–2520.
88. M. M. Gaspar, S. Neves, F. Portaels, J. Pedrosa, M. T. Silva and M. E. Cruz, Therapeutic efficacy of liposomal rifabutin in a *Mycobacterium avium* model of infection, *Antimicrob. Agents Chemother.* **44** (2000) 2424–2430.
89. A. Agarwal, H. Kandpal, H. P. Gupta, N. B. Singh and C. M. Gupta, Tuftsin-bearing liposomes as rifampin vehicles in treatment of tuberculosis in mice, *Antimicrob. Agents Chemother.* **38** (1994) 588–593.
90. C. O. Onyeji, C. H. Nightingale, D. P. Nicolau and R. Quintiliani, Efficacies of liposome-encapsulated clarithromycin and ofloxacin against *Mycobacterium avium*-M. intracellular complex in human macrophages, *Antimicrob. Agents Chemother.* **38** (1994) 523–527.
91. M. H. Cynamon, S. P. Klemens and C. E. Swenson, TLC G-65 in combination with other agents in the therapy of *Mycobacterium avium* infection in beige mice, *J. Antimicrob. Chemother.* **29** (1992) 693–699.
92. M. S. Webb, N. L. Boman, D. J. Wiseman, D. Saxon, K. Sutton, K. F. Wong, P. Logan and M. J. Hope, Antibacterial efficacy against an *in vivo* *Salmonella typhimurium* infection model and pharmacokinetics of a liposomal ciprofloxacin formulation. *Antimicrob. Agents Chemother.* **42** (1998) 45–52.

93. M. Stevenson, A. J. Baillie and R. M. Richards, Enhanced activity of streptomycin and chloramphenicol against intracellular *Escherichia coli* in the J774 macrophage cell line mediated by liposome delivery, *Antimicrob. Agents Chemother.* **24** (1983) 742–749.
94. R. M. Khalil, F. E. Murad, S. A. Yehia, M. S. El-Ridy and H. A. Salama, Free versus liposome-entrapped streptomycin sulfate in treatment of infections caused by *Salmonella enteritidis*, *Pharmazie* **51** (1996) 182–184.
95. P. R. Gangadharam, D. A. Ashtekar, N. Ghori, J. A. Goldstein, R. J. Debs and N. Duzgunes, Chemotherapeutic potential of free and liposome encapsulated streptomycin against experimental *Mycobacterium avium* complex infections in beige mice, *J. Antimicrob. Chemother.* **28** (1991) 425–435.
96. P. R. Gangadharam, D. R. Ashtekar, D. L. Flasher and N. Duzgunes, Therapy of *Mycobacterium avium* complex infections in beige mice with streptomycin encapsulated in sterically stabilized liposomes, *Antimicrob. Agents Chemother.* **39** (1995) 725–730.
97. R. Schiffelers, G. Storm and I. Bakker-Woudenberg, Liposome-encapsulated aminoglycosides in pre-clinical and clinical studies, *J. Antimicrob. Chemother.* **48** (2001) 333–344.
98. N. Duzgunes, V. K. Perumal, L. Kesavalu, J. A. Goldstein and R. J. Debs, Enhanced effect of liposomes-encapsulated amikacin on *Mycobacterium avium*-M. intracellular complex infection in beige mice, *Antimicrob. Agents Chemother.* **32** (1988) 1404–1411.
99. R. M. Fielding, L. Moon-McDermott and R. O. Lewis, Bioavailability of a small unilamellar low-clearance liposomal amikacin formulation after extravascular administration, *J. Drug Targ.* **6** (1999) 415–426.
100. I. A. Bakker-Woudenberg, A. F. Lokerse, F. Roerdink, D. Regts and M. F. Michel, Free versus liposome-entrapped ampicillin in treatment of infection due to *Listeria monocytogenes* in normal and athymic nude mice, *J. Infect. Dis.* **55** (1985) 917–924.
101. J. V. Desiderio and S. G. Campbell, Intraphagocytic killing of *Salmonella typhimurium* by liposome encapsulated cephalothin, *J. Infect. Dis.* **148** (1983) 563–570.
102. I. S. Barsoum and M. Reich, The effect of liposome-entrapped penicillin G on *Staphylococcus aureus* infection in mice, *Pharmacology* **10** (1982) 358–363.
103. P. Bonventre and G. Gregoriadis, Killing of intraphagocytic *Staphylococcus aureus* by dihydrostreptomycin entrapped within liposomes, *Antimicrob. Agents Chemother.* **13** (1978) 1049–1051.
104. K. Yanagihara, Design of anti-bacterial drug and anti-mycobacterial drug for drug delivery system, *Curr. Pharm. Des.* **8** (2002) 475–482.
105. D. Kerridge, Mode of action of clinically important antifungal drugs, *Adv. Microb. Physiol.* **27** (1986) 1–72.
106. J. P. Utz, Amphotericin B toxicity: general side effects, *Ann. Intern. Med.* **61** (1964) 340–343.
107. G. Bodey, B. Bueltmann and W. Duguid, Fungal infections in cancer patients: an international autopsy survey, *Eur. J. Clin. Microbiol. Infect. Dis.* **11** (1992) 99–109.
108. D. Michaud, Liposomal amphotericin B for the treatment of severe fungal infection, *Dynamics* **12** (2001) 17–21.
109. B. Dupont, Overview of the lipid formulations of amphotericin B, *J. Antimicrob. Chemother.* **49** (2002) 31–36.
110. S. Arikan and J. H. Rex, Lipid-based antifungal agents: current status, *Curr. Pharm. Des.* **7** (2001) 393–415.
111. J. Adler-Moore and R. T. Proffitt, AmBisome®: liposomal formulation, structure, mechanism of action and pre-clinical experience, *J. Antimicrob. Chemother.* **49** (2002) 21–30.
112. R. T. Proffitt, A. Satorius, S. M. Chiang, L. Sullivan and J. P. Adler-Moore, Pharmacology and toxicology of a liposomal formulation of amphotericin B (AmBisome®) in rodents, *J. Antimicrob. Chemother.* **28** (1991) 49–61.

113. J. P. Adler-Moore, S. M. Chiang, A. Satorius, D. Guerra and B. McAndrews, Treatment of murine candidosis and cryptococcosis with a unilamellar liposomal amphotericin B formulation (AmBisome®), *J. Antimicrob. Chemother.* **28** (1991) 63–71.
114. R. Chopra, S. Blair, J. Strang, P. Cervi, K. G. Patterson and A. H. Goldstone, Liposomal amphotericin B (AmBisome®) in the treatment of fungal infections in neutropenic patients, *J. Antimicrob. Chemother.* **28** (1991) 93–104.
115. F. Meunier, H. G. Prentice and O. Ringden, Liposomal amphotericin B (AmBisome®): safety data form and phase- II/III clinical trial, *J. Antimicrob. Chemother.* **28** (1991) 83–91.
116. O. Ringden, F. Meunier, J. Tollemar, P. Ricci, S. Tura, E. Kuse, M. A. Viviani, N. C. Gorin, J. Klastersky and P. Fenaux, Efficacy of amphotericin B encapsulated in liposomes (AmBisome®) in the treatment of invasive fungal infections in immunocompromised patients, *J. Antimicrob. Chemother.* **28** (1991) 73–82.
117. P. C. Johnson, L. J. Wheat, G. A. Cloud, M. Goldman, D. Lancaster, D. M. Bamberger, W. G. Powderly, R. Hafner, C. A. Kauffman and W. E. Dismukes, Safety and efficacy of liposomal amphotericin B compared with conventional amphotericin B for induction therapy of histoplasmosis in patients with AIDS, *Ann. Intern. Med.* **137** (2002) 105–109.
118. O. Ringden, Ten years experience with liposomal amphotericin B in transplant recipients at Huddinge University Hospital, *J. Antimicrob. Chemother.* **49** (2002) 51–55.
119. R. N. Kotwani, P. V. Bodhe, B. G. Kirodian, K. P. Mehta, U. S. Ali and N. A. Kshirsagar, Treatment of neonatal candidiasis with liposomal amphotericin B(L-AMP-LRC-1): phase II study, *Indian Pediatr.* **40** (2003) 545–550.
120. A. M. Hillery, Supramolecular lipidic drug delivery systems: From laboratory to clinic. A review of the recently introduced commercial liposomal and lipid-based formulations of amphotericin B, *Adv. Drug Del. Rev.* **24** (1997) 345–363.
121. K. Moribe, K. Maruyama and M. Iwatsuru, Encapsulation characteristics of nystatin in liposomes: effects of cholesterol and polyethylene glycol derivatives, *Int. J. Pharm.* **188** (1999) 193–202.
122. K. Moribe, K. Maruyama and M. Iwatsuru, Spectroscopic investigation of the molecular state of nystatin encapsulated in liposomes, *Int. J. Pharm.* **201** (2000) 37–49.
123. P. S. Sarin, R. C. Gallo, D. I. Scheer, F. Crews and A. S. Lippa, Effects of a novel compound (AL 721) on HTLV-III infectivity *in vitro*, *N. Eng. J. Med.* **313** (1985) 1289–1290.
124. A. Rios, M. Rosenblum, G. Crofoot, R. P. Lenk, A. Hayman and G. Lopez-Berestein, Pharmacokinetics of liposomal nystatin in patients with human immunodeficiency virus infection, *J. Infect. Dis.* **168** (1993) 253–254.
125. J. Sinha, S. Mukhopadhyay, N. Das and M. K. Basu, Targeting of liposomal andrographolide to *L. donovani*-infected macrophages *in vivo*, *Drug Deliv.* **7** (2000) 209–213.
126. F. Frezard, M. S. Michalick, C. F. Soares and C. Demicheli, Novel methods for the encapsulation of meglumine antimoniate into liposomes, *Braz. J. Med. Biol. Res.* **33** (2000) 841–846.
127. S. Mukherjee, L. Das, L. Kole, S. Karmakar, N. Datta and P. K. Das, Targeting of parasite-specific immunoliposome-encapsulated doxorubicin in the treatment of experimental visceral leishmaniasis, *J. Infect. Dis.* **189** (2004) 1024–1034.
128. R. I. Mahato, A. Rolland and E. Tomlinson, Cationic lipid-based gene delivery systems: Pharmaceutical perspectives, *Pharm. Res.* **14** (1997) 853–859.
129. R. G. Crystal, Transfer of genes to humans: early lesions and obstacles to success, *Science* **270** (1995) 404–410.
130. M. C. Pedroso de Lima, S. Neves, A. Filipe, N. Duzgunes and S. Simoes, Cationic liposomes for gene delivery: from biophysics to biological applications, *Curr. Med. Chem.* **10** (2003) 1221–1231.
131. P. L. Felgner, T. R. Gadek, M. Holm, R. Roman, H. W. Chan, M. Wenz, J. P. Northrop, G. M. Ringold and M. Danielsen, Lipofectin: a highly efficient, lipid mediated DNA transfection procedures, *Proc. Natl. Acad. Sci. USA* **84** (1987) 7413–7417.

132. P. L. Felgner, R. H. Zangg and J. A. Norman, Synthetic recombinant DNA delivery for cancer therapeutics, *Cancer Gene Ther.* **2** (1995) 61–65.
133. P. L. Felgner and G. M. Ringold, Cationic liposome-mediated transfection, *Nature* **377** (1989) 387–388.
134. X. Gao and L. Huang, Cationic liposome mediated gene transfer, *Gene Ther.* **2** (1995) 710–722.
135. H. Ishiwata, N. Suzuki, S. Ando, H. Kikuchi and T. Kitagawa, Characteristics and biodistribution of cationic liposomes and their DNA complexes, *J. Contr. Rel.* **69** (2000) 139–148.
136. A. Kim, E. H. Lee, S. H. Choi and C. K. Kim, *In vitro* and *in vivo* transfection efficiency of a novel ultradeformable cationic liposome, *Biomaterials* **25** (2004) 305–313.
137. J. Yoshida, M. Mizuno, M. Fujii, Y. Kajita, N. Nakahara, M. Hatano, R. Saito, M. Nobayashi and T. Wakabayashi, Human gene therapy for malignant gliomas (glioblastoma multiforme and anaplastic astrocytoma) by *in vivo* transduction with human interferon beta gene using cationic liposomes, *Human Gene Ther.* **15** (2004) 77–86.
138. D. Lasic, Liposomes – an industrial view, *Chem. Ind.* **1996**, 210–213.
139. E. Tomlinson and A. P. Rolland, Controllable gene therapy: pharmaceuticals of non viral gene delivery systems, *J. Contr. Rel.* **39** (1996) 357–372.
140. A. Sharma and U. S. Sharma, Liposomes in drug delivery: progress and limitations, *Int. J. Pharm.* **154** (1997) 123–140.
141. Y. Zou, G. Zong, Y. H. Ling and R. Perez-Soler, Development of cationic liposome formulations for intratracheal gene therapy of early lung cancer, *Cancer Gene Ther.* **7** (2000) 683–696.
142. P. Bandyopadhyay, B. T. Kren, X. Ma and C. J. Steer, Enhanced gene transfer into HuH-7 cells and primary rat hepatocytes using targeted liposomes and polyethylenimine, *Biotechniques* **25** (1998) 282–284.
143. K. Shimizu, Y. Maitani, K. Takayama and T. Nagai, Formulation of liposomes with a soybean-derived sterylglucoside mixture and cholesterol for liver targeting, *Biol. Pharm. Bull.* **20** (1997) 881–886.
144. C. Kawaura, S. Hasegawa, N. Hirashima and M. Nakanishi, Monosialoganglioside containing cationic liposomes with a cationic cholesterol derivative promote the efficiency of gene transfection in mammalian culture cells, *Biol. Pharm. Bull.* **23** (2000) 778–780.
145. S. Kawakami, F. Yamashita, K. Nishida, J. Nakamura and M. Hashida, Glycosylated cationic liposomes for cell-selective gene delivery, *Crit. Rev. Ther. Drug Carrier Syst.* **19** (2002) 171–190.
146. S. Y. Joo and J. S. Kim, Enhancement of gene transfer to cervical cancer cells using transferrin-conjugated liposome, *Drug Dev. Ind. Pharm.* **28** (2002) 1023–1031.
147. N. Shi and W. M. Pardridge, Noninvasive gene targeting to the brain, *Proc. Natl. Acad. Sci. USA* **97** (2000) 7567–7572.
148. N. Shi, Y. Zhang, C. Zhu, R. J. Boado and W. M. Pardridge, Brain-specific expression of an exogenous gene after i.v. administration, *Proc. Natl. Acad. Sci. USA* **98** (2001) 12754–12759.
149. R. L. Juliano and G. Lopez-Berestein, New lives for old drugs-liposomal drug delivery systems reduce the toxicity but not the potency of certain chemotherapeutic agents, *Pharm. Int.* **45** (1985) 15–28.
150. G. J. Nabel, A. E. Chang, E. M. Hersh, N. J. Voegtzang, J. Rubin, H. Silver, S. Stah and A. B. Schreiber, Combined experience from Phase I studies with Allovectin-7, a direct gene transfer immunotherapeutic, 31st Annual Meeting of American Society of Clinical Oncology, May 20–23, 1995, Los Angeles; *Proc. Annu. Meet. Am. Soc. Clin. Oncol.* **14** (1995) 582A.
151. I. Ono, T. Yamashita, H. Y. Jin, Y. Ito, H. Hamada, Y. Akasaka, M. Nakasu, T. Ogawa and K. Jimbow, Combination of porous hydroxyapatite and cationic liposomes as a vector for BMP-2 gene therapy, *Biomaterials* **19** (2004) 4709–4718.

152. E. Mathew, G. E. Hardee, C. F. Bennett and K. D. Lee, Cytosolic delivery of antisense oligonucleotides by listeriolysin O-containing liposomes, *Gene Ther.* 10 (2003) 1105–1115.
153. G. Gregoriadis, R. Saffie and J. B. de Souza, Liposome-mediated DNA vaccination, *FEBS Lett.* 402 (1997) 107–110.

S A Ž E T A K

Liposomski sustavi za isporuku lijekova – Klinička primjena

PARVEEN GOYAL, KUMUD GOYAL, SENGODAN GURUSAMY VIJAYA KUMAR, AJIT SINGH, OM PRAKASH KATARE i
DINA NATH MISHRA

Od 1970. godine liposomi se intenzivno istražuju kao nosači ljekovitih tvari za isporuku u određene dijelova organizma. Unapređenje i razvoj liposoma omogućili su njihovu kliničku primjenu u terapiji određenih bolesti. Na tržištu je prisutan određen broj ljekovitih oblika na bazi liposoma, a dio je još u kliničkim pokusima. Svi su oni rezultat boljeg razumjevanja raspodjele liposoma i interakcija između lipida i ljekovite tvari. Moguće je pripremiti liposome koji se mogu usmjeriti u određena tkiva, stanice ili međustanične prostore, sa ili bez vezanih molekula za prepoznavanje na površini membrane. Ovaj revijalni članak uglavnom obuhvaća liposome za terapiju karcinoma, bakterijskih i gljivičnih infekcija jer je u tim područjima primjena liposoma najviše opravdana i stoga najviše istraživana. Osim toga, pravilnim izborom vektora za prijenos gena i načina isporuke može se povećati i učinkovitost prijenosa gena.

Ključne riječi: liposomi, klinička primjena, terapija karcinoma, genska terapija, amfotericin B, doksorubicin

Department of Pharmaceutics, Lord Shiva College of Pharmacy, Near Civil Hospital, Post Box No. 63, Sirsa-125055, Haryana, India

Department of Pharmaceutical Sciences, Guru Jambheshwar University, Hisar-125001, Haryana, India

Department of Pharmaceutics, University Institute of Pharmaceutical Sciences, Punjab University, Chandigarh-160014, India