

Clinical development of liposome-based drugs: formulation, characterization, and therapeutic efficacy

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Abstract: Research on liposome formulations has progressed from that on conventional vesicles to new generation liposomes, such as cationic liposomes, temperature sensitive liposomes, and virosomes, by modulating the formulation techniques and lipid composition. Many research papers focus on the correlation of blood circulation time and drug accumulation in target tissues with physicochemical properties of liposomal formulations, including particle size, membrane lamellarity, surface charge, permeability, encapsulation volume, shelf time, and release rate. This review is mainly to compare the therapeutic effect of current clinically approved liposome-based drugs with free drugs, and to also determine the clinical effect via liposomal variations in lipid composition. Furthermore, the major preclinical and clinical data related to the principal liposomal formulations are also summarized.

Keywords: PEGlated liposomes, temperature sensitive liposomes, therapeutic efficiency, virosomes, cationic liposomes

Introduction

The clinical utility of most conventional chemotherapeutics is limited either by the inability to deliver therapeutic drug concentrations to the target tissues or by severe and harmful toxic effects on normal organs and tissues. Liposomes are small, spherical, and enclosed compartments separating an aqueous medium from another by phospholipid bilayer. Many hundreds of drugs, including anticancer and antimicrobial agents, chelating agents, peptide hormones, enzymes, proteins, vaccines, and genetic materials, have been incorporated into the aqueous or lipid phases of liposomes, with various sizes, compositions, and other characteristics, to provide selective delivery to the target site for in vivo application. Several techniques, such as the Bangham, detergent-depletion, ether/ethanol injection, reverse phase evaporation, and emulsion methods, have been reported for preparing liposomes with high-entrapment efficiency, narrow particle size distribution, and long-term stability.¹⁻⁷ Recently, some alternative methods including dense gas and supercritical fluid techniques have been introduced for liposome preparation without using any organic solvent.^{1,7-9} Due to the differences in preparation methods and lipid compositions, liposomes can be classified according to their lamellarity (uni- and multilamellar vesicles), size (small [≤ 100 nm], intermediate [$100-250$ nm], or large [≥ 250 nm]), and surface charge (anionic, cationic, or neutral).¹⁰⁻¹² In clinical studies, liposomes show improved pharmacokinetics and biodistribution of therapeutic agents and thus minimize toxicity by their accumulation at the target tissue.^{13,14}

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Liposomes were first discovered by Bangham in 1965 and the first liposomal pharmaceutical product, Doxil[®], (Ben Venue Laboratories, Inc Bedford, OH) received US Food and Drug Administration (FDA) approval in 1995 for the treatment of chemotherapy refractory acquired immune deficiency syndrome (AIDS)-related Kaposi's sarcoma.¹³⁻¹⁵ Currently, there are about twelve liposome-based drugs approved for clinical use and more are in various stages of clinical trials (Tables 1 and 2).¹³⁻⁶² Most liposomal drug formulations, such as Doxil and Myocet[™] (GP-Pharm, Barcelona, Spain), are approved for intravenous application.⁶³ Other administration routes such as intramuscular delivery have been approved for delivery of surface antigens derived from the hepatitis A or influenza virus (Epaxal[®] [Berna Biotech Ltd, Berne Switzerland] and Inflexal[®] V [Berna Biotech España SA, Madrid, Spain]).^{37,38} Oral delivery has also been examined; however, this is more troublesome due to the potential for liposome breakdown following exposure to bile salts.⁶⁴

Storage of liposomes: lyophilization

Liposomes dispersed in aqueous solution generally face physical and chemical instabilities after long-term storage.⁶⁵ Hydrolysis and oxidation of phospholipids and liposome aggregation are the common cause of liposome instabilities. According to the literature, many methods have been investigated for the stabilization of liposomes, such as lyophilization, freezing, and spraying drying. In commercial liposome-based drugs (Table 1), AmBisome[®] (Gilead Sciences, Inc, San Dimas, CA), Amphotec[®] (Ben Venue Laboratories, Inc, Bedford, OH), Myocet, Visudyne[®] (Novartis Pharma AG, Basel, Switzerland), and LEP-ETU (liposome-entrapped paclitaxel easy-to-use; NeoPharm, Inc, Lake Bluff, IL) are all lyophilized products. In general, freeze-drying increases the shelf-life of liposomal formulations and preserves them in dried form as lyophilized cakes to be reconstituted with water for injection prior to administration.⁶⁶ Furthermore, cryoprotectants need to be added to maintain particle size distribution of liposomes after the freeze-drying-rehydration cycle. Various types and concentrations of sugars have been investigated for their ability to protect liposomes against fusion and leakage during lyophilization processes.⁶⁶ In commercial liposome lyophilized products, lactose has been used as a cryoprotectant in the formulations of Amphotec, Myocet, and Visudyne, and sucrose was added in the formulations of AmBisome and LEP-ETU to increase liposome stability during lyophilization. Interestingly, these commercial lyophilized products showed similar shelf-life in comparison with other liposome products (eg, suspension and emulsions) and hence lyophilization may not have the expected effect on liposome

stability. In 1998, Clemons and Stevens compared the potency and therapeutic efficacy among the different lipid-based formulations of amphotericin B (Amphotec, AmBisome, and Abelcet[®] (Sigma-Tau PharmaSource, Inc, Indianapolis, IN)) for the treatment of systemic and meningeal cryptococcal disease.⁶⁷ Their work indicated that the therapeutic efficacy of Amphotec and AmBisome was superior to that of Abelcet, by up to ten-fold, in survival and in clearing infection from all organs. In these three commercially available lipid-based formulations of amphotericin B, Amphotec and AmBisome are both lyophilized products and Abelcet is formulated as a suspension. Therefore, lyophilization may not extend the shelf-life of products but may increase therapeutic efficacy in vivo. Similar results were also reported in our previous studies.⁷⁰ We investigated the stability of the siRNA-loaded liposomes in suspension and lyophilized powder form up to 1 month postmanufacture.⁶⁸ Following formulation, the siRNA-loaded liposomes were stored at either 4°C or room temperature. The particle size and zeta potential of siRNA-loaded liposomes remained unchanged in both storage conditions. However, siRNA entrapment efficiencies were observed to have decreased slightly after 1 month in storage for both suspension (90% → 83%) and lyophilized powder (94% → 84%) forms. Surprisingly, the gene-silencing efficiency of siRNA-loaded liposomes in aqueous solution showed 80% reduction following 1 month of storage at either 4°C or room temperature. This was in contrast to liposomes prepared in the lyophilized powder form where 100% of the gene-silencing efficiency was retained following storage at either 4°C or room temperature for 1 month. Although therapeutic efficiency of liposome-based drugs may vary depending on the choice of lipids, the preparation technique, physico-chemical characteristics of the bioactive materials, and overall charge of the liposome, lyophilization is useful for the long-term storage of liposome-based drugs.

Clinical studies of liposomal-based anticancer drugs: doxorubicin

Liposome delivery systems offer the potential to enhance the therapeutic index of anticancer drugs, either by increasing the drug concentration in tumor cells or by decreasing the exposure in normal host tissues. Doxorubicin is an anthracycline widely used to treat solid and hematological tumors, but its major drawback is its related cardiotoxicity. In cardiotoxicity, positively charged doxorubicin's affinity for negatively charged cardiolipin, a lipid abundant in heart tissue, is thought to be involved in drug localization in the heart tissue.⁶⁹ Therefore, doxorubicin-loaded liposomes were developed to combat

Table 1 Liposome-based drugs on market

Product name	Route of injection	Drug	Particle type/size	Drug form/Storage time	Lipid composition	Approved indication	Ref
Ambisome	Intravenous	Amphotericin B	Liposome	Powder/36 months	HSPC, DSPG, cholesterol, and amphoteracin B (2:0.8:1:0.4 molar ratio)	Sever fungal infections	13,17,18
Abelcet	Intravenous	Amphotericin B	Lipid complex	Suspension/24 months	DMPC and DMPG (7:3 molar ratio)	Sever fungal infections	19,20
Amphotec	Intravenous	Amphotericin B	Lipid complex	Powder/24 months	Cholesteryl sulfate	Sever fungal infections	21,22
DaunoXome	Intravenous	Daunorubicin	Liposome	Emulsion/12 months	DSPC and cholesterol (2:1 molar ratio)	Blood tumors	13,23–25
Doxil	Intravenous	Doxorubicin	PEGylated liposome	Suspension/20 months	HSPC, cholesterol, and PEG 2000-DSPE (56:39:5 molar ratio)	Kaposi's sarcoma, Ovarian/breast cancer	13,14,26
Lipo-dox	Intravenous	Doxorubicin	PEGylated liposome	Suspension/36 months	DSPC, cholesterol, and PEG 2000-DSPE (56:39:5 molar ratio)	Kaposi's sarcoma, ovarian/breast cancer	27
Myocet	Intravenous	Doxorubicin	Liposome	Powder/18 months	EPC and cholesterol (55:45 molar ratio)	Combination therapy with cyclophosphamide in metastatic breast cancer	13,14,28
Visudyne	Intravenous	Verteporfin	Liposome	Powder/48 months	EPG and DMPC (3:5 molar ratio)	Age-related molecular degeneration, pathologic myopia, ocular histoplasmosis	29–31
Depocyt	Spinal	Cytarabine	Liposome	Suspension/18 months	Cholesterol, Triolein, DOPC, and DPPG (11:1:7:1 molar ratio)	Neoplastic meningitis and lymphomatous meningitis	13,32
DepoDur	Epidural	Morphine sulfate	Liposome	Suspension/24 months	Cholesterol, Triolein, DOPC, and DPPG (11:1:7:1 molar ratio)	Pain management	33,34
Epaxal	Intramuscular	Inactivated hepatitis A virus (strain RG-SB)	Liposome	Suspension/36 months	DOPC and DOPE	Hepatitis A	35–37
Inflexal V	Intramuscular	Inactivated hemagglutinine of Influenza virus strains A and B	Liposome	Suspension/12 months	DOPC and DOPE	Influenza	38,39

Abbreviations: DOPE, dioleoylphosphatidylethanolamine; DOPC, dioleoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol; HSPG, hydrogenated soy phosphatidylcholine; DSPG, distearoylphosphatidylglycerol; EPC, egg phosphatidylcholine; DSPC, distearoylphosphatidylcholine; DMPC, 1- α -dimyristoylphosphatidylcholine; DMPG, 1- α -dimyristoylphosphatidylglycerol; EPG, egg phosphatidylglycerol; PEG 2000-DSPE, polyethylene glycol 2000-distearoylphosphatidylethanolamine.

Table 2 Liposome-based drugs in clinical trials

Product name	Route of injection	Drug	Lipid composition	Approved indication	Trial phase	Ref
LEP-ETU (powder/12 months)	Intravenous	Paclitaxel	DOPC, cholesterol, and cardiolipin (90:5:5 molar ratio)	Ovarian, breast, and lung cancers	Phase I/II	13,40
LEM-ETU	Intravenous	Mitoxantrone	DOPC, cholesterol, and cardiolipin in 90:5:5 molar ratio	Leukemia, breast, stomach, liver, ovarian cancers	Phase I	13,41
EndoTAG-1 (powder/24 months)	Intravenous	Paclitaxel	DOTAP, DOPC, and paclitaxel (50:47:3 molar ratio)	Anti-angiogenic properties, breast cancer, pancreatic cancer	Phase II	13, 42-44
Arikace	Portable aerosol delivery	Amikacin	DPPC and cholesterol	Lung infection	Phase III	45,46
Marqibo	Intravenous	Vincristine	Cholesterol and egg sphingomyelin (45:55 molar ratio)	Metastatic malignant uveal melanoma	Phase III	13, 47,48
ThermoDox	Intravenous	Doxorubicin	DPPC, MSPC, and PEG 2000-DSPE (90:10:4 molar ratio)	Non-resectable hepatocellular carcinoma	Phase III	49,50
Atragen	Intravenous	Tretinoin	DMPC and soybean oil	Acute promyelocytic leukemia, hormone-refractory prostate cancer	Phase II	13
T4N5 liposome lotion	Topical	Bacteriophage T4 endonuclease 5	Unknown	Xeroderma pigmentosum	Phase III	51
Liposomal Grib-2	Intravenous	Grib2 antisense oligodeoxynucleotide	Unknown	Acute myeloid leukemia, chronic myelogenous leukemia, acute lymphoblastic leukemia	Phase I	52
Nyoctran LE-SN38	Intravenous	Nystatin	DMPC, DMPC, and cholesterol	Systemic fungal infections	Phase I/II	13
Aroplatin	Intravenous	SN-38, the active metabolite of irinotecan	DOPC, cholesterol, and cardiolipin	Metastatic colorectal cancer	Phase I/II	13,53
Liprostin	Intravenous	Cisplatin analog (L-NDDP)	DMPC and DMPC	Metastatic colorectal carcinoma	Phase II	54
StimuMax	Subcutaneous	Prostaglandin E1	Unknown	Peripheral vascular disease	Phase II/III	55
SPI-077	Intravenous	BLP25 lipopeptide (MUC1-targeted peptide)	Monophosphoryl lipid A, cholesterol, DMPC, and DPPC	Cancer vaccine for multiple myeloma developed encephalitis	Phase III	56,57
Lipoplatin (suspension/36 months)	Intravenous	Cisplatin	SHPC, cholesterol, and DSPE-PEG	Head and neck cancer, lung cancer	Phase I/II	13
S-CKD602	Intravenous	Cisplatin	SPC, DPPG, cholesterol, and mPEG 2000-DSPE	Pancreatic cancer, head and neck cancer, mesothelioma, breast and gastric cancer, and non-squamous non-small-cell lung cancer	Phase III	13,58
OSI-211	Intravenous	Camptothecin analog	DPSC and DSPE-PEG (95:5 molar ratio)	Recurrent or progressive carcinoma of the uterine cervix	Phase I/II	13, 59,60
INX-0125	Intravenous	Lurtotecan	HSPC and cholesterol (2:1 molar ratio)	Ovarian cancer, head, and neck cancer	Phase II	25,61
INX-0076	Intravenous	Vinorelbine	Cholesterol and egg sphingomyelin (45:55 molar ratio)	Advanced solid tumors	Phase I	13,62
Liposome-annamycin (powder)	Intravenous	Topotecan	Cholesterol and egg sphingomyelin (45:55 molar ratio)	Advanced solid tumors	Phase I	13
Liposome-annamycin (powder)	Intravenous	Annamycin	DPSC, DSPG, and Tween	Advanced solid tumors	Phase I/II	13

Abbreviations: DOPC, 1,2-Dioleoyl-sn-glycero-3-phosphocholine; MSPC, monostearylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DOTAP, 1,2 dioleoyl-3-trimethylammonium-propane; SPC, soy phosphatidylcholine; mPEG 2000-DSPE, methoxy-polyethylene glycol-distearoyl phosphatidylethanolamine; DSPE, distearoylphosphatidylethanolamine.

aggressive tumors, like breast and ovary metastatic cancers and Kaposi's sarcoma. Myocet and Doxil were the first-approved liposome-based drugs for cancer treatment. Both products contain doxorubicin but are different, particularly in the presence of polyethylene glycol (PEG) coating (Figure 1). In pharmacokinetic studies of doxorubicin-loaded liposomes, free doxorubicin had an elimination half-life of 0.2 hours and an area under the plasma concentration–time curve (AU) of $4 \mu\text{g h mL}^{-1}$ in patients as compared with 2.5 hours and $45 \mu\text{g h mL}^{-1}$ for Myocet and with 55 hours and $900 \mu\text{g h mL}^{-1}$ for Doxil, respectively.²⁵ The particle size of Myocet is about 190 nm and

Doxil is about 100 nm. Both liposome products have longer circulating half-life in blood as compared with the free drug, but Doxil has a much longer circulation time in blood than Myocet. Generally, the blood circulation time of liposomes ($T_{1/2}$) increases with decreasing size, negative charge density, and fluidity in the bilayer or PEG surface coating. In a Phase III head-to-head comparison of free doxorubicin vs Myocet in patients with metastatic breast cancer, similar results were presented in first-year survival rate (64% vs 69%) and progression-free survival (3.8 vs 4.3 months), but Myocet had low incidence of cardiac events (13% vs 29%),

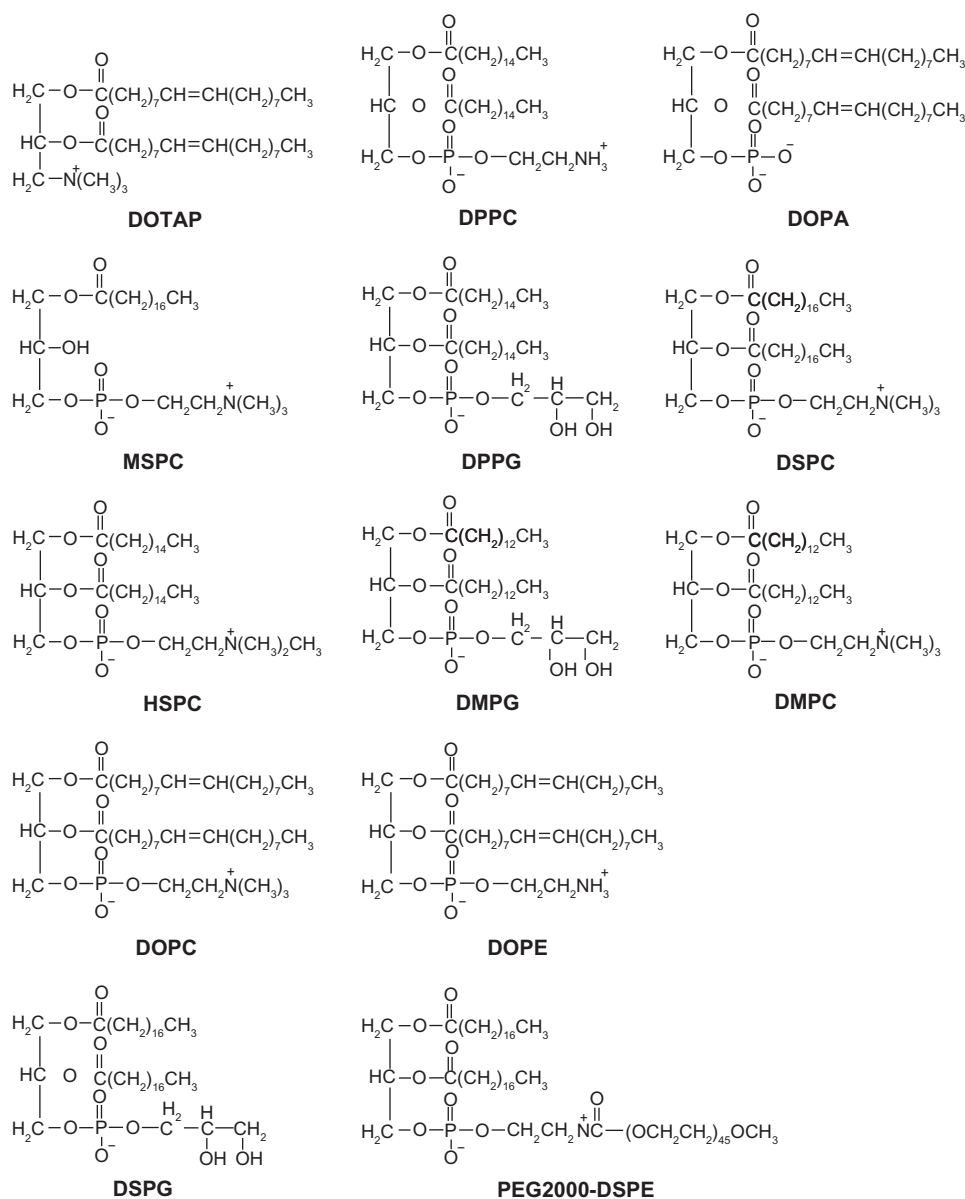


Figure 1 Chemical structures of lipids in liposome formulations.

Abbreviations: DOTAP, 1,2-dioleoyl-3-trimethylammonium propane; DPPC, dipalmitoylphosphatidylcholine; DOPA, 1,2-Dioleoyl-sn-Glycero-3-Phosphate; MSPC, monostearoylphosphatidylcholine; DPPG, dipalmitoylphosphatidylglycerol; DSPC, distearoylphosphatidylcholine; HSPC, hydrogenated soy PC; DMPG, 1- α -dimyristoylphosphatidylglycerol; DMPC, 1- α -dimyristoylphosphatidylcholine; DOPC, 1,2-Dioleoyl-sn-glycero-3-phosphocholine; DOPE, dioleoyl phosphatidylethanolamine; DSPG, distearoylphosphatidylglycerol; PEG2000-DSPE, polyethylene glycol 2000-distearoylphosphatidylethanolamine.

mucositis/stomatitis (8.6% vs 11.9%), and nausea/vomiting (12.3% vs 20.3%).^{70,71} Therefore, Myocet tends to reduce drug-related toxicity (eg, cardiotoxicity) rather than to enhance antitumor efficacy. Similar to Myocet, Doxil had a better safety profile, including reduction of cardiotoxicity (3.9% vs 18.8%), neutropenia (4% vs 10%), vomiting (19% vs 31%), and alopecia (20% vs 66%) in a Phase III trial of metastatic breast cancer, whereas its progression-free survival times (6.9 vs 7.8 months) and overall survival times (21 vs 22 months) demonstrated equivalent efficacy to conventional doxorubicin.⁷² However, palmar-plantar erythrodysesthesia (48% vs 2%), stomatitis (22% vs 15%), and mucositis (23% vs 13%) were found to be more often associated with Doxil than free doxorubicin.

Lipo-dox[®] (TTY Biopharm Company Ltd, Taipei Taiwan) is the second generation of PEGylated liposomal doxorubicin, composed of distearoylphosphatidylcholine (DSPC) and cholesterol with a surface coating of PEG.²⁷ DSPC, which has two completely saturated fatty acids (both stearic acids), has high phase-transition temperature (T_m), 55°C, and good compatibility with cholesterol. Normally, lipid bilayer has two thermodynamic phases: gel or liquid-crystal phase. At temperature < T_m , the lipid membrane is in the gel phase, which is relatively rigid and tight because the lipid molecules have lower energy of random motion and the hydrocarbon chains are fully extended and closely packed. Liposomes composed of phospholipids like DSPC have higher stability compared with others containing unsaturated fatty acid (egg phosphatidylcholine [PC]) or fatty acids of shorter or not uniform carbon chains like hydrogenated soy PC (HSPC). In a Phase I clinical study, Lipo-dox achieved the most prolonged circulation half-life (65 hours).⁷³ However, Tseng et al demonstrated that there were no differences in survival between free doxorubicin only (median survival time of 23 days) and Lipo-dox (medium survival time of 23.5 days) in a murine B-cell lymphoma model.⁷⁴ In patients with metastatic breast cancer, the median time to disease progression of 163 days represented the result of Lipo-dox treatment and the median duration of response in responding patients (286 days) are comparable with those of Doxil treatment.⁷⁵ Neutropenia, stomatitis, and skin toxicity were reported in many cases of Lipo-dox administration. Moreover, stomatitis became the new dose-limiting toxicity of PEGylated liposomal doxorubicin. For Lipo-dox, stomatitis appeared at doses of 30 mg/m² and reached dose limit at 50 mg/m².²⁷ In contrast, Doxil reached dose limit at 80 mg/m² and hence Lipo-dox had higher incidence of severe stomatitis than Doxil. In comparison with Myocet (the non-PEGylated form of liposomal doxorubicin), Doxil and Lipo-dox (both PEGylated forms of liposomal doxorubicin) both showed

significant incidence of stomatitis and this is mainly due to the long circulation properties of PEGylated liposomes.^{27,71,72}

The new generation of doxorubicin-loaded liposomes are thermosensitive liposomes (TSLs), which release their encapsulated drugs in regions where local tissue temperatures are elevated.⁷⁶ Compared with non-TSLs that remain stable and do not release drug in the physiologic temperature range, TSLs undergo a gel-to-liquid crystalline phase change when heated that renders the liposomes more permeable, releasing their encapsulated drugs. ThermoDox[®] (Celsion Corporation, Lawrenceville, NJ), a proprietary TSL encapsulation of doxorubicin, has recently begun Phase III clinical trials for the treatment of hepatocellular carcinoma.⁷⁷ ThermoDox is composed of dipalmitoylphosphatidylcholine (DPPC), monostearoylphosphatidylcholine (MSPC), and polyethylene glycol 2000-distearoylphosphatidylethanolamine (PEG 2000-DSPE) in 90:10:4 molar ratio.^{49,50} In the design of TSLs, it is necessary to choose a phospholipid that has a gel-to-liquid crystalline phase transition temperature (T_c) in the temperature range of clinically attainable local hyperthermia (41°C–42°C). The mechanism behind TSLs is the temperature-induced membrane instability at the T_c of the used lipids. DPPC with a $T_c = 41.5^\circ\text{C}$ is an ideal lipid according to temperature-triggered technology.^{78,79} For liposomes composed of DPPC alone, the rate of release and the amount released are relatively small. By incorporating a small amount of lysolipids, such as MSPC or monopalmitoylphosphatidylcholine, into DPPC liposomes, T_c is shifted down slightly and membrane instability and drug release rate is significantly enhanced at T_c . In vitro release studies, monopalmitoylphosphatidylcholine-containing TSLs released about 45% of encapsulated doxorubicin in bovine serum at 42°C in a few seconds (20 seconds), while pure DPPC liposomes released only 20% over 1 hour.⁷⁹ Banno et al demonstrated that the presence of MSPC, rather than PEG 2000-DSPE, in DPPC liposomes would give rise to the rapid drug-release profile in vitro, suggesting that lysolipid is the more important component in determining the rate of TSL content release.⁸⁰ Indeed, Banno's in vivo data showed that the presence of 9.6 mol% MSPC in TSL could result in more rapid elimination of the encapsulated doxorubicin ($T_{1/2} = 1.29$ h), compared with the formulation without lysolipid ($T_{1/2} = 2.91$ h). In 2007, Dromi et al compared the accumulation of doxorubicin in mice tumors among free doxorubicin, Doxil, and ThermoDox.⁵⁰ Results showed that over time, doxorubicin gradually increased in tumors when both Doxil and ThermoDox were used but not with free doxorubicin. At 24 hours after administration, doxorubicin concentrations in tumors were found to be significantly higher

with Doxil than ThermoDox. ThermoDox is currently under evaluation in clinical trials and hence the therapeutic efficacy of ThermoDox is still unknown.

Clinical studies of liposomal-based anticancer drugs: daunorubicin

Daunorubicin is classified as an anthracycline anticancer drug in the treatment of leukemia and a wide variety of solid tumors, but its major drawbacks are myelosuppression and cardiotoxicity.⁸¹ Daunorubicin has also been incorporated into liposomes for the formulation of liposomal anticancer chemotherapy drugs. DaunoXome[®] (Gilead Sciences, Inc) is a commercial liposomal formulation of daunorubicin in which the drug is entrapped into small unilamellar vesicles (45 nm) composed of DSPC and cholesterol in 2:1 molar ratio. In animal studies with tumor models in mice, DaunoXome increased tumor uptake of daunorubicin ten-fold when measured against free drug (2470.5 vs 245.1 $\mu\text{g}\cdot\text{hr}/\text{mL}$ for 0–48 hours).⁸² Furthermore, clinical pharmacokinetic studies have demonstrated that DaunoXome was 36-fold higher in AUC (375.3 vs 10.33 $\mu\text{g}\cdot\text{hr}/\text{mL}$) in comparison with conventional daunorubicin.⁸³ In a Phase III trial of DaunoXome versus a conventional combination of doxorubicin, bleomycin, and vincristine (ABV) in AIDS-related Kaposi's sarcoma, the efficacy of DaunoXome was comparable to that of vincristine. Response rates (25% vs 28%), time to treatment failure (115 vs 99 days), and overall survival (369 vs 342 days) were similar on both treatment arms.⁸⁴ Moreover, patients treated with DaunoXome experienced less alopecia (8% vs 36%) and neuropathy (13% vs 41%) and their cardiac function remained stable. Therefore, DaunoXome may provide another comparable but safer chemotherapy.

Clinical studies of liposomal-based anticancer drugs: paclitaxel

Taxol[®] (paclitaxel) is a marketed product for the treatment of ovarian, breast, non-small cell lung cancer, and AIDS-related Kaposi's sarcoma.⁴⁰ However, paclitaxel is only sparingly soluble in water and, therefore, intravenous administration depends on the use of the non-ionic surfactant Cremophor EL[®] (polyethoxylated castor oil) to achieve a clinically relevant concentrated solution. Unfortunately, Cremophor EL increases toxicity and leads to hypersensitivity reactions in certain patients.⁸⁵ The LEP-ETU formulation of paclitaxel is being developed to potentially reduce toxicities associated with Taxol by eliminating the drug formulation component polyoxyethylated castor oil. LEP-ETU formulations composed of 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine

(DOPC), cholesterol, and cardiolipin in 90:5:5 molar ratio were prepared by the modified thin-film hydration method. DOPC, a zwitterionic natural phospholipid, is chosen as one of the lipid components in the LEP-ETU formulation because of a low T_c (-22°C) and hence DOPC can form more flexible liposomes to entrap highly hydrophobic molecules. Moreover, cholesterol is included in LEP-ETU formulations to increase the liposome stability. Liposomes containing cardiolipin reportedly reduced cardiotoxicity associated with doxorubicin by altering the pharmacokinetics and tissue distribution of the drug and hence cardiolipin may also exert similar results in LEP-ETU.⁸⁶ Fetterly et al evaluated the maximum tolerated dose, dose-limiting toxicities, and pharmacokinetics of liposome-encapsulated paclitaxel (LEP-ETU) in comparison with Taxol.^{87,88} The maximum tolerated dose of LEP-ETU was 325 mg/m^2 in a Phase I study of patients with locally advanced or metastatic carcinoma.^{88,89} This dose is higher than that achieved with Taxol, which is typically delivered at a dose range of 135 to 200 mg/m^2 . The major toxicity to administration of paclitaxel is neuropathy. In the Phase I study, neurotoxicity occurred in 5 of 12 patients (42%) treated with LEP-ETU at ≥ 325 mg/m^2 . Although a direct comparison with Taxol is not possible, neutropenia was seen in 53% of metastatic breast cancer patients treated with 250 mg/m^2 Taxol as demonstrated by Winer et al.⁸⁹ Therefore, the neuropathy caused by LEP-ETU appears to be no worse than that reported for Taxol within 3 weeks of treatment. Following LEP-ETU administration, the AUC of paclitaxel in patients with advanced or metastatic carcinoma was improved (8.2 to 6.16 $\mu\text{g}\cdot\text{hr}/\text{mL}$) with increasing dose (135 to 375 mg/m^2), which is similar to Taxol. Although similarities exist between the plasma pharmacokinetics of the two formulations, the clinical evidence obtained from the Phase I study shows LEP-ETU can be administered safely at higher doses than Taxol.^{88,89}

Another liposome formation of paclitaxel is EndoTAG-1.^{42–44} The formulation of EndoTAG-1 is prepared by 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), DOPC, and paclitaxel in 50:47:3 molar ratio. DOTAP is a cationic synthetic lipid, which comprises one positive charge at the head group. The use of cationic lipids to enhance gene delivery has been studied extensively, but their application in clinic is relatively unexplored. Recently, there has been great interest in cationic liposomes, mainly due to their inherent ability to selectively target tumor vasculature. This selective affinity of cationic liposomes to tumor vasculature provides an opportunity for the development of many anti-angiogenic and anticancer formulations based on cationic

liposomes.⁴² EndoTAG-1 is the first formulation of cationic liposomes carrying paclitaxel in clinical trial. For commercial storage, EndoTAG-1 formulations are lyophilized, and they are reconstituted with water for injection directly prior use. In preclinical programs, EndoTAG-1 inhibited tumor growth also in Taxol-resistant animal tumor models such as B16 melanoma and Sk-Mel 28 melanoma. EndoTAG-1 demonstrated a strong antivascular effect on the preexisting tumor vasculature and affected several tumor microcirculatory parameters. In a Phase II trial of patients with pancreatic adenocarcinoma who were not candidates for surgery, EndoTAG-1 in combination with gemcitabine substantially extended overall survival compared with gemcitabine alone.⁹⁰ Median survival in patients who received gemcitabine alone was 7.2 months, whereas it was up to 9.4 months in those who received combination treatment of EndoTAG-1 plus gemcitabine. After 6 and 12 months of treatment, survival rate was superior for all EndoTAG-1 doses plus gemcitabine compared with gemcitabine alone. The 12-month survival rates in patients given the two higher doses of EndoTAG-1 (22 and 44 mg/m² plus gemcitabine) were 36% and 33%, respectively, compared with 17.5% in those given gemcitabine alone. Combination treatment with EndoTAG-1 plus gemcitabine was well tolerated and led to substantially more prolonged survival rates compared with standard therapy in this Phase II trial. Further clinical studies are warranted to demonstrate a statistically significant survival benefit associated with EndoTAG-1 plus gemcitabine in advanced pancreatic cancer.

Liposome application in vaccine formulation: Epaxal and Inflexal V

The incorporation of viral membrane proteins or peptide antigens into liposomes has been shown to potentiate cell-mediated and humoral immune response, and generate solid and durable immunity against the pathogen. Virosomes are reconstituted virus liposomes, constructed without the genetic information of the virus making them unable to replicate or cause infection.^{91,92} The lipid layers of virosomes, composed of dioleoyl phosphatidylethanolamine (DOPE) and DOPC, are employed to mimic viral membrane for vaccine delivery. Epaxal and Inflexal V are both vaccine products using the virosome-based antigen delivery system for commercial use (Table 1). For the production of Inflexal V, the influenza viruses, grown in hens' eggs, are first inactivated with beta-propiolactone. The influenza surface antigens, hemagglutinin and neuraminidase, are then purified and mixed with the phospholipid lecithin to form virosomes.

Due to the virosomal technology, hepatitis A virus (HAV) vaccine Epaxal, and influenza vaccine Inflexal V are highly efficacious by mimicking natural viral infection. The use of virosomes to deliver hepatitis A or influenza antigens stimulates a strong immune response of immunocompetent cells. In contrast to other commercially available HAV vaccines, Epaxal is an aluminium-free vaccine based on formalin-inactivated hepatitis A (strain RG-SB) antigen-incorporated virosomes. In a clinical study by Usonis et al, seroprotection rates were 100% in all infants and children at 1 and 12 months after primary vaccination with Epaxal.³⁵ In contrast, the seroprotection rate after vaccination with the aluminium-containing vaccine Havrix™ (GlaxoSmithKline Biologicals Rixensart, Belgium) was 67.7% in infants with pre-existing maternal anti-HAV antibodies, and a booster vaccination was required for complete seroprotection. Moreover, Epaxal was generally well tolerated by infants and children, with no serious systemic or local events reported after either primary or booster vaccination.

For Inflexal V, most studies have shown inferior efficacy or ineffectiveness on clinical parameters for these vaccines compared with the nonadjuvanted, split-virus, or subunit seasonal vaccines.⁹³ Kanra et al, compared the immunogenicity and safety of Inflexal V in children with a split influenza vaccine, Fluarix (GlaxoSmithKline Biologicals, Dresden, Germany).⁹⁴ Both vaccines were well tolerated and could induce effective immune responses in children. Interestingly, the virosome-adjuvanted influenza vaccine showed greater immunogenicity (88.8% seroconversion rates for H3 N2) over the split influenza vaccine (77.5% seroconversion rates for H3 N2) in unprimed children. In essence, virosomal techniques may not be able to give superior protective immunity in clinic but play an important role in preventing morbidity and lethality associated with vaccine.

Liposomal formulations in ophthalmology: Visudyne®

Verteporfin is a hydrophobic chlorin-like photosensitizer, which has been shown to be highly effective for photodynamic therapy in vivo. However, verteporfin also has a tendency to undergo self-aggregation in aqueous media, which can severely limit drug bioavailability to biological systems. It is important to introduce verteporfin into the bloodstream in its monomeric form and hence verteporfin was encapsulated in liposomes (Visudyne) for intravenous drug delivery.²⁹⁻³¹ The lipid layers of Visudyne are composed of unsaturated egg phosphatidylglycerol and dimyristoyl phosphatidyl choline in 3:5 molar ratio. Visudyne was the

only drug approved by the FDA for the photodynamic treatment of age-related macular degeneration. Visudyne treatment prevents the growth of the destructive blood vessels without hurting the surrounding tissues. Phase I and II clinical trials were conducted for 609 patients with age-related macular degeneration.^{95,96} After 12 months of treatment, the group treated with Visudyne (6 mg/m² body surface area) had statistically better visual acuity, contrast sensitivity, and fluorescein angiographic outcomes than those who had placebo treatment (5% dextrose in water). At the examination 12-months posttreatment, 246 (61%) of 402 eyes assigned to verteporfin compared with 96 (46%) of 207 eyes assigned to placebo had lost fewer than 15 letters of visual acuity from baseline. In subgroup analyses, the visual acuity benefit of verteporfin therapy was clearly demonstrated (67% vs 39%) when the area of choroidal neovascularization, caused by age-related macular degeneration, occupied 50% or more of the area of the entire lesion. However, Chowdhary et al reported that Visudyne was readily destabilized in the presence of relatively low concentrations of plasma.²⁹ Therefore, the aim of future investigation of liposomal formulations in ophthalmology is to develop stable liposome structures for extending the plasma circulation time following intravenous injection.

Future approaches

Since the first liposomal pharmaceutical product, Doxil, received FDA approval in 1995, liposomes have been widely applied as drug carriers in clinic. Until now, several important types of liposomes, such as PEGylated liposomes (Doxil and Lipo-dox), temperature sensitive liposomes (ThermoDox), cationic liposomes (EndoTAG1-1), and virosomes (Expal and Inflexal V) have been investigated for clinic use. In contrast with liposomal-based drugs on the market (Table 1), liposome-based drugs in clinical trials (Table 2) focused more on the types of delivered drugs (eg, Cisplatin, BLP25 lipopeptide, Grb2 antisense oligodeoxynucleotide, Bacteriophage T4 endonuclease 5, etc) and therapeutic applications (from topical delivery systems to portable aerosol delivery systems). New liposomal formulations, such as PEGylated liposomes, may extend blood circulation time, vary drug distribution in the body, and hence reduce the possible side effects related to the drugs (eg, cardiotoxicity). However, PEGylated liposomes (Doxil and Lipo-dox) displayed significant incidence of stomatitis in clinical trials, which may be related to PEGylation. Moreover, some of the new generation liposomes showed only comparable or even poor therapeutic efficiency compared with free drug or conventional vesicles

in clinical trials. In comparison with Doxil, ThermoDox displayed significantly weaker doxorubicin accumulation in mice tumors at 24 hours after administration.⁵⁰ EndoTAG-1 plus gemcitabine and EndoTAG-1 plus paclitaxel achieved excellent therapeutic effect in two Phase II clinical trials in pancreatic cancer and triple receptor-negative breast cancer, but EndoTAG-1 therapy alone in triple receptor-negative breast cancer resulted in poor survival rate (34%) and median progression-free survival time (3.4 months) in comparison with paclitaxel (48% and 3.7 months).⁹⁷ SPI-077, the first liposomal formulation of cisplatin, had limited clinical efficacy in a Phase II clinical trial of advanced non-small cell lung cancer, even though SPI-077 demonstrated enhanced cisplatin tumor accumulation in preclinical models.⁹⁸ Similar to SPI-077, a Phase II study of liposomal annamycin in the treatment of doxorubicin-resistant breast cancer had no detectable antitumor activity.⁹⁹ Although new liposomal-based drugs have been well studied in preclinical animal models, these liposomal pharmaceutical products may be unable to provide promising therapeutic effects in clinical trials. For future development of liposomal-based drugs, the comparison of drug circulation time in blood, drug accumulation in tissues, and possible toxicity between conventional vesicles and new generations of liposomes should be investigated in preclinical animal models. Furthermore, there should also be focus on the clinical therapeutic effects and toxic side effects of liposomal lipid composition.

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Disclosure

The authors report no conflicts of interest in this work.

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