

Clinically-Proven Liposome-Based Drug Delivery: Formulation, Characterization and Therapeutic Efficacy

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Abstract

Liposomes in their various forms have the possibility of providing therapeutic efficacy in the area of drug delivery. This review mainly addresses therapeutic effect of current clinical approved liposomal formulations, preparation techniques, storage aspects, as well as lipid compositions. Researches on liposome formulations have progressed from conventional vesicles to new generation liposomes, such as cationic liposomes, temperature-sensitive liposomes and virosomes, by modulating the preparation method and lipid composition. Furthermore, the major pre-clinical and clinical data relating the principal liposomal formulations are also summarized.

Keywords: Liposomes; Temperature-sensitive liposomes; Lyophilization; Virosomes

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. Controlled drug delivery systems have been attempted to overcome these problems by providing selective delivery to the affected area. Liposomes are small, spherical and enclosed compartments separating an aqueous medium from another by phospholipid bilayer and liposomal formulations are one of advanced drug delivery systems in clinical application. Due to differences in preparation methods and lipid compositions, liposomes can be classified according to their lamellarity (uni- and multi-lamellar vesicles), size (small, intermediate, or large) and charge (anionic, cationic and neutral) [1-3]. Liposomes are able to encapsulate lipophilic or hydrophilic drugs with their lipidic layers or in their aqueous core respectively and deliver those to target site for in vivo application. Moreover, liposome delivery system can increase the solubility of hydrophobic drugs and stabilize a variety of therapeutic agents such as peptides, proteins and nucleotides in bloodstream [3,4]. In clinic studies, liposomes show improved pharmacokinetics and biodistribution of therapeutic agents and thus minimize toxicity by their accumulation at the target tissue [5]. Liposomes were first discovered by Bangham in 1965 and the first liposomal pharmaceutical product, Doxil, received FDA approval in 1997 for the treatment of chemotherapy refractory AIDS-related Kaposi's sarcoma [4,5]. Currently there are about 12 liposome-based drugs approved for clinical use and more are in various stages of clinical trials (Table 1 and 2) [5-46]. Most of liposomal drug formulations, including Ambisome, Doxil and Myocet, are approved for intravenous application. Other administration routes such as intramuscular delivery have also been approved for delivery of surface antigens derived from the hepatitis A or influenza virus. Oral delivery has been examined however this is more troublesome due to the potential for liposome breakdown following exposure to bile salts [47].

Liposomal encapsulation technology

Many hundreds of drugs, including anti-cancer and antimicrobial agents, chelating agents, peptide hormones, enzymes, other proteins, vaccines and genetic materials, have been incorporated into the

aqueous or lipid phases of liposomes with various sizes, compositions and other characteristics by different preparation techniques. An ideal method of liposome formulation is preparing liposome with high entrapment efficiency, narrow particle size distribution and long term stability. Numbers of techniques have been reported for preparation of liposomes such as Bangham method, the detergent depletion method, the ether/ethanol injection method, the reverse phase evaporation and the emulsion method [48]. The majority of liposome preparation methods require using organic solvents to dissolve lipids but these organic solvents are harmful to the environment and human body. Recently, some alternative methods including dense gas and supercritical fluid techniques have been introduced for liposome preparation without using any organic solvent [48-50]. Despite the clear advantages of dense gas or supercritical fluid liposome production, there are also problems with the known processes for liposome formation. The dense gas or supercritical fluid processes generally require elevated pressures of at least 1,000 psi and the conditions commonly used are 3000-4500 psi and temperatures of 60°C for liposome production. For drug encapsulation, both anti-solvent methods successfully encapsulated low molecular weight drugs such as paclitaxel, camptothecin and betulinic acid in liposomes [50]. However, high temperature, pressure, and shearing forces presenting in liposome processing condition with dense gas or supercritical fluid potentially denature high molecular weight drugs, such as peptides or proteins and result low production and encapsulation efficiency [51]. Physicochemical properties of liposomal formulations, including size, membrane lamellarity, surface charge, permeability, and encapsulation volume, are depending on the lipid composition (cationic, anionic, and neutral lipid species). The major function of liposome preparation

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techniques is to obtain efficient drug entrapment and increase stability of the liposome products [52]. Most of drugs listed in Table 1 using liposomes as carrier to increase the drug solubility in aqueous solution and also decrease drug toxicity in human body. Ambisome, Doxil and Myocet are the examples for improving therapeutic index by reducing the toxicities associated with the free drugs.

Storage of Liposomes: lyophilization

Liposomes dispersed in aqueous solution generally face physical and chemical instabilities after long term storage [53]. 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, Amphotec, Myocet, Visudyne and LEP-ETU are all lyophilized products. In general, freeze-drying increases the shelf-life of liposomal formulations and preserves it in dried form as a lyophilized cake to be reconstituted with water for injection prior to administration [54]. Furthermore, cryoprotectants need to be added to maintain particle size distribution of liposomes after 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 [54]. In commercial liposome lyophilized products, lactose was used as a cryoprotectant in the formulation of Amphotec, Myocet and Visudyne and sucrose was added in the formulation 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 expected effect on liposome stability. In 1998, Clemons et al. [55] compared the potency and therapeutic efficacy among the different lipid-based formulations of amphotericin B (Amphotec, AmBisome and Abelcet) for the treatment of systemic and meningeal cryptococcal disease. Their work indicated that the therapeutic efficacy of Amphotec and AmBisome was superior to that of Abelcet by up to 10-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 form. 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. We investigated the stability of the siRNA-loaded liposomes in suspension and lyophilized powder form up to 1 month post manufacture [56]. 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 for both storage conditions. However, siRNA entrapment efficiencies for both storage conditions were observed to have decreased slightly over time. Surprisingly, the gene-silencing efficiency of siRNA-loaded liposomes in aqueous solution was almost completely abolished 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

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 amphotericin B in 2:0.8:1:0.4 molar ratio	Sever fungal infections	[5,7]
Abelcet	Intravenous	Amphotericin B	Lipid complex	Suspension/24 months	DMPC and DMPG in 7:3 molar ratio	Sever fungal infections	[8,9]
Amphotec	Intravenous	Amphotericin B	Lipid complex	Powder/ 24 months	cholesteryl sulfate	Sever fungal infections	[10,11]
DaunoXome	Intravenous	Daunorubicin	Liposome	Emulsion/12 months	DSPC and cholesterol (2:1 molar ratio)	Blood tumors	[5, 12-14]
Doxil	Intravenous	Doxorubicin	PEGylated Liposome	Suspension/20 months	HSPC: cholesterol: PEG 2000-DSPE (56:39:5 molar ratio)	Kaposi's sarcoma, Ovarian/Breast Cancer	[5, 15,16]
Lipodox	Intravenous	Doxorubicin	PEGylated Liposome	Suspension/36 months	DSPC: cholesterol: PEG 2000-DSPE (56:39:5 molar ratio)	Kaposi's sarcoma, Ovarian/Breast Cancer	[17]
Myocet	Intravenous	Doxorubicin	Liposome	powder/ 18months	EPC: cholesterol (55:45 molar ratio)	Combination therapy with cyclophosphamide in metastatic breast cancer	[5,15,18]
Visudyne	Intravenous	Verteporfin	Liposome	Powder/48 months	EPG:DMPC in 3:5 molar ratio	Age-related molecular degeneration, , pathologic myopia, ocular histoplasmosis	[19-21]
Depocyt	Spinal	Cytarabine	Liposome	Suspension/18 months	Cholesterol: Triolein: DOPC: DPPG in 11:1:7:1 molar ratio	neoplastic meningitis and lymphomatous meningitis	[5,8]
DepoDur	Epidural	Morphine sulfate	Liposome	Suspension/24 months	Cholesterol: Triolein: DOPC: DPPG in 11:1:7:1 molar ratio	Pain management	[8,22]
Epaxal	intramuscular	Inactivated hepatitis A virus (strain RG-SB)	Liposome	Suspension/36 months	DOPC/DOPE in 75:25 molar ratio	Hepatitis A	[23-25]
Inflexal V	intramuscular	Inactivated hemaglutinine of Influenza virus strains A and B	Liposome	Suspension/12 months	DOPC/ DOPE in 75:25 molar ratio	Influenza	[23,24,26]

Abbreviations: DOPE: Dioleoylphosphatidylethanolamine; DOPC: Dioleoylphosphatidylcholine; DPPG: Dipalmitoylphosphatidylglycerol; HSPC: Hydrogenated Soy Phosphatidylcholine; DSPG: Distearoylphosphatidylglycerol; EPC: Egg Phosphatidylcholine; DSPC: Distearoylphosphatidylcholine; DMPC: α-Dimyristoylphosphatidylcholine; DMPG: 1-α-Dimyristoylphosphatidylglycerol; EPG: Egg Phosphatidylglycerol; PEG 2000-DSPE: Polyethylene glycol 2000- Distearoylphosphatidylethanolamine

Table 1: Liposome based drugs in market.

temperature for a month. Although therapeutic efficiency of liposome-based drug may vary depending on the choice of lipids, the preparation technique, the physico-chemical characteristics of the bioactive, and the overall charge of the liposome, lyophilisation is absolutely essential for the long term storage of liposome-based drugs.

Liposomal anti-cancer drug researches: Doxorubicin

Liposome delivery systems offer the potential to enhance the therapeutic index of anticancer drugs, either by increasing the drug concentration in tumor cells and 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 the onset of resistance. Therefore, doxorubicin-loaded liposomes were developed to combat aggressive tumors, like breast and ovary metastatic cancers and Kaposi's sarcoma. Myocet and Doxil were first approved liposome-based drugs for cancer treatment. Both products contain doxorubicin but different particularly in the presence of Poly Ethylene Glycol (PEG) coating (Figure 1). In pharmacokinetic studies of doxorubicin-loaded liposomes, free doxorubicin had an elimination half-life time of 0.2 hours and an AUC (area under the curve) of $4 \mu\text{g h ml}^{-1}$ in patients as compared with 2.5 hour 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 [15]. Both liposome products showed longer circulating half-life as compared with free drug. In phase III head to head comparison of free doxorubicin vs Myocet in patients with metastatic breast cancer, similar results presented in response rates (26% for both) and progression-free survival times (4 months for both) but Myocet had low incidence of cardiac events (29 vs 13%) and of congestive heart failure (8 vs 2%) [57]. 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 the reduce of cardiotoxicity, myelosuppression, vomiting and alopecia in phase III trial of metastatic breast cancer whereas its response rates, progression-free survival times and overall survival times demonstrated equivalent efficacy to conventional doxorubicin.

Lipo-dox is the second generation of PEGylated liposomal doxorubicin and which is composed of Distearoyl Phosphatidylcholine (DSPC) and cholesterol with surface coating with PEG [17]. Liposomes composed of phospholipids like DSPC (Figure 1), which has two completely saturated fatty acids (stearic acid), have higher stability compared with others containing unsaturated fatty acid (egg PC) or fatty acids of shorter or not uniform carbon chains like Hydrogenated Soy Phosphatidylcholine (HSPC). In phase I clinical study, Lipo-dox has achieved the most prolonged circulation half-life (65 hours). However, the antitumor activity of Lipo-dox for hepatocellular carcinoma is not higher than free doxorubicin. Moreover, stomatitis became the new dose-limiting toxicity of PEGylated liposomal doxorubicin. For Lipo-dox, stomatitis appeared at doses of 30mg/m^2 and reached dose limit at 50mg/m^2 . In contrast, Doxil reached dose limit at 80mg/m^2 and hence Lipo-dox had higher incidence of serve stomatitis than Doxil. PEGylated liposomal doxorubicin, Doxil and Lipodox (both PEGylated form of liposomal doxorubicin) have significantly more side effects than Myocet (the non PEGylated form of liposomal doxorubicin) and this is mainly due to the long circulation properties of PEGylated liposomes.

The new generation of doxorubicin-loaded liposomes is Thermosensitive Liposomes (TSL) which releases their encapsulated drugs in regions where local tissue temperatures are elevated.

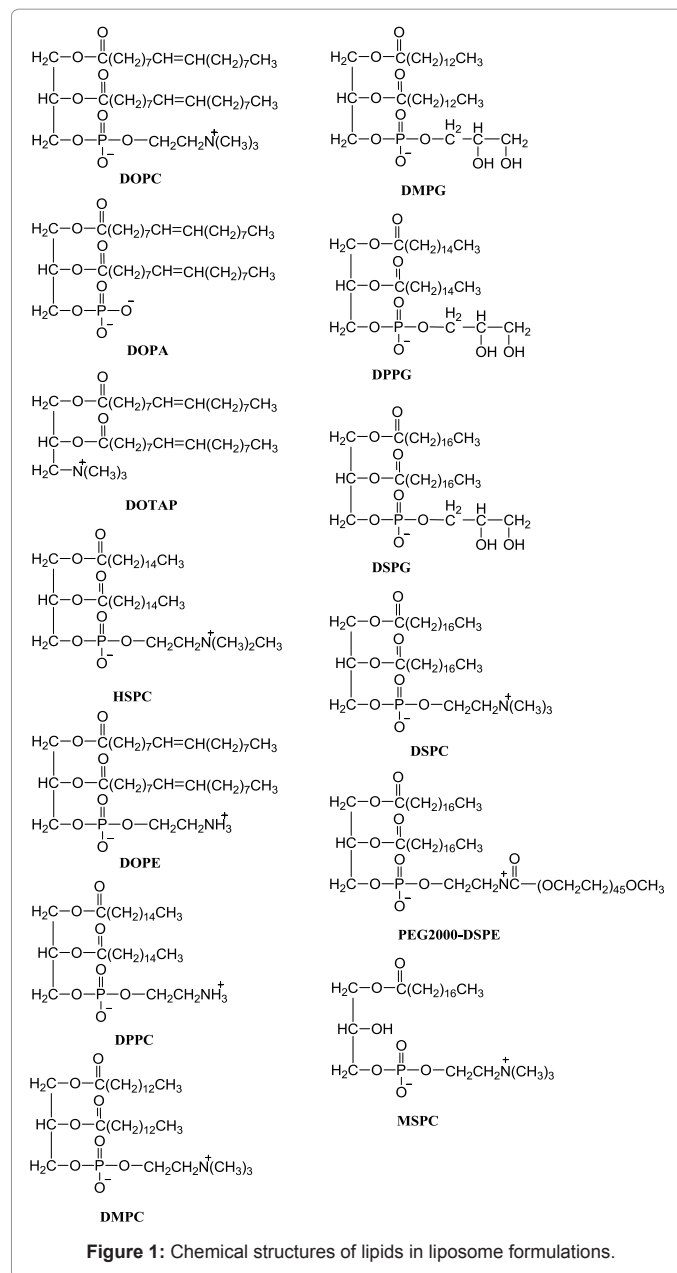


Figure 1: Chemical structures of lipids in liposome formulations.

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[®], a proprietary TSL encapsulation of doxorubicin, recently is in phase III clinical trials for the treatment of hepatocellular carcinoma. ThermoDox[®] is composed of Dipalmitoylphosphatidylcholine (DPPC), Monostearoylphosphatidylcholine (MSPC) and PEG 2000-DSPE in 90:10:4 molar ratio [35-36]. In the design of TSL, 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- 42°C). The mechanism behind TSL 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 [58]. 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 (MPPC), into DPPC liposomes, Tc is shifted down slightly and membrane instability and drug release rate is significantly enhanced at Tc. Banno et al. [59] demonstrated that the presence of MSPC, rather than DSPE-PEG2000, in DPPC liposomes would give rise to the rapid drug release profile in vitro and that represents lysolipid is the more important component in determining TSL contents release. 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 ($T_{1/2}=1.29\text{h}$), compared to the formulation without lysolipid ($T_{1/2}=2.91\text{h}$). In 2007, Dromi et al. [36] compared the accumulation of doxorubicin in mice tumors among free doxorubicin, Doxil and ThermoDox. 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.

Liposomal anti-cancer drug researches: daunorubicin and paclitaxel

Daunorubicin and paclitaxel have also incorporated into liposomes for the formulation of liposomal anti-cancer chemotherapy drugs. DaunoXome is a commercial liposomal formulation of daunorubicin in which the drug is entrapped into small unilamellar vesicles composed of Distearoyl phosphatidylcholine (DSPC) and cholesterol in 2:1 molar ratio. LEP-ETU and EndoTAG-1 (previously called MBT-0206) are the potential liposomal formulations of paclitaxel and both are in clinical trials (Table 1 and 2). In comparison with conventional daunorubicin, DaunoXome was 36-fold higher in AUC and in vivo experiments

Product Name	Route of injection	Drug	Lipid composition	Approved indication	Ref
LEP-ETU (Powder/12 months)	Intravenous	Paclitaxel	DOPC, cholesterol and cardiolipin in 90:5:5 molar ratio	ovarian, breast and lung cancers	[5,27]
LEM-ETU	Intravenous	Mitoxantrone	DOPC, cholesterol and cardiolipin in 90:5:5 molar ratio	Leukemia, breast, stomach, liver, ovarian cancers	[5,27]
EndoTAG-1 (Powder/ 24 months)	Intravenous	Paclitaxel	DOTAP, DOPC and paclitaxel in 50:47:3 molar ratio	Anti-angiogenic properties, breast cancer	[5,28-30]
EndoTAG-2	Intravenous	camptothecin	DOTAP	Metastatic cancer	[28,30]
Arikace	portable aerosol delivery	Amikacin	DPPC and cholesterol	Lung infection	[31,32]
Marqibo	Intravenous	Vincristine	cholesterol and egg sphingomyelin in 45:55 molar ratio	metastatic malignant uveal melanoma	[5,33,34]
ThermoDox	Intravenous	Doxorubicin	DPPC, MSPC and PEG 2000-DSPE in 90:10:4 molar ratio	non-resectable hepatocellular carcinoma	[35,36]
Antragen	Intravenous	Tretinoin	DMPC and soybean oil	advanced renal cell carcinoma	[5]
T4N5 liposome lotion	Topical	Bacteriophage T4 endonuclease 5	unknown	xeroderma pigmentosum.	[37]
Liposomal Grb-2	Intravenous	Grb2 antisense oligodeoxynucleotide	unknown	Acute Myeloid Leukemia, Chronic Myelogenous Leukemia, Acute Lymphoblastic Leukemia	[38]
Nyotran	Intravenous	Nystatin	DMPC, DMPG and cholesterol	systemic fungal infections.	[5]
□LE-SN38	Intravenous	SN-38, the active metabolite of irinotecan	DOPC, cholesterol and cardiolipin	metastatic colorectal cancer	[5,39]
Aroplatin	Intraleural	Cisplatin Analog (L-NDDP)	DMPC and DMPG	Malignant Pleural Mesothelioma	[40]
Liprostin	Intravenous	Prostaglandin E1	unknown	Peripheral Vascular Disease	[41]
Stimuvax	subcutaneous	BLP25,MUC1-targeted peptide	unknown	Cancer vaccine for multiple myeloma developed encephalitis	[42]
SPI-077	Intravenous	Cisplatin	SHPC, cholesterol and DSPE-PEG	Head and neck cancer, Lung cancer	[5]
Lipoplatin (suspension /36 months)	Intravenous	Cisplatin	SPC, DPPG and cholesterol	Several cancer type	[5,43]
S-CKD602	Intravenous	Camptothecin analog	unknown	Several cancer type	[5]
OSI-211	Intravenous	Lurtotecan	HSPC and cholesterol in 2:1 molar ratio	Ovarian cancer, head and neck cancer	[44,45]
INX-0125	Intravenous	Vinorelbine	cholesterol and egg sphingomyelin in 45:55 molar ratio	Breast, colon and lung cancer	[5,46]
INX-0076	Intravenous	Topotecan	cholesterol and egg sphingomyelin in 45:55 molar ratio	Advanced cancer	[5]
Liposome-Annamycin (powder)	Intravenous	Annamycin	DSPC, DSPG and Tween	Breast caner	[5]

* DOPC: 1,2-Dioleoyl-*sn*-Glycero-3-Phosphocholine; MSPC: Monostearoylphosphatidylcholine ; DPPC: Dipalmitoylphosphatidylcholine; DOTAP: 1,2 Dioleoyl-3-Trimethylammonium-Propane)

Table 2: Liposome based drug in clinical trials.

indicated increased uptake of daunoXome in tumour tissue at 24 h. In phase III trial of DaunoXome versus vincristine 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 and hence DaunoXome may provide another safe and effective chemotherapy [60].

Taxol® (paclitaxel) is a marketed product for the treatment of ovarian, breast, non-small cell lung cancer and AIDS-related Kaposi's Sarcoma [27]. It is one of the most effective anticancer drugs available on the market. 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. 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 zwitter ionic natural phospholipid, is first chosen as one of the lipid components in LEP-ETU formulation because of a low T_c (−22°C), and which forms more flexible liposomes to entrap highly hydrophobic molecules. Moreover, cholesterol is included in LEP-ETU formulations to increase the liposome stability. 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 [61]. 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. Fetterly et al. [62,63] evaluated the Maximum Tolerated Dose (MTD), Dose-Limiting Toxicities (DLT), and pharmacokinetics of Liposome-Encapsulated Paclitaxel (LEP-ETU) in comparison to Taxol®. The MTD of LEP-ETU was 325 mg/m² in phase I study of patients with locally advanced or metastatic carcinoma. This dose is higher than that achieved with Taxol, which is typically delivered at a dose range of 135 to 200 mg/m². 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/m². Although a direct comparison with Taxol® is not possible, neutropenia was seen in 53% of metastatic breast cancer patients treated with 250 mg/m² Taxol® as demonstrated by Winer et al. [64]. 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, paclitaxel blood concentrations declined polyexponentially and AUC of paclitaxel was less than proportional with increasing dose, 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®.

Another liposome formation of paclitaxel is Endo TAG-1 [28-30]. The formulation of Endo TAG-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 [28]. Endo TAG-1 is the first formulation of cationic liposomes carrying paclitaxel in clinical trial. Endo TAG-1, which is currently tested in clinical studies, comprises about 3 mol% paclitaxel in a DOTAP/DOPC lipid matrix. For commercial storage, Endo TAG-1 formulations are lyophilized, and they are reconstituted with water for injection directly prior use. In preclinical programs, EndoTAG1-1 inhibited tumor growth also in Taxol-resistant animal tumor models, as for example, B16 melanoma and Sk-Mel 28 melanoma. EndoTAG1-1 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 [65]. 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 prolonged survival rates compared to 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 [23-24]. 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 (HA) and Neuraminidase (NA), 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 immuno competent cells. In contrast to other commercially available Hepatitis A Virus (HAV) vaccines, Epaxal is an aluminium-free vaccine based on formalin-inactivated hepatitis A (strain RG-SB) antigen incorporated virosomes. In clinical study by Usonis et al. [66], seroprotection rates were 100% in all infants and children at 1 and 12 months after primary vaccination with Epaxal. In contrast, the seroprotection rate after vaccination with aluminium containing vaccine Havrix 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 [67]. Kanra et al. [68] compared the immunogenicity and safety of Inflexal V in children with a split influenza vaccine, Fluarix. 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 H3N2) over the split influenza vaccine (77.5% seroconversion rates for H3N2) in unprimed children. In essence, virosomal techniques may not be able to give superior protective immunity in clinic but it has given humankind the time to prepare for a potential public health inflection.

Liposomal formulations in ophthalmology: Visudyne

Verteporfin is a hydrophobic chlorin-like photosensitizer, which has been shown to be a 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 [19-21]. The lipid layers of Visudyne are composed of unsaturated Egg Phosphatidyl Glycerol (EPG) and Dimyristoyl Phosphatidyl Choline (DMPC) in 3:5 molar ratio. Visudyne was 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 studies were conducted for 609 patients with age-related macular degeneration [69-70]. After 12 month treatment, the group treated with Visudyne (6 mg/m² body surface area) had statistically better visual acuity, contrast sensitivity, and fluorescein angiographic outcomes than did those who had placebo treatment (5% dextrose in water). At the month-12 examination, 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, Visudyne was readily destabilized in the presence of relatively low concentrations of plasma as results reported by Chowdhary et al. [19]. Therefore, the future investigation of liposomal formulations in ophthalmology is to stable liposome structures for extending the plasma circulation time following intravenous injection.

Conclusions

Since the first liposomal pharmaceutical product, Doxil, received FDA approval in 1995, liposomes are widely applied as drug carriers in clinic. Until now, several important types of liposomes, like temperature sensitive liposomes (ThermoDox) and cationic liposomes (EndoTAG1-1) have been extensively studied for clinic use. New liposomal formulations may extend plasma circulation time, vary drug distribution in body and hence reduce the possible side effects related to the drugs. However, these new generation liposomes have only comparable or poor therapeutic efficiency to conventional vesicles in clinical trials. Therefore, new liposomal pharmaceutical

products should focus more on the types of delivered drugs (from small hydrophobic anti-cancer drugs to influenza surface antigens) and therapeutic applications (from anti-cancer chemotherapy to vaccination) than formulation design.

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