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Review

Development of liposomal formulations: From concept to clinical investigations

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ABSTRACT

Liposome is one of the most successful drug delivery systems applying nanotechnology to potentiate the therapeutic efficacy and reduce toxicities of conventional medicines. Since the first doxorubicin-loaded liposome reached the market, numerous researches have been carried out to develop new liposomal formulations over the past decade and have given birth to a series of commercial products. Therapeutic agents, most of which are anti-cancer drugs, are encapsulated in the aqueous core or lipid bilayers of liposomes to improve their delivery to the targeted tissue. There are several liposomal formulations, such as EndoTAG-1 (paclitaxel-loaded cationic liposomes), Lipoplatin (cisplatin-loaded long circulating liposomes) and Stimuvax (a cancer vaccine), showing promising therapeutic value in clinical studies. Besides, new designs including environmentally sensitive liposomes, liposomal drug combinations and liposomal vaccines are now tested in clinical trials.

1. Introduction

The efficacy of therapeutic molecules is often limited by the insufficient delivery, accumulation in target tissues or undesirable side effects even severe toxicities in healthy organs. In recent years, nanoparticles such as polymeric micelles [1,2], liposomes [3–5] and conjugated nanoparticles [6–8] have inspired the drug development. Among these delivery systems, liposomes appear promising because of their biocompatible composition as well as superior efficacy, especially the significant improvement in drug circulation and biodistribution materialized by PEGylation [9].

Liposomes are round bubbles consisting of an aqueous core encapsulated by natural or synthetic phospholipids. This structure turns liposomes into ideal drug carriers, since hydrophilic drugs tend to be entrapped in the core; while hydrophobic ones will be entrapped within the lipid bilayers. The encapsulation is partially dependent on the partition coefficient or LogP of a certain drug [10]. Prepared by different methods, liposomes vary with size, lamellarity and surface charge. Generally, liposomes can be classified as unilamellar or multilamellar. Unilamellar liposomes are further divided into small size (SUV, 50–100 nm) or large size (LUV, 100–250 nm). SUV and LUV are both composed of a single lipid

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Table 1 – Approved liposomal formulations.

Drug	Product name	Type	Lipid composition	Route of administration	Approved treatment	Reference
Amphotericin B	Ambisome	Liposome	HSPC, DSPG and cholesterol	Intravenous	Sever fungal infections	[3]
Doxorubicin	Myocet	Liposome	EPC and cholesterol	Intravenous	Metastatic breast cancer	[11,12]
	Doxil	PEGylated liposome	HSPC, cholesterol and DSPE-PEG_{2000}	Intravenous	Kaposi's sarcoma, ovarian and breast cancer	[5,13]
	Lipo-dox	PEGylated liposome	DSPC, cholesterol and DSPE-PEG_{2000}	Intravenous	Kaposi's sarcoma, ovarian and breast cancer	[14]
Daunorubicin	DaunoXome	Liposome	DSPC and cholesterol	Intravenous	Blood cancer	[4,15]
Verteporfin	Visudyne	Liposome	EPG and DMPC	Intravenous	Age-related molecular degeneration	[16–18]
Cytarabine	Depocyt	Liposome	DOPC, DPPG, cholesterol and triolein	Spinal	Neoplastic meningitis and lymphomatous meningitis	[19]
Morphine sulfate	DepoDur	Liposome	DOPC, DPPG, cholesterol and triolein	Epidural	Pain	[20,21]
Vincristine sulfate	Marqibo	Liposome	Egg sphingomyelin and cholesterol	Intravenous	Acute lymphoblastic leukemia	[22,23]

bilayer and a large aqueous core, thus suitable for loading hydrophilic drugs; while multilamellar liposomes (MLV), usually with a diameter of 1–5 μm, are composed of several lipid bilayers and a limited aqueous space, thus suitable for loading hydrophobic drugs.

From the first liposomal pharmaceutical product-Doxil approved in 1995 to the latest Marqibo in 2012, there are now a few successful liposomal formulations (Table 1). Most of them have to be administrated intravenously due to the degradation of lipids in the gastrointestinal tract. However, some recent formulations such as Arikace (see Table 2) can be subcutaneously injected or inhaled as aerosols. Apart from a broadened range of drugs being investigated for liposomal formulations, new strategies such as environmental sensitivity and combination therapy have been applied to the development process to achieve better efficacy. Moreover, liposomes could be

Table 2 – Liposomal formulations in clinical trials.

Drug	Product name	Lipid composition	Route of administration	Treatment under investigation	Trial phase	Reference
Paclitaxel	LEP-ETU	DOPC, cholesterol and cardiolipin	Intravenous	Ovarian, breast and lung cancers	I	[32,33]
	EndoTAG-1	DOTAP and DOPC	Intravenous	Anti-angiogenesis, breast and pancreatic caners	II	[34]
Doxorubicin	ThermoDox	DPPC, MSPC and DSPE-PEG_{2000}	Intravenous	Non-resectable hepatocellular carcinoma	III	[35,36]
Cisplatin and its analog	SPI-077	HSPC, cholesterol and DSPE-mPEG	Intravenous	Lung, head and neck cancers	I/II	[37,38]
	Lipoplatin	SPC, DPPG, cholesterol and DSPE-mPEG	Intravenous	Pancreatic cancer, head and neck cancer, mesothelioma, breast cancer, gastric cancer and non-small-cell lung cancer.	III	[39,40]
	Aroplatin	DMPC and DMPG	Intrapleural/ intravenous	Malignant pleural mesothelioma and advanced colorectal carcinoma	II	[41,42]
Mitoxantrone	LEM-ETU	DOPC, cholesterol and cardiolipin	Intravenous	Leukemia, breast, stomach, liver and ovarian cancers	I	[43,44]
Topotecan	INX-0076	Egg sphingomyelin and cholesterol	Intravenous	Advanced solid tumors	I	[9]
Vinorelbine	INX-0125	Egg sphingomyelin and cholesterol	Intravenous	Breast, colon and lung cancers	I	[9,45]
Lurtotecan	OSI-211	HSPC and cholesterol	Intravenous	Ovarian, head and neck cancers	II	[46,47]
Amikacin	Arikace	DPPC and cholesterol	Inhaled as aerosol	Lung infection	III	[48,49]
BLP25 lipopeptide	Stimuvax	Monophosphoryl lipid A, cholesterol, DMPG and DPPC	Subcutaneous	Non-small-cell lung carcinoma	III	[50]
All-trans retinoic acid	Atragen	DMPC and soybean oil	Intravenous	Advanced renal cell carcinoma	I/II	[51]
Annamycin	Liposome-annamycin	DSPC, DSPG and tween	Intravenous	Breast cancer	I/II	[52,53]
Cytarabine and daunorubicin	CPX-351	DSPC, DSPG and cholesterol	Intravenous	Acute myeloid leukemia	II	[54]
Irinotecan HCL and floxuridine	CPX-1	DSPC, DSPG and cholesterol	Intravenous	Colorectal cancer	II	[55,56]

successfully applied to areas other than cancer therapy, such as vaccines. This review will introduce the approved liposomal products, while focus more on the species under clinical trials and new tendencies in their development.

2. Development of liposomal drugs: a typical example of doxorubicin

Doxorubicin, a kind of anthracyclines, is a potent and broad-spectrum anti-cancer drug and has been used as a "first-line" medicine in cancer therapy [24]. Two main mechanisms of action are involved for the drug: (1) it inhibits DNA and RNA synthesis by inserting in base pairs of DNA strands, thus preventing the replication and transcription in rapidly-growing cancer cells; (2) it inhibits the enzyme topoisomerase II, which is an additional way for blocking DNA transcription and replication. However, the positively charged doxorubicin is also of high affinity to negatively charged cardiolipin, which is abundant in heart tissue [25]. This damage results in the dangerous cumulative dose-dependent cardiotoxicity (i.e. irreversible congestive failure), which considerably limits the tolerable dose range of doxorubicin. Other side effects of doxorubicin include severe myelosuppression, nausea and vomiting and mucocutaneous toxicities [5].

Therefore, liposomal formulation is proposed to overcome these toxicities. Initially, liposomal doxorubicin was prepared to be negatively charged, medium-size oligolamellar liposomes, in which the drug was passively entrapped by the lipid hydration method [26]. However, this formulation failed in following clinical trials mainly due to the rapid drug release and clearance by reticuloendothelial system *in vivo*. "Remote loading" was then used to improve the drug loading efficiency and formulation stability, bringing about Myocet and Doxil in which doxorubicin was loaded by a pH or ammonium gradient, respectively. The morphology and structure of Doxil is shown in Fig. 1. A major advancement of Doxil over Myocet is the coating with PEG, which significantly improves its pharmacokinetic profile. So in a pharmacokinetic study of doxorubicin-loaded liposomes, free doxorubicin had an elimination half-life of 0.2 h and an area under the plasma concentration–time curve (AUC) of 3.81 μg h/ml, compared with 2–3 h and 46 μg h/ml for Myocet and with a further increase to 41–70 h and 902 μg h/ml for Doxil [27]. Both Myocet and Doxil significantly reduce the toxic effects of doxorubicin. In a Phase III comparison of free doxorubicin with Myocet, patients treated with Myocet had low incidence of cardiac events (13% vs. 29%), mucositis/stomatitis (8.6% vs. 11.9%), and nausea/vomiting (12.3% vs. 20.3%) [28]. Similar results were found in another Phase III trial of Doxil, in which the reduction of cardiotoxicity (3.9% vs. 18.8%), neutropenia (4% vs. 10%), vomiting (19% vs. 31%), and alopecia (20% vs. 66%) were found [29]. However, equivalent survival rates between liposomes and free drugs were found in these studies, suggesting the advantage of Myocet and Doxil lay only in the reduction of toxicities.

Lipo-dox is the third approved liposomal formulation for doxorubicin. The major improvement is the application of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), a kind of lipid consists of saturated fatty acids and has high phase-transition temperature (T_m). The high T_m makes Lipo-dox less likely to leak drugs and thus more stable. Since Lipo-dox is also PEGylated, significantly long *in vivo* circulation is found, as its half-life is about 65 h [30]. However, no further improvement in therapeutic efficacy is achieved by Lipo-dox. When investigated in patients with metastatic breast cancer, Lipo-dox induced the overall response rate of 41.2%, the median time to disease progression of 163 days, and the median duration of response in responding patients of 286 days, all of which were similar to those of Doxil [31]. Moreover, the major side effects of Lipo-dox were stomatitis and skin toxicity, which were also found during Doxil treatment [5,29]. In fact, although the cardiotoxicity is reduced by PEGylation, the long circulation time often results in skin toxicity referred as Palmar Plantar Erythrodysthesia (PPE) [5], which is a drawback of PEGylated doxorubicin-loaded liposomes and remains to be overcome.

3. Clinical studies of liposomal formulations

Some liposomal formulations currently under clinical trials are summarized in Table 2. Model drugs range from conventional chemotherapeutic agents to lipopeptide. But the application of newly developed liposomes is more and more focused on cancer therapy. In addition, synthetic lipids are more widely used.

3.1. *Paclitaxel liposomes*

Paclitaxel is a potent anti-cancer drug used in various tumors, including ovarian, breast and non-small-cell lung carcinoma [57]. Due to the poor water solubility of paclitaxel, the surfactant Cremophor EL is used in its marketed product Taxol. However, Cremophor EL increased the toxicity and led to hypersensitivity

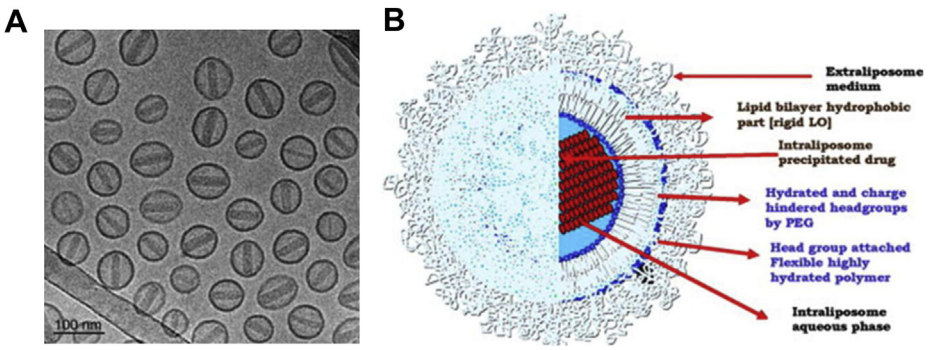

Fig. 1 – (A) Morphology of Doxil by cryo-TEM. (B) Illustration of the structure of Doxil [5].

reactions in patients [58]. Therefore, LEP-ETU, a liposomal formulation of paclitaxel, was developed to eliminate the severe toxicity of Taxol. In a Phase I pharmacokinetic/pharmacodynamic study, the maximum tolerated dose of LEP-ETU was 325 mg/m^2, which was higher than the typical dose range of 135–200 mg/m^2 permitted with Taxol [33]. Neutropenia was found to be the major side effect of LEP-ETU, but it was not worse than that observed with Taxol treatment [59]. Therefore, LEP-ETU achieved higher doses of paclitaxel treatment with neither the allergic reaction nor new severe toxicities.

EndoTAG-1 is another liposomal formation of paclitaxel. The cationic lipid, DOTAP, is firstly used for constructing liposomal paclitaxel. In addition to the anti-tumor effect of paclitaxel, EndoTAG-1 also inhibits tumor vasculature, which is possibly due to the vascular targeting by cationic liposomes [60]. In both orthotopic pancreatic cancer and subcutaneous Lewis lung carcinoma models, EndoTAG-1 was delivered primarily to tumor epithelium, whereas Taxol was distributed in the interstitial region. There was a synergetic effect between EndoTAG-1 and cisplatin chemotherapy, and the metastasis of pancreatic cancer could be inhibited by the combinational therapy of EndoTAG-1 and gemcitabine [61].

3.2. *Cisplatin liposomes*

Cisplatin is another widely used anti-cancer drug, which induces DNA lesions and mitochondrial apoptosis. However, often observed toxicities including nephrotoxicity, neurotoxicity and ototoxicity [62] demand new formulations to be developed to reduce toxicities and potentiate efficacy. SPI-077 is the liposomal cisplatin with long circulating profile. In a Lewis lung tumor model, SPI-077 achieved a 28-fold higher tumor AUC than cisplatin, while a 4-fold reduction of cisplatin delivered to kidneys [63]. Moreover, SPI-077 exhibited the equivalent anti-tumor efficacy at only half the dose of cisplatin. However, in a following Phase I–II study, SPI-077 did not show appreciable efficacy for patients with inoperable head and neck cancer [38].

Lipoplatin is an alternative liposomal formulation of cisplatin. Similar to SPI-077, Lipoplatin also reduces cisplatin-induced toxicities [64]. Besides, Lipoplatin is efficacious against several tumors, such as breast cancer, non-small-cell lung cancer, pancreatic cancer and head and neck cancer. In a recent Phase II study, patients with metastatic breast cancer were treated with Lipoplatin and vinorelbine in combination. The objective response rate and median survival time were 53.1% and 22 months, respectively, while the side effects observed were acceptable [65]. Therefore, this regimen was proposed as a first-line therapy for metastatic breast cancer. In another Phase III study, 202 patients with non-small-cell lung carcinoma were treated with paclitaxel combined with Lipoplatin or cisplatin. It was found the response rate was 59.22% of Lipoplatin group versus 42.42% of cisplatin group. After 18 months, the number of surviving patients was double with Lipoplatin treatment versus cisplatin treatment [66].

Aroplatin encapsulates cis-bis-neodecanoato-trans-R,R-1,2-diaminocyclohexane platinum (II) (L-NDDP), a structural analog of oxaliplatin. It has been investigated in patients with malignant pleural mesothelioma in a Phase II study. Following the intrapleural administration, a response rate of 42% and a median survival time of 11.2 months were observed [41]. In another Phase II study concerning advanced colorectal cancer, both anti-tumor efficacy and tolerable toxicity were found after the intravenous administration of Aroplatin [42].

3.3. *New tendencies in the development of liposomal formulation*

3.3.1. *Environmentally sensitive liposomes in cancer therapy*

High efficiency of drug release from liposomes in tumor tissue is an important premise for successful therapies. Although the size and PEGylation of liposomes have realized the sufficient delivery to tumor, drug release has to be triggered by either inner stimulations such as changes of pH and oxygen level, or outer stimulations such as the local heating. Thermosensitive liposome is a new kind of liposomal formulation, which can immediately release encapsulated drugs in the tumor region with local heating owing to the gel-to-liquid crystalline phase change of lipids used to construct the formulation. This strategy has already been applied to the next generation of doxorubicin-loaded liposomal product, called ThermoDox. DPPC and MSPC are key lipid components of ThermoDox. The T_m of DPPC is 41.5 °C, ensuring a phase change at about 42 °C, a clinically attainable temperature for local hyperthermia [59]. However, the drug release was still relatively slow for liposomes composed of DPPC alone. So, the addition of small amount of MSPC, a kind of lysolipid inducing a slight decrease of T_m but the significant instability of lipid membrane at T_m, could further accelerate drug release [67]. In a previous study using local hyperthermia, ThermoDox was found to achieve higher concentration of doxorubicin in tumor as well as stronger anti-tumor effect than non-thermosensitive liposomes [36]. There is an ongoing Phase III trial for the treatment of hepatocellular carcinoma with ThermoDox [68].

3.3.2. *Liposomal combinations in cancer therapy*

Combination chemotherapy, i.e. the use of drugs with different mechanisms of action and non-overlapping side effects, appears promising in the treatment of malignant cancer and has also been applied to the development of nanomedicines. Two sets of liposomal drug combinations have entered clinical trials. CPX-351 is the liposome product co-encapsulating cytarabine and daunorubicin in a molar ratio of 5:1 for leukemia therapy. This combination ratio was found to achieve the greatest degree of synergy in a panel of 15 tumor cell lines as well as 3 animal models [69]. The maximum tolerated dose and the lowest response dose for the combination have been determined in a completed Phase I trial and there is a Phase II trial ongoing [54].

Another product entering Phase II trial is CPX-1 in which irinotecan HCl and floxuridine are co-encapsulated at 1:1 molar ratio. The liposomal drug combination achieved better response rate (7.7%) and progression-free-survival (3.9 months) than either floxuridine (4%, 2.5 months) or irinotecan (4.2%, 2.6 months) alone for the treatment of advanced colorectal cancer; while the adverse effects were similar to those of irinotecan [55].

3.3.3. *Liposomal vaccines*

Liposomal vaccines, also known as virosomes [70], are constructed with viral surface antigens and synthetic lipids such as

DOPC, DOPE or DPPC, which simulate viral membrane for vaccine delivery. Compared with conventional vaccines, virosomes exhibit the excellent immunogenicity as well as better biocompatibility and safety. To date, two liposomal vaccines, Epaxal and Inflexal V, have been approved for clinical use.

Epaxal is a hepatitis A virus (HAV) vaccine. In a clinical study, the efficacy of Epaxal was compared with a conventional HAV vaccine in infants and children. The seroprotection rate was 100% in all participants after primary vaccination with Epaxal. In conventional vaccine treatment groups, this rate dropped to 67.7% in infants with pre-existing maternal anti-HAV antibodies [71]. Due to the absence of aluminum, which was widely used in conventional HAV vaccines, there were fewer local reactions and side effects caused by Epaxal [72].

Inflexal V is an influenza vaccine which has been used worldwide for fifteen years. In a clinical study involving 453 children, Inflexal V achieved a significantly higher seroprotection rate (88.8%) for H_3N_2 virus than that of a conventional influenza vaccine (78.3%), indicating the better immunogenicity [73]. Moreover, the superior purity and biocompatible nature of Inflexal V constituents resulted in a significantly reduced rate of unwanted side effects [74].

Liposomal vaccines are also investigated in cancer treatment currently. Stimuvax containing BLP25 lipopeptide is a cancer vaccine that targets the MUC1 tumor-associated antigen [75]. In Phase II studies, the 3-year survival rate of patients with non-small-cell carcinoma was 31% for the Stimuvax treatment group and 17% for the control group [76]. No severe toxicities were observed during Stimuvax treatment [77]. Recently, there began a further Phase III study on Stimuvax for the stage III non-small-cell carcinoma [50].

4. Conclusion

Discovered in 1965, liposomal formulations have been investigated for almost half a century. The successful Doxil, which is the first approved liposomal product, has inspired explorations for new liposome delivery systems and brought about various products as well as numerous clinical trials. Conventional chemotherapeutic drugs, such as doxorubicin, paclitaxel and cisplatin, have been developed with liposomal formulations in order to improve efficacy, and more importantly to reduce toxicities. Besides, new technology has continuously been applied to the formulation development process. For example, PEGylation used in Doxil has significantly prolonged the *in vivo* circulation time and improved the tumor delivery; DSPC used in Lipo-dox has enhanced the formulation stability; and DPPC used in ThermoDox has realized a stimulative drug release in tumor with local heating strategy. Moreover, newly developed liposomal combinations and liposomal vaccines have shown promising results in clinical studies. In all, liposomal formulations are still important and successful accesses for the clinical application of nanomedicines.

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